NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Characterising the scale and significance of persistent organic pollutants in South African sediments

Claudine Roos
12568473

Thesis submitted for the degree Doctor of Philosophy in Environmental Sciences at the Potchefstroom Campus of the North-West University

Promoter:	Dr. R. Pieters
Co-promoter:	Prof. H. Bouwman

November 2010

“In an age when man has forgotten his origins and is blind even
to his most essential needs for survival,
water along with other resources has become victim of his
indifference”

~ Rachel Carson: Author - Silent Spring ~

1

Preface

According to the National State of the Environment Report (DEAT, 1999), South Africa is a water-poor country with only 8.6% of its rainfall available as surface water. Groundwater resources are also relatively limited compared to that of other countries. Currently, South Africa's available freshwater resources are nearly fully-utilised and under severe stress. The quantity and quality of available water will increasingly become a limited resource, and supply will become a major factor restricting the country's future socio-economic development. Many of our freshwater environments (rivers, dams and wetlands) are polluted by industrial, domestic and commercial effluents, sewage, acid mine drainage, agricultural runoff and litter, and the majority of South Africa's rivers have eutrophication problems. Poor water quality does not only affect associated sediments and aquatic life, but has an effect on terrestrial ecosystems and even the economy as well. In 2009, South Africa's agricultural sector nearly suffered a severe knock when possible restrictions were announced on exports of fruit and vegetables to key markets due to the concerns about produce being irrigated with contaminated water. Polluted water may also pose significant health threats to recreational and domestic water users.

This study focusses on a group of highly persistent, toxic pollutants, ubiquitous in terrestrial and aquatic environments all over the world. Here, the research characterises the scale and significance of certain organic pollutants (OPs), especially persistent organic pollutants (POPs) in selected water bodies of South Africa, specifically targeting sediments, which are the main reservoirs of these pollutants in aquatic environments.

Acknowledgements

The following institutions contributed financially towards this study:

- The Water Research Commission (WRC) of South Africa (K5/1561),
- The South African/Norwegian Bilateral Scientific Agreement administrated by the National Research Foundation of South Africa and the Research Council of Norway (UID 64489) and
- The National Research Foundation (NRF) (grant-holder linked bursary).

I wish to extend my sincerest gratitude towards the following persons and institutions:

- My supervisor and co-supervisor, Dr. Rialet Pieters and Prof. Henk Bouwman. Thank you for setting a great example. I appreciate your guidance, patience and invaluable contributions.
- The School for Environmental Sciences and Development and its staff.
- The Norwegian Institute for Air Research (NILU), in particular to Prof. Henrik Kylin, Dr. Anuschka Polder and Ellen-Katrin Enge.
- The National Metrology Institute of South Africa (NMISA), especially Mrs. Jayne de Vos.
- My colleague and friend, Miss Laura Quinn, for her assistance and friendship.

A special thanks to my family and friends, especially my husband, Wentzel Roos, and my parent, Danie and Marcelle van Vuuren. Thank you for your love, support and encouragement, and for having faith in my abilities.

Lastly, and most importantly, I owe my deepest and greatest gratitude to my Creator, for presenting me with many great opportunities. Without His blessing, none of this would have been possible.

Soli Deo Gloria.

Summary

Characterising the scale and significance of persistent organic pollutants in South African sediments

Water resources in South Africa are scarce, and should therefore be protected against pollutants, also from persistent organic pollutants (POPs). POPs are a global concern due to their ubiquitous presence, persistence and toxicity. This is emphasised by the Stockholm Convention on POPs, which aims at reducing and ultimately eliminating them. South Africa signed and ratified the treaty, and it became international law on 17 May 2004, but there is still a lack of information regarding POPs in South Africa.

This study focussed on establishing the levels of POPs and other organic pollutants, which included various organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), dioxin-like compounds (DLCs), non-dioxin-like polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether (PBDE). Sampling regions included the industrial cities – Cape Town, Richards Bay, Durban and Bloemfontein, and low-income, high density residential areas surrounding a wetland in Soweto/Lenasia and Botshabelo. Additionally, rivers flowing into neighbouring countries, rivers in the vicinity of paper and pulp producers and high altitude rivers were included. Sediment samples were firstly screened for the presence of DLCs by the H4IIE-*luc* bio-assay, whereafter sites eliciting quantifiable responses were selected for further chemical analysis by high resolution gas chromatography-high resolution mass spectrometry (HRGC/HRMS).

Of the 96 sites, only 23 had quantifiable levels of DLCs. These sites were mainly of industrial, semi-industrial or low-income residential nature. PAHs were the predominant class of compounds at most of the sites, while OCPs and PCBs were present in moderate concentrations and PBDEs in minor concentrations. The concentration of pollutants measured in South African soils and sediments were intermediate when compared to the levels measured in some European, Asian and Scandinavian countries, with the exception of a few sites where exceptionally high levels of compounds were measured. The carbon content normalized concentrations of certain compounds at some of the sites exceeded the Canadian sediment quality guidelines. The estimated cancer risk associated with dermal absorption of OCPs measured in this study was negligible when compared to the background cancer risk expected for South Africans due to life style factors. However, it was estimated that dermal exposure to PCBs, DLCs and PAHs may lead to severe increases in cancer cases, and may seriously impact on human health.

***Keywords**: Persistent Organic Pollutants, South Africa, sediment, H4IIE-luc bio-assay, HRGC/HRMS.*

Opsomming

Karakterisering van die graad en belangrikheid van persisterende organiese besoedelstowwe in Suid-Afrikaanse sediment

Waterhulpbronne in Suid-Afrika is skaars en moet daarom teen besoedelstowwe, ook teen persisterende organiese besoedelstowwe (POBs), beskerm word. Hierdie stowwe wek wêreldwye kommer as gevolg van hulle alomteenwoordigheid, blywendheid en toksisiteit. Die belang van die probleem word onderstreep deur die Stockholmkonvensie vir POBs, wat dit ten doel stel om POBs te verminder, en uiteindelik te elimineer. Suid-Afrika het die verdrag onderteken en bekragtig, en op 17 Mei 2004 het die Konvensie internasionale wet geword, maar daar is steeds ’n tekort aan inligting met betrekking tot POBs in Suid-Afrika.

Hierdie studie het daarop gefokus om die vlakke van POBs en ander organiese besoedelstowwe soos verskeie organochloor pestisiede (OCPe), polisikliese aromatiese koolwaterstowwe (PAKe), dioksienagtige verbindings, nie-dioksienagtige poligechloreerde bifeniele (PCBe) en poligebromineerde difeniel-eters (PBDEs) te bepaal. Sedimentmonsters is uit verskeie industriële stede en lae-inkomste, hoë-digtheid nedersettings versamel. Die volgende gebiede is tydens die studie geteiken: Kaapstad, Richardsbaai, Durban, Bloemfontein, ’n vleiland wat deur Soweto en Lenasië vloei, en Botshabelo. Verder is riviere wat na buurlande vloei, wat naby aan papier-en-pulp industrieë geleë is, en riviere wat hoog bo seevlak geleë is, ook geteiken. Sedimentmonsters is eerstens met die H4IIE-*luc* biosiftingstoets vir die teenwoordigheid van dioksienagtige verbindings geanaliseer, waarna die monsters wat kwantifiseerbare lesings met die siftingstoets gelewer het, weggestuur is vir verdere chemiese analise met hoë resolusie gaschromatografie en massaspektrometerie (HRGC/HRMS).

Van die 96 monsters het slegs 23 kwantifiseerbare vlakke van dioksienagtige verbindings gehad. Hierdie monsters is hoofsaaklik van industriële, deels-industriële of lae-inkomste residensiële oorsprong. PAKe het die hoogste vlakke by die meeste van die monsternemingsareas verteenwoordig, terwyl die OCPe en PCBe in matige konsentrasies, en PBDEs in lae konsentrasies teenwoordig was. In vergelyking met ander Europese, Asiese en Skandinawiese lande, was die vlakke van die besoedelstowwe in Suid-Afrikaanse sediment gematig, behalwe vir ’n paar van die monsternemingsareas waar vlakke van besoeldelstowwe uitermate hoog was. Die koolstof genormaliseerde konsentrasies van sekere verbindings was hoër as die kwaliteitsriglyne vir Kanadese sediment. Die geskatte kankerrisiko geassosieer met die dermale absorpsie van die OCPe gemeet in hierdie studie, is heelwat laer as die normale kankerrisiko wat as gevolg van lewensstylfaktore vir Suid-Afrikaners verwag word. Die kankerrisiko geassosieer met die PCBe, dioksienagtige verbindings en PAKe is egter aansienlik hoër en mag ernstige gesondheidsgevolge hê.

<u>Sleutelwoorde</u>:* *Persisterende Organiese Besoedelstowwe, Suid-Afrika, sediment, H4IIE-luc biosiftingstoets, HRGC/HRMS.

Acronyms and abbreviations

Please take note that a separate fold out sheet with the site abbreviations is added to the back of this thesis. Site abbreviations are, however, also included in the alphabetical list to follow.

A

ACGIH	American Conference of Governmental Industrial Hygienists
AFskin	Dermal absorption factor
AhR	Aryl hydrocarbon receptor
Arnt	Aryl hydrocarbon nuclear translocator
ASE	Accelerated Solvent Extractor
ATSDR	Agency for Toxic Substances and Disease Registry

B

B(a)P-EQs	Benzo(a)pyrene equivalents
BF	Bloemfontein (sampling sites)
BFR	Brominated flame retardants
BO	Botshabelo (sampling sites)
BM	Body mass

C

CBD	Central business district
CCME	Canadian Council of Ministers of the Environment
CLRTAP	Convention on Long-range Transboundary Air Pollution
COP	Conference of the Parties
CR	Cancer risk
Cs	Concentration of carcinogenic substance in sediment
Croc	Crocodile River (sampling sites)
CSIR	Council for Scientific and Industrial Research
CT	Cape Town (sampling sites)
CV	Coefficient of variation

D

D	Durban (sampling sites)
D_1	Days per week exposed to carcinogenic substance
D_2	Weeks per year exposed to carcinogenic substance
D_3	Total years exposed to carcinogenic substance
DAD	Dermal absorbed dose
DCM	Dichloromethane
DDA	Bis(dichlorodiphenyl) acetic acid
o,p'-DDD	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
p,p'-DDD	1,1,-Dichloro-2,2-bis(*p*-chlorophenyl)ethane
o,p'-DDE	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene
p,p'-DDE	1,1-Dichloro-2,2-bis(*p*-chlorophenyl)ethylene
o,p'-DDT	1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane
p,p'-DDT	1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane
DEAT	Department of Environmental Affairs and Tourism
DLCs	Dioxin-like compounds
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethyl sulphoxide
DRE	Dioxin response element
Drknberg	Drakensberg rivers (sampling sites)
dw	dry weight
DWAF[1]`	Department of Water Affairs and Forestry
DWA	Department of Water Affairs

E

EC_{20-80}	Effective concentrations eliciting 20%, 50% and 80% response in cells
ECHA	European Chemical Agency
EDC	Endocrine disrupting chemicals
EF	Exposure frequency
EU	European Union

F

FBS	Foetal bovine serum

---

[1] Please note: The Department of Water Affairs and Forestry (DWAF) is now known as the Department of Water Affairs (DWA).

G

GC	Gas chromatography
GC/MS	Gas chromatography/mass spectrometry
GC-TOF-MS	Gas chromatography-time of flight-mass spectrometry
GDP	Gross domestic product
GHS	Globally Harmonized System
GPC	Gel permeation chromatography

H

HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HEPA	High efficiency particulate air
HEPES	[4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid] buffer
HMM-PAH	High molecular mass polycyclic aromatic hydrocarbons
HPLC	High pressure liquid chromatography
HRGC/HRMS	High resolution gas chromatography high resolution mass spectrometry
HSP	Heat shock proteins

I

IARC	International Agency for Research on Cancer
INCHEM	International Programme on Chemical Safety
ISQG	Interim sediment quality guidelines
I-TEFs	International toxic equivalency factors
IUPAC	International Union of Pure and Applied Chemistry

K

Komati	Komati River (sampling sites)
KZN Riv	KwaZulu-Natal rivers (sampling sites)

L

LE	Life expectancy
LEL	Lowest effect level
Lim	Limpopo River (sampling sites)
LMM-PAHs	Low molecular mass polycyclic aromatic hydrocarbons

LOD	Limit of detection
logK_{OW}	log *n*-octanol-water coefficient
M	
MS	Mass spectrometry
MTT	3-[4,5-dimethyltiazol-2yl]-2,5-diphenyl tetrazolium bromide
N	
NADH	Nicotinamide-adenine dinucleotide
NADPH	Nicotinamide-adenine dinucleotide phosphate
ND	Not detected
NILU	Norwegian Institute for Air Research
NMISA	National Metrology Institute of South Africa
NTP	United States National Toxicology Program
O	
OCPs	Organochlorine pesticides
Olif	Olifants River (sampling sites)
OXC	Oxidizable organic carbon
P	
PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenylether
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzo-*p*-dioxins
PCDF	Polychlorinated dibenzofurans
PEL	Probable effect level
Pongola	Pongola River (sampling sites)
POPs	Persistent Organic Pollutants
R	
RB	Richards Bay (sampling sites)
REP	Relative potency
RLU	Relative light/luminescence units

S

SA	South Africa
SA_H	Skin surface area exposed available for contact.
SCCP	Short-chained chlorinated paraffins
SCPOPs	Stockholm Convention on Persistent Organic Pollutants
SEL	Severe effect level
S/L	Soweto/Lenasia (sampling sites)
SL_H	Sediment loading to exposed skin
SvC	Solvent control

T

TCDD	2,3,7,8-tetrachloro dibenzo-*para*-dioxin
TCDD-EQ	TCDD-equivalent
TEF	Toxic equivalency factors
TEQ	Toxic equivalent quotient
TOC	Total organic carbon

U

UNEP	United Nations Environment Programme
US EPA	United States Environmental Protection Agency
UV	Ultra violet

W

WHO	World Health Organization
WHO-TEFs	World Health Organization toxic equivalency factors
WWTW	Waste water treatment works

Table of contents

Chapter 4 – Materials and Methods 51 - 89

2

Introduction

2.1. South Africa's water crisis

South Africa is an arid to semi-arid region that receives a mean annual rainfall of less than 500 mm, compared to a world mean of approximately 860 mm (South African Weather Service, 2009). The Department of Water Affairs and Forestry (DWAF; now the Department of Water Affairs - DWA) predicted that the water demand of the country will exceed the water supply by 2040 (DWAF, 1986), but others estimate that it might happen by as early as 2025 (Institute for Futures Research, 2009), or even by 2013 (Ogutu, 2007) if the current demand situation persists. It is not only the quantity of water that is important, but the quality of water is of equal significance. Anthropological practices generating pollutants could and does have an immense impact on water quality, and it is therefore vital that this scarce resource is protected, to prevent a severe water crisis in the country.

In South Africa, many water quality surveys have been conducted, mainly focusing on trace elements and heavy metals such as copper, arsenic, mercury and lead (Binning & Baird, 2001; Jackson *et al*., 2001; Pretorius *et al*., 2001; Van Aardt & Erdmann, 2004), and other industrial and agricultural pollutants (Du Preez *et al.,* 2005; Gravelet-Blodin *et al*., 1997), but not much is known about organic pollutants, especially persistent organic pollutants (POPs).

2.2. Persistent organic pollutants (POPs)

POPs are stable, toxic compounds that persist in the environment by resisting photolytic, biological and chemical degradation. Many POPs can be lethal in high concentrations, but their greatest detrimental effects lie in their chronic toxicity, leading to dermal effects, liver and kidney disease, defects of the immune-, reproductive-, nervous-, and endocrine systems, and even cancer (Schecter *et al.*, 2006). As a result of their lipophilic nature, these pollutants tend to accumulate in matrices rich in organic matter, such as soil, sediment and biota, and can bio-accumulate in food webs (Schecter *et al.*, 2006). Their physical and chemical characteristics enable the compounds to undergo long-range transport, allowing the pollutants to become widely distributed geographically, even to regions where they have never been used or produced (Ritter *et al.,* 2005).

POPs have been a global focus of social and scientific concern, and to take action against these pollutants, the United Nations Environment Programme (UNEP) initiated the Stockholm Convention on Persistent Organic Pollutants (SCPOPs) in May 1995 (UNEP, 2001).

2.3. The Stockholm Convention on POPs (SCPOPs)

The SCPOPs is an international, legally binding treaty initially focusing on the reduction and elimination of the twelve most harmful POPs, also known as the “dirty dozen” (UNEP, 2001). These POPs include certain chlorinated pesticides [aldrin, chlordane, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (DDT), dieldrin, endrin, heptachlor, mirex and toxaphene], two groups of industrial chemicals, hexachlorobenzene (HCB) and polychlorinated biphenyls (PCB), and unintentional combustion by-products known as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (UNEP, 2005a). At its fourth meeting held from 4 to 8 May 2009, the Conference of the Parties (COP) adopted amendments to Annexes A, B and C of the SC POPs to list nine additional chemicals as POPs. These include α-hexachlorocyclohexane (HCH), β-HCH and γ-HCH (lindane), chlordecone, hexabromobiphenyl, hexa- and heptabromodiphenyl ether, pentachlorobenzene, perfluorooctane sulonic acid, its salts and perfluorooctane sulfonyl fluoride, and tetra- and pentabromodiphenyl ether (of the polybrominated diphenyl ether – PBDE family).

Almost all of these pollutants have been banned in most countries of the world, although the use of DDT still occurs in developing countries, among them South Africa, mainly for the control of malaria. There are, however, many sites that are already contaminated with these chemicals that continue to release POPs to the environment (UNEP, 2005a).

The SCPOPs is best understood as having five essential **objectives** or **aims**:

- The first aim of the Convention is to **terminate** the **release and use** of the POPs included in the SCPOPs. The Convention **bans and limits** the **production and use** of the **intentionally produced POPs** and it aims at **reducing releases** of the **unintentionally produced POPs,** which are formed as by-products of combustion and industrial processes (UNEP, 2005a).

- Secondly, the Convention **supports** the **replacement** of **harmful POPs** with **safer**, **cost-effective alternatives**. This process may pose a challenge to developing countries, as they may lack the financial and technological resources to use and manufacture safer chemicals and develop or implement new techniques or technologies. The Convention calls on developed nations to share their knowledge and lend financial support to developing countries in aiding their transition to more suitable alternatives (UNEP, 2001).

- In addition to the POPs listed in Section 2.3 of this thesis, there might be other POPs harming human health and the environment. The third aim of the SCPOPs is to **identify** these **additional POPs** and to aim at the reduction and elimination of these substances (UNEP, 2005a). A formal body of the Convention, the POPs Review Committee, has several other compounds on their agenda considered for inclusion in the SCPOPs, which include short-chained chlorinated paraffins (SCCPs) and endosulfan, amongst others (SCPOPs, 2009).

- Fourthly, the Convention aims at **cleaning up stockpiles** and **equipment containing POPs**. Stockpiles and waste sites should be identified and managed in an environmentally safe manner (UNEP, 2005a).

- The fifth aim of the Convention is to **increase public awareness** and **provide information regarding these pollutants** through educational programmes and other national action plans. The Convention calls on industries, public interest groups, politicians and scientists to work together to establish a global partnership as a component of the SCPOPs (UNEP, 2005a).

South Africa played a major role in the negotiations and implementation of the SCPOPs. The final text of the Convention (SCPOPs, 2009) was successfully negotiated in Johannesburg in December 2000, and on 22 to 23 May 2001 the world's governments held a conference in Stockholm, Sweden, and adopted the SCPOPs. South Africa ratified the treaty on 4 September 2002, and the Convention entered into force on 17 May 2004 (Bouwman, 2004).

As a party to the Convention, South Africa is legally obligated to abide by the objectives of the treaty, and is encouraged to support research on POPs.

Despite the fact that South Africa has the largest economy and industrial base in Africa (World Bank, 2009), the levels of organic pollutants in the environment, biota, and humans are not well-known. Some research have been done on the pesticide POPs, such as DDT (Bouwman *et al.,* 2006), and the intentionally produced PCBs (Greichus *et al*., 1977; Grobler *et al.,* 1996), but there is still much to learn about POPs in the country. This study will therefore contribute towards a better understanding of POPs and other organic pollutants in major South African fresh- and estuarine waters.

2.4. Scope and aims of the study

The main aim of this study is to assess the scale and significance of certain organic pollutant and POP pollution in South African waters. Previous studies have indicated the presence of some POPs and other organic pollutants in South African soils and sediments, with the highest levels of pollutants mainly associated with industrial and high-density residential areas (Vosloo & Bouwman, 2005; Nieuwoudt, 2006; Nieuwoudt *et al*., 2009; Quinn *et al*., 2009). Thus, the current study focusses on water bodies located in these areas.

Water bodies from several provinces were targeted to be representative of South Africa. The sampling regions included Soweto and Lenasia (Gauteng), Cape Town (Western Cape), Durban and Richards Bay (KwaZulu-Natal), and Bloemfontein and its associated high-density, low-income residential area, Botshabelo (Free State). The scope of the study also included high altitude rivers to assess the role of long-range transport (the Mzimkhulu- and Mkomazi Rivers in the Drakensberg), and rivers in the vicinity of paper and pulp manufacturers, a known source of POP pollution (the Mhlathuze-, Tugela- and Umvoti Rivers). International rivers shared with South Africa and neighbouring countries (the Limpopo- Olifants-, Crocodile-, Komati- and Pongolo Rivers and their tributaries) were also targeted to estimate the scale of POP transport from South Africa to certain adjacent countries. Detailed descriptions of the sampling sites follow in Sections 4.1 and 4.2.

Because POPs tend to associate with particulate matter and accumulate in sediment rather than staying in solution in water, sediment, and in some instances associated soil samples, were collected and analysed. The analytes included in the study are the organochlorine pesticides (OCPs), hexachlorobenzene (HCB), HCH, aldrin, heptachlor, chlordane and its metabolites oxychlordane, and chlordene, nonachlor, mirex, DDT and its metabolites DDD and DDE; and the industrially associated pollutants, PBDEs, PCBs, dioxin-like compounds (PCDDs, PCDFs and dioxin-like PCBs) and PAHs.

For some of the compounds that were measured, such as the brominated flame retardant class, PBDE, it was the first such analysis done for sediment in South Africa. The majority of the pollutants analysed are POPs according to SCPOPs. Although PAHs are not listed in the SCPOPs, they are included in the Convention on Long-range Transboundary Air Pollution (CLRTAP), and the sixteen most frequently-occurring and/or dangerous PAHs are classified as priority pollutants by the United States Environmental Protection Agency (US EPA) (Zhang & Tao, 2009). Even though South Africa cannot be part of the CLRTAP (a regional MEA), we should aim to reduce our emissions of these troublesome pollutants.

The aim of the study is to determine the levels of selected organic pollutants, especially POPs, in the aquatic environment of South Africa.

The following objectives were set:

○ To assess the scale of the occurrence of POPs and certain other organic pollutants sediments and associated soils of South Africa.

○ To understand the distribution and fate of selected POPs and certain other organic pollutants in sediments and associated soils of selected aquatic systems.

○ To determine the possible sources of POPs and certain other organic pollutants to sediments and associated soils of selected aquatic systems.

○ To assess the potential effects of POPs and certain other organic pollutants on human health, by determining the potential cancer risk of exposed communities.

3

Literature Review

3.1. Organochlorine pesticides (OCPs)

Of the OCPs included in the study, only HCB, HCH, heptachlor, mirex and DDT were present at measurable levels at the majority of the sites. It is for this reason that only these are treated in the following paragraphs. The physical and chemical properties, sources, environmental fate, and toxicity of HCB, HCH, heptachlor, mirex and DDT are discussed briefly in the sub-sections to follow.

Pre-empting the results in anticipation of the literature review, it can be noted that although the majority of this study's sampling sites were situated in areas where these OCPs have not been applied for many years, residues may still remain due to the persistent nature and long-range transport of these chemicals.

3.1.1. Hexachlorobenzene (HCB)

Until 1965 HCB (C_6Cl_6) was widely used as a fungicide on seed of onions, sorghum, wheat and other grains. It was also used in the production of fireworks, ammunition and synthetic rubber (Sala *et al.*, 2001). Currently, its production is banned in most countries and it is included in the SCPOPs (UNEP, 2005a).

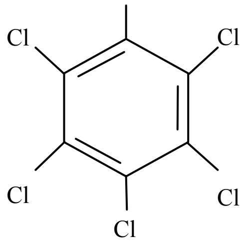


Figure 3.1. Chemical structure of HCB

3.1.1.1. Physical and chemical characteristics

HCB is an organochlorine compound (chlorinated hydrocarbon) (Fig. 3.1) that is widespread in the environment, lipophilic and bio-accumulative. Technical agricultural grade HCB contains 98% HCB, 1.8% pentachlorobenzene and 0.2% tetrachlorobenzene (ATSDR, 2002a). It has a molecular mass of 284.76 and is practically insoluble in water (0.005 mg/l). HCB has a vapour pressure of 2.3×10^{-3} Pa at 25 °C and a log *n*-octanol-water coefficient (log K_{OW}) of 3.93 to 6.42 (ATSDR, 2002a).

3.1.1.2. Sources

Although its use as a fungicide was banned in the 1960's, HCB is still used as an industrial chemical and it is an unintended by-product of several processes, such as during the production of chlorinated solvents (Bailey, 2001). Presently, the major sources of HCB are emissions from metal industries, combustion processes and chemical processes such as perchloroethylene-, chlorobenzene-, and chlorinated organics production (Euro Chlor, 2002). It is also a trace contaminant in certain pesticides and may remain in the environment due to historic use as a fungicide (ATSDR, 2002a).

3.1.1.3. Environmental fate

Since HCB is lipophilic and persistent, the compound is relatively stable in soil with halflives ranging from 2.7 to 7.5 years (Augustijn-Beckers *et al.*, 1994). The compound may degrade aerobically and anaerobically, but its low water solubility causes HCB to have a low mobility in the soil environment. Once in the aquatic environment, HCB is broken down rapidly. Experimental results on hydro-soil have shown almost complete degradation of HCB to pentachlorophenol and related compounds in less than 5 days (Augustijn-Beckers *et al.*, 1994). Breakdown in vegetation also seems to be rapid (only 1% of initial amount remaining after 15 days; Beall, 1976).

3.1.1.4. Toxicity

The most prominent adverse health effects caused by HCB are reproductive toxicity. Jarrel and Gocmen (2000) reported on the effects of HCB on a Turkish population accidentally ingesting HCB-treated seeds. Their most pertinent observation was the absence of children below the age of 5 years in some villages, which would qualify HCB as one of the most potent reproductive toxicants. While some human reproductive health studies have shown a positive correlation between HCB exposure and spontaneous abortion, decreased birth mass, decreased crown-rump length, and reduced gestational period (Fenster *et al.*, 2006; Jarrel *et al.*, 1998; Schade & Heinzow, 1998). Others have reported no or non-linear associations

(Gladen *et al.*, 2003; Khanjani & Sim, 2006; Sagiv *et al.*, 2007). Although no neurological symptoms have been reported for humans, rodents exhibit symptoms such as tremors, paralysis, muscle incoordination, weakness and convulsions at high doses of HCB (Edwards *et al.*, 1991).

Increased lung-, thyroid-, liver- and spleen tumours were noted in animals chronically exposed to HCB, but the potential for the chemical to cause carcinogenic effects in humans is not known (ATSDR 2002a; Edwards *et al.*, 1991).

3.1.2. Hexachlorocyclohexane (HCH)

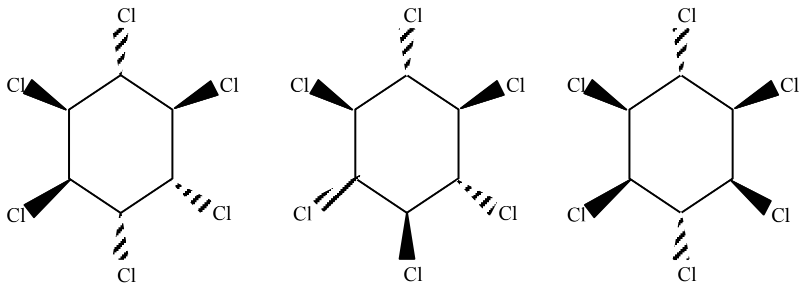
HCH ($C_6H_6Cl_6$) was previously used as an insecticide to control cotton insects, leaf hoppers, stem borers and wireworms on cotton, cereals, sugar beets, oilseed and hardwood logs (INCHEM, 2001). It is also used to treat head and body lice and scabies. The technical product consists of a mixture of isomers (α-, β-, γ-, δ- and ε-HCH) (Fig. 3.2) of which γ-HCH (also known as lindane) is the major component and the only isomer that possesses effective insecticidal activity (Willet *et al.*, 1998). Although not initially included in the SCPOPs (UNEP, 2005a), the α-, β- and γ-isomers have since May 2009 been included in the Convention and should therefore eventually be banned.

3.1.2.1. Physical and chemical characteristics

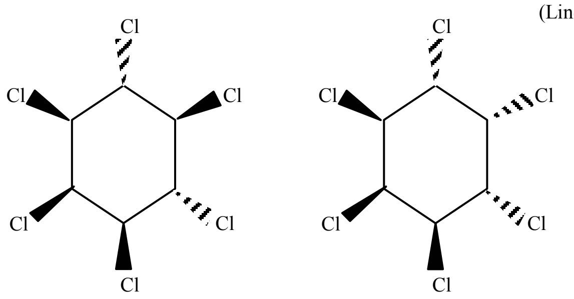
HCH is a cyclohexane substituted with six chlorine atoms (Fig. 3.2). The compound is volatile, hydrophobic, and bio-accumulative (ATSDR, 2007a). Technical HCH is a white or yellowish powder or solid flakes, with a persistent musty odour (INCHEM, 2001). It consists of approximately 40 – 45% of the γ-isomer, and 20 - 22%, 18 – 22%, 4% and 1% of the δ-, α-, β- and ε-isomers respectively (INCHEM, 2001). The isomers are differentiated by variations in the axial-equatorial positions of chlorine around a ring of 6 carbons (Willet *et al.*, 1998) (Fig. 3.2). Compared to other OCPs, HCH is more water soluble and more volatile (Table 3.1).

Table 3.1. Physical and chemical properties of α-, β- and γ-HCH (from Willet *et al.*, 1998).

HCH-isomer	Molecular mass	Water solubility (mg/l)	Vapour pressure (mmHg)	Log K_{OW}
α-HCH	290.8	10	0.02	3.80
β-HCH		5	0.005	3.78
γ-HCH (lindane)		7.3	9.4×10^{-6}	3.61 – 3.72



α-Hexachlorocyclohexane β-Hexachlorocyclohexane γ-Hexachlorocyclohexane (Lindane)



δ-Hexachlorocyclohexane ε-Hexachlorocyclohexane

Figure 3.2. Chemical structures of the HCH-isomers

3.1.2.2. Sources

Despite the restricted use of technical grade HCH and lindane, its residues are still entering the environment. HCH remains in the environment due to its extensive historic use, present use of lindane (for the control of lice and mites) in several countries, unused stockpiles from earlier manufacturing, as well as from leaching from waste disposal sites (ATSDR, 2007a; Bhatt *et al.*, 2009).

3.1.2.3. Environmental fate

HCH is persistent in the environment. In air, HCH can exist in the vapour phase or may be bound to particulate matter, such as soil particulates or dust. It may undergo long-range transport, but the vapour phase is degraded more rapidly than the particulate phase (ATSDR, 2007a). Because of its low polarity, HCH tends to associate with soil and sediment (Andreu & Picó, 2004), where it is persistent (halflife of approximately 15 months), but eventually breaks down to less toxic substances by algae, fungi and bacteria. In the aquatic environment, HCH is stable and resistant to photodegradation (ATSDR, 2007a; Wauchope *et al.*, 1992).

3.1.2.4. Toxicity

γ-HCH is considered to be the most toxic of the HCH-isomers (Smith, 1991). The main target organ of HCH is the central nervous system (CNS). While α- and γ-isomers are CNS stimulants, the β- and δ-isomers are CNS depressants (Gosselin *et al.*, 1984). Acute negative health effects associated with high level HCH exposure include impairment of the CNS, excitation, clonic and tonic convulsions, increased respiratory rate, and hyper-irritability (Smith, 1991). Other health effects associated with HCH exposure include blood disorders, dizziness, headaches, seizures, and changes in the levels of sex hormones (ATSDR, 2007a).

Although experimental results are contradictory, some tests on animals suggest that lindane and other HCH isomers are “reasonably anticipated to be human carcinogens” (ATSDR, 2007a; Smith, 1991).

3.1.3. Heptachlor

Heptachlor ($C_{10}H_5Cl_7$) was extensively used in the 1960s and 1970s to control termites, ants and soil insects on seed grains and crops. It was also used by exterminators and home owners to kill termites. In South Africa, its registration was withdrawn in 1976 (South African Department of Agriculture, 2008) and its use is currently banned in most countries (ATSDR, 2007b).

3.1.3.1. Physical and chemical characteristics

Heptachlor is a chlorinated dicyclopentadine (Fig. 3.3). Technical heptachlor consists of approximately 72% heptachlor and about 28% related compounds, such as *trans*-chlordane and *trans*-nonachlor. Its available formulations included dusts, wettable powders, emulsifiable concentrates, and oil solutions (ATSDR, 2007b).

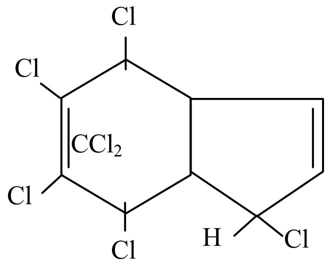


Figure 3.3. Chemical structure of heptachlor

Heptachlor (molecular mass = 373.32) has a water solubility of only 0.056 mg/l, and it is soluble in acetone, alcohol, benzene, cyclohexanone, paraffin and xylene (Kidd & James, 1991). It has a vapour pressure of 3.99×10^{-2} Pa at 25 °C, and a log K_{OW} of between 6.1 and 6.13 (Simpson *et al.*, 1995).

3.1.3.2. Sources

There are no natural sources of heptachlor, but heptachlor epoxide is formed by abiotic or biotic transformation of heptachlor in the environment (WHO, 2006). As with the other banned OCPs, heptachlor still exists in the environment due to historic use, unused stockpiles, and in leachates from disposal sites (ATSDR, 2007b). Additionally, heptachlor is also a component in plywood glues, and a constituent of the pesticide chlordane (which, although banned in most parts of the world, is still used for the control of termites) (WHO, 2006).

3.1.3.3. Environmental fate

Heptachlor is broken down to heptachlor epoxide in the environment. The metabolite is more likely to be found in the environment than heptachlor (ATSDR, 2007b) and is resistant to biodegradation, photolysis, oxidation, and hydrolysis (Smith, 1991).

Heptachlor and heptachlor epoxide are subjected to long-range environmental and biotic transport, and are removed from the atmosphere by wet and dry deposition (WHO, 2006). Soil and sediment are the predominant environmental compartments for heptachlor. Both the parent compound and the metabolites are moderately bound to, and persistent in soils and sediments (Augustijn-Beckers *et al.*, 1994). Depending on the soil type, halflives for heptachlor may range between 150 and 290 days (Augustijn-Beckers *et al.*, 1994). The major route of loss of heptachlor from soil surfaces is via volatilisation. Because heptachlor is almost insoluble in water, it may enter surface waters mainly through surface runoff. In the aquatic environment, heptachlor is rapidly degraded to heptachlor epoxide by hydrolysis and degradation by micro-organisms (Augustijn-Beckers *et al.,* 1994). Volatilisation, adsorption to sediments and photodegradation may also contribute towards the loss or bio-availability of heptachlor and heptachlor epoxide from the water environment (Matsumura, 1985; Smith, 1991).

3.1.3.4. Toxicity

Like most OCPs, heptachlor may interfere with nerve transmission (Ecobichon, 1991). The negative health effects associated with heptachlor epoxide may be greater than the effects associated with heptachlor. Health effects due to exposure to heptachlor or its metabolites may include hyperexcitation of the CNS, liver damage, lethargy, tremors, convulsions, stomach cramps, and coma (ATSDR, 2007b, Smith, 1991). Exposure to heptachlor or heptachlor epoxide may also cause reproductive effects. Studies have shown infertility and improper development of offspring in mice and rats (Smith, 1991). Some experiments suggest that heptachlor may promote the development of tumours in rats (Smith, 1991), but evidence is insufficient to assess the potential of heptachlor to cause cancer in humans.

3.1.4. Mirex

Mirex ($C_{10}Cl_{12}$) is a chlorinated hydrocarbon that was used as an insecticide to control fire ants and leaf cutter ants (mostly in South America), mealybugs (Hawaii), and harvester termites (South Africa; ATSDR, 1995) . Its use was prohibited in 1976 by the US EPA and it is included as a POP in the Stockholm Convention (UNEP, 2005a).

3.1.4.1. Physical and chemical characteristics

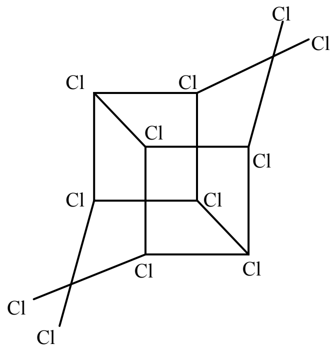


Figure 3.4. Chemical structure of mirex

Similar to the other OCPs, mirex is persistent, toxic, and resistant to degradation (ATSDR, 1995). The compound is a white crystalline solid, which is a derivative of cyclopentadiene (C_5H_6). It is odourless, inflammable, and practically insoluble in water (0.6 mg/l at 25 °C; Kenaga, 1980). It is, however, soluble in dioxane, xylene, benzene and methyl ethyl ketone (ATSDR, 1995). Mirex has a vapour pressure of 3 x 10^{-7} mmHg at 25 °C and a log K_{OW} of 5.28 (Verschueren, 1983).

3.1.4.2. Sources

Mirex does not occur naturally in the environment. It is produced by the dimerization of hexachlorocyclopentadiene in the presence of an aluminium chloride catalyst (Sittig, 1980). Although mirex is mostly known for its insecticidal properties, it was also extensively used as a flame retardant in plastics, rubber, paint, paper and electrical equipment (ATSDR, 1995). Its use as an insecticide and fire retardant was banned in the 1970's, but residues of this compound may still remain in the environment due to historical use, disposal, accidental spillages, fires, and volatilisation or leaching from old stockpiles.

3.1.4.3. Environmental fate

Mirex binds strongly to organic matter in soil, sediment and water. When bound to particulate matter, it can be transported for long distances before partitioning into a different phase. Adsorption and volatilisation are the most important environmental fate processes for mirex, while atmospheric transport may also play a role (ATSDR, 1995). Due to its lipophilic nature (high log K_{OW}) and persistence, mirex is bio-accumulated and bio-magnified in food webs.

The compound is resistant to chemical and biological degradation in soil and sediment (half life of >10 years). The primary process responsible for the degradation of mirex (to photomirex) is photolysis (Carlson *et al*., 1976). During anaerobic degradation mirex in soil and sediment are dechlorinated to the monohydro- derivative, while aerobic biodegradation plays a minor role (Carlson *et al.*, 1976).

3.1.4.4. Toxicity

Data on human health effects is lacking. Animal studies linked mirex exposure to harmful effects on the stomach, intestines, liver, kidneys, eyes, thyroid, nervous system and reproductive system (ATSDR, 1995). In rats, mirex exhibits toxic effects on foetuses, including cataract formation and liver hypertrophy (UNEP, 2002). It is classified as a Group 2B possible human carcinogen by the US EPA, but the few experimental results are inconclusive.

3.1.5. DDT

Although its use was banned in 1983 (South African Department of Agriculture, 2008), the application of DDT is still permitted in certain parts of South Africa to control the disease carrying mosquito, *Anopheles sp*., the vector of the malaria parasite (Bouwman, 2004).

3.1.5.1. Physical and chemical characteristics

DDT ($C_{14}H_9Cl_5$), DDE ($C_{14}H_8Cl_4$) and DDD ($C_{14}H_{10}Cl_4$) are organochlorine substances consisting of two attached aromatic phenyl rings with chlorine atoms covalently bonded in the *ortho*- or *para* positions (Fig. 3.5). Commercial DDT is a mixture of these closely related compounds, with *p,p'*-DDT being the principal component (65 to 80%), and *o,p'*-DDT and *p,p'*-DDD present in smaller amounts (15 to 21%, and about 4%, respectively; Beard, 2006). In its pure form, DDT is a colourless crystalline solid with a weak, chemical odour (ATSDR, 2002b). The pesticide is available in several different forms including aerosols, dustable powders, emulsifiable concentrates, granules, and wettable powders (ATSDR, 2002b).

DDT has a low volatility and high log K_{OW} values. DDT and its metabolites are insoluble in water, making these chemicals persistent in soils and aquatic sediments (Table 3.2; ATSDR, 2002b). It is lipophilic and soluble in most organic solvents, fats and oils, and therefore has the potential to bio-concentrate and bio-accumulate in humans and biota (Beard, 2006; Zhu *et al.,* 2005). DDT is stable under most environmental conditions and relatively resistant to degradation. Its less toxic metabolite, DDE, has a stability equal to, or greater than, the parent compound. Half lives reported for DDT range between 2 and 15 years for soil and as much as 150 years in the aquatic environment (ATSDR, 2002b; Hooper *et al.*, 1997).

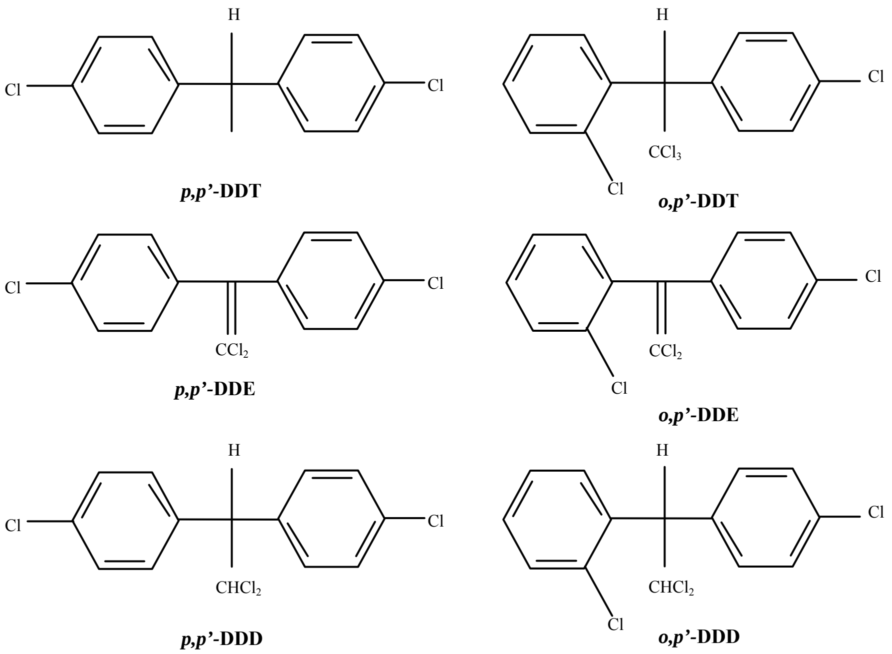


Figure 3.5. Chemical structures of *o,p'*- and *p,p'*-DDT, -DDE and –DDD

Table 3.2. IUPAC names and physical and chemical properties of DDT and its metabolites (adapted from ATSDR, 2002b)

Compound	IUPAC name	Molecular mass	Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
p,p'-DDT	1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)-ethane	354.49	0.025	1.6×10^{-7}	6.91
o,p'-DDT	1,1,1-Trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-ethane	354.49	0.085	1.1×10^{-7}	6.79
p,p'-DDE	1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene	318.03	0.12	6.0×10^{-6}	6.51
o,p'-DDE	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene	318.03	0.14	6.2×10^{-6}	6.00
p,p'-DDD	1,1,-Dichloro-2,2-bis(*p*-chlorophenyl)-ethane	320.05	0.09	1.35×10^{-6}	6.02
o,p'-DDD	1,1-Dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane	320.05	0.1	1.94×10^{-6}	5.87

3.1.5.2. Sources

DDT is produced by the reaction of chloral (CCl_3CHO) and chlorobenzene (C_6H_5Cl) in the presence of sulphuric acid as catalyst (ATSDR, 2002b). It was first synthesized in 1874 by a chemist named Zeidler, but its insecticidal properties were only discovered in 1939 by P.H. Mueller (US EPA 1975; WHO, 1979). DDT was initially used by the military during the second World War for public health purposes to control malaria, typhus, body lice, and bubonic plague (WHO, 1979). In addition to its public health uses, DDT was also applied to a variety of food crops, including beans, cotton, soybeans, sweet potatoes, peanuts, cabbage, tomatoes, cauliflower, corn, and other crops (Casida & Quistad, 1998).

Due to the concern over carcinogenicity, bio-accumulation, and adverse health effects on wildlife (Kumar *et al*., 2008; Lee *et al*., 2001), the use of DDT is prohibited in most countries, but is still legally manufactured for the use in malaria-endemic areas. In South Africa, the widespread use of DDT was banned in in the early 1980's (DEAT, 2005), but it is currently applied for malaria vector control in confined areas in the northern and eastern parts of Limpopo, the north-eastern parts of Mpumalanga and northern KwaZulu-Natal (Bouwman *et al*., 1992; Coetzee & Hunt, 1998; Sharp & Le Sueur, 1996). Most of the DDT found in the environment in areas where use had been banned is due to the persistent nature of the chemical. Traces of DDT measured in areas where the substance has never been applied or produced can be ascribed to the compound's potential for long-range transport (Gong *et al*., 2007; Hung *et al*., 2007).

Because the processes used to synthesise DDT and dicofol are similar, dicofol is often contaminated with DDT. Dicofol, a non-systemic acaricide used for the control of mites on crops and orchards, is still registered for use in South Africa and could therefore be an additional source of DDT contamination (Clark *et al*., 1990; Qiu *et al.,* 2005).

3.1.5.3. Environmental fate

DDT is persistent in the environment, and because it tends to associate with organic matter, DDT is relatively immobile in soils. Routes of loss and degradation in the terrestrial environment include runoff, volatilisation, photolysis, and biodegradation (Beard *et al*., 2000). However, this will only happen over long periods of time (ATSDR, 2002b). DDE and DDD are major metabolites and breakdown products of DDT in the environment. The metabolites are also persistent and their physical and chemical characteristics are similar to that of DDT (ATSDR 2002b, Table 3.2). DDT released into water adsorbs to particulate matter in natural water, and sediment is the main sink for DDT in the aquatic environment (Zeng *et al.,* 1999). Its lipophilic property, combined with a long halflife, is responsible for its high potential for bio-accumulation in aquatic organisms. DDT progressively bio-magnifies in food webs (Ford & Hill, 1991). DDT, DDE, and DDD present in water may be transformed by hydrolysis, photodegradation, and biodegradation (Coulson, 1985).

DDT enters the atmosphere via emission or volatilisation. Volatilisation of DDT, DDE, and DDD is known to account for considerable losses of these compounds from soil surfaces and water (Wania & MacKay, 1993). Volatilisation loss will depend on the amount of DDT applied, proportion of soil organic matter, proximity to the soil-air interface (depth), and the amount of sunlight (Zhu *et al.,* 2005). In the atmosphere, approximately 50% of DDT is adsorbed to particulate matter and 50% exists in the vapour phase (Bidleman, 1988). Long-range transport of DDT in the atmosphere is dependant on airflow patterns. DDT is removed from the atmosphere by precipitation, wet and dry deposition, and diffusion into water bodies, or degraded photochemically by hydroxyl radical reactions (Woodwell *et al*., 1971).

3.1.5.4. Toxicity

DDT is slowly transformed to DDE and DDD in the human body. Although DDD is excreted rapidly, DDE and DDT are stored in the fatty tissue, excreted slowly and may bring about adverse health effects. DDT and its metabolites are ultimately transformed into bis(dichlorodiphenyl) acetic acid (DDA) and excreted via the urine (ATSDR, 2002b).

Acute effects due to low to moderate exposure to DDT may include nausea, diarrhoea, increased liver enzyme activity, irritation, depression, and excitability. Higher doses may lead to tremors and convulsions (Van Ert & Sullivan, 1992). Studies on experimental animals have shown that DDT may cause chronic effects on the nervous system, liver, kidneys, and immune system (ATSDR, 2002b). There is also evidence that DDT may cause reproductive effects due to endocrine disruption (Zeng *et al*., 1999). According to Bornman and co-workers (2009) a study conducted during 2004 to 2006 in the Limpopo Province in South Africa revealed that women who lived in villages sprayed with DDT gave birth to 33% more boys with urogenital birth defects than women in unsprayed villages. Studies on rats and mice have shown decreased embryo implantation, miscarriage and decreased foetal mass as a result of DDT exposure (Chowdhury *et al*., 1990). It appears that DDT may have the potential to cause genotoxic effects in humans. Blood cell cultures of men occupationally exposed to DDT showed an increase in chromosomal damage (ATSDR, 2002b). The evidence regarding the carcinogenicity of DDT is unclear. It has been shown to cause increased production of tumours of mainly the liver and lung in test animals. Significant association between DDT exposure and pancreatic cancers in chemical workers has been found (ATSDR, 2002b).

DDT has also been shown to have negative impacts on animals, especially birds, where it was directly linked to eggshell thinning, and it is toxic to many aquatic invertebrate species (Beard, 2006).

3.2. Industrially associated organic pollutants

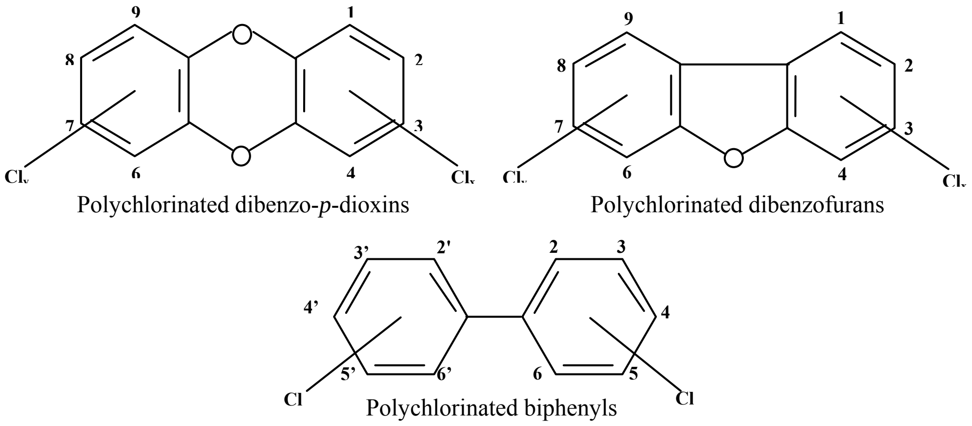
3.2.1. Unintentionally produced organic pollutants

PCDDs ($C_{12}H_{8-x}Cl_xO_2$), PCDFs ($C_{12}H_{8-x}Cl_xO$) and co-planar PCBs ($C_{12}H_{10-x}Cl_x$), collectively known as dioxin-like compounds (DLCs), as well as PAHs, are not currently produced deliberately for any purpose except scientific research. They are formed as by-products of incomplete combustion during industrial and thermal processes (Schecter *et al.*, 2006). PCBs were manufactured for industrial purposes since the early 1930s, but their production and use were banned in the 1980s. The deliberately produced non-dioxin-like PCBs (discussed in section 3.2.2.2) are still released into the environment due to historical use and releases from stockpiles, while dioxin-like PCBs are formed unintentionally similarly to PCDD/Fs (Koppe & Keys, 2001). The physical and chemical characteristics, sources, environmental fate, and toxicity of DLCs (PCDD/Fs and PCBs) and PAHs are discussed in sections 3.2.1.1 and 3.2.1.2.

3.2.1.1. Dioxin-like compounds: Polychlorinated dibenzo-*p*-dioxins (PCDDs), -dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (PCBs)

3.2.1.1.1. Physical and chemical characteristics

PCDDs and PCDFs are two related groups of planar tricyclic compounds with similar chemical structures (Fig. 3.6) and properties. They consist of twelve carbon atoms forming two aromatic phenyl rings, attached to one another by two oxygen bonds in dioxins and by one oxygen bond and one carbon-carbon bond in furans. Both PCDDs and PCDFs may contain between one and eight chlorine atoms in the hydrogen atom position (Fig. 3.6) (Schecter *et al.*, 2006). PCBs are aromatic compounds formed by two benzene rings bonded by a single carbon-carbon bond. The hydrogen atoms on the biphenyl molecule may be replaced by one to up to ten chlorine atoms. The two benzene rings can rotate along the carbon-carbon bridge axis, enabling PCBs to assume a propeller-like conformation or a co-planar conformation similar to PCDDs (Fig. 3.6) (Schecter *et al.*, 2006). Co-planar PCBs will also be referred to as “dioxin-like PCBs” from hereon.



Polychlorinated dibenzo-*p*-dioxins

Polychlorinated dibenzofurans

Polychlorinated biphenyls

Figure 3.6. Chemical structures of PCDDs, PCDFs and PCBs

The physical and chemical characteristics (Table 3.3), and therefore also the fate and toxicity of these compounds, are determined by the number and structural position of chlorine atoms on the molecules. DLCs are generally relatively insoluble in water, sorb strongly to soil and organic matter and have a high potential for bio-accumulation.

Table 3.3. Physical and chemical properties of PCDD/Fs and PCBs (information from Henry & De Vito, 2003; Sinkkonen & Paasivirta, 2000; Syracuse, 2007; Whylie *et al.*, 2003)

PCDD/F and PCB congeners	Molecular mass		Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
	PCDD	PCDF			
Monochlorodibenzo-*p*-dioxins/ dibenzofurans	218.6	212.6	2×10^{-10} - 2.78×10^{-1}	1.09×10^{-10} - 5.3×10^{-1}	4.52 - 13.37
Dichlorodibenzo-*p*-dioxins/ dibenzofurans	253.1	237.1			
Trichlorodibenzo-*p*-dioxins/ dibenzofurans	287.5	271.5			
Tetrachlorodibenzo-*p*-dioxins/ dibenzofurans	322	306			
Pentachlorodibenzo-*p*-dioxins/ dibenzofurans	356.4	340.4			
Hexachlorodibenzo-*p*-dioxins/ dibenzofurans	390.9	374.9			
Heptachlorodibenzo-*p*-dioxins/ dibenzofurans	425.3	409.3			
Octachlorodibenzo-*p*-dioxins/ dibenzofurans	459.8	443.8			
	PCBs				
PCB 81 - 3,4,4',5-TeCB	292				6.3
PCB 77 - 3,3',4,4'-TeCB	292		1.8×10^{-1}	4.4×10^{-7}	6.0 - 6.6
PCB 126 - 3,3',4,4',5-PeCB	326.43				7.0
PCB 169 - 3,3',4,4',5'-PeCB	326.43		3.6×10^{-5} - 1.2×10^{-2}	4.0×10^{-7}	7.4
PCB 105 - 2,3,3',4,4'-PeCB	326.43		3.4×10^{-3}	6.5×10^{-6}	7.0
PCB 114 - 2,3,4,4',5-PeCB	326.43		1.6×10^{-2}	5.5×10^{-6}	7.0
PCB 118 - 2,3',4,4',5-PeCB	326.43		1.3×10^{-2}	9.0×10^{-6}	7.1
PCB 123 - 2',3,4,4',5-PeCB	326.43				7.0
PCB 156 - 2,3,3'4,4',5-HxCB	360.88		5.3×10^{-3}	1.6×10^{-6}	7.6
PCB 157 - 2,3,3',4,4',5'-HxCB	360.88				7.6
PCB 167 - 2,3',4,4',5,5'-HxCB	360.88		2.2×10^{-3}	5.8×10^{-7}	7.5
PCB 189 - 2,3,3',4,4',5,5'-HpCB	395.32		7.5×10^{-4}	1.3×10^{-7}	8.3

As the degree of chlorination increases, DLCs become less soluble and volatile, but more lipophilic and strongly associated with organic matter, making the higher chlorinated compounds more stable and persistent in the environment (Mackay *et al.*, 1992).

Theoretically, there are 75, 135 and 209 possible congeners for PCDDs, PCDFs and PCBs, respectively, but only the seven PCDD, ten PCDF and twelve PCB congeners listed in Table 3.4 (in Section 3.2.1.1.4) are of toxicological interest (WHO, 1997).

3.2.1.1.2. Sources

DLCs are formed as unintentional by-products of industrial and thermal processes, in the presence of carbon, oxygen, and chlorine, and one or more catalysts at temperatures ranging between 400 and 700 °C. These conditions commonly occur during incomplete combustion processes (Fiedler *et al.*, 1996). In developing countries where regulations have not yet been established for all of the potential sources of PCDD/Fs and co-planar PCBs, mixed waste incineration is identified as the largest contributor towards DLC releases, with the most significant sources being the combustion of municipal, hazardous and medical waste (Ritter *et al.*, 2005). In developed countries, where regulations to properly manage incineration processes and other possible sources of POPs have been established and enforced, uncontrolled combustion processes seem to be the major culprit. A survey conducted by the US EPA during 2002 to 2004 revealed that backyard trash burning was the largest source of DLCs, contributing towards an estimated 56% of the total DLC pollution in the US. The other 44% comprised of incineration processes, the paper and pulp production, residential and industrial wood burning, and vehicle exhaust emissions. According to the New York State Department of Environmental Conservation (2004), the lack of control regulations on backyard trash burning attributed to the problem, emphasising the importance of establishing and enforcing regulations on POP emissions.

In summary, the most common sources of DLCs are waste incineration, ferrous and non-ferrous metal production, cement production, power generation and heating, mining and the production of mineral products, vehicle exhaust emissions, uncontrolled combustion, chemical- and petrochemical industry, and paper and pulp manufacturing. Smaller non-point sources include burning wood in stoves and fireplaces, landfill fires, open burning on the ground, and natural processes such as forest fires and volcanoes (Ritter *et al.*, 2005). A comprehensive list of sources and emissions is provided in the *Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases* (UNEP, 2005b).

3.2.1.1.3. Environmental fate

PCDD/Fs and dioxin-like PCBs are distributed ubiquitously in the environment (Carey *et al.*, 1998). These compounds are semi-volatile and can occur in both the gaseous and particulate phase in the atmosphere. DLCs are not atmospherically persistent in the vapour phase, but they are persistent when associated with particulate matter, and can be transported over long distances before being removed from the atmosphere by wet and dry deposition (Mackay *et al.*, 1992).

In terrestrial and aquatic compartments, DLCs are also associated with particulate matter. In soils, they are bound to the solid phase and therefore relatively immobile, resistant to degradation, and are persistent. The more volatile, less chlorinated PCDD/Fs and dioxin-like PCBs are lost from soils to the atmosphere through volatilisation (Gao *et al.*, 2005) or may be broken down by photodegradation (Isosaari, 2004). Due to their high soil-water partition coefficients and low water solubilities (Table 3.3), PCDD/Fs and PCBs in aquatic environments tend to associate with organic matter in sediments. Photolysis and biological degradation are the main transformation processes affecting the persistence of DLCs in water, but once associated with sediments, they are more resistant to degradation and persistent (Henry & De Vito, 2003). Aquatic sediments may therefore be an important environmental sink for PCDD/Fs and PCBs. (The degradation of DLCs will be discussed more comprehensively in Chapter 5).

PCDD/Fs and PCBs can be redistributed from abiotic compartments to bio-accumulate in lipid-rich tissues of biota (Carey *et al.*, 1998; Fiedler, 2003). The more chlorinated congeners (tetra- and above) generally have higher bio-concentration and bio-accumulation factors, and the presence of DLCs in human breast milk (Fuerst *et al.,* 1987; Kayama *et al.*, 2003; Schecter *et al.*, 2002) and in animals at the top of the food web (Malisch & Baum, 2007; Scott *et al.*, 2009; Storelli & Marcotrigiano, 2003;) demonstrate that these substances can bio-magnify. Humans are mainly exposed to PCDD/Fs and PCBs through their diet. Once food is digested, these substances bind to lipoproteins in blood and are transported to different parts of the body. DLCs have a tendency to bio-accumulate in fatty tissues as well as the liver, bone marrow, and cerebral tissue of mammals, and may cause significant negative health effects (Carey *et al.*, 1998).

3.2.1.1.4. Toxicity

Lateral chlorines (in the 2, 3, 7 and 8 positions) are essential for toxicity (Carey *et al.,* 1998), and the PCDD/Fs and PCBs listed in Table 3.4 is seen as toxicologically important (WHO, 1997). These congeners have assigned toxic equivalency factors (TEFs) to indicate their toxic potencies relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), the most toxic dioxin. TEFs are used to calculate the toxic potential of these DLCs on birds, mammals and fish (Schecter *et al*., 2006).

Table 3.4. IUPAC names of the most toxic PCDD/F and PCB congeners (WHO, 1997)

Specific isomers	Compound name	Specific isomers	Compound name
PCDDs		**PCBs**	
2,3,7,8 –TCDD	Tetrachlorodibenzo-*p*-dioxin		
1,2,3,7,8-PeCDD	Pentachlorodibenzo-*p*-dioxin	**Non-*ortho* substituted PCBs**	
1,2,3,4,7,8-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,3’,4,4’-TeCB	Tetrachlorobiphenyl (PCB 77)
1,2,3,4,6,7,8-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,4,4’,5-TeCB	Tetrachlorobiphenyl (PCB 81)
1,2,3,7,8,9-HxCDD	Hexachlorodibenzo-*p*-dioxin	3,3’4,4’,5-PeCB	Pentachlorobiphenyl (PCB 126)
1,2,3,4,6,7,8-HpCDD	Heptachlorodibenzo-*p*-dioxin	3,3’,4,4’,5,5’-HxCB	Hexachlorobiphenyl (PCB 169)
1,2,3,4,5,6,7,8-OCDD	Octachlorodibenzo-*p*-dioxin		
PCDFs			
2,3,7,8-TeCDF	Tetrachlorodibenzofuran	**Mono-*ortho* substituted PCBs**	
1,2,3,7,8-PeCDF	Pentachlorodibenzofuran	2,3,3’,4,4’-PeCB	Pentachlorobiphenyl (PCB 105)
2,3,4,7,8-PeCDF	Pentachlorodibenzofuran	2,3,4,4’,5-PeCB	Pentachlorobiphenyl (PCB 114)
1,2,3,4,7,8-HxCDF	Hexachlorodibenzofuran	2,3’,4,4’,5-PeCB	Pentachlorobiphenyl (PCB 118)
1,2,3,6,7,8-HxCDF	Hexachlorodibenzofuran	2’,3,4,4’,5-PeCB	Pentachlorobiphenyl (PCB 123)
1,2,3,7,8,9-HxCDF	Hexachlorodibenzofuran	2,3,3’,4,4’,5-HxCB	Hexachlorobiphenyl (PCB 156)
2,3,4,6,7,8-HxCDF	Hexachlorodibenzofuran	2,3,3’,4,4’,5’-HxCB	Hexachlorobiphenyl (PCB 157)
1,2,3,4,6,7,8-HpCDF	Heptachlorodibenzofuran	2,3’,4,4’,5,5’-HxCB	Hexachlorobiphenyl (PCB 167)
1,2,3,4,7,8,9-HpCDF	Heptachlorodibenzofuran	2,3,3’,4,4’,5,5’-HpCB	Heptachlorobiphenyl (PCB 189)
1,2,3,4,5,6,7,8-OCDF	Octachlorodibenzofuran		

The toxic responses of DLCs are through the aryl hydrocarbon receptor (AhR) inside the cytoplasm of animals (Carey *et al*., 1998). DLCs are lethal in high concentrations, but their greatest detrimental effects lie in their chronic toxicity, leading to dermal effects, defects of the immune-, reproductive-, nervous-, and endocrine systems, and even cancer (Schecter *et al*., 2006).

A condition commonly seen in industrial workers chronically exposed to DLCs is chloracne. This condition is perceived as the hallmark of dioxin exposure and can be described as the visible acne-like eruption of blackheads, cysts and pustules occurring on the cheeks, behind the ears, in the armpits and in the groin region (Schecter *et al*., 2006). Other dermal effects, such as hyperpigmentation and hypertrichosis (excessive hair growth) can also be associated with exposure to DLCs.

PCDD/Fs and dioxin-like PCBs are also classified as endocrine disrupting chemicals (EDCs). Because they are structurally similar to natural hormones, EDCs can interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones, disrupting a number of natural reactions in various systems (Kavlock *et al.*, 1996). By exerting their effects on the endocrine system, DLCs largely affect the reproduction and growth of animals and humans (Birnbaum, 1995). A study on rats has shown that relatively high doses of TCDD and related chemicals can cause testicular and ovarian degeneration. PCDD/F and PCB exposure have also been linked to other reproductive effects such as the inability to maintain pregnancy, decreased fertility, reduced sperm counts, increased endometriosis, and lowered testosterone levels (Birnbaum, 1995; Moore *et al.*, 1985).

Thyroid hormones, which are critical for normal growth and differentiation of cells, also appear to respond to dioxin exposure. DLCs interfere with normal growth regulation by decreasing thyroid hormone levels, causing several developmental deficits in animals and humans (Koppe *et al.*, 1991). The developmental effects of PCDD/Fs and PCBs are especially evident on the unborn embryo or foetus (Koppe *et al.*, 1991). Studies done on infants from Japan and Taiwan whose mothers consumed DLC contaminated rice oil while pregnant, showed premature births, decreases in birth mass, discolouration of the skin and nails, and abnormal teeth and gums. Along with these obvious physical differences, many of the children showed neurological and behavioural changes, such as unresponsiveness and lowered short-term memory, when compared to normal, unaffected children (Koppe *et al.*, 1991; Tanabe, 1988).

Other effects of PCDD/Fs and PCBs on the nervous system may include decreases in visual recognition, learning deficits, and changes in brain activity, which may lead to depression and personality changes (Corsolini *et al.*, 2002). In addition to the neurological effects of PCDD/Fs and PCBs, these substances can have serious effects on the immune system. Studies on Rhesus monkeys, which have immune systems similar to humans and other animals, revealed that DLC exposure can cause a decrease in the thymus gland, reducing the ability to produce cell-killing T-lymphocytes, which is important for immunity (Brouwer *et al.*, 1998).

Lastly, some DLCs might have the ability to cause cancer. In 1985, the US EPA characterised mixtures of dioxin-like substances as “probable human carcinogens”, but their reassessment in 2003 concluded that dioxins are better characterised as “likely human carcinogens”. The most toxic and best studied member of the dioxin family, 2,3,7,8-TCDD, is classified as a “known human carcinogen” by the International Agency for Research on Cancer (US EPA, 2008a). Although a number of cancerous effects, such as liver, lung and bladder tumours have been reported in animals exposed to DLCs, it is more difficult to prove that these substances cause cancer in humans (Birnbaum, 1995).

3.2.1.2. Polycyclic aromatic hydrocarbons (PAHs)

3.2.1.2.1. Physical and chemical characteristics

PAHs are a large group of semi-volatile organic compounds consisting of fused aromatic rings in linear, angular or clustered arrangements (Nadal *et al.*, 2004) (Fig. 3.7). They have a lipophilic nature and have a high affinity for organic matter (Morillo *et al.*, 2007). As shown in Table 3.5, individual PAHs differ significantly in their physical and chemical characteristics with properties such as water solubility and vapour pressure ranging in five and twelve orders of magnitude respectively (Lundstedt, 2003). Low molecular mass PAHs (LMM-PAHs) are generally more volatile and more water soluble than the high molecular mass PAHs (HMM-PAHs) (Table 3.5). HMM-PAHs, on the other hand, have higher hydrophobicity and lipophilicity, making them less susceptible to degradation and more persistent in the environment [as shown by their K_{OW} values] (Table 3.5).

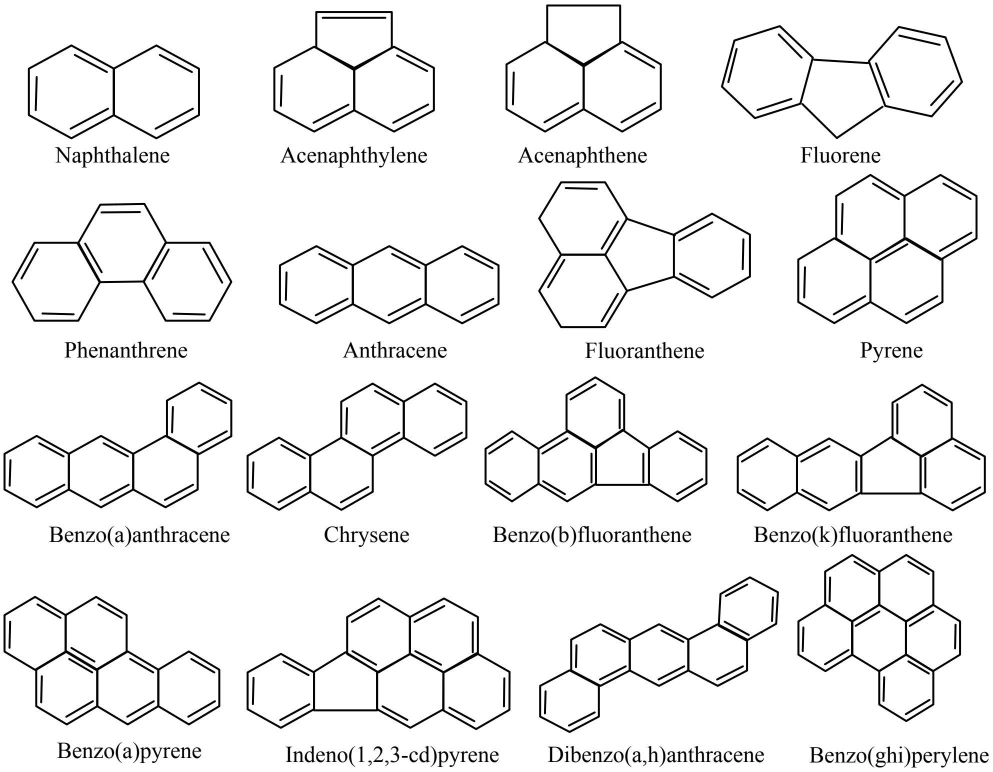


Figure 3.7. Representation of the chemical structures of the 16 US EPA PAHs

There are more than a hundred different PAHs, but for the purpose of this study, we only focuss on the 16 PAHs identified by the US EPA as requiring priority monitoring action within the framework of environmental quality control (Zhang & Tao, 2009). The structural representations and physical and chemical properties of these US EPA PAHs are shown in Figure 3.7 and Table 3.5.

Table 3.5. Molecular mass and some physical and chemical characteristics of the 16 US EPA PAHs (adapted from Lundstedt, 2003, originally from Mackay *et al.,* 1992)

		Molecular mass	**Water solubility (mg/l)**	**Vapour pressure (Pa)**	**Log K_{OW}**
2-ringed PAHs	Naphthalene	128	31	1.0×10^{2}	3.37
3-ringed PAHs	Acenaphthylene	152	16	9.0×10^{-1}	4.00
	Acenaphthene	154	3.8	3.0×10^{-1}	3.92
	Fluorene	166	1.9	9.0×10^{-2}	4.18
	Phenanthrene	178	1.1	2.0×10^{-2}	4.57
	Anthracene	178	0.045	1.0×10^{-3}	4.54
4-ringed PAHs	Fluoranthene	202	0.26	1.2×10^{-3}	5.22
	Pyrene	202	0.13	6.0×10^{-4}	5.18
	Benzo(a)anthracene	228	0.011	2.8×10^{-5}	5.91
	Chrysene	228	0.006	5.7×10^{-7}	5.91
5-ringed PAHs	Benzo(b)fluoranthene	252	0.0015	_	5.80
	Benzo(k)fluoranthene	252	0.0008	5.2×10^{-8}	6.00
	Benzo(a)pyrene	252	0.0038	7.0×10^{-7}	5.91
6-ringed PAHs	Indeno(1,2,3-cd)pyrene	276	0.00019	_	6.50
	Dibenzo(a,h)anthracene	278	0.0006	3.7×10^{-10}	6.75
	Benzo(ghi)perylene	276	0.00026	1.4×10^{-8}	6.50

3.2.1.2.2. Sources

Anthropogenic activities are generally recognised as the most important source of PAH release to the environment (Nadal *et al*., 2004). The PAHs measured during this study are mainly of *petrogenic* or *pyrogenic* origin. This means that they are generally formed as by-products during processes such as petroleum refining, burning of fossil fuels, and other petrochemical processes, or as a result of inefficient combustion of organic materials such as wood, coal and oil. Domestic heating, power generation, incineration or vehicle exhaust emissions are also sources of PAHs. Some of the lighter PAHs such as acenapthene, fluorene and anthracene are constituents of creosote, commonly used for the treatment of wood (Culotta *et al.,* 2006; Masih & Taneja, 2006).

Perylene, retene and phenanthrene homolgues are generally formed by natural processes such as fires and volcanic eruptions. Some homologues can also be derived from biogenic precursors, such as pigments and steroids, under anaerobic conditions (Culotta *et al.*, 2006).

3.2.1.2.3. Environmental fate

The semi-volatility of PAHs makes them mobile through the environment by deposition and re-volatilisation between air, soil, water, and sediments (Nadal *et al*., 2004). The LMM two- and three-ringed PAHs are mainly present in gaseous form, while the heavier four- to sixringed PAHs are largely associated with particulate matter. Both gaseous and particulate forms are transported in the atmosphere over short and long distances (Chen *et al.*, 2004). Their semi-volatile nature and aqueous solubility make LMM-PAHs susceptible to various degradation processes, volatilisation and leaching. In contrast, HMM-PAHs are less available for degradation because they are primarily associated with particles in the atmosphere and water (Wild & Jones, 1995). In aquatic environments, PAHs tend to become rapidly associated with organic matter in sediments. The degree of sorption generally increases as the number of benzene rings in the PAH molecule increases, since this implies higher lipophilicity (Weissenfels *et al.*, 1992). Due to their hydrophobicity and persistence, PAHs may be retained in sediments for many years. Therefore, sediment represents the most important reservoir of PAHs in aquatic environments (Culotta *et al.,* 2006).

Degradation of PAHs in the environment occurs through biological, chemical and photochemical processes. Biological degradation, where micro-organisms such as bacteria and fungi transform PAHs to inorganic end products, appears to be the main process responsible for the breakdown of PAHs in soils and sediments (Wilson & Jones, 1993). However, oxidation and photochemical reactions may also be important degradation pathways (Kochany & Maguire, 1994).

3.2.1.2.4. Toxicity

One of the greatest concerns regarding PAH exposure is the fact that some of these compounds are mutagenic and carcinogenic. Exposure of humans to PAHs may lead to elevated levels of DNA mutation, reproductive defects, and an increased risk of cancer and other adverse health effects (US EPA, 2002; Zhang & Tao, 2009). Epidemiological studies have shown that exposure to some PAHs may cause increased incidences of leukemia, bone-, brain-, bladder- and scrotal cancers, and adverse pregnancy outcomes (Mastrangelo *et al.*, 1996; Nadal *et al.*, 2004). Especially studies done on exposed workers of aluminium smelters and coke ovens have shown increased incidences of lung and bladder cancer (Boffetta *et al.*, 1997; Mastrangelo *et al.*, 1996; Negri & Vecchia, 2001).

The carcinogenic effects of PAHs depend on the specific structure and physical and chemical properties of the PAH. HMM-PAHs such as benzo(a)pyrene, benzo(a)anthracene, dibenzo(a,h)anthracene are more potent carcinogens than the majority of the LMM-PAHs (Eickhoff *et al.*, 2003). The US EPA classified benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene and indeno(1,2,3-c,d)pyrene as Group B2 probable human carcinogens (US EPA, 2002).

3.2.2. Intentionally produced industrial chemicals

PBDEs and PCBs are intentionally produced industrial chemicals. PBDEs are brominated flame retardants (BFR), which are applied to various combustible materials such as plastics, wood, paper and textiles to increase the product's fire resistance (De Wit, 2002). They are additive flame retardants, which are simply blended, not binded, with the polymers and therefore likely to be released out of the products (Alaee *et al.*, 2003).

PCBs were produced for industrial purposes as insulating materials in electrical equipment, plasticizers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants and dielectrics in transformers (Koppe & Keys, 2001). Due to their toxicity, the production and use of PCBs were banned in the 1980's, but they are still released into the environment via accidental spillages, fires, and volatilisation from old stockpiles (Koppe & Keys, 2001). Co-planar or dioxin-like PCBs are still unintentionally formed in the same way as PCDD/Fs, as described in section 3.2.1.1, but this section will focus only on the non-dioxin-like PCBs.

Information on the physical and chemical characteristics, sources, environmental fate and toxicity of PBDEs and PCBs follows in sections 3.2.2.1 and 3.2.2.2.

3.2.2.1. Polybrominated diphenyl ethers (PBDEs)

3.2.2.1.1. Physical and chemical characteristics

PBDEs ($C_{12}H_{10-x}Br_xO$) consist of two benzene rings linked with an oxygen atom, with one to ten bromine atoms attached to the benzene rings (Hellström, 2000) (Fig. 3.8). Theoretically, there are 209 possible PBDE congeners, divided into ten congener groups (monoBDE to decaBDE).

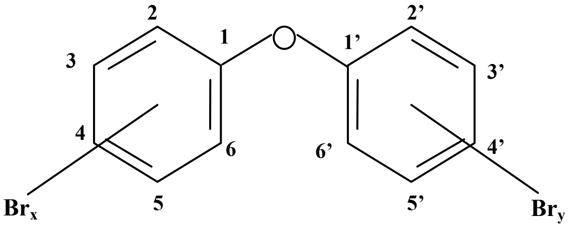


Figure 3.8. Chemical structure of PBDEs

The majority of BFRs are higher brominated (penta to deca) compounds, and PBDEs with less than four bromine atoms are generally not found in commercial PBDE products (Darnerud *et al*., 2001). Penta-, octa- and decaBDE are the main congeners in technical (commercial) mixtures of PBDEs, but it also contains different mixtures of tetra- to decaBDE (De Boer *et al*., 2003). Consequently, little environmental data is available on mono-, di-, tri-, hexa- and nona-BDEs.

Table 3.6. Physical and chemical characteristics of PBDEs (Darnerud *et al.,* 2002; De Wit, 2002; ECHA, 2008; Hellström, 2000; Hunziker *et al*., 2004)

Compound	Molecular mass	Water solubility (µg/l)	Vapour pressure (Pa)	Log K_{OW}
PBDEs				
Tetra-BDE	485.8	10.9	$2.7 - 3.3 \times 10^{-4}$	5.9 – 6.2
Penta-BDE	564.8	2.4	$2.9 - 7.3 \times 10^{-5}$	6.5 – 7.0
Octa-BDE	801.5	0.5	$1.2 - 2.7 \times 10^{-7}$	8.4 – 8.9
Deca-BDE	959.2	<0.1	$4.6 \times 10^{-6} - 5.8 \times 10^{-11}$	10

PBDEs are hydrophobic and resistant to environmental degradation processes. Because of their low volatility, low water solubility (Table 3.6), and strong adsorption to sediments, the higher brominated compounds are less mobile in the environment (Watanabe & Tatsukawa, 1990). With an increasing degree of bromination, the aqueous solubility and vapour pressure of BDE congeners decrease, while the hydrophobicity increases (Zegers *et a*l., 2003) (Table 3.6).

3.2.2.1.2. Sources

PBDE products are produced by brominating diphenyl ether in the presence of a catalyst (De Wit, 2002). The various PBDE technical products are applied to different materials as flame retardants. PentaBDE are mainly used in epoxy resins, phenol resins, polyesters, polyurethane foam and textile. OctaBDE are generally applied to acrylonitrile butadiene styrene, polycarbonate and thermosets; while decaBDE products are used in most types of synthetic materials, including textiles and polyester used in circuit boards (De Wit, 2002).

The most evident sources of BFRs into the environment are effluents from plants producing BFRs, flame-retarded polymers, and other plastic products (Sellström & Jansson, 1995). Because they are "additive flame retardants", which are mixed into the polymers and not chemically bound to the plastics or textiles, PBDEs may separate and be released from the surface of their product applications into the environment. Polyurethane foam and electronic equipment, such as television sets and computers, may be significant sources of BFRs (Watanabe & Sakai, 2003). Additionally, the sources responsible for the formation of polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans could also be responsible for the formation of BFRs. Possible emission sources include municipal, hospital and hazardous waste incineration, facilities recycling plastics and metals from electronic equipment, accidental fires, and disposal sites (WHO, 1998).

3.2.2.1.3. Environmental fate

Commercial PBDEs are relatively resistant to physical, chemical and biological degradation (Darnerud *et al*., 2001). PBDEs are only slightly volatile (Table 3.6), and have a low potential to evaporate from aqueous surfaces. They have a high adsorption potential to suspended matter, and especially the higher brominated BFRs tend to accumulate in sediments or particulate matter near their sources of emission (ECHA, 2008). On the other hand, the lower brominated compounds, especially the breakdown products of PBDE, such as polybrominated and mixed brominated/chlorinated dibenzo-*p*-dioxins and dibenzofurans, may be transported farther from their sources of emission (Watanabe & Sakai, 2003).

While the higher brominated BFRs are persistent in the environment, the lower brominated PBDEs are more susceptible to degradation. Photodegradation and microbiologic degradation are the main means of PBDE breakdown, but incineration may also play a significant role (Darnerud *et al*., 2001). Due to their low water solubilities, hydrolysis is not likely to be a significant route of environmental degradation (ECHA, 2008).

3.2.2.1.4. Toxicity

Although their greater molecular size may inhibit movement across biological membranes, the similarity in molecular structure of PBDEs with that of PCBs and PCDD/Fs, and their resistance to degradation, gave rise to concerns that they may lead to similar environmental problems and health effects (Allchin *et al*., 1999). Toxicological data have demonstrated that some BFRs may lead to serious adverse health effects, such as thyroidogenic, estrogenic and dioxin-like activities (Bergman & Ulrika, 2001). Studies on mammals also showed effects on the liver and reduced embryonic development (Hellström, 2000).

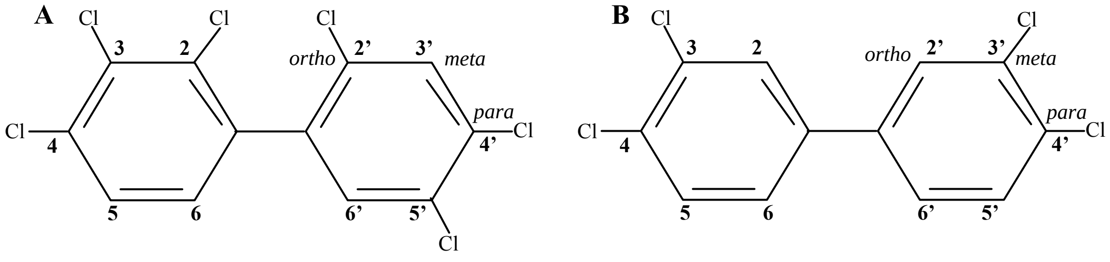
Documented effects of PBDE on human health are lacking. PBDEs may cause chloracne and have effects on the liver. Deca-BDEs have been classified as “possibly carcinogenic to humans” by the US EPA, while tetra-, penta- and octa-BDEs have been classified as not carcinogenic (Hellström, 2000). There is evidence linking PBDE exposure to the induction of cancer by a non-mutagenic mechanism (Helleday *et al*., 1999). PBDEs may also have negative effects on the thyroid hormone system and may cause developmental neurotoxic effects such as aberrations in spontaneous behaviour, learning, memory and function (Eriksson *et al.,* 2002).

3.2.2.2. Polychlorinated biphenyls (PCBs)

The 197 PCB congeners that were not listed in Table 3.4 are collectively referred to as "non-dioxin-like", "non-coplanar" or "*ortho*-substituted" congeners, and are discussed separately from the dioxin-like PCBs because of their deliberate production.

3.2.2.2.1. Physical and chemical characteristics

Although dioxin-like and non-dioxin-like PCBs have similar physical and chemical characteristics (referred to in section 3.2.1.1.1 and summarized in Table 3.7), they can be distinguished in terms of their sources, structure and mechanism of action (Fischer *et al.*, 1998). In short, dioxin-like PCBs are unintentionally produced as by-products of industrial processes, while the non-dioxin-like PCBs were purposefully manufactured in the past for industrial purposes (Ritter *et al.*, 2005). Regarding their structures, coplanar or dioxin-like PCBs lack *ortho* substitution, whereas non-coplanar PCBs contain chlorine atoms in one or more of the four *ortho* positions (closest to the biphenyl bond; Fig. 3.9) (Fischer *et al.* 1998). Where coplanar PCBs bind with affinity to the AhR, non-coplanar PCBs are poor ligands for the AhR and the mechanism of their biological effects are in many cases unknown (Rogan & Gladen, 1992).



Non-coplanar/*ortho*-substituted/non-dioxin-like PCBs.

Coplanar/non-*ortho*-substituted/dioxin-like PCBs.

Figure 3.9. Chemical structures of non-dioxin-like and dioxin-like PCBs, with 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138) being representative of non-dioxin-like PCBs (A), and 3,3',4,4'-tetrachlorobiphenyl (PCB 77) being representative of dioxin-like PCBs (B)

Table 3.7. Physical and chemical properties of PCBs (adapted from Henry & De Vito, 2003; Sinkkonen & Paasivirta, 2000; Syracuse, 2007; Whylie *et al.*, 2003)

PCB congeners	Molecular mass	Water solubility (mg/l)	Vapour pressure (Pa)	Log K_{OW}
Monochlorobiphenyl	188.65	$1 \times 10^{-5} – 1 \times 10^{-7}$	$2.1 \times 10^{-4} – 4 \times 10^{-3}$	4.30 - 8.26
Dichlorobiphenyl	233.1			
Trichlorobiphenyl	257.54			
Tetrachlorobiphenyl	292			
Pentachlorobiphenyl	326.43			
Hexachlorobiphenyl	360.88			
Heptachlorobiphenyl	395.32			
Octachlorobiphenyl	429.77			
Nonachlorobiphenyl	464.21			
Decachlorobiphenyl	498.66			

In summary, PCB congeners that contain more chlorine atoms and have fewer *ortho* substitutions are less volatile, less soluble in water, have a higher potential to bind to organic matter, and are more susceptible to anaerobic dechlorination processes (Carey *et al.*, 1998). These congeners typically have a higher potential for bio-accumulation, and are more abundant in soils and sediments and less abundant in water and the atmosphere (Henry & De Vito, 2003). On the other hand, the congeners that contain less chlorine atoms and have more *ortho* substitution are more volatile, more water soluble, and are more readily metabolized by biota. These congeners are therefore more prominent in the atmosphere and surface waters, than in soils and sediments (Henry & De Vito, 2003).

3.2.2.2.2. Sources

PCBs had been manufactured from the early 1930s for industrial purposes. These compounds are chemically stable, resistant to heat, non-flammable and have low vapour pressures and high dielectric constants (Ritter *et al.*, 2005), which made them excellent as insulating materials in electrical equipment, plasticisers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants, and dielectrics in transformers (Koppe & Keys, 2001). Although their production and use were banned, they are still released into the environment as a consequence of historical use and disposal via accidental spillages, fires, and releases from old stockpiles (Henry & De Vito, 2003).

3.2.2.2.3. Environmental fate

In the environment, PCBs are always found as mixtures of both dioxin-like and non-dioxin-like PCBs. The environmental fate of PCBs is dependant on their physical and chemical characteristics (Ritter *et al.*, 2005), as discussed in section 3.2.1.1.3.

3.2.2.2.4. Toxicity

Although the mechanism of toxicity and the toxicological effects of dioxin-like PCBs are well known, it was originally thought that *ortho*-substituted, non-dioxin-like PCBs are biologically inactive, but is now known that they can exhibit toxic effects (Fischer *et al*., 1998). Their exact mechanism of action is uncertain, but it is known that toxic effects of non-dioxin-like PCBs occur via multiple toxicity pathways not involving the AhR (Henry & De Vito, 2003). Studies have shown that certain *ortho*-substituted PCB congeners are partially responsible for neurotoxic effects, which include decreased catecholamine levels in certain regions of the brain, and it may cause behavioural changes (Schantz *et al*., 1995; Shain *et al.,* 1991) and learning deficiencies (Chen & Hsu, 1994). Other effects of PCBs include the stimulation of insulin (Rogan & Gladen, 1994) and an increase in neuro-endocrine, endocrine, immunological, and carcinogenic effects. Endocrine disruptive effects of non-dioxin-like PCBs include abnormal thyroid gland development, a decrease in serum thyroxine concentrations and various effects on the reproductive system (Brouwer *et al*., 1998).

3.3. Environmental transport of organic pollutants and POPs

Once POPs or organic pollutants (discussed in sections 3.1 and 3.2) are formed, they may be released into atmospheric, aquatic, and terrestrial compartments, depending on their emission sources. When present in one of these compartments they can cycle among the compartments, because their physical and chemical characteristics allow them to partition between gaseous, liquid, and particulate phases (Carey *et al*., 1998). POPs and other organic pollutants may be transported through various routes including ocean currents, rivers, strong winds, and migratory animals such as birds, whales, dolphins, and salmon, as well as anthropogenic practices such as trade (Krümmel *et al*., 2003). Due to the great volumes involved and their massive movements, oceanic- and riverine transport may contribute significantly in transferring pollutants all over the world. Although the majority of the organic pollutants included in the study are insoluble in water, these substances adsorb onto sediment particles or are taken up by biota, which carry them over long distances (Whylie *et al.,* 2003). Since the majority of POPs are semi-volatile they are capable of long-range transport, leading to their occurrence in places where they were never produced (Henry & De Vito, 2003), and thereby making POPs a global issue.

In polar regions where the production and emission sources of POPs are limited, significant levels of these substances are found in breast milk of women, and in fatty tissues of dolphins and whales. This phenomenon can be explained by the "cold condensation" or "grasshopper" effects (Corsolini *et al.*, 2002) – as explained by Figure 3.10.

In regions with higher temperatures, POPs evaporate into the atmosphere because of their volatility. Once in the atmosphere, these substances are carried by wind and air currents. When there are alterations in atmospheric conditions, POPs can return to the surface as precipitation or impactation. In this process, POPs are transferred further north (Fig. 3.10) (Corsolini *et al.*, 2002).The cycle repeats itself and in the colder polar regions or high altitude mountains, POPs return to the surface in rain or snow. The low temperatures at these locations limit subsequent evaporation, trapping POPs at these regions.

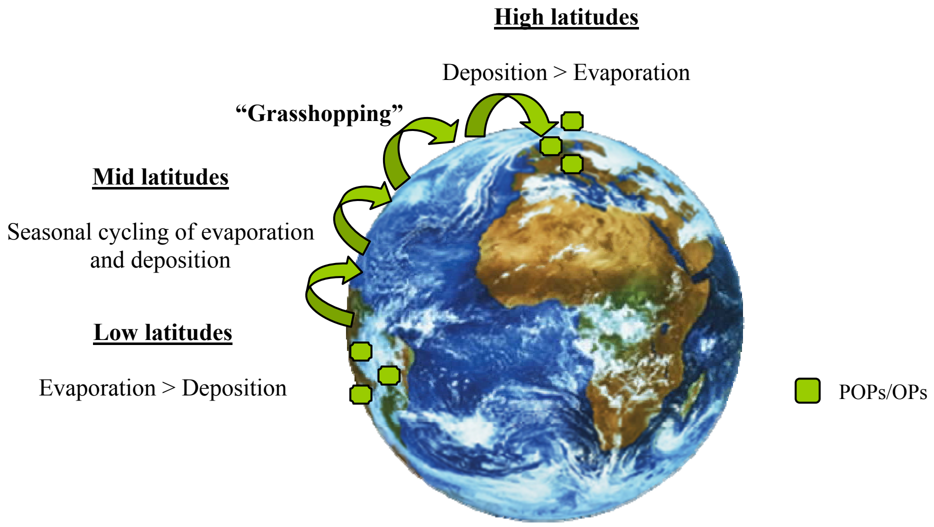


Figure 3.10. Diagrammatic representation illustrating long-range transport of organic pollutants or POPs known as the "grasshopper-effect" (adapted from Corsolini *et al.*, 2002)

This sequence of events may be repeated several times (Corsolini *et al.*, 2002). During the "grasshopper-effect" a part of the pollutant load may be lost through degradation, deposition in sediment or soil, absorption into vegetation, or algae taking up pollutants (Tysklind *et al.*, 1993).

3.3.1. Organic pollutants in sediment

Due to its hydrophobic nature organic pollutants tend to associate with organic material in sediment in the aquatic environment. Sediment is a naturally-occurring material derived from surface erosion and comprises a mineral component, arising from the erosion of bedrock, and an organic component arising during soil-forming processes (which include biological and microbiological production and decomposition) (Ongley *et al.*, 1990).

For the purposes of aquatic monitoring, sediment can be classified as “deposited” or “suspended”. Deposited sediment (or bed load as it is also referred to) is found on the bed of a river or lake that is transported by rolling, sliding and/or saltating. Suspended sediment (or wash load) is the finer portion of sediment that is found in the water column where it is transported by water movements (Ashworth & Ferguson, 1989).

Particle sizes ranging from fine clay to cobbles and boulders may exist in a river. The grain-size distribution of aquatic sediments is an important factor controlling pollutant sorption (Smith *et al.,* 1988). Studies of riverbed sediment report increasing pollutant concentration with a decreasing grain size (Witkowski *et al.,* 1986), with high contaminant concentrations generally found in fine-grained bed sediments.

Since only the top 5 to 10 cm sediment layer was sampled in this project, the underlying assumption is that most of the sediment samples would have been from the finer portion of sediment that is transported by the water column. Particle-size, which is a determining factor of pollution load, was not measured for this study but the total organic carbon for each sample was determined. This was deemed a good enough characterisation of the nature of a particular sediment sample since it is the determinant for sorption of the pollution under investigation. In a paper by Karichkhoff and co-authors (1979) if was proven that the sand fraction of sediment was a less effective sorbent than the fine fraction and the differences in sorption within in the silt and clay fractions were largely related to differences in organic carbon content. In another study on the sorption of nine pesticides Gao *et al.* (1998) proved that although the sorptive capacity of sediment is directly related to both its organic carbon content and particle size, the differences in sorption are largely dependent on the organic carbon content of each particle fraction.

3.4. Methods used to determine the concentrations of organic pollutants during this study

Presently, gas chromatography mass spectrometry (GC/MS) or high-resolution GC high-resolution MS (HRGC/HRMS) are the most generally applied chemical methods used for the detection and quantification of POPs. The major advantage of GC/MS and HRGC/HRMS techniques is their ability to determine the identity and concentrations of numerous individual chemicals and congeners with reasonable precision (Safe, 1995). However, the technique is laborious and expensive for a developing country such as South Africa. Also, chemical analyses merely determine the concentrations of targeted congeners and do not account for interactions among chemicals, which can alter the toxic potential of complex mixtures that may involve many more compounds than just the targeted chemicals (Hilscherova *et al*., 2000).

Due to the shortcomings in standard chemical methods, biological methods such as bio-markers, cell- or organ-based bio-assays and protein binding assays have been introduced during the last decade (Behnisch *et al*., 2001). For the purpose of this study, a cell-based bio-assay, the H4IIE-*luc* reporter gene bio-assay was used. This bio-assay is rapid, sensitive, and relatively cost-effective in comparison to chemical analysis. A disadvantage of the assay is its inability to distinguish between individual compounds with precision. However, they measure the toxic effects of specific mixtures on organism level accurately (Chutter, 1998). The assay also integrates possible interactions among chemicals, detecting potential dioxin-like impacts on organisms and the environment that might have been missed by performing chemical analysis alone (Hilscherova *et al*., 2000).

When bio-assays are used to measure the amount of DLCs, the toxicity is reported as *TCDD-equivalents* (TCDD-EQs). TCDD-EQs are the equivalent to toxic equivalency quotients (TEQs) calculated from chemical analysis results (Schecter *et al*., 2006). To calculate the TEQ of a compound using chemical analysis, the concentration of each isomer is multiplied with its TEF value. The TEF values for all isomers are ultimately added to give the total TEQ for the sample (Safe, 1995). There is generally a corresponding trend and a strong correlation between bio-assay and chemical analysis results (Carbonnelle *et al*., 2004; Nieuwoudt *et al*., 2009; Vanderperren *et al*., 2004; Van Wouwe *et al*. 2004) although the bioassay results are often one to two orders of magnitude higher than when the TEQ is calculated based on the chemical analysis.

Results gathered from this bio-assay do not give information as to the exact compounds responsible for the results, and therefore the source of the pollution can not be identified. This is one of the reasons why HRGC/HRMS is needed. Knowing what was responsible for the pollution may help decide on the management plan to prevent similar pollution events. However, the TCDD-EQ results from the assay may be used to inform users of a particular stretch of river or dam not to eat fish from that waterbody (if the TCDD-EQ's were determined on fish tissues from that particular area) or not to convert a particular area into a school's playground if soil from that area had high TCDD-EQs. It is also true that these advisories can only be made available if there is a guideline against which the TCDD-EQs can be compared, such as a sediment quality guideline (which does not exist yet for South Africa).

Since both chemical and biological methods have limitations and advantages, it is best to use these methods in combination with one another (Kannan *et al.*, 2001). For the purpose of this study, all samples were firstly screened with the H4IIE-*luc* bio-assay to determine the amount of DLCs present in each sample, since bio-analysis is a cost-effective means to screen so many samples. Following, only the samples eliciting quantifiable responses with the assay were selected for chemical analysis for other organic pollutants (pesticides, PCBs, PBDEs and PAHs). Although the H4IIE-*luc* bio-assay only detects DLCs (PCDD/Fs, dioxin-like PCBs and PBDEs), it is considered to be an effective method to eliminate the sites with insignificant amounts of industrial pollutants, since PAHs and PCBs have sources (process- and product associated) common with PCDD/Fs.

It is realized and it should be noted that some sites with insignificant amounts of PCDD/Fs, but containing significant amounts of PAHs, BFRs and PCBs, might have been eliminated for further analysis. Nonetheless, bearing in mind the financial implications of chemically analysing a large amount of samples, this was considered as the best possible means of selecting samples for further analysis. Since chemical analysis for DLCs are costly, and accredited analytical facilities for these substances are limited in South Africa, chemical analysis were not done for PCDD/Fs and dioxin-like PCBs. Only the H4IIE-*luc* bio-assay results will be reported and discussed for these compounds.

Although the H4IIE-*luc* bio-assay only screens for the presence of DLCs, and indirectly for industrially-associated pollutants, the same samples that had high levels of DLCs were chemically analysed for pesticides as well. It is acknowledged that some sites with high levels of OCPs could have been missed, but the financial implications of chemical analysis were the motivation for using this approach. Also, although of interest, OCPs were not the main concern of this study, since some South African data already exists for many of the selected compounds.

3.5. Mechanism of the H4IIE-*luc* tissue culture bio-assay

The H4IIE-*luc* bio-assay measures the effects of DLCs on rat hepatoma cells, stably transfected with a luciferase reporter gene under control of dioxin-response elements (DREs) (Hilscherova *et al.*, 2000). This bio-assay indirectly measures cytochrome P450 induction, which is an endpoint in an AhR-mediated response and an indicator of toxic exposure (Stegeman, 1992). Luciferase was inserted down-stream of the P450 gene. The AhR, also known as the dioxin receptor, is a transcription factor, which is a member of the basic helix-loop-helix family of transcription regulators. This receptor is complexed with heat shock proteins (HSP) and is located in the cytosol of cells (Carey *et al.*, 1998). Upon binding of the ligand (in this case DLCs) to the AhR, conformational changes occur, resulting in the translocation of the AhR-ligand complex to the nucleus and dissociation of HSP from the receptor (Elferink, 2003) (Fig. 3.11).

In the nucleus, the AhR-ligand complex heterodimerises with the AhR nuclear translocator (Arnt) protein (Pocar *et al.*, 2005). The binding of the ligand-AhR-Arnt transcriptionally active complex to the DRE, results in increased transcription of cytochrome P450 (Eisen *et al.*, 1983; Fiedler, 2003) and in the case of the H4IIE-*luc* cell line, it results in an up-regulation of luciferase transcription (Fig. 3.11).

Once luciferin is added to the cells, a light-producing reaction is catalysed, which is equivalent to the cells' toxicant exposure and can be measured with a luminometer (Denison *et al.*, 1996; Hilscherova *et al.*, 2000).

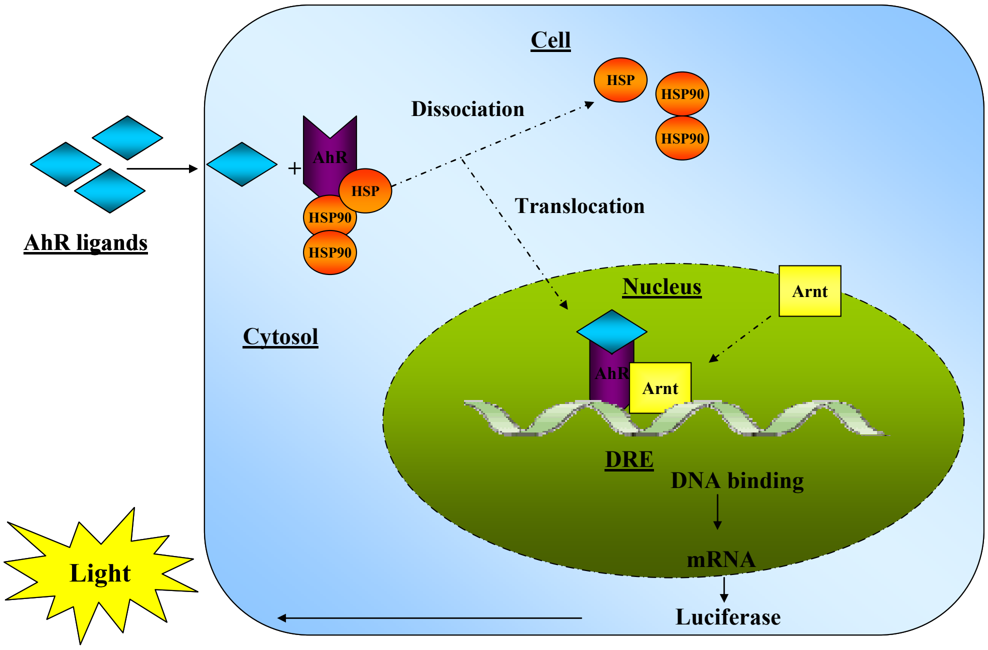


Figure 3.11. Mechanism of the H4IIE-*luc* bio-assay

The method of the H4IIE-*luc* bio-assay is described in section 4.5.1.

4

Materials and Methods

4.1. Site selection

Based on the results of previous studies (Nieuwoudt, 2006; Nieuwoudt *et al.* 2009; Quinn *et al.,* 2009; Vosloo & Bouwman, 2005), it is apparent that POPs and other industrial pollutants are present in South African soils and sediments. Although some of these pollutants, especially PCDD/Fs, PCBs and some OCPs, may generally be present in low concentrations, hot-spots remain, mostly associated with industrial centres and high-density residential areas, which are the main focus of this study. Also included in the study are water bodies located in the vicinity of paper mills, rivers flowing into neighbouring countries, and high-altitude rivers. The motivation for selecting these sites has been given in Section 2.4 and is elaborated in Section 4.2. The sampling regions or locations included in each of these categories are listed in Table 4.1.

Table 4.1. The site categories and the locations, regions or rivers included in each category

Sampling site categories	Locations/regions/rivers
Industrial areas	Soweto and Lenasia, Cape Town, Richards Bay, Bloemfontein and its associated high-density residential area, Botshabelo.
Low-income, high-density areas	Soweto and Lenasia, Cape Town, Richards Bay, Bloemfontein and its associated high-density residential area, Botshabelo.
Rivers flowing to neighbouring countries	Limpopo-, Komati-, Pongola-, Olifants- (Mpumalanga) and Crocodile (east) Rivers
High-altitude rivers	Drakensberg area: Mzimkhulu- and Mkomazi Rivers, and one stream of which the name is uncertain.
Paper mills	KwaZulu-Natal: Mhlathuze-, Tugela- and Mvoti Rivers

A brief description as well as the location of each of the sites is provided in Section 4.2.

4.2. Site descriptions

4.2.1. South Africa in general

South Africa has an estimated population of 48.7 million (Statistics South Africa, 2008) and has the largest economy and industrial base of any country in Africa (World Bank, 2009). The country's key sectors include chemical industry, mining and minerals, automobiles, and agriculture (mainly maize, ground-nuts, sunflowers, cotton and fruit). Gauteng (33.3%), followed by KwaZulu-Natal (KZN) (16.7%) and the Western Cape (14.4%) are responsible for the largest contributions towards the country's gross domestic product (GDP), with the Northern Cape (2.2%) contributing least to the GDP (South African Info, 2009). The areas that are included in the study are indicated in Figure 4.1, and include the provinces of Gauteng, Limpopo, Mpumalanga, KZN, Free State and the Western Cape.

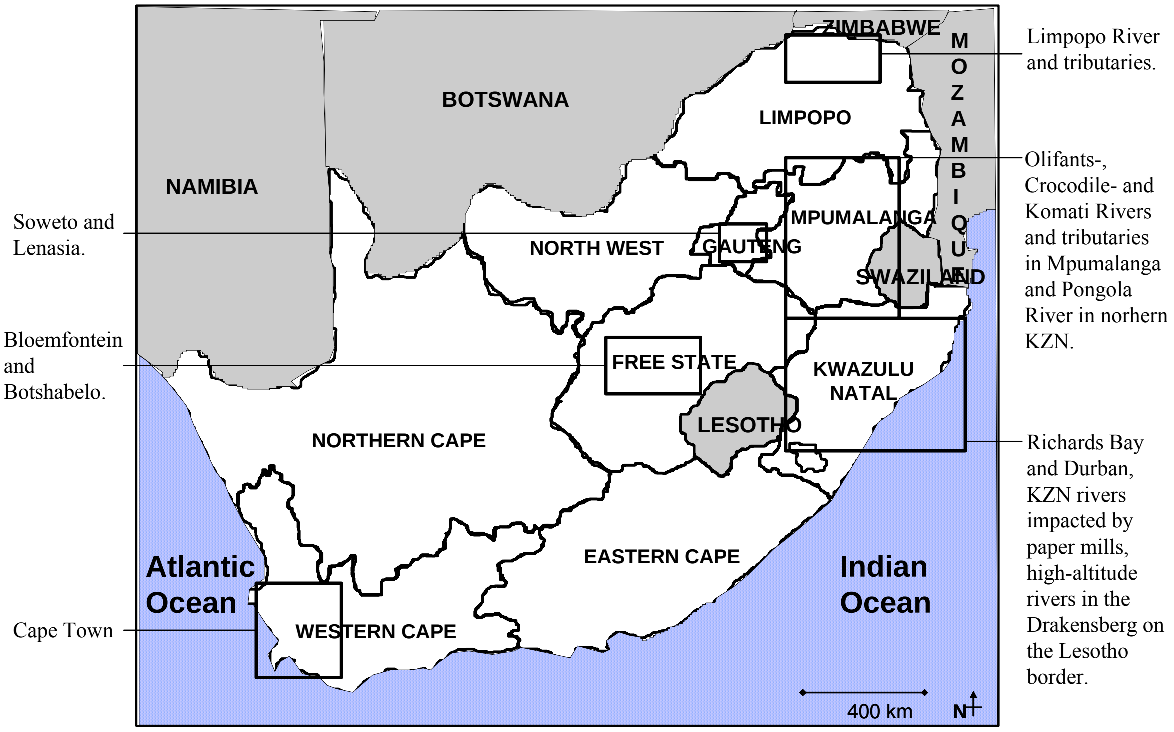


Figure 4.1. A map of Southern Africa indicating the approximate locations of sampling areas

Key for Figures 4.1 to 4.13:

🟦	Water bodies (Dams, pans and oceans)	🟩	Parks/Game Reserves/Nature Reserves	▨	Wetlands
□	Developed/Built-up areas	□	Open/undeveloped areas	▨	Industrial areas
▩	Neighbouring countries	+-+-+-+	Railways	—	Gravel roads
—	Secondary roads	—	National roads	~	Rivers/streams
▲	Sampling sites	↑N	North	⊢—⊣ 50 km	Map scale

4.2.2. Soweto and Lenasia

With a population of nearly one million, Soweto is the most densely populated black urban residential area in South Africa (Statistics South Africa, 2008). The area is mainly residential, with a few small industries. Lenasia, on the other hand, is more industrial with the main industries specialising in the manufacture of plastic, resin, furniture and steel products. The Klip River wetland system, possibly one of the most economically important wetlands in Africa, meanders through the western parts of Soweto, and the northern and eastern parts of Lenasia (McCarthy *et al*., 2007). Preliminary results of a previous, unpublished study conducted during 2005 and 2006, show the highest levels of DLCs thus far recorded for South African sediments (380 ng TCDD-EQ/kg, dw, determined by the H4IIE-*luc* bio-assay) in sediment collected from this wetland in an area situated south of Soweto and south-east of Lenasia (Fig. 4.2, S/L 9 and 12). It was therefore decided that a section of the wetland (further referred to as the "Soweto & Lenasia wetlands" or S/L) should be included in the current study to determine the scale and significance of selected organic pollutants in this wetland more extensively.

The first samples were collected north and up-stream of Soweto (S/L1 to 3). Samples were also collected in the residential and industrial areas of Soweto and Lenasia (S/L5 to 8, and 11), and finally at a location down-stream from both areas (S/L9, 10, 12 and 13) (Fig. 4.2, Table 4.2). Although Soweto and Lenasia are not particularly industrialised, the wetland receives polluted effluent from the western section of the Witwatersrand urban-industrial-mining complex (McCarthy *et al*., 2007). Domestic activities such as open burning for cooking or heating may also contribute to organic pollutants in the wetland.

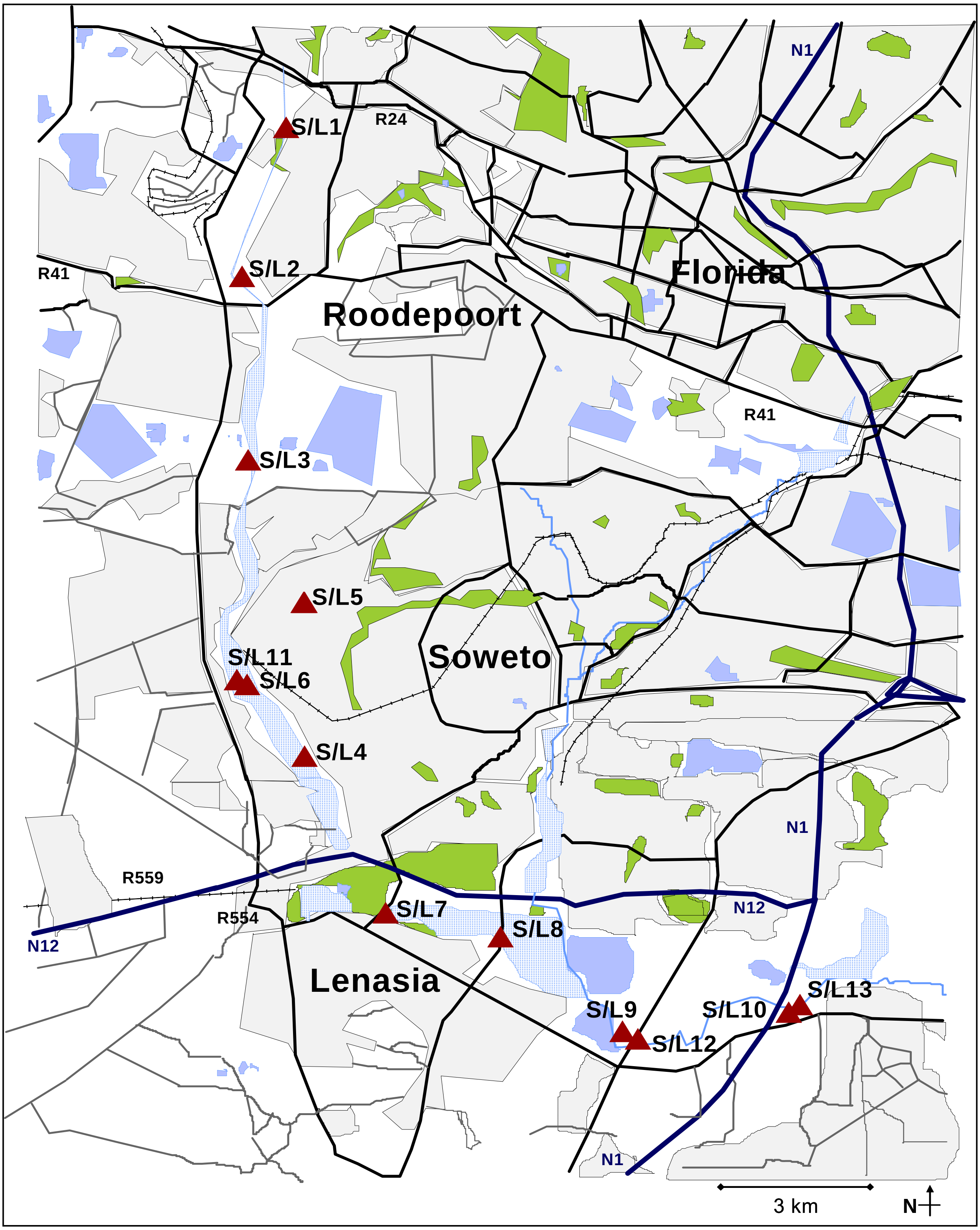


Figure 4.2. Sediment and soil sampling sites in the Soweto/Lenasia wetland

Table 4.2. Description of sediment and soil sampling sites from Soweto and Lenasia (S/L) wetlands

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
S/L1	17/01/07	S26°08'10.6" E27°49'24.5"	Roodepoort: Mindalore, east of Chamdor Road (R558)	Residential area, near old gold mine tailings dams.	Wetland reeds
S/L2	17/01/07	S26°10'09.0" E27°49'03.1"	Witpoortjie. North-east of R558-R41 intersection	Residential and several industries	Wetland reeds
S/L3	17/01/07	S26°12'35.1" E27°48'50.9"	Parallel with Adcock Street (R558) near Jameson Raid monument. North-west of Thulani.	Approximately 300 – 400 m away from residential area, near a gold mine tailings dam	Wetland reeds
S/L4	17/01/07	S26°16'26.4" E27°49'39.2"	Protea Glen: Protea Boulevard, east of R558-Protea Blvd intersection.	Residential area. No industries.	Wetland reeds
S/L5	17/01/07	S26°14'25.3" E27°49'37.1"	Naledi: Milkplum Street, next to Adcock Street (R558)	Residential area. No industries.	Wetland reeds
S/L6	17/01/07	S26°15'30.5" E27°48'51.5"	Naledi: Pitse Street, next to Adcock Street (R558)	Residential area. No industries.	Wetland reeds
S/L7	17/01/07	S26°18'26.3" E27°50'43.7"	Nirvana Drive bridge. R554 crossing.	Residential area. Coal burning	Wetland reeds
S/L8	17/01/07	S26°18'48.2" E27°52'22.0"	M10: Klip Spruit Valley bridge	Down-stream of residential area. Small industries.	Wetland reeds, next to river
S/L9	17/01/07	S26°20'08.3" E27°54'09.5"	On the R553 near bridge: Approximately 200 m north of the R554-R553 intersection.	Downstream of sewage works.	Wetland reeds

Table 4.2. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
S/L10	17/01/07	S26°19’44.2” E27°56’34.1”	On the R554: East of the R554-N1 intersection.	Near an old bridge, in the vicinity of farm/small-holdings	Wetland reeds, next to river
S/L11*	17/01/07	S26°15’29.3” E27°48’46.8”	Naledi: Pitse Street, next to Adcock Street (R558)	Residential area	Grass/rocky area next to S/L 6 wetland
S/L12*	17/01/07	S26°20’08.3” E27°54’09.5”	On the R553 near bridge: Approximately 200 m north of the R554-R553 intersection.	Downstream of sewage works.	Soil
S/L13*	17/01/07	S26°19’44.2” E27°56’34.1”	On the R554: East of the R554-N1 intersection.	Near an old bridge, in the vicinity of farm/small-holdings	Soil

*Associated soil samples

4.2.3. Cape Town

Cape Town is the second most populous city in South Africa (Statistics South Africa, 2008) and has a large industrial complex consisting of oil refining, diamond cutting, shipbuilding and -repair, food processing, printing, and the production of paper, chemicals, fertilizers, cement, clothing, plastics, and leather goods. The city also has a large national railway, an airport and a harbour complex. Unfortunately, the steep incline and depth of the harbour makes it inaccessible for sampling. The nuclear power station, Koeberg, provides electricity for the majority of Cape Town's needs. The Western Cape is well-known as an agricultural region, generating 25% of the total gross income of the country’s agricultural sector (South African Info, 2009). Sediments were sampled from industrially-, residentially- and agriculturally impacted areas to determine the scale and significance of POPs pollution associated with these areas. The sites CT2, 4 to 8, 10, 14, 19 and 20 were selected because of their close proximity to industry, mainly focusing on a petrochemical plant, a paper and pulp treatment plant, an oil- and gas refinery, manufacturers of chemicals and fertilizers, and other smaller industries (Fig. 4.3 – 4.5, Table 4.3).

CT1, 9 and 17 were situated in medium- to high-income-, and high-density, low-income residential areas, whereas sites 11, 12, 15, 16 and 18 were selected in areas that would give an indication of the impacts associated with industrial-residential combinations. Finally, CT3 and 13 were located in the vicinity of agriculturally impacted areas, mostly vineyards (Fig. 4.3 – 4.5, Table 4.3). Information regarding the sampling sites is listed in Table 4.3, and site locations are indicated in Figures 4.3 to 4.5.

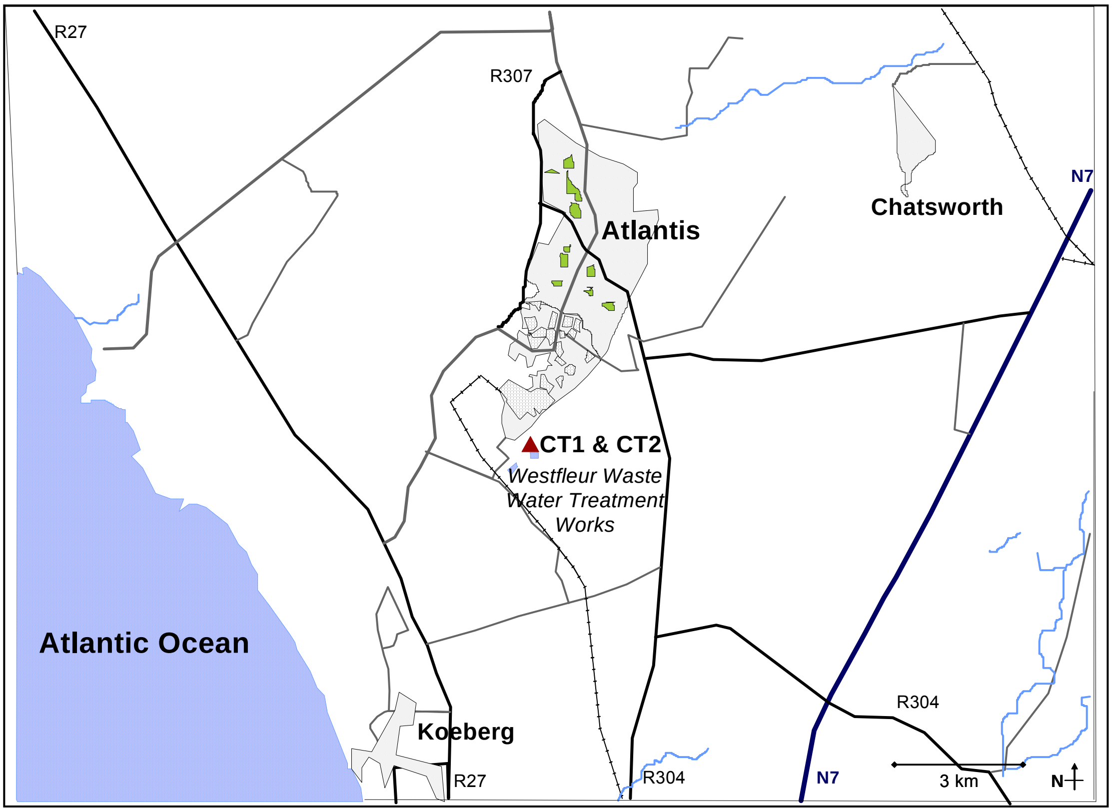


Figure 4.3. Sampling locations of CT1 and CT2 [Atlantis and Koeberg are situated north of Bloubergstrand (Fig. 4.4)]

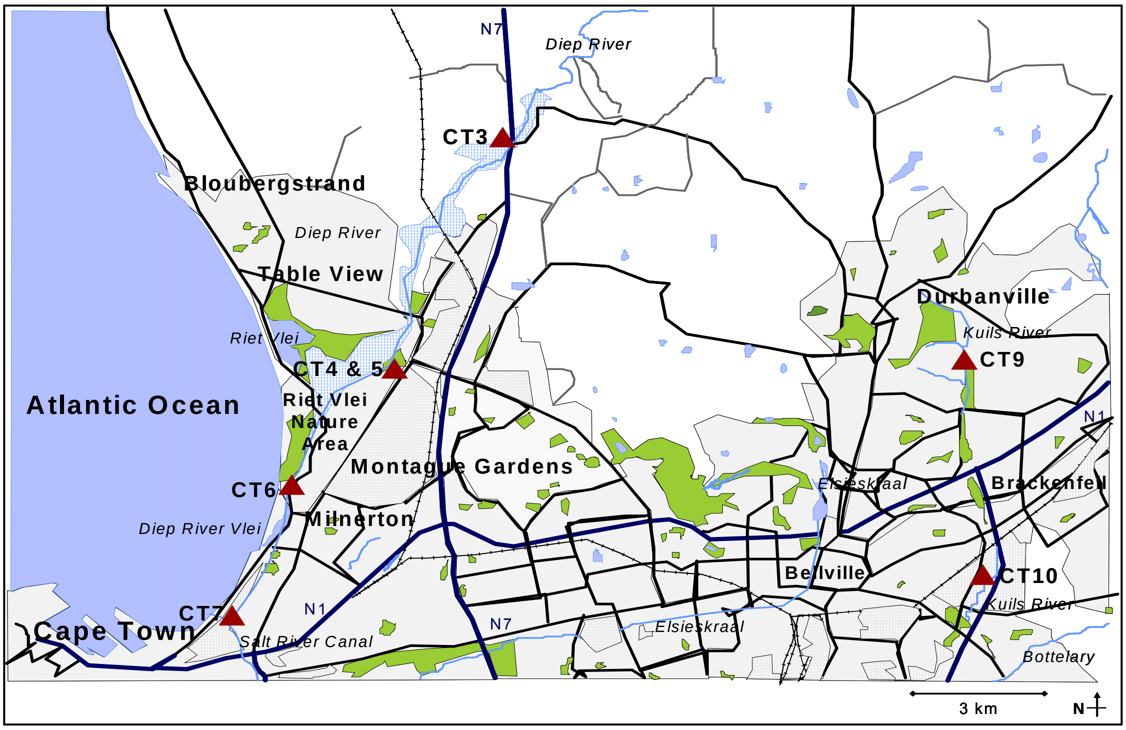


Figure 4.4. The northern parts of Cape Town and its suburbs indicating the sampling locations of CT3 to 7 and CT9 and 10

Figure 4.5. The southern parts of Cape Town and its suburbs indicating the sampling locations of CT8 and CT11 to 20

Table 4.3. Description of sediment sampling sites from Cape Town (CT) and its suburbs

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT1	16/04/07	S33°36'34.8" E18°28'41.2"	Atlantis: Westfleur waste water treatment works (WWTW).	Final sewage effluent containing **domestic waste**.	Sediment settled in cement canal leading to waste water pond – drains to aquifer which supplies drinking water.
CT2	16/04/07	S33°36'34.8" E18°28'41.2"	Atlantis: Westfleur WWTW	Same system as mentioned above at CT1, but contains **industrial waste**.	Same as CT1
CT3	16/04/07	S33°47'25.6" E18°32'52.8"	Vissershok: Vissershok Road off the N7.	Upper part of Diep River. Agriculturally impacted (river flows through vineyards).	River was fairly dry (seasonal flow) and wetland-like with many reeds.
CT4	16/04/07	S33°50'53.4" E18°31'10.8"	Theo Marais Park/Sports fields. Off Koeberg Road (M5).	Highly industrialised area collecting waste water from many industries, including a paper and pulp treatment plant.	Unlined canal collecting industrial waste water and storm water drainage.
CT5	16/04/07	S33°50'53.4" E18°31'10.8"	Theo Marais Park/Sports fields. Off Koeberg Road (M5).	Located in the same area as, and only a few metres away from CT4. Also collects industrial waste water, but from other industries including a fertilizer manufacturer and an oil-and gas refinery.	Unlined canal collecting industrial waste water and storm water. The two canals (CT4 & CT5) join at a point where it flows into the Diep River.
CT6	16/04/07	S33°52'38.0" E18°29'28.1"	Milnerton: Next to the R27, adjacent to Milnerton Golf club and the Canoe club.	Located down-stream of the Riet Vlei Nature area, a few kilometres down-stream of CT4 and CT5.	Lower part of Diep River (wide and deep), just up-stream of the estuary.
CT7	16/04/07	S33°54'29.8" E18°28'36.5"	Paarden Eiland industrial. Vrystaat Road.	Highly industrialised area located near a petrochemical plant.	Part of the Diep River Vlei. Wetland-like area with many hyacinths.

Table 4.3. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT8	16/04/07	S33°57’08.6” E18°29’42.7”	Mowbray: Rondebosch Golf course.	The sample was taken on the Golf course from the Black River, down-stream of Athlone WWTW and other industries.	Black River (slow flow).
CT9	17/04/07	S33°50’46.4” E18°40’06.9”	Dubanville: On Fairview Road off Fairtrees Road.	Open field near residential area. Only residential areas and vineyards are located up-stream of this site.	Kuils River (slow flow). Contains a lot of reeds and water plants.
CT10	17/04/07	S33°53’58.6” E18°40’21.0”	Kaymor Industria: Cilmor Street off the M31.	Industrial area. A new storm water canal was being built at the time with a steep downward slope leading to river. May receive industrial storm water.	Kuils River. Sample was collected between reeds in the shallow part of the river – near the riverbed.
CT11	17/04/07	S33°57’03.4” E18°40’10.2”	Kalkfontein/Gersham: Residential area north-west of Nooiensfontein Road/ Stellenbosch Road intersection.	Residential area. Down-stream of Bellville’s WWTW, industries and a residential area. Beyond (down-stream) the Bottelary- and Kuils River confluence.	Kuils River (wide). Cattle feeding and drinking at site.
CT12	17/04/07	S34°04’10.5” E18°45’52.3”	Macassar Beach: Off Macassar Road – at pump station.	Industrial area, down-stream of industries and residential areas and Macassar WWTW. Beyond the Kuils- and Eerste River confluence.	Eerste River (medium flow) after Kuils River confluence.
CT13	17/04/07	S34°03’54.4” E18°46’19.1”	Faure area: R102	Mainly vineyards up-stream.	Eerste River (slow flow) before confluence with Kuils River.

Table 4.3. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
CT14	17/04/07	S33°58'16.3" E18°34'42.7"	Airport Industria: Manhattan Street. Open lot surrounded by industries.	Highly industrialised area which may receive a lot of storm water due to downwards slope leading to the pond.	Relatively stationary artificial pond. Wetland-like and enclosed by reeds.
CT15	17/04/07	S34°00'04.9" E18°33'05.6"	Sand Industria: Near M7 off ramp to the M9. Edith Stephens Wetland Park.	Down-stream of residential and industrial areas. The wetland is used to control storm drainage and to prevent flooding.	Wetland. Relatively stationary with many hyacinths.
CT16	17/04/07	S34°00'37.7" E18°31'36.5"	Hanover Park: Springfield Street.	The river flows through many industries and low-income, high-density residential areas.	Ottery River. Flows through a canal that is lined with bricks. Water from this river is used for the irrigation of vegetables.
CT17	17/04/07	S34°04'22.0" E18°31'06.9"	Zeekoevlei: Zeekoevlei Rd.	Surrounded by low-income residential areas. Sediment was collected near the Cape Flats WWTW. Receives flow from the Bottelary River and receives discharge from the Rondevlei.	Zeekoevlei: Large vlei with slow water flow. Frequent blue-green algal blooms.
CT18	17/04/07	S34°04'34.9" E18°31'06.5"	Muizenberg: Uxbridge Road. Zandvlei yacht club.	Residential area. Vlei receives flow from rivers draining industrial areas.	Zandvlei: Tidal flow - estuary area that forms a vlei.
CT19	17/04/07	S34°04'34.9" E18°27'41.8"	Frogmore Estate: Aberfeldy Road.	Residential area situated directly down-stream of Retreat Industrial Area. The river flows directly through this industrial area.	River with medium flow. Beyond Spaanschemat- and Keysers River confluence.
CT20	17/04/07	S34°02'50.9" E18°28'26.2"	Heathfield: Willowmere Road.	Residential area surrounded by industries in. Receives flow from the Diep River.	Little Princess vlei: Vlei with many reeds and water plants.

4.2.4. Durban

Durban has a large and diverse economy consisting of industrial, tourism, and transportation sectors, and one of South Africa's foremost harbours. The harbour is a major importer of raw materials and an exporter of many products such as manganese, chrome ore, coal, sugar and grain. Significant industries in Durban include the textile industry, railroad repair, machine works, oil refineries, and the manufacturing of soap, paint, dye, and fertilizer. Many high-density, low-income residential areas are located in the vicinity of the city. Umlazi is one of the biggest of these types of settlements, accommodating more than 300 000 inhabitants (Statistics South Africa, 2008).

In Durban, several river systems were targeted to represent highly polluted, less polluted and theoretically "pristine" areas. Sediment was sampled from several industrial areas (D1 to 5, 8 to 11 and 14) and close to the harbour at the yacht club (D6), as well as near the dry docks (D7) where old paint is stripped from ships. Reference samples were also collected from rivers flowing through residential areas (D12 and 13), expected to receive minimal industrial effluent due to the lack of industries up-stream of these areas (Fig. 4.6). Site descriptions follow in Table 4.4

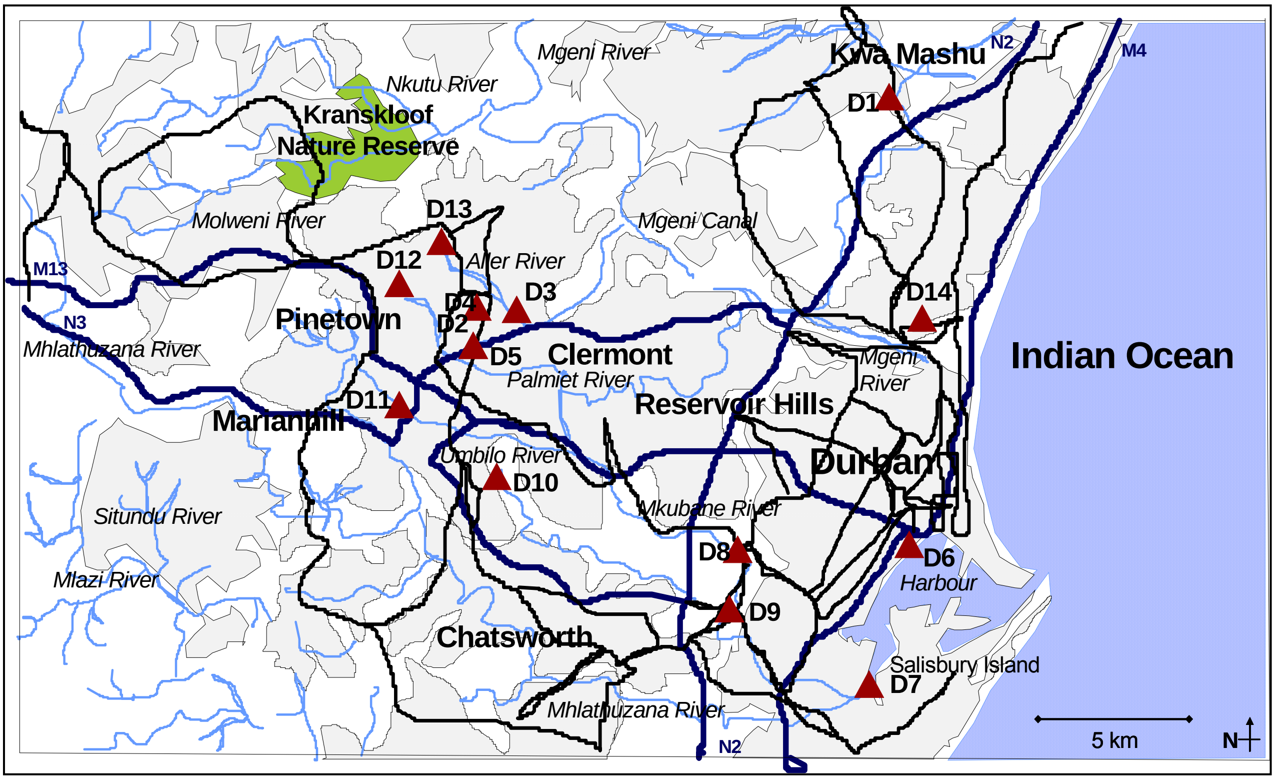


Figure 4.6. Map of Durban and its suburbs indicating the sampling sites of this area (D1 – 14)

.

Table 4.4. Description of sediment sampling sites from Durban (D) and its suburbs

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
D1	20/02/07	S29°45’03.8’’ E31°00’36.7”	Inanda/Phoenix: On R102. Stone age cement construction site	Draining various industries, mainly textile and building materials manufacturers.	Umhlagane River. Medium/slow flowing stream.
D2	20/02/07	S29°48’21.9’’ E30°53’49.3”	New Germany WWTW (downstream of effluent)	Across from low-income residential area. Receiving effluent from various industries including steelworks and textile industry.	Aller River. Medium/slow flowing stream receiving treated sewage effluent.
D3	20/02/07	S29°48’21.9’’ E30°53’49.3”	New Germany WWTW (upstream of effluent)	(See D2)	Aller River. Medium/slow flowing stream sampled up-stream of effluent pipes.
D4	20/02/07	S29°48’17.2’’ E30°53’04.5”	Falcon industrial park. Off of Eskom Road at Valleyview Road.	Mainly textile industry. Up-stream of New Germany WWTW.	Aller River. Slow, narrow stream.
D5	20/02/07	S29°48’53.2’’ E30°52’55.6”	Down-stream of Pinetown and New Germany industria. Genie Sand distributors.	Various industries.	Palmiet River. Medium/rapid flowing stream.
D6	21/02/07	S29°51’56.3’’ E31°01’03.4”	Durban Harbour: At yacht club across from Wilson wharf.	Down-stream of industrial areas.	Sea – harbour.
D7	21/02/07	S29°53’58.5’’ E31°00’17.2”	Durban Harbour: Bayhead Park. Bayhead Road 550. Trawlers wharf.	Down-stream of dry docks and industrial areas.	Harbour.
D8	21/02/07	S29°52’04.2’’ E30°57’53.0”	Umkumbaan: Next to Cato Manor Sports Field. Wentlock Road.	Down-stream of various industries. Historically used as crematoria.	Mkubane River tributary. Slow flowing, narrow river stream.

Table 4.4. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
D9	21/02/07	S29°52'55.9'' E30°57'46.5"	Carrington Heights: Wright Place. Under bridge off Francois Road-Grosvenor Road intersection.	Industrial area.	Mkubane and Umbilo River confluence. Slow flowing, narrow stream.
D10	21/02/07	S29°50'51.4'' E30°53'22.0"	Pinetown: Umbilo WWTW.	Down-stream of WWTW, receiving effluent from textile and dye industries.	Umbilo River. Slow flowing river stream.
D11	21/02/07	S29°49'44.5'' E30°51'30.8"	Pinelands: Trim Park (recreational park). Winston Churchill Drive – Merrifield Road crossing.	Directly down-stream of various industries.	Umbilo River. Shallow river stream.
D12	21/02/07	S29°47'56.8'' E30°51'36.9"	Pinetown: Padfield residential area. Padfield Road.	Residential area. Palmiet River "reference site". Site is up-stream of industries and expected to be "pristine".	Palmiet River. Slow flowing, narrow river stream
D13	21/02/07	S29°47'17.5'' E30°52'22.2"	Reservoir Hills residential area.	Residential area. Aller River "reference site". Site is up-stream of industries and expected to be "pristine".	Aller River. Shallow river stream.
D14	21/02/07	S29°48'28.2'' E31°01'19.4"	Umgeni Mouth: Umgeni Park (Riverside). Riverside Road, between Butter and Brown's Drive turn-offs.	Umgeni River mouth: After confluence of Palmiet-, Aller- and Mbongokazi Rivers with Umgeni. May contain various industrial effluents.	Umgeni River mouth. Deep, wide river mouth.

4.2.5. Richards Bay

Richards Bay is home to the country's largest harbour, which was converted into a deep water harbour in 1976. The city has an extensive industrial complex composed of aluminium smelters, a fertiliser plant, paper and pulp manufacturers and woodchip producers. Iron ore, titanium oxide and zircon are mined from sand dunes located close to the estuary. The city's exports include coal, aluminium, titanium, granite, ferrochrome, paper, pulp, woodchips and phosphoric acid (Statistics South Africa, 2008).

Only nine samples were collected from Richards Bay, since the area is concentrated with water bodies situated close to one another. Sediment was sampled from the harbour and its surrounding areas, including the small craft harbour (RB5), Naval Island recreational area (RB6) and water canals under harbour authority (RB2 and 3) (Fig. 4.7). Where accessible, samples were also collected from other industrially impacted areas, specifically targeting the two aluminium smelters, a fertilizer plant, a woodchip manufacturer and other less significant industries (RB1 and 4) (Fig. 4.7). Sediment from RB1 was black and had a distinct noxious odour. The site was located in the direct vicinity of a large industrial complex and seemed to be heavily polluted with industrial effluent. Sites RB7 to 9 were chosen in residential areas (Fig. 4.7). Table 4.5 contains site descriptions and other information on the sampling areas.

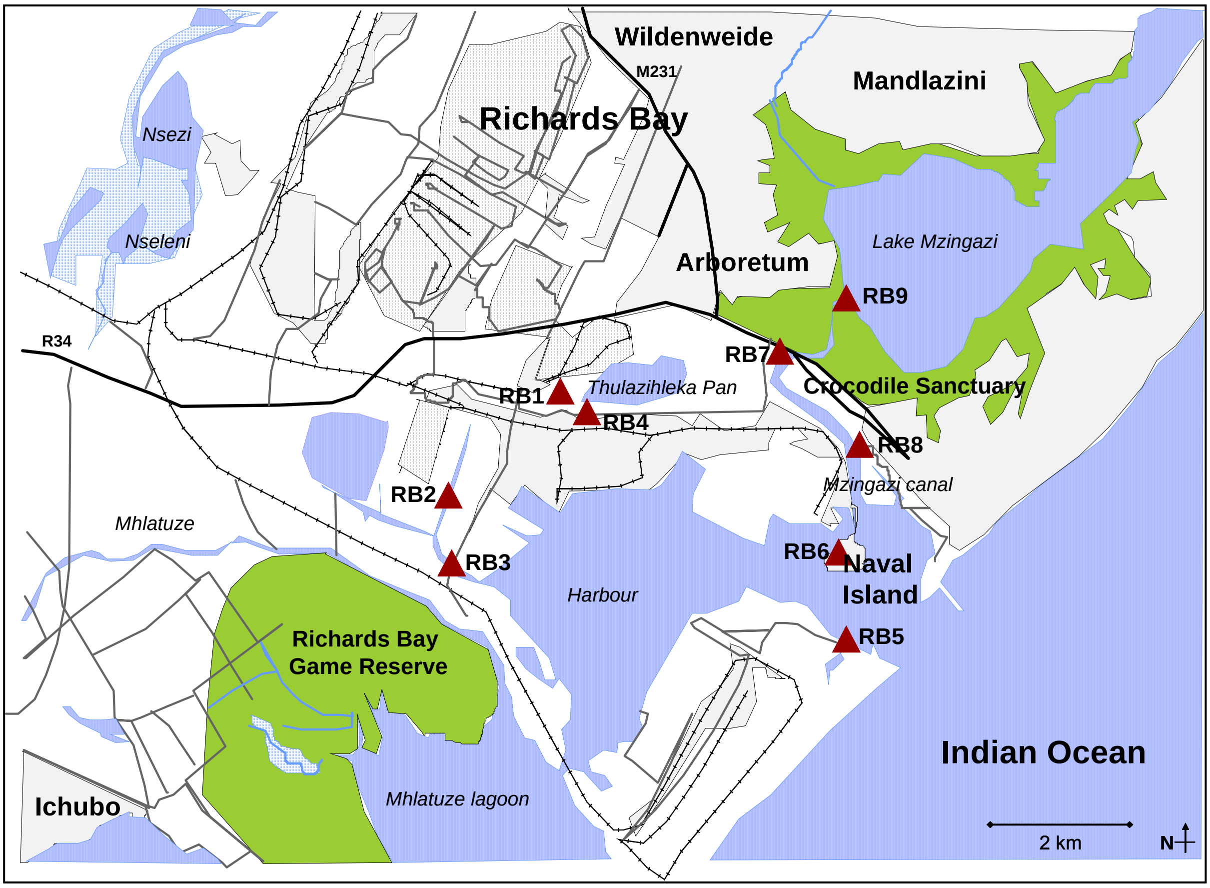


Figure 4.7. Map of Richards Bay indicating sediment sampling sites

Table 4.5. Description of sediment sampling sites from Richards Bay (RB)

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
RB1	19/02/07	S28°46’44.2’’ E32°02’07.9”	Richards Bay industrial: Gravel road off Harbour Arterial.	Industrial area. Includes woodchip production, aluminium smelters, fertilizer plant etc.	Wetland area with many reeds.
RB2	19/02/07	*S28°47’33.40’’ E32°01’03.32”	Manzamyana Canal off Urania Road, close to railway.	Various industries. In close proximity of aluminium smelters.	# Manzamyana Canal.
RB3	19/02/07	*S28°48’03.48’’ E32°01’05.21”	Urania Road. After confluence of Manzamyana- and Bhizolo Canals.	Down-stream of various industries. Near aluminium smelters.	# Manzamyana- and Bhizolo Canal confluence.
RB4	19/02/07	S28°46’54.0’’ E32°02’22.5”	Thulazihleka Pan: Bird Sanctuary.	Down-stream of various industries. Near woodchip producers, aluminium smelters etc.	Pan. Sample was taken near reeds.
RB5	19/02/07	*S28°48’37.70’’ E32°04’51.15”	Richards Bay Harbour. Sample taken at location between small craft harbour and combi terminal.	Near industrial area. Sample taken inside harbour.	Harbour (Sea).
RB6	18/02/07	S28°47’58.5’’ E32°04’45.4”	Naval Island. Recreational area.	Near harbour.	Lagoon-like, tidal area.
RB7	18/02/07	S28°46’26.6’’ E32°04’09.4”	Ngodweni Canal. After Medway Road, before joining with Mzingazi Canal.	Residential development.	# Ngodweni Canal.
RB8	18/02/07	S28°47’09.4’’ E32°04’55.9”	Mzingazi canal. Meerensee boatclub.	Residential area.	# Canal.
RB9	18/02/07	S28°46’02.2’’ E32°04’46.9”	Lake Mzingazi: Private Road.	Upstream from future residential area under construction.	Lake.

\# Unlined canals, which look like natural streams.

* GPS coordinates uncertain due to poor satellite reception.

4.2.6. Bloemfontein and Botshabelo

As with the other cities included in the study, Bloemfontein has a large industrial centre containing railroad workshops, metal works, food-processing plants, and manufacturers of clothing, furniture, plastics and glassware. The city is home to a power generation plant and has many transport services, including a railway and an airport. Mining, livestock farming (cattle and sheep) and dry-land maize and wheat cultivation are the main occupations in the area surrounding the city.

In total, ten sediment samples were collected from Bloem Spruit and the Modder River, as well as from other dams and ponds in the area. The sampling locations once again included industrially- (BF4 to 6 and 8), residentially/recreationally- (BF3, 7, 9 and 10) and agriculturally (BF1 and 2) impacted areas (Table 4.6; Fig. 4.8 & 4.9).

Botshabelo, the largest township in the Free State Province, is located about 50 km east of Bloemfontein along the N8 national road (Fig. 4.8). Botshabelo is mainly residential, but it has an industrial complex consisting of more than 150 factories specialising in the manufacture of plastics and textile, amongst others. Samples were taken from a wetland system meandering through the township, passing through industrial- and residential areas (BO1 to 4) (Fig. 4.8, Table 4.7). During the sampling period, it was evident that many parts of the township had poor municipal services with inadequate water supply and sanitation.

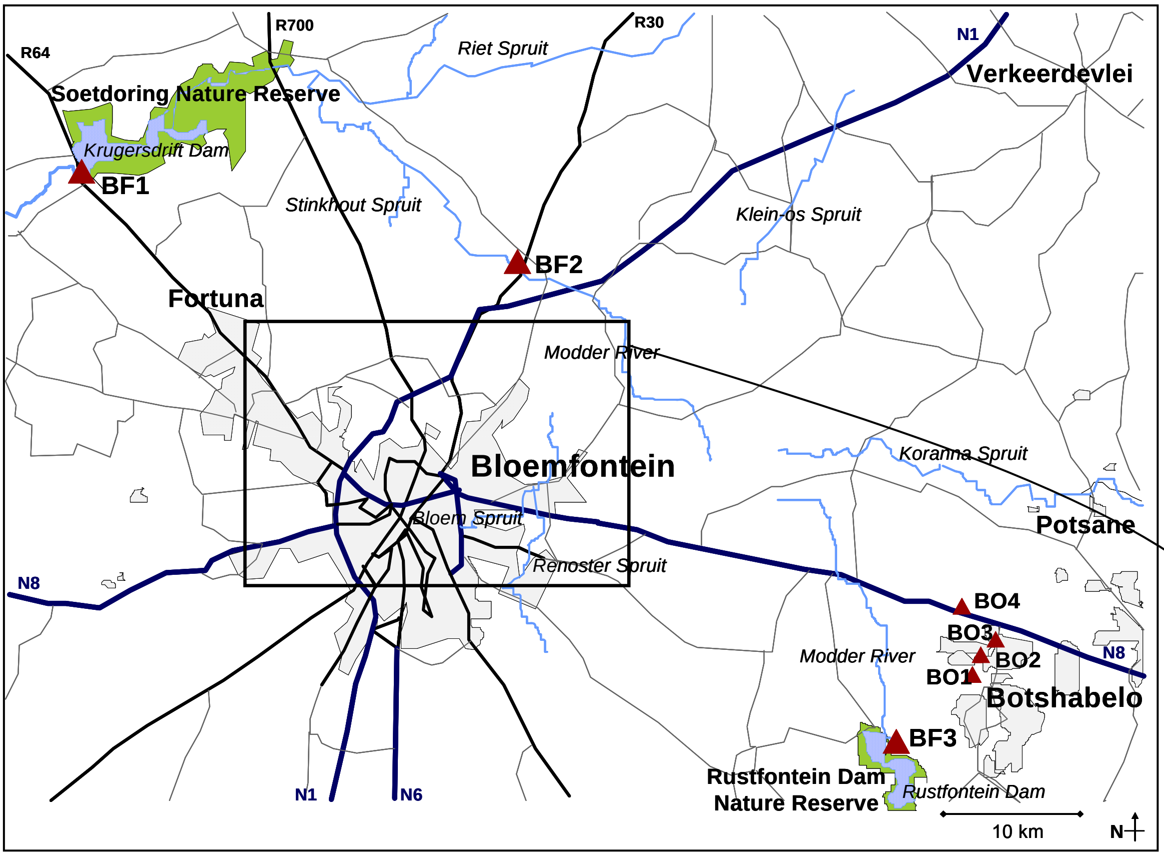


Figure 4.8. Map of Bloemfontein and its surrounding areas indicating the sites sampled from Botshabelo and the outskirts of Bloemfontein

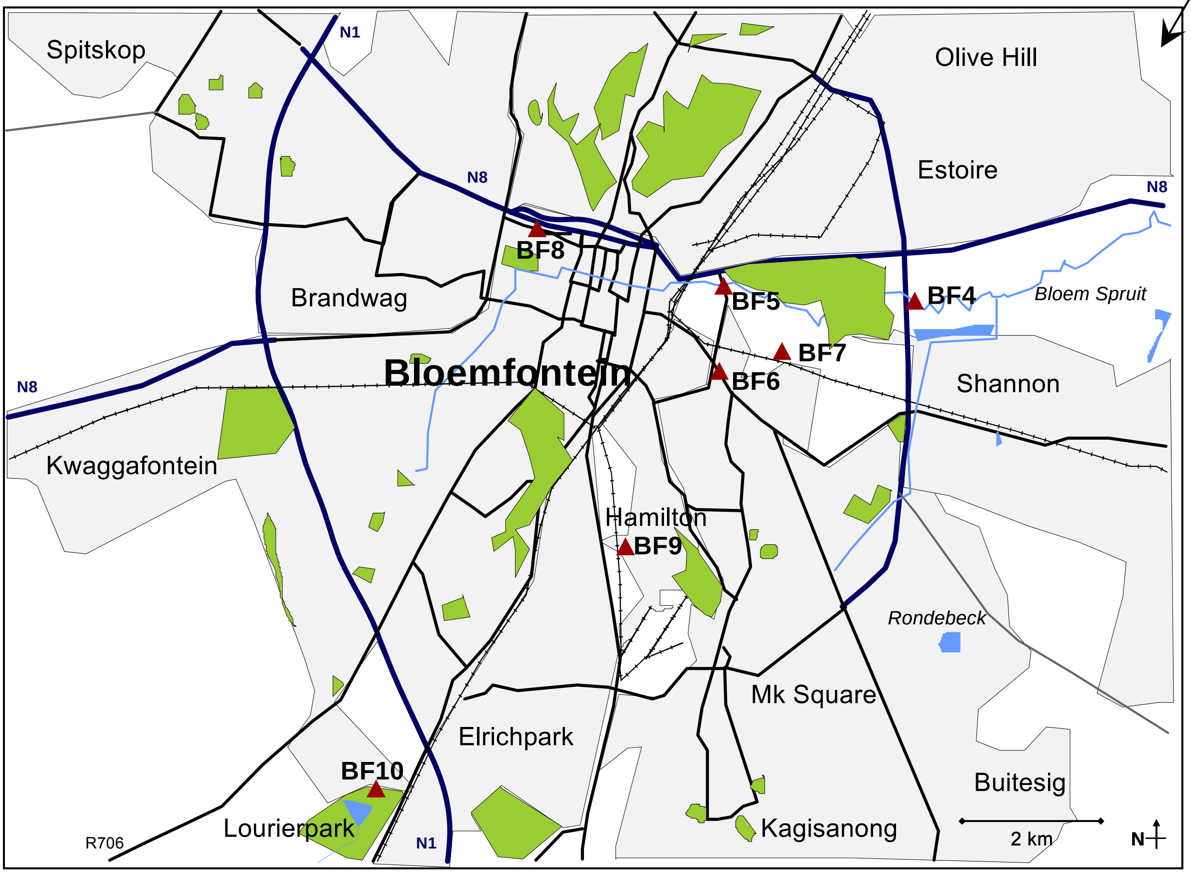


Figure 4.9. Map of Bloemfontein indicating sediment sampling sites

Table 4.6. Description of sediment sampling sites from Bloemfontein (BF)

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BF1	19/04/07	S28°53'06.5" E25°57'18.3"	On the R64 near the wall of the Modder Dam/Krugersdrift Dam.	Agricultural area.	Modder River tributary that flows from the Modder Dam. Rocky riverbed with many reeds and little sediment.
BF2	19/04/07	S28°56'44.8" E26°19'01.5"	On the R30 off the N1.	Undeveloped/agricultural area.	Modder River tributary down-stream of Krugersdrift Dam. Slow flowing tributary with clayish sediment.
BF3	19/04/07	S29°16'42.9" E26°37'27.5"	Off the N8 Rustfontein turn off.	Rustfontein Dam recreational area. No industries in close proximity. May be impacted on by Botshabelo.	Large dam. Used for recreational purposes, including fishing.
BF4	19/04/07	S29°07'20.1" E26°16'05.0"	Of the M10 (Rudolf Greyling) south of the intersection with the N8 in Bloemfontein.	Across from Bloemfontein Golf Club, near Ooseinde industrial area.	Bloem Spruit: River tributary flowing through deep gully.
BF5	19/04/07	S29°07'12.2" E26°14'15.6"	Buitesig: McGreggor Street.	River flows through the CBD, many industries and a low-income residential area.	Bloem Spruit: Shallow, narrow, rapid flowing river with many rocks..
BF6	19/04/07	S29°07'53.5" E26°14'15.2"	Off McGreggor Street, turn into Dr. Belcher Street.	The stream flows through an industrial- and a low-income residential area.	Unknown river stream than joins with Bloem Spruit at a down-stream locality. Shallow river with medium to slow water flow.
BF7	19/04/07	S29°07'44.1" E26°14'48.9"	Ooseinde: McKenzie Street.	Industrial area with an informal settlement located on the river bank.	Part of the same system as BF6. The stream is narrow, shallow and rocky.

Table 4.6. *Continued*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BF8	19/04/07	S29°06’47.5” E26°12’26.5”	Loch Logan: Across from the waterfront on municipal grounds.	Situated within Bloemfontein’s CBD. Near a shopping mall. Mostly businesses located near the area.	Loch Logan: Pond. Receiving water from inflow pipes.
BF9	19/04/07	S29°09’14.2” E26°13’18.2”	Hamilton: Mill Street	Low-income, high-density residential area (Bochabella*) and a large industrial complex (Hamilton).	Blou Dam: relatively large dam with many reeds and abundant bird life. The area was flooded during the time of sampling.
BF10	19/04/07	S29°11’09.5” E26°10’53.1”	Lourierpark: Geelhout Street	Residential/Recreational area.	Rooi Dam: Large Dam with abundant bird life and many plants.

* Not to be confused with Botshabelo, the large township situated 50 km east of Bloemfontein.

Table 4.7. Description of sediment sampling sites from the township of Botshabelo (BO)

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
BO1	19/04/07	S29°13’47.1” E26°41’31.8”	Off of main road, open field.	Residential/industrial area.	Wetland with a narrow stream. Raw sewage was present in water.
BO2	19/04/07	S29°13’02.1” E26°42’01.2”	Off of main road on right hand side.	Residential area.	Wetland. Sampled from a small pond-like area.
BO3	19/04/07	S29°12’18.8” E26°42’31.7”	Main Road close to the Shell garage.	Industrial area.	Wetland area – a narrow stream with many reeds.
BO4	19/04/07	S29°11’28.9” E26°41’35.6”	On the N8 – near a small bridge.	Near national road (N8) surrounded by grasslands.	Wetland area with narrow stream..

4.2.7. International and other rivers

One of the project's objectives is to estimate the scale of POPs transport to neighbouring countries. For this purpose, several international rivers were sampled, including the Olifants- (Mpumalanga), Crocodile- (Mpumalanga), Komati-, Pongolo-, and Limpopo Rivers. The Olifants- (Fig. 4.11), Crocodile- (Fig. 4.11), and Pongola (Fig. 4.12) Rivers flow from South Africa into Mozambique, and the Komati River (Fig. 4.12) runs into Swaziland. The Limpopo River (Fig. 4.10) is a large river system running through South Africa and Mozambique, forming a border between South Africa and Zimbabwe, and South Africa and Botswana. Samples were collected from three to six locations distributed within each river system, and at least one sediment sample was taken as close as possible to the borders of neighbouring countries. The majority of sites were located down-stream of, or in close proximity to industries such as paper and pulp manufacturers, petrochemical plants and brick works. Many sites were situated in the vicinity of agricultural lands, primarily sugar cane plantations.

Sediment was sampled from the Mzimkhulu- and Mkomzi Rivers and one unknown stream located in the Drakensberg, a high altitude mountain in South Africa (Fig. 4.13) to determine if selected POPs are transported via long-range transport and deposited in the colder climates of high altitude areas. No major industries are located in the area, and the main agricultural practice is farming with cattle and crops, therefore; no industrial POPs are expected to be found in these sediments. If these pollutants are present in sediments collected from these localities, it could possibly be a consequence of long-range transport. However, chlorinated pesticides, which may be applied to crops, were expected in these sediments.

En route from Richards Bay to Durban opportunistic samples were taken from three water bodies: the Mhlathuze-, Tugela- and Umvoti Rivers. These samples were collected down-stream of, or in close proximity to paper and pulp manufacturers to assess the potential contribution of these industries to the presence of organic pollutants in sediments. The samples were taken in Felixton, Mandini and Aldinville (Fig. 4.13).

Site descriptions are listed in Table 4.8 and sites maps are given in Figures 4.10 to 4.13.

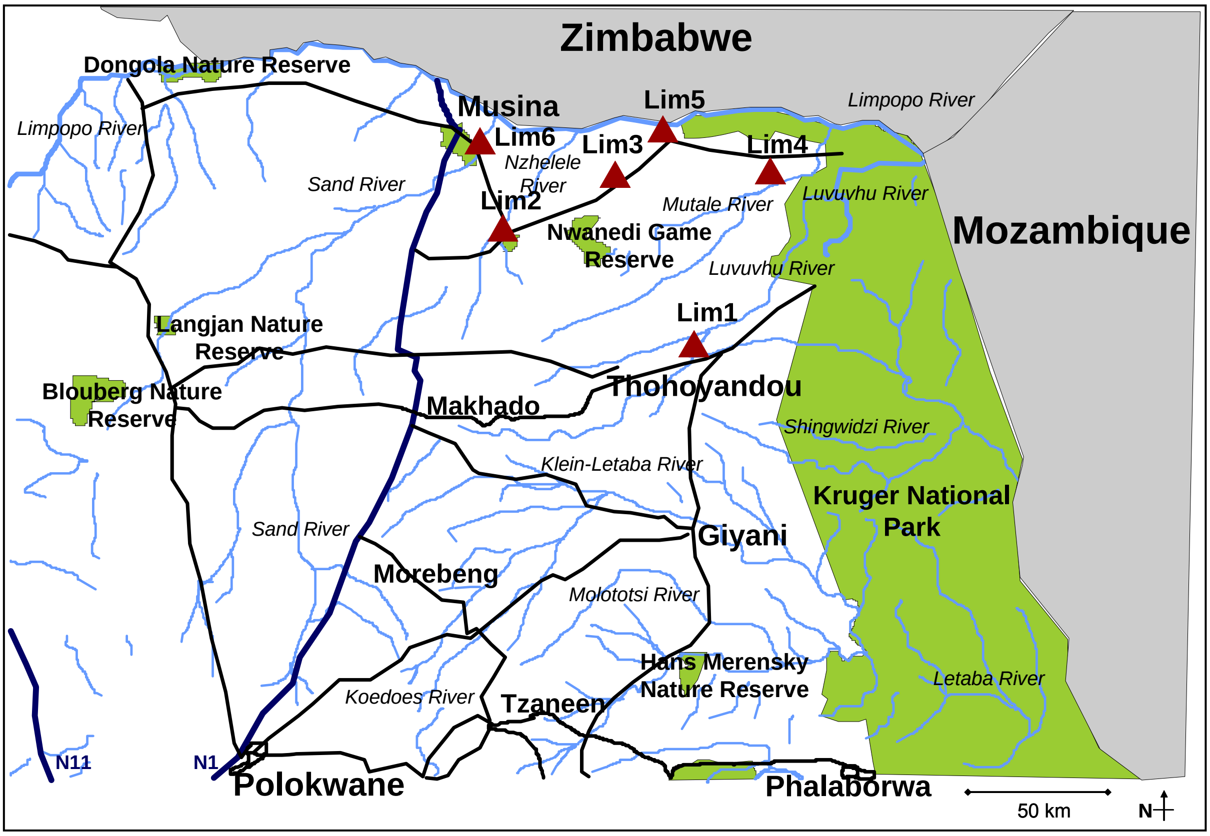


Figure 4.10. Sediment sampling sites in the Limpopo River

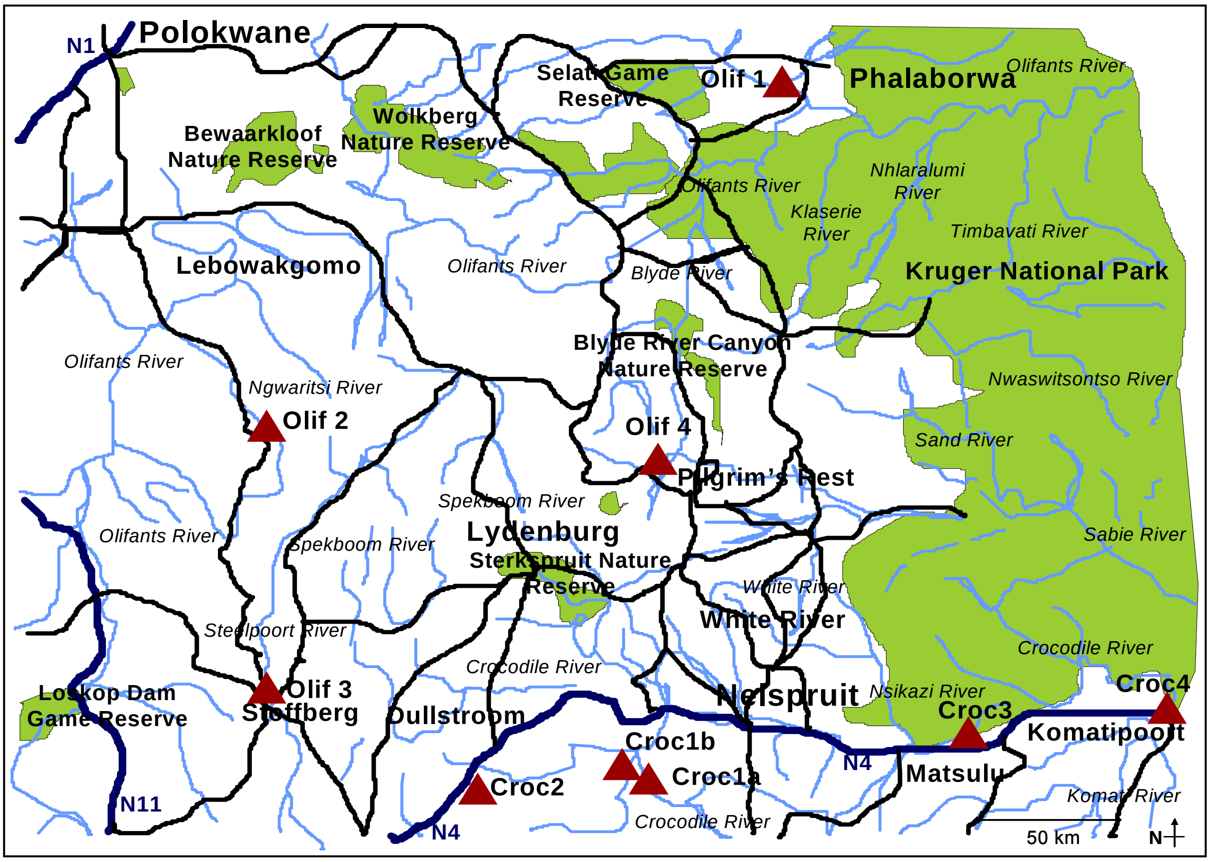


Figure 4.11. Sediment sampling sites in the Crocodile- and Olifants Rivers

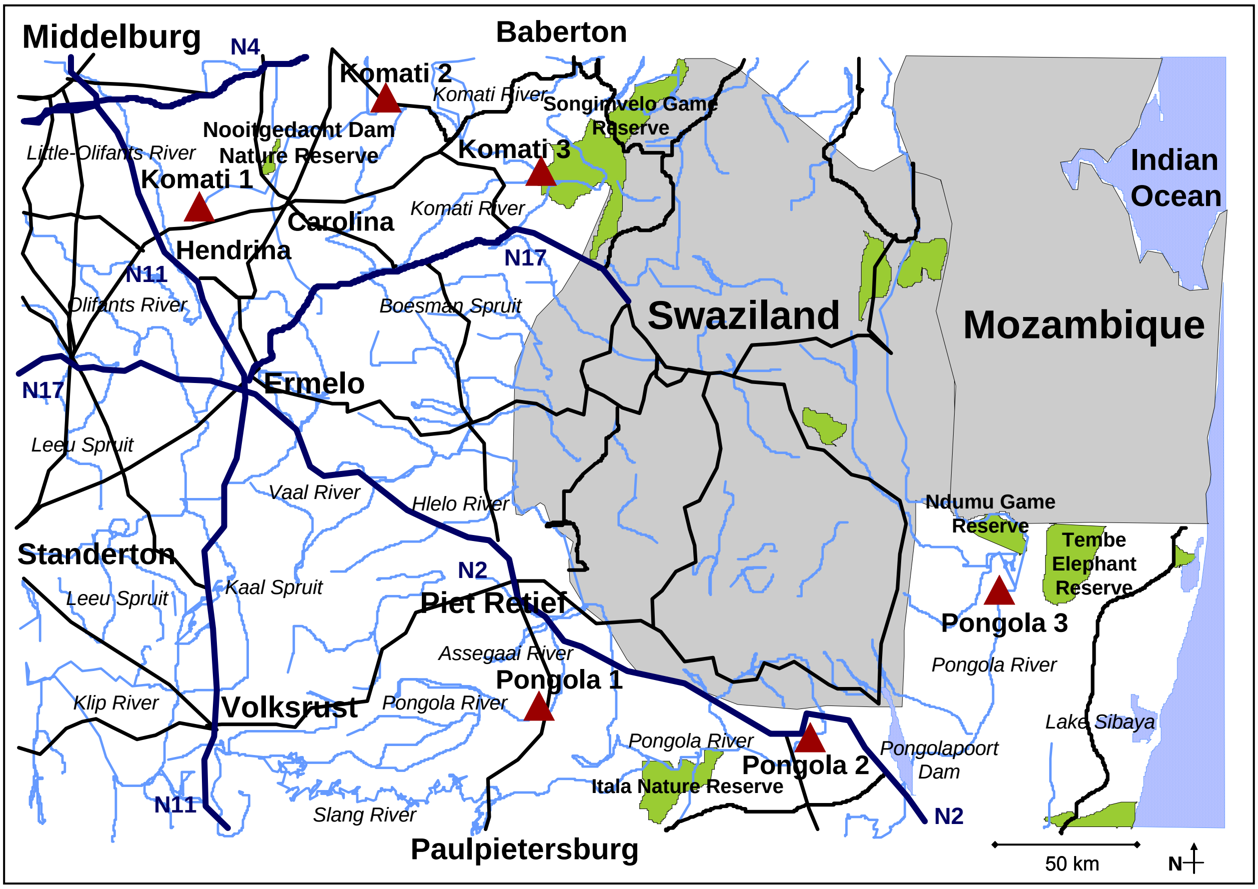


Figure 4.12. Sediment sampling sites in the Komati- and Pongola Rivers

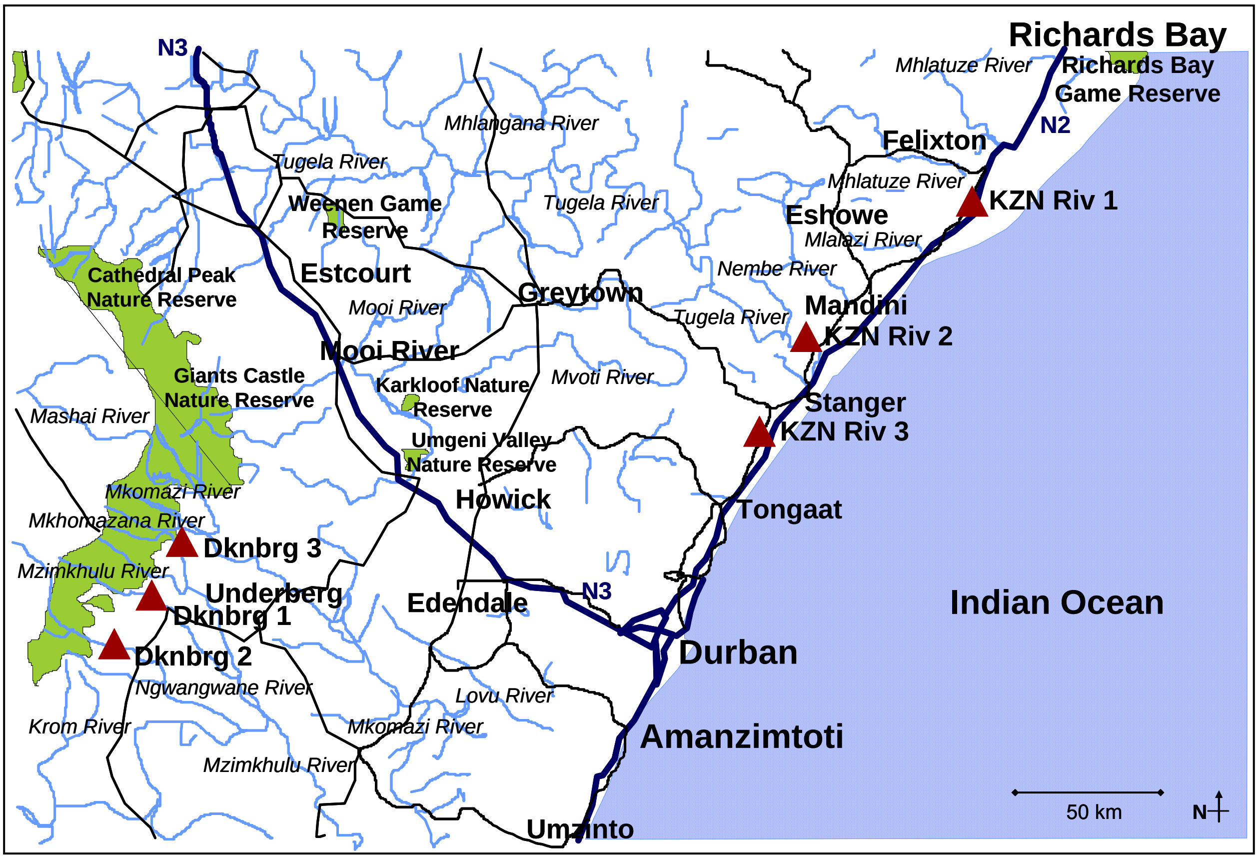


Figure 4.13. High-altitude rivers and rivers sampled in the vicinity of KZN paper mills

Table 4.8. Description of sediment sampling sites from international- [Limpopo- (Lim), Olifants- (Olif), Crocodile- (Croc), Komati- and Pongola Rivers] and high-altitude rivers (Drknberg), and KwaZulu-Natal (KZN) rivers associated with paper mills

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Lim1	07/03/07	S22°54’071” E30°41’729”	North east of Thohoyandou on R524.	Agricultural (cattle drinking place). Local housing 50 km away.	Luvuvhu River.
Lim2	08/03/07	S22°36’524” E30°09’942”	Near Tshipise on R508, north of R525-R508 intersection.	Agricultural (citrus farms).	Nzhele River.
Lim3	08/03/07	S22°28’260” E30°27’796”	On R525 – North of Nwanedi Game Reserve.	Bridge and traffic crossing.	Nwanedi River.
Lim4	07/03/07	S22°28’407” E30°52’823”	Gravel road south of Masisi.	Local housing in the vicinity agricultural activities. Laundry and bathing in river.	Mutale River.
Lim5	08/03/07	S22°21’063” E30°35’489”	Gravel road north of R525.	Gholf course, lucern and palm tree plantation. Fishing spot and game farms.	Nwanedi River.
Lim6	08/03/07	S22°23’931” E30°05’950”	On R508: South-east of Messina.	Adjacent to Musina Nature Reserve.	Sand River.
Olif1	15/02/07	S23°58’35.5’’ E31°04’26.5”	On R530 near Phalaborwa, 46 km from Mica.	Industrial area. In close proximity of oil and gas refinery, power plant and mines.	Olifants River: Slow/stationary river tributary.
Olif2	15/02/07	S24°47’00.0’’ E29°50’05.5”	On R579 near Jane Furse. Bridge over road.	Agricultural land and close to many informal settlements.	Olifants River: Rapid flowing, rocky river tributary.
Olif3	15/02/07	S25°23’55.0’’ E29°49’31.9”	2 km north of Stoffberg. Under bridge 2871, Blinkwater.	Agricultural area. Steel and coal works.	Olifants River: Relatively rapid flowing water river tributary.

Table 4.8. *Continued.*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Olif4	16/02/07	S24°52’38.4’’ E30°45’36.6”	Pelgrim’s Rest:Secondary Road. (Blyde River)	Paper and pulp plantations and mining industries.	Olifants River tributary: Very rocky river with rapid flow..
Croc1a	17/02/07	S25°35’57.0’’ E30°45’30.5”	Secondary road north-west of Kaapsehoop. Water under road.	Paper mill – downstream of Ndogwana paper and pulp plant. Many plantations in area.	Crocodile River: Wetland-like tributary. Slow flowing with a lot of plant material.
Croc1b	17/02/07	S25°34’04.8’’ E30°39’43.9”	Ngodwana paper mills. Tar road off of bridge.	Ndogwana paper mills.	Crocodile River: Fast flowing tributary. Soil and sediment composite.
Croc2	17/02/07	S25°38’22.0’’ E30°19’37.6”	Watervalboven: Bridge located in Abattoir Road.	Town, agricultural lands and plantations.	Crocodile River: Rocky, fast flowing river.
Croc3	16/02/07	S25°29’17.7’’ E31°30’11.9”	Malelane: Monte Vista Estate	Sugar cane and plantations.	Crocodile River: Relatively slow flowing river stream.
Croc4	16/02/07	S25°26’31.8’’ E31°57’50.6”	Komatipoort: Bridge over river. Right turn-off of Rissik Street. .	Sugar cane and plantations.	Crocodile River: Slow, wide river with many reeds.
Komati1	17/02/07	S26°07’58.8’’ E29°54’09.8”	On the R38 between Carolina and Hendrina. (Vaalbank)	Agricultural area with coal mines.	Komati River tributary. Almost dry, very slow flowing river.
Komati2	17/02/07	S25°49’35.1’’ E30°25’23.9”	On R541 approximately 15 km north of Badplaas.	Agricultural.	Komati River tributary: Rocky, rapid flowing tributary.
Komati3	16/02/07	S26°00’32.4’’ E30°52’09.7”	Josefdal. Off R40 from Baberton. Mlomati Sappi plantations.	Sugar cane and paper plantations.	Komati River tributary: Slow flowing tributary with many reeds.

Table 4.8. *Continued.*

Site nr	Sampling date	Coordinates	Location	Description of area	Type of water body
Pongola1	17/02/07	S27°18’33.8’’ E30°53’53.1”	On R33 near Commondale.	Sappi plantations.	Komati River: Wide, deep river with sandy sediment.
Pongola2	18/02/07	S27°25’01.5’’ E31°42’50.7”	Illovo farms near Pongola.	Sugar refinery. Agricultural, mainly sugar cane plantations.	Komati River: Wide river with heavy flow and sandy soil.
Pongola3 ***Sample lost***	18/02/07	S27°02’12.7’’ E32°15’56.4”	50 km north of Jozini on Khosi-bay road bridge.	Agricultural. Many informal settlements and brick works near site.	Komati River: Wide river with medium flow. (Swimming and bathing)
Drknberg1	22/02/07	S29°46’27.9’’ E29°28’29.2”	Drakensberg: On R617. At farm 4 km South of Underburg.	Farming/Plantations.	Upper part of Mzimkhulu River.
Drknberg2	22/02/07	S29°54’20.3’’ E29°20’11.6”	Drakensberg: Right turn-off off R617. Approximately 20 km from Bushmen’s neck.	Farming.	Mountain stream.
Drknberg3	22/02/07	S29°39’10.7’’ E29°32’45.8”	Secondary gravel road. 20 km north of Himeville.	Farming.	Upper part of Mkomazi River.
KZN Riv1	20/02/07	S28°50’19.3’’ E31°54’02.7”	Felixton. Paper mills.	Down-stream of paper and pulp plant. Many agricultural lands (mainly sugar cane) in the area.	Mhlathuze River. Wide river, with medium to rapid flow.
KZN Riv2	20/02/07	S29°09’05.9’’ E31°24’35.1”	Mandini: Off R102, road into town by bridge.	In close proximity of paper mills and sewage works.	Tugela River. Narrow part of river, with slow to medium flow.
KZN Riv3	20/02/07	S29°22’59.1’’ E31°15’05.9”	Aldinville: South of Stanger. Gravel road, under bridge.	In close to paper mills, plantations, brickworks and sand mining.	Umvoti River. Wide river, with medium flow.

4.3. Sediment sampling

4.3.1. Planning

Prior to sampling, several mapping programs, including Garmin MapSource and Google Earth were used to select the most suitable sampling locations within each of the selected areas.

The results of a previous South African study focusing on PCDD/Fs and PCBs in sediments (Nieuwoudt, 2006) indicate that wetland sediments generally contained higher levels of these organic pollutants than river sediments. This is probably because wetlands are more lotic than rivers, preventing pollutants from being “washed” from its sediments, and because wetland sediments have a more organic nature with the potential to “capture” more pollutants. It was therefore decided that:

- Lotic (stationary) water bodies (preferably wetlands) should be targeted as a first option to minimise the effect of water flow on sediments, and
- When sampling from rivers, sediment should be collected at a location where flow would have a negligible or small effect on the movement of sediment i.e. target areas where sediment are deposited and not easily disturbed by the flow of the river.

Finally, the accessibility of the water bodies played an important role in site selection, and sites were chosen at locations that were accessible with relative ease: on foot in waders.

4.3.2. Method of sediment sampling

Of the 96 samples collected, only four were soil samples. The sampling method for soil was similar to the method used for sediment sampling. Sediment was collected from the surface layers (top 1 to 10 cm) by means of a brass grab sampler or a metal spade and deposited into stainless steel containers. Composite samples were assembled by combining equal volumes of sediment from five random points within each of the aquatic sites. The sub-samples were mixed together briskly for approximately one minute to obtain a homogenous sample and transferred to labelled glass containers. The lids of the containers were lined with aluminium foil to prevent sample contamination from contact with the plastic lining of the lid. To prevent ultra violet (UV) breakdown and bio-degradation of the compounds of interest, the containers were covered in brown paper bags and stored at -4 °C until air-dried prior to extraction (Hilscherova *et al.,* 2003).

Sampling was done with pre-cleaned steel or glass equipment, according to US EPA method 1613 (US EPA, 1994). This entails that each of the containers and utensils that came into contact with the sample was washed with phosphate-free soap (Liquinox, Merck) and rinsed with tap- and ultra pure water (18 MΩ) prior to sampling. The containers and utensils were also rinsed with high pressure liquid chromatography-grade (HPLC-grade) acetone and hexane (both from Burdick & Jackson^{TM}) to remove polar and non-polar particles respectively. Glass or stainless steel equipment was used at all times, avoiding plastic to prevent sample contamination (Koh *et al*., 2005).

4.4. Sample extraction and clean-up

All the samples were first screened for the presence of DLCs with the H4IIE-*luc* reporter gene bio-assay at the North-West University in Potchefstroom. Only those samples eliciting significant responses in the assay were selected for chemical analysis, assuming that the compounds included in the study have similar sources to DLCs. Chemical analysis were done by the National Metrology Institute of South Africa (NMISA) [based on the grounds of the Council for Scientific and Industrial Research (CSIR), Pretoria] and Norwegian Institute for Air Research (NILU) in Kjeller, Norway.

4.4.1. Sample extraction and clean-up for analysis with the H4IIE-luc bio-assay

Prior to extraction, the samples were air-dried for a week in an isolated, darkened room that was free from any other activity (Mai *et al.,* 2007). Small stones, leaves and twigs were carefully removed from the dried sediment or soil. The samples were ground with pre-cleaned mortars and pestles to increase the reactivity surfaces, and homogenized with a copper sieve (0.5 mm mesh size). An Accelerated Solvent Extractor (ASE^{TM}) (Dionex, ASE 100) was used to extract DLCs from the samples. The ASE operates on the principle of pressurised fluid extraction by using elevated temperatures and pressure to achieve rapid, effective extraction (Grochowalski & Maślanka, 2003).

The method of extraction can be summarized as follows: A 40 g sub-sample was mixed with an equal volume of anhydrous sodium sulphate (Na_2SO_4) (Merck, UniVAR) to remove residual moisture (Hilscherová *et al*., 2003). Depending on the volume of the sample, it was packed in a 66 ml or 100 ml stainless steel ASE extraction cell, sandwiched between two cellulose filters (30 mm, Dionex). A 3:1 (v/v) mixture of dichloromethane (DCM) and hexane (HPLC grade; Burdick and Jackson^{TM}) was used to extract the samples, using two cycles with the following parameters: 10 342 kPa, 100 °C, 5 minute static and heat time, 60% flush volume and 100 seconds nitrogen purge (Hölscher *et al*., 2004; McCant *et al*., 1999). The extracts were collected in pre-cleaned collection bottles and allowed to cool, whereafter they were evaporated to 1 ml under a gentle stream of nitrogen gas (ultra pure grade, Afrox) at 40 °C inside a TurboVap® II (Caliper).

The volumes were adjusted to 10 ml with hexane and the extracts were subjected to an acid clean-up to prevent non-target compounds from contributing to the response elicited from the H4IIE-*luc* cells (US EPA, 1999; Vondráček *et al*., 2001). [Unfortunately, PAHs, one of the target groups of compounds, are also removed from samples by the acid-wash]. The extract and approximately 15 ml concentrated sulphuric acid (H_2SO_4) (98%, Merck) were added to separation funnels, carefully mixed, ventilated often and left to stand for at least an hour for the two phases to separate. The acid was tapped off and fresh H_2SO_4 was added, until the acid phase was clear (3 to 5 times) (US EPA, 1996a). To remove all traces of acid from the extract, it was washed with a 5% sodium chloride (NaCl) (Fluka, Sigma-Aldrich) solution and left for an hour for the phases to separate. This step was followed by a treatment with 20% potassium hydroxide (KOH) (Saarchem, UniVAR) solution. KOH is a strong base with the ability to break down some of the compounds of interest; therefore, the solution was tapped off the moment the phases separated. The extracts were subjected to a second NaCl-solution treatment and filtered through anhydrous sodium sulphate to remove all traces of water (Koh *et al*., 2005). Cellulose filters and Na_2SO_4 were pre-cleaned with a 3:1 mixture of DCM and hexane in a Soxhlet apparatus for 16 to 24 hours.

Since elemental sulphur may be toxic to the H4IIE-*luc* cells, it was removed from the extracts by gel permeation chromatography (GPC) or by treatment with activated copper. Exclusion of sulphur by GPC was the preferred method, but when the instrument was temporarily out of order, activated copper was used.

A GPC system equipped with a Waters 2487 Dual λ Absorbance Detector, Waters 717plus Autosampler, Waters 1515 Isocratic HPLC Pump and a Waters Fraction Collector III, running on Breeze^{TM} software was used. The system makes use of two Envirogel^{TM} GPC clean-up columns (19 x 150 mm and 19 x 300 mm, Waters) connected in series, with a guard column (4.6 x 30 mm) in-line in front of the column set. The columns are packed with high-performance, fully-porous, highly cross-linked styrene divinylbenzene copolymer particles (100 Å pore size, 15 µm nominal particle size). A reference mixture containing corn oil (Sigma-Aldrich), bis(2-ethylhexyl)phthalate (PESTANAL®, Riedel-de-Haën), methoxychlor (PESTANAL®, Riedel-de-Haën), perylene (OEKANAL®, Riedel-de-Haën) and elemental sulphur (PESTANAL®, Riedel-de-Haën) was prepared, and 2 000 µl of the mixture was injected into the GPC to determine the retention time of each compound. DCM was used as the carrier solvent and the system was set at a flow rate of 5 ml/min for 30 minutes. The initial peak for corn oil appeared approximately 10 to 12 minutes after injection, and the final peak for sulphur appeared almost 22 to 25 minutes after injection. The retention times for phthalate, methoxychlor and perylene were between 12 and 14 minutes, 14 and 15.5 minutes, and 19 and 21 minutes, respectively.

The extracts were evaporated to almost dryness and reconstituted to 2 ml with DCM, subsequent to the acid clean-up in order to exclude sulphur from the samples. After being pre-filtered through a 1 µm glass fibre filter (Ascrodisc®, LifeSciences), the extract was injected into the GPC using the same parameters as for the reference mixture. The extract was collected in TurboVap® II flasks from 0 to 22 minutes after injection to eliminate sulphur.

Freshly activated copper shavings (Merck) were added to the samples immediately after extraction (prior to the acid clean-up) to remove sulphur (US EPA, 1986). The copper shavings were activated by covering it in hydrochloric acid (HCl) (32%, Merck), adding fresh HCl until the shavings were clean and shiny. The shavings were rinsed with deionised water, acetone and hexane before use. Approximately 1 g of copper shavings was added to the extracts, swirled, mixed and allowed to stand for approximately ten minutes. Copper was added until it did not turn black any longer. The extracts were shaken overnight to ensure proper exposure of the samples to the copper shavings. Following the copper treatment, the extracts were evaporated and quantitatively transferred to separation funnels to perform the acid treatment.

The extracts were finally evaporated to 1 ml and stored in amber coloured gas chromatography (GC) vials at –4 °C until used in the bio-assay. On the day of the bio-assay, a two-times dilution series was prepared from the extracts using hexane (Khim *et al.*, 1999).

All utensils and apparatus used during sample extraction and clean-up were washed beforehand with phosphate-free soap, rinsed with ultra-pure water (18MΩ) and rinsed with HPLC-grade acetone and hexane (Vondráček *et al.*, 2001).

4.4.2. Sample extraction and clean-up for chemical analysis

Due to limited sample being available for extraction, S/L 13 was not analysed for OCPs, PCBs, and PBDEs, and the high-alitude reference sites, Drknberg1 and 3, were not analysed for PAHs.

4.4.2.1. Extraction and clean-up method for OCPs, PBDEs and PCBs

Sample extraction and analysis for OCPs, PBDEs and intentionally produced PCBs were done under Norwegian accreditation by NILU in Kjeller, Norway. Analytical details are given by Knutzen *et al*. (2003) and Bengtson Nash *et al.* (2008). In short, dried sediment samples were spiked with ^{13}C-labelled analogs of the analytes (NILU-POPI[2] and NILU-BromI[3]) and extracted with cyclohexane. The majority of the sample matrix was removed with size exclusion chromatography, followed by clean-up on silica and alox columns. Sulphuric acid was also used for clean-up prior to analysis. Suitable recovery control standards were added to each sample before quantification.

4.4.2.2. Extraction and clean-up method for PAHs

The extraction and clean-up for PAHs were done at the North-West University, Potchefstroom, and the extracts sent to the National Metrology Institute of South Africa (NMISA), Pretoria, for analysis. Extraction and clean-up were done using a similar method as depicted in Section 4.4.1. In summary, 40 g of dried sample were mixed with an equal volume of Na_2SO_4, and spiked with perdeuterated surrogate standards[4] prior to extraction.

The samples were extracted with a 3:1 mixture of DCM and hexane with the ASE^{TM} (Dionex), using the same parameters as listed in Section 4.1.1. Elemental sulphur was removed from the extracts with the GPC (Waters), after which the extracts were cleaned on activated Florisil® PR (60 – 100 mesh, Fluka) columns (US EPA, 1996b). Suitable recovery control standards were added to each sample before quantification.

---

[2] NILU-POPI standard contained the following ^{13}C-labelled analogs: PCB 28, -52, -101, -105, -114, -118, -123, -138, -153, -156, -157, -167, -180, -189 and 209, α-HCH, β-HCH, γ-HCH, *p,p*-DDE, *p,p*-DDT, PeCB, HpCl-epoxid, tans-nonachlor, trans-chlordane, dieldrin, mirex, Endosulfan I, Endosulfan II, Endosulfan sulphate, trifluralin, cis-nonachlor, aldrin, endrin, oxychlordane, isodrin, cis-chlordane, delta-BH and Heptachlor.

[3] NILU-Brom I standard contained the following ^{13}C-labelled analogs: BDE-28, -47, -99, -153, -183, -209, and the following d^{18}-labelled analogs: γ-HBCD and α-HBCD.

[4] The PAH standard contained a cocktail of perdeuterated PAHs including d^{8}-naphthalene, d^{8}-acenaphthylene, d^{10}-acenaphthene, d^{10}-fluorene, d^{10}-phenanthrene, d^{10}-anthracene, d^{10}-fluoranthene, d^{10}-pyrene, d^{12}-benz(a)anthracene, d^{12}-chrysene, d^{12}-benzo(b)fluoranthene, d^{12}-benzo(k)fluoranthene, d^{12}-indeno(123-cd)pyrene, d^{12}-benzo(ghi)perylene and d^{14}-benz(a,h)anthracene.

4.5. Sample analysis

4.5.1. Biological analysis of samples by means of the H4IIE-luc bio-assay

4.5.1.1. Maintenance, culturing and passaging of the H4IIE-luc rat hepatoma cell line

Sterile techniques were employed during maintenance, culturing and passaging of the cell line, working in laminar-flow bio-safety hoods and incubators with high efficiency particulate air (HEPA)-filters. All of the equipment used were autoclaved and cleaned with 70% ethanol, and all cell media were filtered through Vacucap® filter units (0.8/0.2 µm, LifeSciences) prior to use.

The H4IIE-*luc* bio-assay uses a rat hepatoma cell line stably transfected with a firefly luciferase reporter gene. The cells were a gift from Prof. John Giesy in 2003, then from the Michigan State University in the USA. Cells were maintained in a water-jacketed incubator (ThermoForma series II, Labotec) at a temperature of 37 °C in a humidified air:carbon dioxide mixture (95% air/5% CO_2). They were grown in tissue culture dishes (100/20 mm, Greiner Bio-one) using Dulbecco's Modified Eagle's Medium (DMEM) without phenol red (Sigma, cat no. D2906), supplemented with 10% foetal bovine serum (FBS; Hyclone®, Thermo Scientific). The dishes were inspected regularly for cell density, microbial activity and other signs of contamination, such as turbidity of the media, the presence of floating objects in media, and the occurrence of microscopic objects with different morphology than the cells (McFarland *et al*., 1998). Cells were passaged as soon as the tissue culture dish was confluent (McFarland *et al*., 1998; Villeneuve *et al*., 1999).

4.5.1.2. The H4IIE-luc reporter gene bio-assay: Method and processing of results

The bio-assay was performed over a period of five days, during which the cells were grown in DMEM, supplemented with 10% hormone-free foetal bovine serum.

On the first day, 96-micro well plates (400 µl, Nunc) were seeded with a 0.25 ml cell suspension (50 000 cells/ml). The outer wells of each plate were not seeded, but contained 0.25 ml PBS (without Ca^{2+} and Mg^{2+}), acting as a buffer area to prevent a hydrostatic pressure from having an effect on cells. The plates were incubated for 24 hours at 37°C and an air/CO_2 concentration of 95%/5% (Hilscherova *et al*., 2003).

On day two, the cells were dosed in triplicate with 2.5 µl of the reference compound (2,3,7,8-TCDD) or sample extract at six different concentrations. The 2,3,7,8-TCDD were dosed at a four-times dilution series (120.0, 30.0, 7.50, 1.88, 0.47 and 0.12 pg TCDD/well) and the extracts were dosed at a two-times dilution series (1:1, 1:2, 1:4, 1:8, 1:16 and 1:32). A solvent control (SvC) row (containing hexane) and blank row (containing cells only) separated the wells dosed with 2,3,7,8-TCDD from the wells dosed with extracts, to prevent cross-communication of cells (Giesy *et al.*, 1997; Hilscherova *et al.,* 2001). The plates were incubated for 72 hours.

On the fifth and final day of the assay, the plates were inspected microscopically for confluency and viability. Additionally, a viability test (described in Section 4.5.1.3) was performed. The culture medium was removed and the cells were washed with PBS with Ca^{2+} and Mg^{2+}. After adding 100 µl of Luclite™ reagent (Perkin Elmer) to each well, the plates were incubated for 10 minutes and placed in a luminometer (Microplate fluorescence reader FLX 800, Bio-Tek Instruments Inc.) to measure the amount of light emitted by the cells (expressed as relative light units or RLU's). Generally, the sensitivity of the instrument was set at 180, but it was adjusted when necessary to create optimum deviation from the background (Hilscherova *et al*., 2001).

Results obtained from the assay were processed with Microsoft Excel XP, calculating the mean RLU's, standard deviation, coefficient of variation (CV) and the percentage maximal induction relative to 2,3,7,8-TCDD (% TCDD max) for each reference compound- and sample series. Since solvents may contribute to cell response, the mean RLU's of the SvC wells should be subtracted from the RLU's of the samples and TCDD (Besselink *et al.,* 2004). However, when this was done the RLU's produced by samples had negative values and were inadequate; therefore, the effect of the solvent was not taken into consideration. Furthermore, the CV had to be less than, or as close as possible to 20 to have acceptable variation. When this value was greater than 20, one of the RLU's was dropped from the calculations (Whyte *et al*., 2004).

Dose-response curves were plotted for the TCDD-reference compound series with the amount of TCDD (log pg TCDD/well) plotted on the x-axis and % TCDD max on the y-axis. Three data points lying on the linear part of the sigmoid curve (beyond 20% TCDD max, but below the point where the curve flattened) were used to calculate the slope, y-intercept and correlation coefficient (R^2), using the equation $y = mx + c$ (Schramm *et al*., 2001). The corresponding x-intercepts for amount of TCDD responsible for 20%, 50% and 80% response in cells were consequently calculated, yielding the effective concentrations or $EC_{20,}$ EC_{50} and EC_{80}. The data obtained from the sediment or soil samples were processed in the same way as the reference compound, compiling dose-response curves for each sample, with the amount of sample (log µl sample/well) on the x-axis and the % TCDD max on the y-axis. EC_{20-80} values were calculated in the same way as the TCDD EC_{20-80} values. This indirect assay assumes that doses of TCDD and sample that elicit the same magnitude of response, can be defined as equally effective doses (Finey, 1971), or relative effect potencies (REP). REPs were calculated from the reference compound and sample's EC_{20-80} values (using $REP_{20-80} = EC_{20-80\ TCDD}/EC_{20-80\ sample}$) to address the phenomenon of non-parallelism between the reference dose-response curve and the sample dose-response curve. It cannot be assumed that the complete mixtures from the environment will exhibit equal efficacy to TCDD; therefore, the % TCDD max of the samples had to be greater than 20 (Villeneuve *et al*., 2000). Since the levels of DLCs in the South African sediment and soil samples generally did not elicit responses of 50% or greater, only REP_{20} were converted into TCDD-EQ_{20} values by back-calculation, based on the mass of the sample, the volume of the extract assayed, and the degree of concentration during the extraction procedure (Koh *et al.,* 2006).

The limit of detection (LOD) for the bio-assay was calculated by determining the mean EC_0 (TCDD concentration at which no response was elicited from cells) for the entire study's TCDD dose-response curves. The 95% confidence interval was subsequently determined, added to the mean (Thomsen *et al*., 2003), and converted to ng TCDD-EQ/kg, dw.

4.5.1.3. The MTT bio-assay: Method and processing of results

Cell viability and proliferation greatly affect the reliability of results produced by *in vitro* assays. The MTT bio-assay is a reliable approach to examine cell proliferation, determining if cell responses were possibly affected by the sample extract. The bio-assay functions on the principle of tetrazolium salt reduction (Oh *et al*., 2004; Vistica *et al*., 1991). Yellow tetrazolium salt (3-[4,5-dimethyltiazol-2yl]-2,5-diphenyl tetrazolium bromide) or MTT in short, is metabolically reduced by active cells [through nicotinamide-adenine dinucleotide phosphate (NADPH) and nicotinamide-adenine dinucleotide (NADH)] resulting in the formation of intracellular purple formazan, which can be dissolved by a tissue culture medium and quantified spectrophotometrically (Oh *et al.,* 2004).

A duplicate set of plates were prepared alongside the luminescence assay, dosed exactly the same. On the fifth and final day, plates were washed with PBS with Ca^{2+} and Mg^{2+}, and 100 µl freshly prepared MTT solution was added to each cell containing well and to three control wells without cells (Blaha *et al*., 2004). The plates were incubated for 30 minutes at 37 °C and a 5%/95% CO_2/air concentration. The MTT solution was replaced with 200 µl DMSO (Saarchem, uniLAB) per well and left for 30 minutes to allow the crystals to dissolve. The absorbencies were measured at 492 nm and 645 nm (background) with a spectrophotometer (PowerWave X, Bio-Tek Instruments Inc.) (Blaha *et al.*, 2004). Each plate was read thrice at both absorbencies and the mean of the three readings was used to calculate cell viability.

The true absorbance for each well was determined by subtracting the reference absorbance from the absorbencies at 492 nm, as well as the absorbency of MTT (wells with no cells, but MTT only). Viability was expressed as a percentage of the absorbance of the control cells (Vistica *et al.,* 1991). These calculations were performed in Microsoft Excel XP. If the cells were less than 80% viable, it was assumed that cell viability might have affected the credibility of the luminescence assay's results.

4.5.2. Chemical analysis of samples

4.5.2.1. Chemical analysis of samples for OCPs, PBDEs and PCBs

Chemical analysis of samples for OCPs, PBDEs and PCBs were done by NILU in Kjeller, Norway. Analysis were done with HRGC/HRMS using an Agilent 6890 N gas chromatograph coupled to an Autospec (Micromass Waters, Manchester UK) mass spectrometer. The mass spectrometer was operated at a resolution of >10,000 using electron ionization. Procedural blanks were run throughout using the criteria that blank levels of target analytes should be below the detection limit (signal-to-noise ratio 3:1) or less than 10% of the lowest expected sample concentration.

This method is accredited after ISO/IEC 17025 – "General requirements for the competence of testing and calibration methods". The following conditions had to be met for quality assurance for an unequivocal identification and quantification of analytes:
(a) the retention time had to be correct;
(b) the isotope ratio had to be correct;
(3) the signal-to-noise ratio had to be more than 3:1 for identification;
(4) the recovery of internal standard had to be within 40 to 120%; and
(5) blank values had to be determined for the complete clean-up and quantification procedures.

4.5.2.2. Chemical analysis of samples for PAHs

Chemical analysis for PAHs was done by the NMISA based in Pretoria, South Africa. The Pegasus III Gas Chromatography-Time of Flight-Mass Spectrometer (GC-TOF-MS) (Leco®) consisting of an Agilent 7683B series injector, Agilent Technologies 6890N Network GC system, and a Pegasus III time of flight mass spectrometer run on ChromoTof version 3.32 software, were used. The column configuration consisted of a 2 m long column with a 0.53 mm ID deactivated guard column, coupled to a 12 m, 0.25 mm RESTEK column with a film thickness of 0.15 µm, fitted with a press tight fitting. Helium was used as the carrier gas.

For quality control purposes, procedural blanks were run throughout and the acquisition system was adjusted with instrument optimization and automatic leak check.

4.6. Determination of oxidizable and total organic carbon

Because persistent organic pollutants and toxicants preferentially associate with organic carbon particles, the total organic carbon (TOC) content of samples had to be determined (Schumacher, 2002). The oxidizable organic carbon (OXC) content of samples was determined with the Walkley-Black method and converted to TOC, using the following method.

The Walkley-Black method is a quantitative, destructive technique based on the principle of wet-oxidation followed by ferrous ammonium sulphate titration. The method is fairly simple to use and time- and cost-effective (Chan *et al.*, 2001; Schumacher, 2002). During this process carbon is oxidized by the dichromate ion ($K_2Cr_2O_7 + 3C + 16H^+ \rightarrow 4\ Cr^{3+} + 3CO_2 + 2H_2O$), and excess dichromate ions are back titrated with ferrous ions ($6Fe^{2+} + Cr_2O_7{}^{2-} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$). To calculate the OXC content, Schumacher (2002) proposed the following equation:

$$\text{OXC (\%)} = \frac{[Fe(NH_4)_2(SO_4)_2 \text{ used for blank (ml)} - Fe(NH_4)_2(SO_4)_2 \text{ used for sample (ml)}] \times M \times 0.3 \times f}{\text{Mass of sediment sample (g)}}$$

Where: M was the concentration of $Fe(NH_4)_2(SO_4)_2$ of 0.5 mol.dm^{-3}, and
f was the correction factor of 1.4.

The TOC content was ultimately calculated from the percentage OXC, using the equation recommended by Sánchez-Monedero and co-workers (1996): TOC (%) = 1.23(OXC) + 0.35.

The results from biological and chemical analysis were normalized to 1% TOC to allow for comparison with environmental quality guidelines proposed by other countries.

4.7. Statistical analysis

4.7.1. Basic statistics

Microsoft Excel was used to do the fundamental calculations and to graphically represent the data. STATISTICA version 8 was used for basic comparisons between two or more sampling sites, sampling regions and land-use types. Here, the one-way ANOVA with *post-hoc* Tukey tests, Kruskal-Wallis ANOVA, or the non-parametric Mann-Whitney U test was applied, depending on the number and distributions of data. For these tests a p-value of less than 0.05 ($p < 0.05$) was considered as statistically significant.

For correlations, Pearson's correlation coefficient (R^2) was used to determine effect size. The correlation was not significant if $R^2 \leq 0.13$. If R^2 was greater than 0.13, but smaller or equal to 0.3, the correlation was significant; and if R^2 was greater than 0.3, the correlation was practically important or statistically practically significant (following Ellis & Steyn, 2003).

4.7.2. Multivariate data analysis

Principle component analysis (PCA) was performed to determine the distribution patterns of pollutants at the various sites. The software package CANOCO 4.5 was used, taking into account only the sites where chemical analyses results were available.

Individual concentrations were expressed as a portion of the sum of all other pollutant concentrations at each sampling site (Alcock *et al*, 2002; Masunaga *et al.*, 2003). In other words, compositional data were considered. The compositional data were log-transformed according to the method proposed by Howel (2007), prior to applying the PCA. The motivation for the method is as follows: The linear dependence of proportions in compositional data leads to an absence of an interpretable covariance structure. This causes the apparent strength and direction of the association between two proportions to appear to depend on the number of elements in the total composition and to differ for different subsets of elements. The ratio of proportions should, however, remain the same and not be influenced by how many pollutants there are, since compositional data attempts to describe the relative magnitudes of pollutants. To address the absence of this property, the centred log-ratio of each proportion (p) was determined by dividing each proportion by the geometric mean across the sample: $\log(p_{ij}/g(p_j))$ where $g(p_j) = (p_{1j}/p_{2j}\ldots\ldots,p_{dj})^{1/d}$, where d pollutants are measured in n samples so that p_{ij} is the proportion of pollutant i in sample j (Howel, 2007). Howel (2007) showed that principal components for compositional data should be derived from the covariance matrix of the centred log-ratios of proportions (equation above) rather than the covariance or correlation matrix of the proportions. The PCA was performed without any further transformations of the data. Where the levels of compounds were below the detection limit, the half-LODs were used to avoid any zero-values.

The principal component loading plot partially explains the importance of each congener in determining the positions of samples along the principal component axis. The compounds with the highest contributions towards the differences observed between samples are located farthest from the origin of the axes. The greater the distance from the origin of the axes, the more the compound contributes to the variation accounted for along that axis. A negative or positive position along the axis indicates whether the compound is negatively or positively correlated with the principal component (Wenning *et al.*, 1992).

5

Results and Discussion

Chapter 5 first reports on and discusses the results of the H4IIE-*luc* bio-assay (Section 5.1), followed by the chemical analysis results (Section 5.2). Section 5.3 elaborates on South Africa's position on the global POPs issue, comparing this study and the results of previous studies to levels measured elsewhere in the world. The chapter concludes by reporting and discussing the possible cancer risk for humans exposed to the compounds under investigation (Section 5.4).

5.1. Biological analysis results

5.1.1. H4IIE-luc- and MTT bio-assay results and TOC content: A summary

In this section, the results of the H4IIE-*luc*- and MTT bio-assays will be reported. Since the amount of organic carbon present in sediment or soil may affect its potential to retain DLCs (Schumacher, 2002), the OXC- and TOC contents of samples will be reported as well. The correlation between the percentage TOC and amount of DLCs in the samples will be established to verify if a statistically significant relationship exists for the samples investigated during this study. Finally, the levels of DLCs measured at the South African sites will be discussed and compared to one another.

The levels of PCDD/Fs and dioxin-like PCBs measured with the H4IIE-*luc* bio-assay ranged from below the LOD to 103 ng TCDD-EQ/kg, dw. The LOD of the bio-assay was 1.93 ng TCDD-EQ/kg, dw, and where TCDD-EQs could not be quantified the half-LOD (of 0.97 ng TCDD-EQ/kg, dw) is used (Table 5.1). Only 23 of the 96 samples elicited responses beyond 20% TCDD max and could be quantified. Sixteen of these samples were from industrially impacted areas, three from residentially impacted areas (mostly associated with low-income, high-density residential settlements), and the remaining four were associated with residential-agricultural or industrial-residential combinations (Table 4.2 to 4.8 & Table 5.1).

The majority of the samples were of such a nature that the viability of the cells was not affected (80 – 100% viable, in 47 of the 96 cases) or only slightly affected (60 - 79% viable, in 26 of the 96 cases) (Table 5.1). However, in 23 of the 96 cases, the viability of cells were moderately (40 – 59% viable), highly (20 – 39% viable) or severely (0 – 19% viable) affected (Table 5.1).

Figures 5.1 a and b give examples of the H4IIE-*luc* and MTT bio-assay results of a sediment sample of which the cell viability was “not affected” (RB2, Table 5.1), while Figures 5.2 a and b show the results of a sediment sample of which the cell viability was “highly affected” (Komati1, Table 5.1). When comparing the two samples, it became apparent that the “unaffected” (in terms of cell viability) sample RB2 (Fig 5.1 a) showed an increase in the percentage TCDD max at increasing sample concentrations. The contrary was true for Komati1; the down-ward slope in the percentage TCDD max at the two highest sample concentrations (log 0.4 and 0.1 µl sample/well; Fig. 5.2 a) is indicative of cytotoxicity. This is confirmed by the MTT results where the percentage cell viability dropped to below 20% and 70% respectively at the highest and second highest sample concentrations (Fig. 5.1 b).

Table 5.1. The % OXC and TOC, and results from the H4IIE-*luc* and MTT bio-assays

Site abbreviation	Type of land-use	Carbon content		H4IIE-luc bio-assay results		MTT assay results
		OXC (%)	[a]TOC (%)	TCDD-EQ$_{20}$ values (ng TCDD-EQ/kg, dw)	[b]Normalized TCDD-EQ$_{20}$'s (ng TCDD-EQ/kg, dw)	Cell viability
S/L1	Residential	5.10	6.63	**7.56**	**1.14**	Moderately affected
S/L2	Residential	3.75	4.96	*0.97*	*0.20*	Moderately affected
S/L3	Residential	4.25	5.57	*0.97*	*0.17*	Moderately affected
S/L4	Residential	5.91	7.62	*0.97*	*0.13*	Not affected
S/L5	Residential	2.27	3.14	*0.97*	*0.31*	Not affected
S/L6	Residential	4.84	6.30	*0.97*	*0.15*	Not affected
S/L7	Residential	6.03	7.76	**6.27**	**0.81**	Highly affected
S/L8	Semi-industrial	5.43	7.03	**4.33**	**0.62**	Slightly affected
S/L9	Semi-industrial	10.81	13.64	**86.63**	**6.35**	Slightly affected
S/L10	Residential/Agricultural	4.97	6.47	**15.02**	**2.32**	Moderately affected
S/L11	Residential	1.88	2.66	*0.97*	*0.36*	Slightly affected
S/L12	Semi-industrial	4.52	5.91	**13.86**	**2.34**	Slightly affected
S/L13	Residential/Agricultural	7.46	9.52	*0.97*	*0.10*	Not affected
CT1	Residential	1.12	1.73	*0.97*	*0.56*	Slightly affected
CT2	Industrial	4.30	5.64	**16.15**	**2.86**	Slightly affected
CT3	Agricultural	0.36	0.79	*0.97*	*1.23*	Moderately affected
CT4	Industrial	0.10	0.47	*0.97*	*2.08*	Slightly affected
CT5	Industrial	0.49	0.95	*0.97*	*1.02*	Not affected
CT6	Industrial	0.79	1.32	**12.34**	**9.33**	Not affected
CT7	Industrial	3.58	4.75	**11.93**	**2.51**	Slightly affected
CT8	Industrial	0.79	1.32	**11.49**	**8.67**	Moderately affected
CT9	Residential	0.26	0.66	*0.97*	*1.46*	Moderately affected
CT10	Industrial	1.32	1.98	*0.97*	*0.49*	Slightly affected
CT11	Industrial/Residential	1.01	1.59	*0.97*	*0.61*	Highly affected
CT12	Industrial/Residential	1.41	2.08	*0.97*	*0.47*	Highly affected
CT13	Agricultural	0.92	1.48	*0.97*	*0.66*	Severely affected
CT14	Industrial	1.91	2.70	*0.97*	*0.36*	Severely affected
CT15	Industrial/Residential	0.39	0.84	*0.97*	*1.16*	Slightly affected
CT16	Industrial/Residential	1.09	1.68	*0.97*	*0.58*	Moderately affected
CT17	Residential	0.37	0.81	*0.97*	*1.20*	Highly affected
CT18	Industrial/Residential	0.69	1.19	*0.97*	*0.81*	Slightly affected
CT19	Industrial	1.12	1.72	*0.97*	*0.56*	Slightly affected
CT20	Industrial	0.62	1.11	*0.97*	*0.87*	Severely affected
D1	Industrial	0.24	0.65	*0.97*	*1.49*	Not affected
D2	Industrial	0.70	1.22	*0.97*	*0.80*	Not affected
D3	Industrial	0.32	0.74	*0.97*	*1.31*	Not affected
D4	Industrial	0.58	1.07	*0.97*	*0.91*	Not affected
D5	Industrial	0.24	0.64	*0.97*	*1.51*	Not affected
D6	Harbour – industrial	0.13	0.51	*0.97*	*1.89*	Moderately affected
D7	Harbour – industrial	0.26	0.67	*0.97*	*1.46*	Not affected
D8	Industrial	0.32	0.74	*0.97*	*1.30*	Slightly affected
D9	Industrial	0.82	1.36	*0.97*	*0.71*	Not affected
D10	Industrial	0.37	0.81	*0.97*	*1.20*	Moderately affected
D11	Industrial	0.69	1.19	*0.97*	*0.81*	Moderately affected
D12	Residential	0.79	1.32	*0.97*	*0.73*	Slightly affected
D13	Residential	0.71	1.23	*0.97*	*0.79*	Moderately affected
D14	Industrial	2.61	3.56	**28.89**	**8.12**	Not affected

[a]The TOC was converted from the OXC as described in section 4.6.

[b]The TCDD-EQ20s were normalized to 1% of the TOC.

Bold: Measured TCDD-EQ20's.

Italics: The half- LOD was reported for responses below 20% TCDD-max.

Table 5.1. *Continued.*

Site abbreviation	Type of land-use	Carbon content		H4IIE-luc bio-assay results		MTT assay results
		OXC (%)	[a]TOC (%)	TCDD-EQ$_{20}$ values (ng TCDD-EQ/kg, dw)	[b]Normalized TCDD-EQ$_{20}$'s (ng TCDD-EQ/kg, dw)	Cell viability
RB1	Industrial	1.25	1.88	**103**	**54.72**	Moderately affected
RB2	Harbour – industrial	0.29	0.71	**28.01**	**39.49**	Not affected
RB3	Harbour – industrial	0.89	1.44	**18.42**	**12.75**	Not affected
RB4	Industrial	4.53	5.92	**7.48**	**1.26**	Not affected
RB5	Harbour – industrial	0.78	1.31	*0.97*	*0.74*	Not affected
RB6	Harbour – recreational	0.23	0.64	*0.97*	*1.52*	Not affected
RB7	Residential	0.32	0.74	*0.97*	*1.30*	Not affected
RB8	Residential	0.49	0.95	*0.97*	*1.02*	Not affected
RB9	Residential	0.38	0.82	*0.97*	*1.19*	Not affected
BF1	Agricultural	1.92	2.71	*0.97*	*0.36*	Not affected
BF2	Agricultural	0.81	1.34	*0.97*	*0.72*	Not affected
BF3	Recreational	1.13	1.75	*0.97*	*0.56*	Slightly affected
BF4	Industrial	0.72	1.23	*0.97*	*0.79*	Slightly affected
BF5	Industrial	0.41	0.85	*0.97*	*1.14*	Slightly affected
BF6	Industrial	0.73	1.25	**44.32**	**35.46**	Not affected
BF7	Industrial/Residential	1.38	2.05	**45.17**	**22.01**	Not affected
BF8	Industrial	1.20	1.82	**47.45**	**26.03**	Slightly affected
BF9	Industrial/Residential	5.17	6.71	**10.34**	**1.54**	Not affected
BF10	Residential/Recreational	2.61	3.56	*0.97*	*0.27*	Not affected
BO1	Industrial/Residential	1.77	2.53	*0.97*	*0.38*	Slightly affected
BO2	Residential	2.82	3.82	*0.97*	*0.25*	Not affected
BO3	Industrial	3.29	4.40	*0.97*	*0.22*	Not affected
BO4	Industrial/Residential	4.31	5.65	**36.05**	**6.38**	Not affected
Lim1	Agricultural	0.40	0.84	*0.97*	*1.15*	Not affected
Lim2	Agricultural	0.37	0.80	*0.97*	*1.21*	Not affected
Lim3	Residential	0.57	1.05	*0.97*	*0.93*	Slightly affected
Lim4	Residential	2.32	3.21	**22.95**	**7.16**	Not affected
Lim5	Recreational	0.51	0.97	*0.97*	*1.00*	Not affected
Lim6	Recreational	0.33	0.75	*0.97*	*1.29*	Not affected
Olif1	Industrial	1.12	1.73	*0.97*	*0.56*	Not affected
Olif2	Residential/Agricultural	1.03	1.61	*0.97*	*0.60*	Not affected
Olif3	Industrial/Agricultural	0.24	0.65	*0.97*	*1.49*	Not affected
Olif4	Industrial	1.56	2.27	**33.31**	**14.70**	Not affected
Croc1a	Industrial	1.26	1.90	*0.97*	*0.51*	Slightly affected
Croc 1b	Industrial	0.93	1.49	**8.02**	**5.39**	Not affected
Croc2	Agricultural	2.73	3.70	*0.97*	*0.26*	Moderately affected
Croc3	Agricultural	1.68	2.42	*0.97*	*0.40*	Slightly affected
Croc4	Agricultural	1.07	1.67	*0.97*	*0.58*	Highly affected
Komati1	Agricultural	0.21	0.61	*0.97*	*1.58*	Highly affected
Komati2	Agricultural	3.87	5.11	*0.97*	*0.19*	Slightly affected
Komati3	Agricultural	0.51	0.97	*0.97*	*1.00*	Not affected
Pongola1	Agricultural	0.41	0.85	*0.97*	*1.14*	Slightly affected
Pongola3	Residential/Agricultural	0.89	1.45	*0.97*	*0.67*	Not affected
Drknberg1	Agricultural	0.86	1.41	*0.97*	*0.69*	Slightly affected
Drknberg2	Agricultural	0.60	1.08	*0.97*	*0.90*	Not affected
Drknberg3	Agricultural	0.97	1.55	*0.97*	*0.63*	Not affected
KZNRiv 1	Industrial	0.56	1.04	*0.97*	*0.94*	Not affected

[a]The TOC was converted from the OXC as described in section 4.6.　[b]The TCDD-EQ20s were normalized to 1% of the TOC.

Bold: Measured TCDD-EQ20's.　*Italics: The half-LOD was reported for responses below 20% TCDD-max.*

Table 5.1. *Continued.*

Site abbreviation	Type of land-use	Carbon content OXC (%)	 [a]TOC (%)	H4IIE-luc bio-assay results TCDD-EQ$_{20}$ values (ng TCDD-EQ/kg, dw)	 [b]Normalized TCDD-EQ$_{20}$'s (ng TCDD-EQ/kg, dw)	MTT assay results Cell viability
KZNRiv 2	Industrial	0.82	1.35	*0.97*	*0.72*	Not affected
KZNRiv 3	Industrial	0.49	0.96	*0.97*	*1.01*	Slightly affected

[a]The TOC was converted from the OXC as described in section 4.6.

[b]The TCDD-EQ20s were normalized to 1% of the TOC.

Bold: Measured TCDD-EQ20's.

Italics: The half-LOD was reported for responses below 20% TCDD-max.

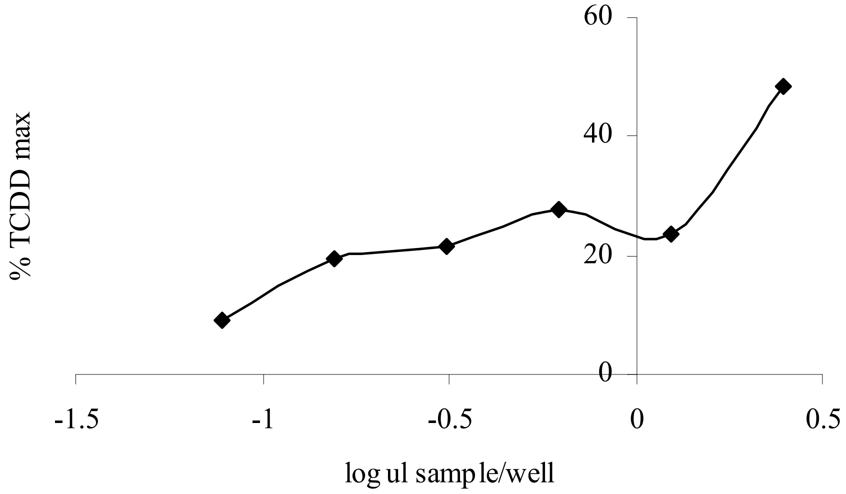


Figure 5.1 a. Dose-response curve for the site “RB2”, with the volume of sample plotted on the x-axis and the percentage TCDD max on the y-axis

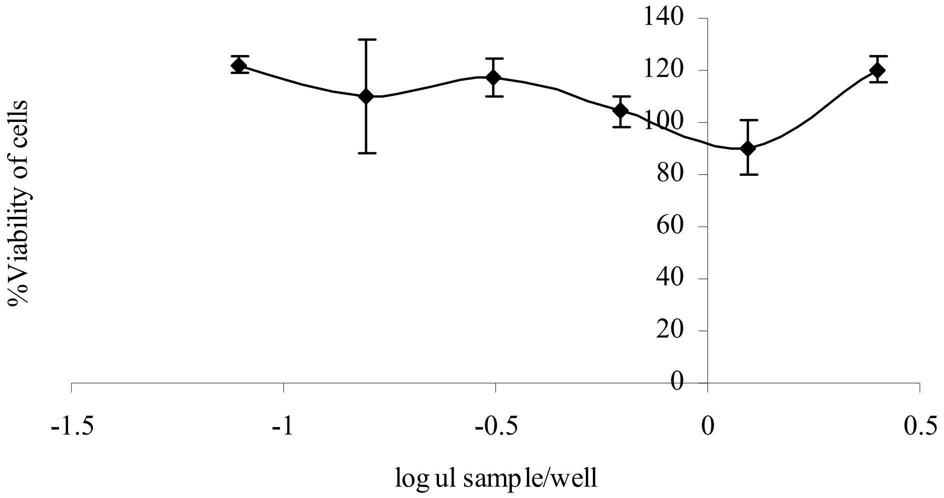


Figure 5.1 b. Dose-response curve for the site “RB2”, with volume of sample plotted on the x-axis and the percentage viability of cells on the y-axis

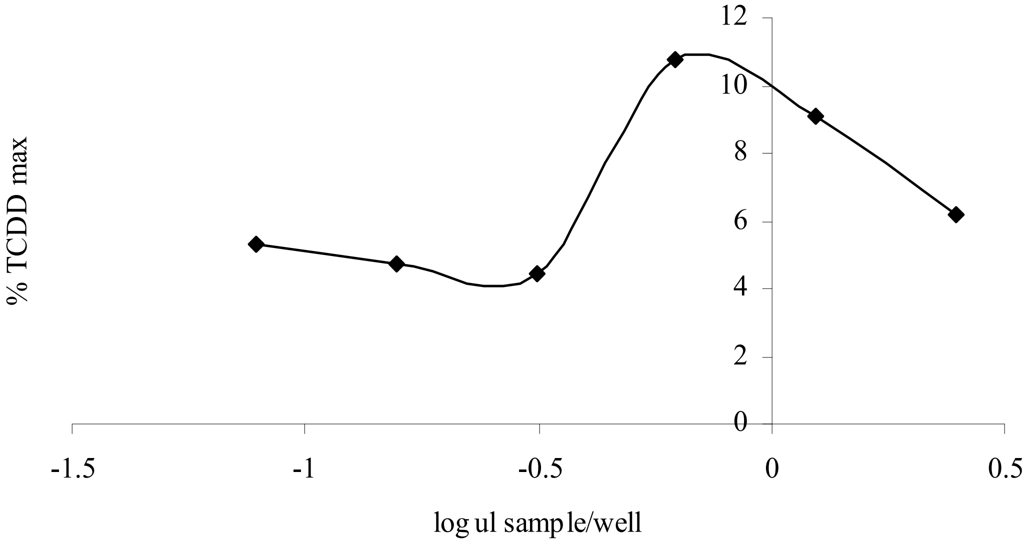


Figure 5.2 a. Dose-response curve for the site “Komati1”, with the volume of sample plotted on the x-axis and the percentage TCDD max on the y-axis

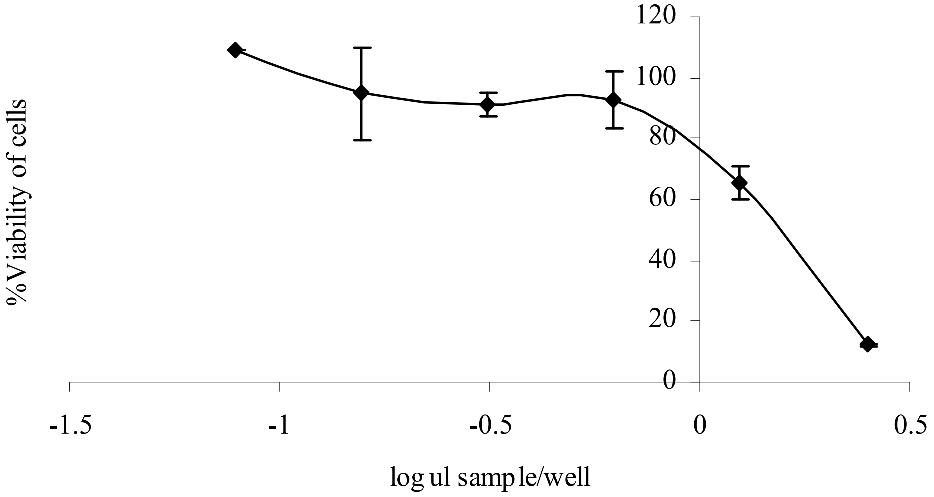


Figure 5.2 b. Dose-response curve for the site “Komati1”, with the volume of sample plotted on the x-axis and the percentage viability of cells on the y-axis

As illustrated in Figures 5.1 and 5.2, decreased cell viability or cytotoxicity could result in false negative results for dioxin-like TCDD-EQ, since wells with fewer living cells would emit less light than 100% viable wells. In such instances, the reduced response in cells (decrease in % TCDD max) would not necessarily be due to little DLCs, but rather to fewer cells. The causes for the loss in cell viability are uncertain. Since only some of the wells, mostly at the highest sample concentrations in a dilution series, were affected, it was likely that a compound or compounds present in the extract was the main culprit. It is known that elemental sulphur and other mixtures of compounds present in sediments may be cytotoxic to H4IIE-*luc* cells (Hilscherova *et al.*, 2000; Yoo *et al.*, 2006), but since sulphur was eliminated from the extracts (section 4.4.1) it is uncertain which compound(s) was/were responsible for cytotoxicity. This indicates the need for further investigations.

In addition to the percentage viability of cells, the TOC content of the samples had to be taken into consideration before comparing sites. A higher organic carbon content may suggest that sediments or soils would have the capacity to adsorb greater concentrations of DLCs than sediments or soils containing less organic carbon (Shimizu *et al.,* 2003). To establish if there was a statistically significant relationship between the percentage TOC and TCDD-EQs of samples, the OXC determined with the Walkley-Black wet-oxidation method was converted to TOC (Table 5.1) (as described in Section 4.6) and the correlation between the amount of organic carbon and levels of DLCs was determined.

In literature, both strong (Shimizu *et al.,* 2003; Wisconsin Department of Natural Resources, 2003; Wevers *et al.*, 2004) and weak (Hilscherova *et al.*, 2003; Nieuwoudt *et al.,* 2009; Suarez *et al.*, 2006) correlations have been found. For the samples included in the current study, the percentage TOC generally correlated weakly with the TCDD-EQ. When all 96 of the samples were included in the calculation (using half-LODs for sites at which the level of DLCs was below the assay's LOD), the $R^2 = 0.0997$, which means that there was only a 9.97% correlation between the amount of organic carbon and the amount of DLCs in the samples (Fig. 5.3). When only the 23 samples eliciting quantifiable responses in cells were considered, the correlation was even weaker ($R^2 = 0.002$, Fig. 5.4). However, when the outlier, RB 1, was removed from the dataset the correlation improved slightly to $R^2 = 0.0435$ (Fig. 5.5), which was still not significant ($p > 0.05$). For this reason, normalized TCDD-EQ_{20}'s will not be used to compare sites to one another. However, the normalized data (Table 5.1) is useful to compare the results to environmental sediment quality guidelines (Wisconsin Department of Natural Resources, 2003).

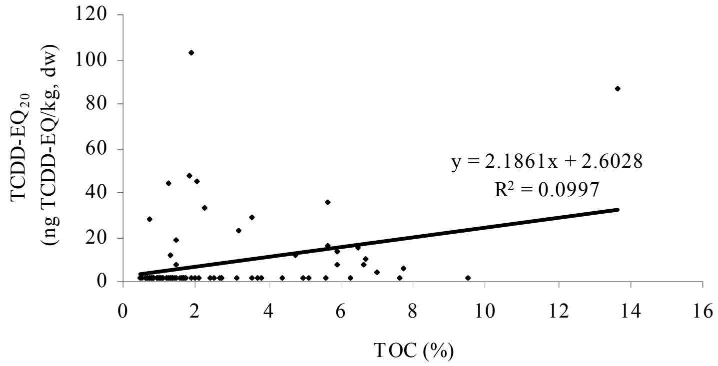


Figure 5.3. Correlation between the TCDD-EQ$_{20}$ and the amount of TOC in samples, with all 96 samples used

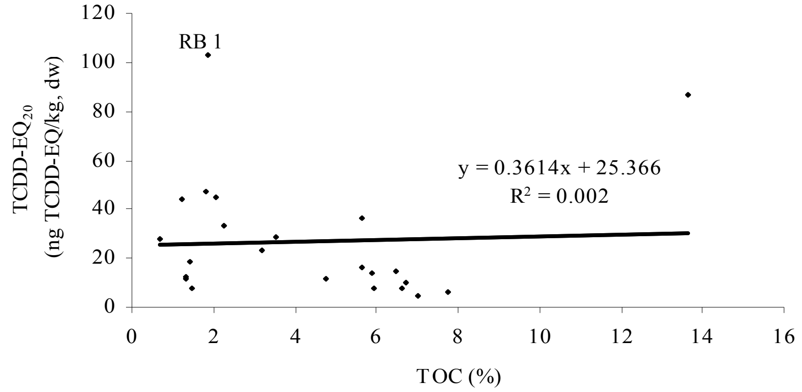


Figure 5.4. Correlation between the TCDD-EQ$_{20}$ and the amount of TOC in samples, with only the 23 samples eliciting quantifiable responses used

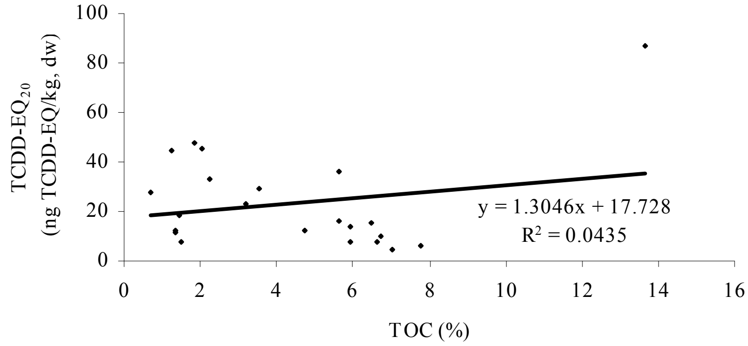


Figure 5.5. Correlation between the TCDD-EQ$_{20}$ and the amount of TOC in samples, with only the 23 samples eliciting quantifiable responses included, and the outlier RB 1 (Fig 5.4) removed

5.1.2. Discussion and comparison of DLC levels in South African sediments

Bar graphs were plotted with the sites arranged in decreasing magnitude of TCDD-EQ$_{20}$ (Fig. 5.6, Table 5.1). The same was done for the data normalised to 1% TOC (Fig. 5.7, Table 5.1). Although the patterns were not identical, some similarities were noted. The non-normalized TCDD-EQ$_{20}$'s were generally higher, with RB1 the highest, and S/L7 and 8 the lowest for both normalized (Fig. 5.7) and non-normalized (Fig. 5.6) data. Furthermore, the industrial and less industrial sites were randomly distributed in the continuum concentrations of DLC pollution; i.e. not all industrial sites had concentrations greater than the residential-, recreational-, industrial/residential- or residential/agricultural sites (Fig. 5.6 & 5.7). This may indicate different sources of DLCs throughout the study area, such as domestic fires and alternative fuel usage for cooking and heating in the low-income residential areas.

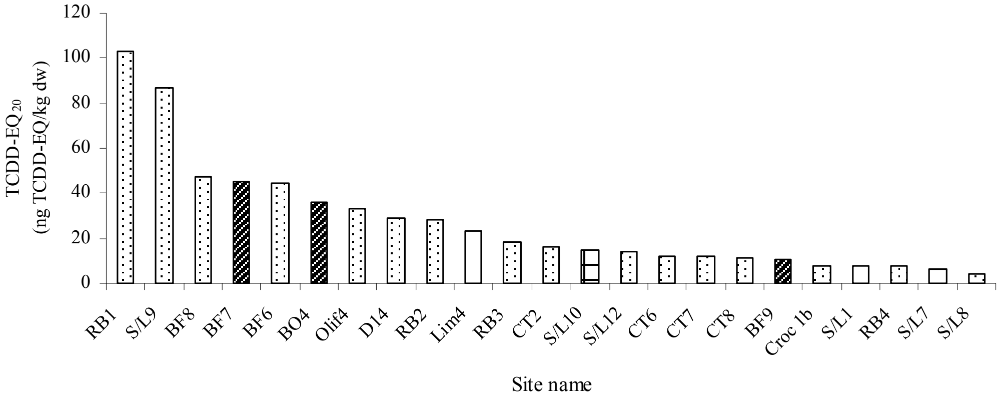


Figure 5.6. The 23 South African samples eliciting quantifiable responses with the bio-assay, arranged from greatest to least TCDD-EQ$_{20}$.

⬚ = Industrially or semi-industrially-, ▨ = industrial/residentially-, □ = residentially-, and ⊞ = residential/agriculturally impacted samples.

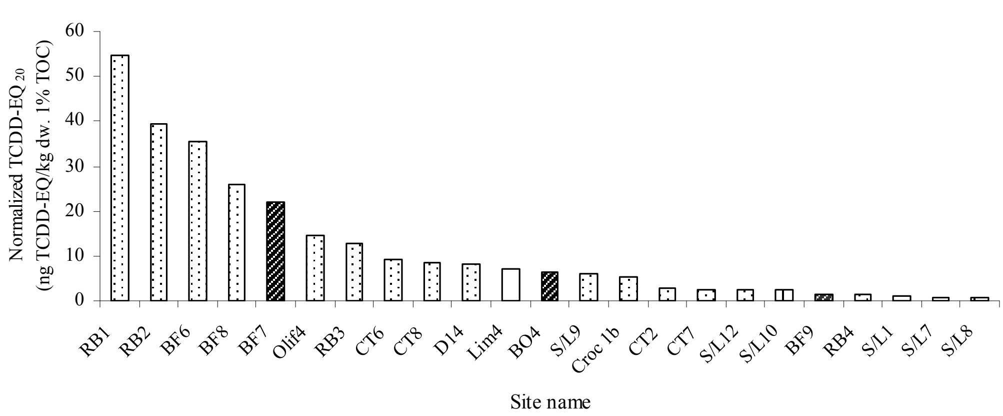


Figure 5.7. The 23 South African samples eliciting quantifiable responses with the bio-assay, arranged from greatest to least normalized TCDD-EQ$_{20}$.

⬚ = Industrially or semi-industrially-, ▨ = industrial/residentially-, □ = residentially-, and ⊞ = residential/agriculturally impacted samples.

When considering all 96 sites, and comparing the mean concentration of TCDD-EQ$_{20}$'s in sediments from the various sampling regions with each other, Richards Bay (18.51 ng TCDD-EQ/kg, dw; n = 9) followed by Bloemfontein (15.89 ng TCDD-EQ/kg, dw; n = 10) and the Soweto/Lenasia wetland (11.32 ng TCDD-EQ/kg, dw; n = 13) had the highest concentrations of DLCs, with the TCDD-EQ$_{20}$'s from the other areas being less substantial. The differences were, however, not significant ($p = 0.54318$, one-way ANOVA, *post-hoc* Tukey test). Since the majority of the towns or cities (except for the reference sites) were selected on the grounds that they are potential hot-spots for DLC pollution, significant differences in the concentration of TCDD-EQ$_{20}$ were not expected.

When only the 23 sites with quantifiable TCDD-EQ$_{20}$ were considered, the mean concentrations of each sampling region were higher, but the ranking of the regions were similar, with Richards Bay (39.23 ng TCDD-EQ/kg, dw; n = 4) having the highest mean concentration, followed by Bloemfontein (36.82 ng TCDD-EQ/kg, dw; n = 5), Durban (28.89 ng TCDD-EQ/kg, dw; n = 1) and the Soweto/Lenasia wetland (22.78 ng TCDD-EQ/kg, dw; n = 6). However, the differences between the mean concentrations of DLCs measured at the sites where still not statistically significant.

It was, nonetheless, anticipated that there would be significant differences in the concentrations of TCDD-EQ$_{20}$ due to different land-uses. Industrial areas, followed by low-income, high-density residential areas, are generally expected to be greater potential sources of PCDD/Fs than agricultural and recreational areas. Incomplete combustion occurring during industrial and thermal processes (which includes smaller non-point sources such as domestic burning of wood, landfill fires and open burning frequently occurring in low-income residential settlements) may continuously produce and emit DLCs, leading to greater concentrations of TCDD-EQ$_{20}$. As anticipated, the industrial areas had slightly greater concentrations of DLCs than the residential, agricultural and recreational areas (see land-use classification in Table 5.1) (12.1 vs 3.5, 1.9 and 1.9 ng TCDD-EQ/kg, dw respectively), but no statistically significant difference was observed ($p = 0.08081$, one-way ANOVA, *post-hoc* Tukey test). It should be noted, though, that all 96 sites were included in the ANOVA, decreasing the mean TCDD-EQ values due to half LODs being used for the majority of the sites.

When only the 23 samples eliciting responses beyond 20% TCDD max were considered (Table 5.2), the industrial and semi-industrial areas were responsible for a statistically significantly higher ($p < 0.05$, Kruskal-Wallis ANOVA) contribution towards the total concentration of DLCs measured at the South African sites.

Industrially impacted areas were responsible for 77% of the contribution towards the total TCDD-EQ concentration measured at the 23 sites, while residential, residential/agricultural- and industrial/residential combinations contributed only 6%, 2%, and 15% respectively (Fig 5.8).

Table 5.2. Summary of the bio-assay data (TCDD-EQ$_{20}$ values), land-use categories, and percentage contribution to the total TCDD-EQ of the 23 sites eliciting quantifiable responses

Land-use category and sites	TCDD-EQ$_{20}$ values (ng TCDD-EQ/kg, dw)	Land-use category and sites	TCDD-EQ$_{20}$ values (ng TCDD-EQ/kg, dw)
Industrial and semi-industrial		**Industrial/residential**	
S/L8	4.33	BF7	45.17
S/L9	86.63	BF9	10.34
S/L12	13.86	BO4	36.05
CT2	16.15	**Sum TCDD-EQ$_{20}$**	**91.56**
CT6	12.34	**Mean TCDD-EQ$_{20}$**	**30.52**
CT7	11.93	**Contribution to total TCDD-EQ$_{20}$**	**15%**
CT8	11.49	**Residential**	
D14	28.89	S/L1	7.56
RB1	103	S/L7	6.27
RB2	28.01	Lim4	22.95
RB3	18.42	**Sum TCDD-EQ$_{20}$**	**36.78**
RB4	7.48	**Mean TCDD-EQ$_{20}$**	**12.26**
BF6	44.32	**Contribution to total TCDD-EQ$_{20}$**	**6%**
BF8	47.45		
Olif4	33.31	**Residential/agricultural**	
Croc1b	8.02	S/L10	15.02
Sum TCDD-EQ$_{20}$	**475.63**	**Sum TCDD-EQ$_{20}$**	**15.02**
Mean TCDD-EQ$_{20}$	**55.96**	**Mean TCDD-EQ$_{20}$**	**15.02**
Contribution to total TCDD-EQ$_{20}$	**77%**	**Contribution to total TCDD-EQ$_{20}$**	**2%**

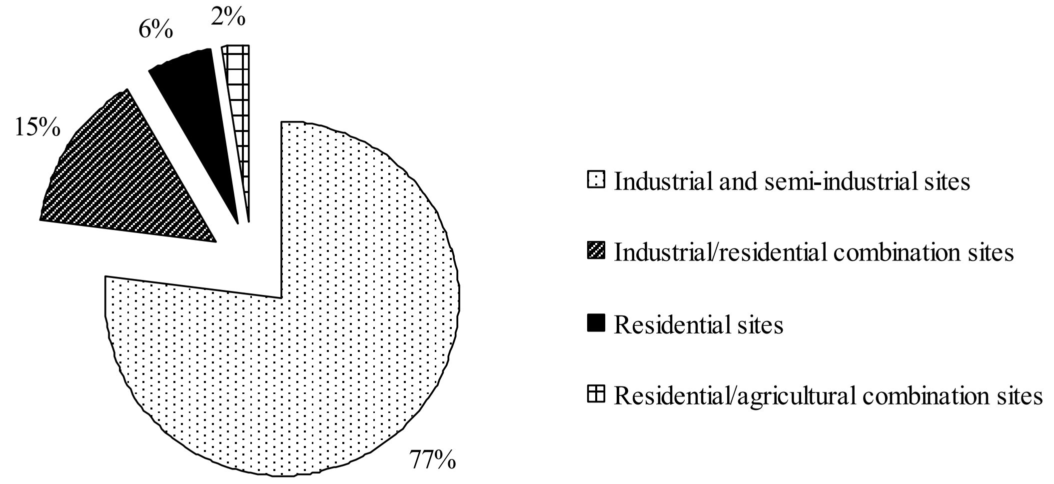


Figure 5.8. The percentage contribution of the various land-use types towards the total concentration of TCDD-EQ20 measured at the 23 quantifiable South African sediment sites

Although the industrially impacted sites dominated in their contribution with respect to the total TCDD-EQ, it was expected that a greater fraction of the 96 sites selected in industrial areas would have measurable TCDD-EQ$_{20}$'s. For example, only one of the 12 industrial sites sampled in Durban elicited a cell response beyond 20% TCDD max (Table 5.1). In the following sub-sections, each of the sampling regions will be considered separately, and the scale and significance of the occurrence of DLCs will be discussed.

5.1.2.1. Soweto and Lenasia sites

As stated earlier, Soweto and Lenasia are mainly residential areas, with only a few industries located in their direct vicinity. Of the 13 samples (ten sediments and three associated soils) collected in the area, six brought about significant cell responses. The highest concentrations of TCDD-EQ$_{20}$ were measured at the semi-industrial site, S/L9, (86.63 ng TCDD-EQ/kg, dw) and its associated soil site, S/L12 (13.86 ng TCDD-EQ/kg, dw) (Fig. 4.2, Table 5.1). A sediment sample collected at the same location during 2005 contained levels of DLCs reaching 380 ng TCDD-EQ/kg, dw, which was the greatest level yet measured for South African sediments. S/L9 and 12 were collected beyond the point where the Klip River, the wetland draining the areas north of Soweto, and the wetland draining the east of Soweto merge (Fig. 4.2). Although the immediate surroundings of S/L9 and 12 are not highly industrialised, the location may potentially receive polluted effluents from some of these areas. The wetlands drain the south of Johannesburg and meanders through the west of the outskirts of Roodepoort, both highly industrialised areas with many industries, such as chemical- and petrochemical plants, ferrous- and non-ferrous metal smelting operations, paper and pulp industries, cement production and fuel combustion – all of which have the potential to produce DLCs. The wetland also receives runoff from urban areas, discharge from sewage treatment plants, and polluted water from the Witwatersrand's mining complexes, mostly contaminated by acid mine drainage (McCarthy *et al*., 2007). S/L8, also collected in a semi-industrial area situated south of Soweto, produced a TCDD-EQ20 of 4.33 ng TCDD-EQ/kg, dw.

Of the residentially associated samples, S/L1 and S/L7 (collected up-stream of S/L9) yielded concentrations of 7.56 and 6.27 ng TCDD-EQ/kg, dw respectively (Table 5.1). A TCDD-EQ$_{20}$ of 15.02 ng TCDD-EQ/kg, dw was measured at S/L10, a residential/agriculturally impacted site. The majority of the residences in the area are well developed and supplied with potable water and electricity, but many of the low-income, high-density residential settlements are not. Residents from those areas are dependent on domestic fires for cooking and heating, which are potential sources of PCDD/Fs and PCBs (UNEP, 2005b), possibly explaining the relatively high levels of DLCs. Sites S/L7 and S/L10 are located in close proximity to two national roads (the N1 and N12) carrying heavy traffic, with vehicle exhaust emissions also probably contributing to DLC pollution (UNEP, 2005b).

Finally, wetlands are known for their filtering and purifying properties (Davies & Day, 1998; Maltby, 1991), trapping pollutants in their sediments. They are generally rich in flora and the abundance of plants helps to stabilise sediments, increasing sediment capture rates (Davies & Day, 1998). Plants may also have the additional function of protecting sediments from UV-radiation, which may degrade DLCs present in exposed sediment. These characteristics of the Soweto and Lenasia wetland might explain the relatively high levels of PCDD/Fs and PCBs at these sites.

5.1.2.2. Cape Town sites

The levels of DLCs measured at the Cape Town sites ranged between 11.49 and 16.15 ng TCDD-EQ/kg, dw, (Table 5.1, Figures 4.3 to 4.5) with only four of the 20 samples eliciting quantifiable responses with the bio-asssay. Agriculturally and residentially impacted samples' TCDD-EQs were generally below the assay's LOD. The samples with measurable TCDD-EQ_{20}'s were collected in industrial areas and it was therefore expected that the sediments would be polluted with DLCs to some extent. It was, however, anticipated that more of the industrially associated sites would produce quantifiable responses from the H4IIE-*luc* cells. Both samples CT4 and 5 were collected from canals containing industrial effluents from a paper and pulp manufacturer, a fertilizer company, chemical industry and an oil and gas refinery. Also CT10, 12, 14, 16 and 20 were sampled in highly industrialised areas or close to low-income, high-density residential areas (Fig. 4.3). The reason for the low concentrations of DLCs at these sites is uncertain. Only a few of the samples (CT12, 14, 16 and 20) were of such a nature that the assay's results could have been affected by a loss in cell viability (Table 5.1). Other possible causes for the relatively low concentrations of DLCs may include seasonal and meteorological conditions, photodegradation and degradation by micro-organisms, which will be explained comprehensively later in this chapter under Section 5.1.3. Although dilution may be a possible contributing factor towards low DLC levels, it was not considered to have a significant effect on Cape Town's sediments, since the CT samples were collected during the dry season (April 2007).

5.1.2.3. Durban sites

Only D14, the sample collected from the Umgeni River mouth, had a quantifiable TCDD-EQ$_{20}$ of 28.89 ng TCDD-EQ/kg, dw. This sample was collected beyond the confluence of the Palmiet-, Aller- and Mbongokazi Rivers with the Umgeni River (Fig. 4.4). This site potentially receives various industrial effluents from the textile industry, oil refineries and soap-, paint-, dye- and fertilizer manufacturers situated up-stream. It is uncertain why so few of the industrially impacted samples had quantifiable levels of DLCs. According to the MTT bio-assay's results (Table 5.1), cell viability generally had little or no effect on the H4IIE-*luc* bio-assay's outcome. Although the samples were preferably collected from rivers or streams with slow to medium flow, sedimentation shifts could have had an effect on DLC levels in sediments. The samples were collected in February 2007 during Durban's rainy season, implying that DLCs bound to sediment may be diluted or washed away to down-stream localities, as large quantities of water enter the system. Other factors, as discussed in Section 5.1.3, could also be potential causes of low DLC levels at the majority of the Durban sites.

5.1.2.4. Richards Bay sites

Richards Bay is a highly concentrated industrial complex with ample industrial activity (Sect. 4.2.4). Four of the nine samples had measurable levels of TCDD-EQ$_{20}$, ranging from 7.48 to 103 ng TCDD-EQ/kg, dw. The area had the highest mean concentration of DLCs of all the sites included in the study, and the second highest TCDD-EQ$_{20}$ measured thus far for South African sediments was measured at RB1 (103 ng TCDD-EQ/kg, dw; Table 5.1). RB1 was collected from a wetland-like area (Fig. 5.9) in the heart of Richards Bay industria (Fig. 4.7), possibly receiving effluent, run-off and/or atmospheric deposition from aluminium smelters, a woodchip producer and a fertilizer plant, all potential sources of DLCs (UNEP, 2005b). The sample had a deep black colour and distinct noxious odour, and the site seemed to be free from any bird life.

Figure 5.9. Photographs of the site RB 1 (A) and its immediate surroundings (B)

RB2 and 3 were collected near the harbour from the Manzamyana Canal, and beyond the Manzamyana Canal's confluence with the Bhizolo Canal (Fig. 4.5). These are man-made canals that are not lined with cement, but packed with loose rocks and stones covered with sediment, appearing similar to natural river tributaries. The canals pass through the industrial area and are closely associated with the aluminium smelter. TCDD-EQ$_{20}$'s measured for RB2 was 28.01 ng TCDD-EQ/kg, dw, and RB3 was 18.42 ng TCDD-EQ/kg, dw. The site RB4 was situated in the Thulazihleka Pan Bird Sanctuary, also located near the industrial area, especially the woodchip manufacturer. A TCDD-EQ$_{20}$ of 7.48 ng TCDD-EQ/kg, dw was measured for sediments from this pan. The other industrially impacted site, RB5, and the recreationally impacted site, RB6, were sampled in lagoon-like tidal areas, not directly associated with the industrial area. The levels of DLCs at RB5 and 6, and the residential sites RB7, 8 and 9 were below the LOD of the bio-assay.

5.1.2.5. Bloemfontein and Botshabelo sites

The agricultural (BF1 and 2) and recreational (BF3 and 10) areas sampled in Bloemfontein generally had below-quantifiable levels of DLCs. However, the levels of DLCs at two of the industrial- (BF6 and 8) and two of the industrial/residential combined sites (BF7 and 9) were quantifiable and ranged between 10.34 and 47.45 ng TCDD-EQ/kg, dw. The impacted residential sites were mostly of a low-income, high-density nature and emissions from domestic fires and fuels used for cooking and heating probably contributed to DLC pollution. At the industrial sites the power generation- and food-processing plants, metal works, and manufacturers of clothing, furniture, plastics and glassware might have been responsible for DLC emissions.

Although the Botshabelo sites seemed polluted (distinct smell and polluted appearance of water and sediment), the pollution must have been from a different kind because only one of the four sites showed a TCDD-EQ$_{20}$ of 36.05 ng TCDD-EQ/kg, dw, BO4. The site is situated next to a national road (the N8, Fig. 4.8) and vehicle exhaust emissions, along with domestic processes, and to a smaller extent industrial activities are suspected to be the main culprits of DLC pollution. It should also be noted that BO4 is the most down-stream of the four Botshabelo sites and that DLCs might have concentrated in the wetland system, leading to elevated levels at this point.

5.1.2.6. International and other rivers

Of the international rivers (Figures 4.10 to 4.12) it was only one site in the Limpopo River (Lim4), one site in the Olifants River (Olif4) and one site in the Crocodile River (Croc1b) that elicited quantifiable TCDD-EQs of 22.95, 33.31, and 8.02 ng TCDD-EQ/kg, dw respectively. The six samples collected from the Limpopo River were from agricultural (Lim1 and 2), residential (Lim3 and 4) and recreational (Lim5 and 6) origin. Little or no DLC pollution was expected for these sites, with the exception of Lim4, which was located near a larger low-income, high-density residential area. Greater concentrations of TCDD-EQ were anticipated for the Olifants River sites, since even the agriculturally associated sites were located in the vicinity of coal and steel works (Olif3) and low-income, high-density residential settlements (Olif2). Olif1 and 4 were sampled in close proximity to various industries, but only Olif4, situated close to plantations and paper and pulp manufacturers, and mining industries elicited a measurable response in the H4IIE-*luc* cells. The samples from the Crocodile River were mostly collected near agricultural lands and sugarcane plantations (Croc2 to 4). Two samples were also collected close to paper mills near Nelspruit (Croc1a and 1b). Croc1b, an associated sediment and soil sample pair collected on the premises of the paper mills, contained quantifiable amounts of DLCs, while sediment from Croc 1a, situated a few kilometres from the plant, did not. The Komati- and Pongola Rivers were mostly sampled in agricultural areas, where there might be quantifiable levels of OCPs, but the levels of DLCs were below the assay’s detection limit.

To assess the possible role of long-range transport from distant sources, sediments were collected from high-altitude rivers in the Drakensberg near the Lesotho border. The three sites (Drknberg1 to 3) were located in agricultural areas, with no industrial activities in the vicinity. The absence of PCDD/Fs and PCBs (Table 5.1) indicated that long-range transport played a negligible role in carrying these compounds from their sources to the high-altitude sites.

The levels of DLCs in sediments sampled from the sites associated with paper and pulp manufacturers (KZN Riv1 to 3), namely the Mhlathuze-, Tugela- and Umvoti Rivers, were also below the LOD of the bio-assay (Table 5.1). This was unexpected, since some level of DLC pollution was expected at sites that are so closely connected to significant potential sources of PCDD/Fs and PCBs (UNEP, 2005b). The low concentrations of TCDD-EQ_{20} might be ascribed to seasonal and meteorological conditions, photodegradation, sedimentation shifts, dilution or degradation by micro-organisms, which will be explained in the following section.

5.1.3. Possible reasons for low levels of DLCs in South African sediments

Although all of the potential sources for PCDD/Fs and PCBs do occur in South Africa, either concentrated in industrial parks or distributed in residential and rural landscapes, the levels of these compounds were generally low in South African sediments. Even though low concentrations were anticipated for the agriculturally and residentially impacted sites, measurable concentrations were expected for the industrial sites (which are known sources of DLCs). The factors possibly contributing towards the low levels of DLCs measured in the majority of the South African sediments will be debated in this section.

5.1.3.1. Seasonal and meteorological conditions

South Africa's climate is characterised by high temperatures, little precipitation and long summers, different from the more moderate conditions in Europe, North America and elsewhere where most of the POPs research has been conducted. These conditions could influence the distribution and degradation of organic pollutants, especially in abiotic matrices, such as soil and sediment (Quinn *et al*., 2009).

Variations in seasonal and meteorological conditions, such as temperature, rainfall, wind speed and direction, solar intensity and humidity influenced the occurrence of DLCs in environmental compartments (Lohmann *et al.,* 1999). Moon and co-workers (2005) established that DLC concentrations in Korean soils and sediments were higher in the winter months than during the summer. Their study was performed in a summer rainfall region with mean ambient temperatures ranging between 4.1 and 14.5 ˚C during the winter, and between 16.2 and 25.3 ˚C during the summer. The typical weather conditions of the area are similar to the climates of the majority of the sampling regions included in our study, except for Cape Town, where precipitation was higher during the winter (Tables 5.3 – 5.5).

Moon and co-workers (2005) concluded that ambient temperature and the amount of precipitation were the most probable factors affecting the seasonal variation in PCDD/F and PCB deposition. High ambient temperatures may facilitate the volatilisation of DLCs from surfaces, and may lower the amount of DLCs available for deposition through chemical degradation processes such as OH^- radical reactions, leading to lowered PCDD/F and PCB concentrations in the environment (Meneses *et al.,* 2003). Therefore, lower temperatures occurring during the winter might be responsible for higher dioxin-loads in sediments and soils. Since DLCs, especially the higher chlorinated compounds, are dependant on wet deposition, the amount of precipitation may also affect the amount of PCDD/Fs and PCBs deposited.

To assess the possible effect of variations in climate on the levels of DLCs in the environment, the meteorological conditions of each of the sampling regions were considered. The daily meteorological data for Johannesburg (reference for the Soweto and Lenasia wetland), Cape Town, Durban, Richards Bay, Bloemfontein, Musina (reference for the Limpopo River sites), Nelspruit (reference for the Crocodile- and Olifants River sites), Middelburg (reference for the Komati River sites), and Himeville (reference for the sites selected in the Drakensberg highlands) for the period 1998 to 2009 were obtained from the South African Weather Service (2009) and used to calculate the mean monthly maximum and minimum temperatures (Tables 5.3 and 5.4), amount of precipitation (Table 5.5), and dominant wind direction (Table 5.6). No meteorological data was available for the area surrounding the Pongola River, and no minimum or maximum temperatures were available for the high altitude rivers.

South Africa generally has warm summers (December to February) with mean maximum temperatures of the sampling regions reaching 22.1 to 33.9 ˚C (Table 5.3). The minimum temperatures occurring during the summer months were also relatively high, ranging from 9.5 to 22.1 ˚C (Table 5.4). Temperatures were mild during spring and autumn, but winter months (June – August) were generally cooler with maximum temperatures varying between 17.2 and 31.1 °C, and minimum temperatures of as low as -2.6 to 17 ˚C often prevailing (Table 5.3 and 5.4, from the South African Weather Service, 2009).

The mean monthly precipitation (rain- or snowfall) varied between the different sampling areas, mainly due to the distinctive geographical setting of each area. The greater part of the sampling regions received most precipitation during the summer (Fig. 5.10 c), but in Cape Town and Nelspruit the amount of precipitation was higher during the winter and spring (Fig. 5.10 b; Table 5.5). In Durban and the Drakensberg area the mean monthly precipitation were nearly equal during all twelve months of the year (Fig. 5.10 c; Table 5.5).

Table 5.3. Mean monthly maximum temperatures (˚C) for each sampling region calculated from meteorological data of the past ten years (1998 - 2009)

Season	Month	Mean monthly maximum temperature (˚C)							
		Soweto/ Lenasia wetland	Cape Town	Durban	Richards Bay	Bloemfontein and Botshabelo	Limpopo River	Crocodile- and Olifants Rivers	Komati River
	Dec	25.1	26.9	25.6	30.0	30.2	33.9	22.1	27.7
Summer	Jan	25.2	27.6	26.0	30.0	29.7	33.5	22.3	26.8
	Feb	24.1	26.2	25.9	29.2	27.8	32.1	23.3	27.1
	Mar	22.2	23.6	25.4	27.3	24.4	30.6	24.6	28.7
Autumn	Apr	19.3	20.6	25.3	25.5	20.4	28.4	24.5	28.9
	May	17.2	18.6	25.4	23.8	18.2	25.9	24.6	29.7
	Jun	17.2	17.8	25.6	23.7	18.3	25.5	25.5	30.1
Winter	Jul	19.9	17.9	25.9	24.9	20.7	28.5	26.1	30.0
	Aug	23.0	19.5	25.9	25.7	24.2	30.7	26.3	31.1
	Sep	24.6	22.3	25.5	26.6	27.1	32.1	25.7	30.7
Spring	Oct	24.8	24.0	25.4	28.2	28.6	33.1	24.6	29.9
	Nov	25.1	25.9	25.4	29.6	29.9	33.4	23.4	29.3

Table 5.4. The mean monthly minimum temperatures (˚ C) for each of the sampling regions calculated from meteorological data of the past ten years (1998 - 2009)

Season	Month	Mean monthly minimum temperature (˚C)							
		Soweto/ Lenasia wetland	Cape Town	Durban	Richards Bay	Bloemfontein and Botshabelo	Limpopo River	Crocodile- and Olifants Rivers	Komati River
Summer	Dec	14.7	16.4	16.0	22.0	14.5	21.5	10.5	12.1
	Jan	14.3	16.6	17.4	22.1	14.5	21.4	9.5	11.0
	Feb	12.9	14.8	18.0	20.9	11.8	19.9	10.7	11.7
Autumn	Mar	10.4	12.5	17.6	18.7	7.4	16.3	11.7	12.9
	Apr	6.6	10.5	16.3	15.6	2.0	11.1	13.3	13.1
	May	4.3	8.4	15.9	13.3	-1.3	9.0	14.3	14.8
Winter	Jun	3.5	7.5	16.0	13.0	-2.6	7.8	15.6	15.4
	Jul	6.0	8.1	17.4	15.1	0.2	11.0	17.0	16.6
	Aug	8.8	9.2	18.0	16.6	4.1	14.6	16.9	18.0
Spring	Sep	11.4	11.3	17.5	18.2	9.1	17.9	16.4	18.1
	Oct	12.8	13.6	16.4	20.1	11.5	20.2	14.4	16.0
	Nov	14.2	15.7	15.9	21.3	13.2	20.7	11.1	14.2

Table 5.5. The mean monthly precipitation (mm) for each of the sampling regions calculated from meteorological data of the past ten years (1998 - 2009)

Season	Month	Mean monthly precipitation (mm)								
		Soweto/ Lenasia wetland	Cape Town	Durban	Richards Bay	Bloemfontein and Botshabelo	Limpopo River	Crocodile- and Olifants Rivers	Komati River	High-altitude rivers
Summer	Dec	125.4	13.1	78.1	87.4	71.0	75.7	20.5	127.3	79.4
	Jan	142.0	11.5	84.6	137.8	94.0	67.2	30.3	147.6	72.8
	Feb	117.6	7.4	75.9	157.9	77.7	72.7	15.7	76.2	76.1
Autumn	Mar	87.4	11.2	107.1	105.5	85.7	50.1	11.9	53.1	77.9
	Apr	33.0	39.9	77.7	102.0	41.5	5.4	11.1	24.1	79.6
	May	25.3	68.5	88.5	54.5	24.5	2.2	43.5	6.3	64.8
Winter	Jun	10.1	75.2	89.7	81.2	10.8	6.8	85.1	6.5	72.9
	Jul	1.6	96.4	83.3	56.8	1.2	2.1	101.8	1.6	85.3
	Aug	8.9	87.0	72.2	42.8	18.6	0.0	112.1	4.0	64.6
Spring	Sep	20.3	51.2	89.2	105.5	19.9	10.2	127.4	13.6	73.0
	Oct	74.8	25.6	73.5	109.7	40.0	16.3	75.0	64.6	85.7
	Nov	91.7	24.1	89.0	166.0	74.6	65.9	57.8	101.2	65.8

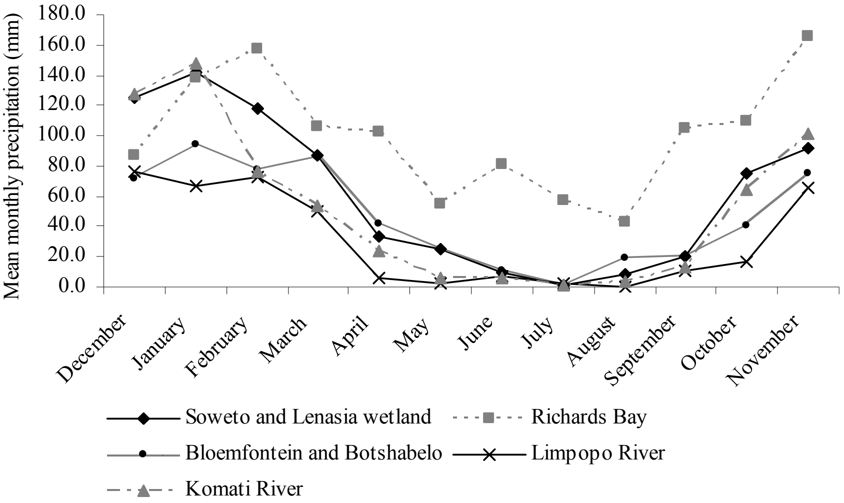


Figure 5.10 a. Mean monthly precipitation (mm) of the predominantly summer rainfall regions (1998 – 2009)

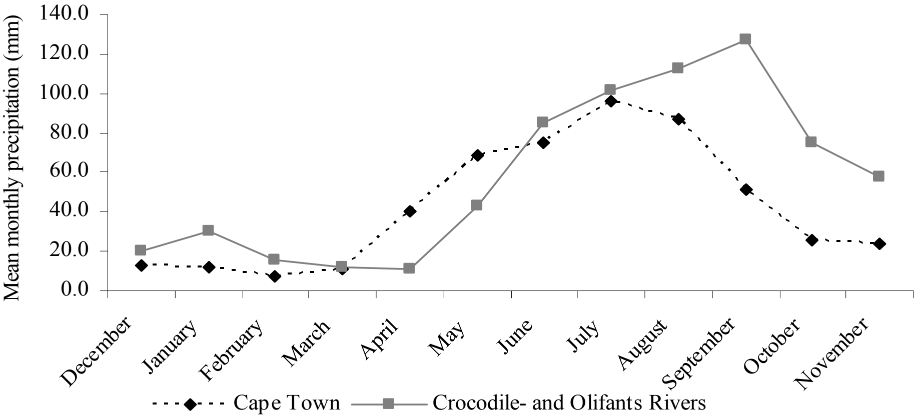


Figure 5.10 b. Mean monthly precipitation (mm) of Cape Town and the Crocodile- and Olifants River Regions (Nelspruit) (1998 – 2009)

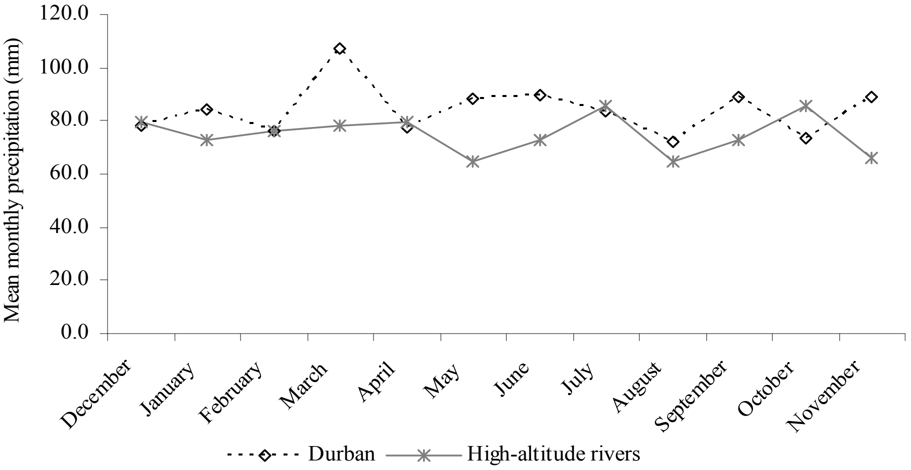


Figure 5.10 c. Mean monthly precipitation (mm) of Durban and the Drakensberg area (1998 – 2009)

Because PCDD/Fs and PCBs can be transported long distances by winds from their emission source before deposition (Lohman & Signeur, 2001), the wind's directional prevalence was considered (Table 5.5). No data was available on the prevalent wind-direction and speed of the Limpopo-, Komati- and Pongola Rivers and the Drakensberg area.

Calm wind conditions prevailed only 2.4 to 19.8% of the time, therefore winds were considered likely to play a role in the transport of PCDD/Fs and PCBs air (emissions, volatilization or particle-borne). In Soweto and Lenasia, Durban, Richards Bay, and Bloemfontein, and Botshabelo, northerly winds were most prevalent, implying that winds would distribute DLCs mainly to the south. In Cape Town and Nelspruit (Crocodile- and Olifants River reference area) southerly and east north-easterly winds were most prevalent, generally carrying DLCs to the north and west respectively.

When considering the combinations of meteorological conditions, generally, the high temperatures prevailing during the summer months were probably responsible for the volatilisation and degradation of DLCs, resulting in low concentrations of these compounds available for deposition when sufficient precipitation occurred. Conversely, during the colder winter months when the conditions were favourable for the deposition of DLCs, there was little or no precipitation to facilitate deposition. This was not the case for the Cape Town, Durban and Nelspruit sites, since precipitation occurred during the cooler winter months.

Finally, wind direction and speed may also have contributed to elevated or reduced levels of DLCs at some sites, since winds may transport compounds from their emission sources for long distances before deposition, leading to relatively low levels of DLCs close to emission sources and levels elevated above expected background at seemingly "unpolluted" areas. However, it seemed that wind direction and speed were less significant factors when considering the distribution of DLCs. The distance from, or the presence of DLC sources seemed to be more directly linked to the distribution of DLCs, as supported by the reference sites with below-quantifiable levels of DLCs.

Table 5.6. Wind directional prevalence (%) and speed (meters/second) of the sampling regions, calculated from mean monthly data from 1998 – 2009

	Prevalent wind-direction (% frequency) and speed (m/s)					
	Soweto/ Lenasia wetland	Cape Town	Durban	Richards Bay	Bloemfontein and Botshabelo	Crocodile- and Olifants Rivers
Frequency of calm conditions	3.1%	2.4%	4.9%	6.8%	15.8%	19.8%
Most prevalent wind direction	North	South	North-east	North north-east	North	East north-east
Frequency of wind from most prevalent direction	13%	23%	13%	16%	9%	13%
Frequency of windspeed from prevalent direction						
0.5 to 4 m/s	6.5%	5%	4%	16%	6%	11.5%
4 to 10 m/s	6.5%	13.5%	7.5%	7%	3%	1.5%
10 to 13 m/s	_	4.5%	1.5%	9%	_	_
13 to 20 m/s	_	1%	_	_	_	_

5.1.3.2. Photodegradation of DLCs

As mentioned in the previous section, South Africa has hot summers with long sunny days, receiving approximately 12 to 13 hours of sunlight on average (South African Weather Service, 2009). This implies that the environment is exposed to solar rays for extended periods of time, potentially leading to the degradation of DLCs through UV radiation.

DLCs have UV absorption wavelengths ranging between 270 nm and 290 nm. UV-rays from sunlight can therefore act as an important factor in the degradation of these compounds through photolysis by cleavage of the carbon-chlorine- or carbon-oxygen bonds (Isosaari, 2004). It has been established that photolysis is one of the few environmentally significant degradation mechanisms for DLCs in water, air, soils and sediments (Isosaari, 2004; Kim & O'Keefe, 2000). UV irradiation leads to the preferential loss of chlorines from the peri positions (positions 1, 4, 6 and 9), rather than from the more stable lateral positions (positions 2, 3, 7 and 8) through carbon-chlorine cleavage. Carbon-oxygen cleavage, on the other hand, is an important degradation pathway for DLCs containing four or less chlorines, producing dihydroxybiphenyls or hydroxydiphenylethers and lower chlorinated PCDD/Fs as end-products, with hydrobenzoic acid being the ultimate aromatic photodegradation product of PCDD/Fs (Kim & O'Keefe, 2000).

Several experiments have been conducted on the photodegradation of DLCs in pure- and environmental waters, such as rivers and ponds. It has been found that the degradation halflives of PCDD/Fs and PCBS in environmental waters (4 to 6 hours) are much shorter than in pure water (1.2 to 6.5 days). This can be attributed to the presence of organics in environmental waters acting as sensitizers, facilitating degradation processes (Isosaari, 2004). Although sunlight easily penetrates water, it only penetrates the top few millimetres of sediments and only the compounds in this layer can be degraded through photolysis. Although the degradation of DLCs in sediments occurs at a slower rate (approximately 5 to 8 days), photodechlorination processes in sediments and water are similar with peri-substituted chlorines being cleft preferentially and lateral chlorines being less reactive (Isosaari, 2004).

Since the sediments in the study areas were subjected to a high degree of UV-radiation, it is possible that DLCs present in the sediments were degraded by photodechlorination or cleavage of the carbon-oxygen bond. For this study, sediment samples were collected from the upper sediment layer (top 1 to 10 cm) at locations that were easily accessible for collection by hand. This implied that samples were generally collected at locations where the water level was about ankle- to knee-deep. At this depth, UV-rays can penetrate the water to irradiate the top sediment layer [top 1 – 4 mm (Garcia-Pichel & Bebout, 1996)], potentially leading to the photodegradation of PCDD/Fs.

5.1.3.3. Sedimentation shifts and effect of dilution

Although precipitation promotes the deposition of DLCs, large amounts of precipitation may lead to lowered levels of DLCs in water and sediments. During periods of high rain- or snowfall, large quantities of water may enter a water body/system, diluting the concentrations of DLCs and shifting sediments to down-stream localities (Davies & Day, 1998). This implies that the DLCs that are bound to sediments are also carried down-stream, decreasing the concentrations of PCDD/Fs and PCBs in the upstream parts of rivers.

The sediment samples were collected during a four month period, extending from January to April 2007. During this period, the mean monthly precipitation for the sampling areas ranged between 7.4 and 157.9 mm (Table 5.4), with the least amount of precipitation occurring in Cape Town and the greatest amount of precipitation occurring in Richards Bay. The majority of the sites (except for Cape Town) were sampled during the rainy season and precipitation could therefore have affected the levels of DLCs in sediments through dilution or sedimentation shifts.

5.1.3.4. Degradation by micro-organisms

Micro-organisms may also be responsible for the degradation of DLCs. Bio-degradation and bio-transformation of PCDD/Fs by micro-organisms had even been considered as bio-remediation options for polluted environments.

Certain aerobic bacteria containing aromatic hydrocarbon dioxygenases have broad substrate specificity and have the ability to degrade the ring structures of PCDDs and related compounds. In addition to this type of bio-degradation, reductive dechlorination by anaerobic micro-organisms and fungal degradation has also been recognized as modes of PCDD bio-transformation (Halden *et al.*, 1999). Microbial reductive dechlorination of PCDD/Fs has been established in sediments and soils. This dechlorination process may degrade highly chlorinated congeners, which are generally barely attacked by aromatic hydrocarbon dioxygenases (Wittich, 2004). Certain strains of the anerobic bacteria, *Dehalococcoides ethenogenes,* have been shown to be able to dechlorinate selected PCDD/F congeners. It is also presumed that members of the *Dehalococcoides* group are capable of dechlorinating a tetra-chlorinated PCB congener, chlorobenzene and trichlorodibenzo-*p*-dioxin. Members of the *Dehalococcoides* group are widely dispersed in nature and play a major role in the transformation of chlorinated substances (Wittich, 2004). *Sphingomonas wittichii* is another bacterial strain with the unique ability to mineralize PCDD/Fs by the key enzyme, dioxin dioxygenase (Halden *et al*., 1999). Many other micro-organisms and fungi may be responsible for the degradation of DLCs. Although water and sediments were not analysed for the presence of micro-organisms during the study, it has to be taken into account that DLCs may possibly be degraded by this pathway.

Any one of the factors discussed in this section, or combinations thereof might have been responsible for the unexpectedly low levels of DLCs measured in sediments associated with highly industrialised areas, containing many potential sources of PCDD/Fs and PCBs.

5.2. Chemical analysis results

In Section 5.2, a summary of the chemical analysis results is firstly given, followed by a discussion of each sampling location's results. Finally, the PCAs of the different groups of compounds are discussed to identify possible differences and similarities in the pollution profiles of the sites.

5.2.1. Chemical analysis results: A summary

To establish the levels of selected OCPs, PAHs, PBDEs, and PCBs present in the samples, 30 of the original 96 samples were chosen for chemical analysis. This included the 23 samples eliciting quantifiable responses with the H4IIE-*luc* bio-assay (in bold in Table 5.1), the two high-altitude reference samples (Drknberg 1 and 3) for minimal industrial pollution, and all of the Soweto and Lenasia wetland sediment samples (Table 5.1). Although only six of the 13 S/L samples produced measurable TCDD-EQ_{20}'s, a full assessment (biological and chemical analysis) was performed on the area's sediments, since it is the location where the highest concentration of DLCs yet recorded for South African sediments was measured, potentially posing a threat to human and environmental health. Furthermore, it was one of the sampling location where the closest interaction existed between humans and presumed contaminated sediments, with many residents (especially low-income) swimming, fishing, playing and washing their clothes in parts of the streams and wetlands of the Klip River system.

The chemical analysis results of the 30 sites are summarized in Tables 5.8 to 5.10. Where the levels of compounds were below the detection limit, the half-LOD was reported (to avoid any zero values, which would be unfavourable in the PCA). Due to limited sample being available for extraction, S/L 13 was not analysed for OCPs, PCBs and PBDEs, and the high-alitude reference sites Drknberg1 and 3 were not analysed for PAHs.

Of the various groups of compounds analysed, only the PAHs were present in all of the samples. The levels of PAHs were statistically significantly higher ($p < 0.05$, Mann-Whitney U test) than the levels of the other pollutants when considering all 30 sites (Table 5.9). OCPs (Table 5.8) and PCBs (Table 5.10) had intermediate concentrations, and PBDEs were the least abundant, with low quantifiable concentrations of the compound at only a few of the sites (Table 5.10). Table 5.7 contains a summary of abbreviations of the chemical compound names that will be used in tables and figures from this point forward.

Table 5.7. Summary of the abbreviations of chemical compound names

OCPs		PAHs	
Compound name	**Abbreviation**	**Compound name**	**Abbreviation**
HCB	HCB	Naphthalene	Naph
α-HCH	α-HCH	Acenaphthylene	Anaphthy
β-HCH	β-HCH	Acenaphthene	Anaphthe
γ-HCH	γ-HCH	Fluorene	Fluor
Heptachlor	Hpchlor	Phenanthrene	Phen
Mirex	Mirex	Anthracene	Anthr
o,p'-DDE	*o,p'*-DDE	Fluoranthene	Fluoran
p,p'-DDE	*p,p'*-DDE	Pyrene	Pyr
o,p'-DDD	*o,p'*-DDD	Benzo(a)anthracene	B(a)A
p,p'-DDD	*p,p'*-DDD	Chrysene	Chrys
o,p'-DDT	*o,p'*-DDT	Benzo(b)fluoranthene	B(b)Fl
p,p'-DDT	*p,p'*-DDT	Benzo(k)fluoranthene	B(k)Fl
		Benzo(a)pyrene	B(a)Pyr
		Indeno(1,2,3-cd)pyrene	In(1,2,3-cd)Pyr
		Dibenz(a,h)anthracene	Dib(a,h)A
		Benzo(ghi)perylene	B(ghi)Per

5.2.1.1. OCP levels

The detection limit for OCPs was 10 ng/kg, and the mean recovery efficiencies were between 46% and 92%. Aldrin and chlordene were not detected at any of the sites, while low levels of nonachlor, chlordane (20 to 120 ng/kg, dw) and oxychlordane (5 to 130 ng/kg) were found at only five of the 30 sites. HCB, HCH and DDT were the predominant OCPs, with concentrations ranging between 50 and 6 800 ng/kg, dw; below the LOD to 2 300 ng/kg, dw; and 5 and 11 000 ng/kg, dw respectively. Heptachlor and mirex were present at lower concentrations (Table 5.8). This can be ascribed to the fact that HCB is still used in industrial applications, and HCH (specifically γ-HCH, but getting phased out) and DDT are presently used as pesticides in some parts of the country, while the use of heptachlor and mirex have long been prohibited in South Africa (Bouwman, 2004; Nel *et al.*, 2002).

Of the HCH-isomers, γ-HCH (lindane) was the most prevalent, contributing between 55 to 99% of the Σ-HCH (Table 5.8). Although lindane was added to the SC POPs in 2009, it is currently registered and used in agricultural and domestic gardens (Nel *et al.*, 2002). Lindane consists of a mixture of all HCH isomers with approximately 90% γ-HCH (Gong *et al.*, 2007). The large contribution of γ-HCH at the sites therefore points to recent applications of lindane, rather than the use of technical HCH (consisting of 55 to 80% α-HCH) or historic inputs, since γ-HCH degrades more rapidly than α-HCH, under aerobic and anaerobic conditions (Wu *et al.*, 1997).

DDT is degraded to DDE and DDD in the environment. To assess whether the DDT measured in sediments was due to recent or historic use, the ratio of DDT to (DDE + DDD) was determined. DDT:(DDE + DDD) ratios greater than 1 would indicate recent application, whilst ratios of less than 1 would indicate historic use (Gong *et al.*, 2007). The ratios were less than 1 at the majority of the sites, indicating historic use of DDT. At S/L8 and Lim4, the ratio exceeded 1 and suggested recent application. Lim 4 was sampled in a malaria endemic area where DDT is sprayed for vector control. The suggestion of recent DDT application at the semi-industrial site, S/L8, was, however, unexpected. It should be noted, though, that the levels of *o,p'*- and *p,p'*-DDT were relatively low at S/L8 when compared to other sites (840 and 470 ng/kg, dw respectively; Table 5.8).

The sites with the highest pesticide loads were D14, the Umgeni River mouth (ΣOCPs = 23 300 ng/kg, dw), followed by a residential site in the Limpopo Province, Lim4 (ΣOCPs = 22 400 ng/kg, dw) and the Soweto wetland site, S/L9 (ΣOCPs = 12 900 ng/kg, dw) (Table 5.8, Fig. 5.11). At Lim4 and D14, ΣDDT were mainly responsible for the high OCP-loads, while HCB, ΣDDT, and HCH almost equally contributed towards the ΣOCPs at S/L9 (Fig 5.11).

5.2.1.2. PAH levels

The concentration of the 16 US EPA priority PAHs were above the detection limit at all of the sites. For two of the sites, RB1 and RB4, acenaphthylene could not be quantified due to interferences on the chromatograms (Table 5.9).

The distribution profiles of PAHs were similar at the majority of the sites (Fig 5.12). In general, 4-ringed PAHs [fluoranthene, pyrene, benzo(a)anthracene and chrysene] were the most abundant, followed by 5-ringed congeners [benzo(b)fluoranthene, benzo(k)fluoranthene and chrysene] in most instances, and finally, either by the 3- [acenaphtylene, acenaphtene, fluorene, phenanthrene and anthracene] or 6-ringed congeners [indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene and benzo(ghi)perylene]. The 2-ringed PAHs were generally the least abundant (except at the sites S/L4, S/L8, S/L13 and Olif4; Fig. 5.12). Due to their physical and chemical properties, HMM-PAHs are less susceptible to biodegradation or loss from soil or sediment through processes such as evaporation, leaching and dissolution (Manoli & Samara, 1999), which might explain the higher prevalence of the HMM-PAH species at the majority of the sites. The concentration ranges were as follows: 2-ringed PAHs: 11 000 to 1 100 000 ng/kg, dw; 3-ringed PAHs: 270 to 700 000 ng/kg, dw; 4-ringed PAHs: 2 600 to 920 000 ng/kg, dw; 5-ringed PAHs: 5 100 to 800 000 ng/kg, dw; and 6-ringed PAHs: 1 100 to 1 600 000 ng/kg, dw (Table 5.9).

The mean recovery for PAHs was between 10% and 60%. The highest concentrations of ΣPAHs were measured at Croc1b, situated on the premises of a papermill (ΣPAHs = 8 992 000 ng/kg, dw), S/L8, situated within a residential area (ΣPAHs = 5 528 000 ng/kg, dw, and CT7, a highly industrialised area in the vicinity of a petrochemical plant (ΣPAHs = 3 040 000 ng/kg, dw; Fig. 5.12). Source characterisation will be dealt with under Section 5.2.2.3.

5.2.1.3. PCB levels

CB-153, -138, -118 and -101 were the predominant PCB congeners, while the others were present at lower concentrations (Fig. 5.13). The concentrations of the seven PCB congeners analysed for ranged between below the LOD and 3 100 ng/kg, dw for CB-28, below the LOD and 1 900 ng/kg, dw for CB-52, below the LOD to 9 400 ng/kg, dw for CB-100, 160 and 14 000 ng/kg, dw for CB-118, 120 and 18 000 ng/kg, dw for CB-138 and CB-153, and below the LOD and 4 700 ng/kg, dw for CB-180 (Table 5.10). The detection limit for PCBs was 10 ng/kg. Recovery efficiencies for these congeners ranged between 58 and 92%. The highly industrialised site, RB1, had the highest concentration of ΣCBs at 57 300 ng/kg, dw, followed by S/L9 (ΣCBs = 48 580 ng/kg, dw), D14 (ΣCBs = 46 180 ng/kg, dw) and RB2 (ΣCBs = 39 790 ng/kg, dw), also associated with industrial activities (Fig. 5.13).

5.2.1.4. PBDE levels

The detection limit for PBDE was 1 ng/kg, dw and recovery efficiencies were between 66 and 72%. BDE-28, -47 and -209 were not detected at any of the sites. The concentrations of BDE-99, -153 and -183 ranged between below the LOD to 60, 110 and 20 ng/kg, dw respectively, with BDE-153 being the predominant PBDE (Table 5.10, Fig. 5.14). The compounds were generally present at low levels, with the highest concentration of ΣBDE measured at D14 being 179 ng/kg, dw. Other sites with otherwise notable concentrations of BDE were CT7 (ΣBDE = 87 ng/kg, dw) and S/L9 (ΣBDE = 60 ng/kg, dw) (Fig. 5.14).

Table 5.8. Concentrations (ng/kg, dw) of certain OCPs measured by chemical analysis

	HCB	**α-HCH**	**β-HCH**	**γ-HCH**	***ΣHCH***	**Hpchlor**	**Mirex**	***o,p'*- DDE**	***p,p'*-DDE**	***o,p'*- DDD**	***p,p'*-DDD**	***o,p'*- DDT**	***p,p'*-DDT**	***ΣDDT***
S/L 1	760	5*	5*	780	*790*	70	40	50	340	5*	80	70	290	*835*
S/L 2	2100	5*	5*	1400	*1410*	20	30	80	650	30	350	30	340	*1480*
S/L 3	540	5*	5*	360	*370*	40	60	30	540	5*	160	40	340	*1115*
S/L 4	140	250	20	330	*600*	50	40	50	590	10	240	50	380	*1320*
S/L 5	100	5*	5*	640	*650*	20	20	70	480	5*	550	30	440	*1575*
S/L 6	160	5*	5*	210	*220*	5*	70	20	410	20	130	70	190	*840*
S/L 7	50	5*	5*	900	*910*	90	50	10	340	20	140	90	190	*790*
S/L 8	230	5*	5*	1100	*1110*	50	10	5*	440	60	110	840	470	*1925*
S/L 9	4100	430	30	2100	*2560*	360	230	120	2600	150	750	380	1300	*5300*
S/L 10	80	5*	5*	1300	*1310*	120	50	70	1100	90	90	140	30	*1520*
S/L 12	3500	5*	5*	1400	*1410*	430	150	110	2900	90	290	420	5*	*3815*
CT2	5700	5*	5*	320	*330*	140	20	90	780	5*	210	190	390	*1665*
CT6	3200	5*	5*	5*	*15*	40	40	40	810	5*	170	200	330	*1555*
CT7	6800	5*	5*	80	*90*	90	10	20	430	5*	130	180	440	*1205*
CT8	560	5*	5*	350	*360*	30	5*	30	660	5*	160	90	330	*1275*
D14	2600	5*	5*	560	*570*	2800	330	190	8600	130	890	1300	4100	*15210*
RB1	5600	800	5*	2100	*2905*	40	20	5*	290	5*	20	20	90	*430*
RB2	1800	5*	5*	1300	*1310*	80	20	5*	190	5*	40	30	140	*410*
RB3	2600	5*	5*	1300	*1310*	30	30	5*	300	5*	5*	5*	50	*370*
RB4	3300	5*	5*	1400	*1410*	80	20	5*	220	5*	5*	5*	100	*340*
BF6	4800	5*	5*	670	*680*	10	20	10	980	20	230	360	750	*2350*
BF7	5300	5*	5*	890	*900*	60	40	30	1500	40	170	140	410	*2290*
BF8	1300	5*	5*	90	*100*	150	80	20	660	40	200	40	490	*1450*
BF9	3600	5*	5*	340	*350*	20	30	50	1200	10	200	30	450	*1940*
BO4	1400	5*	5*	5*	*15*	5*	5*	5*	790	5*	70	140	290	*1300*
Lim 4	60	5*	5*	30	*40*	170	5*	220	7800	180	340	2500	11000	*22040*
Olif 4	960	5*	5*	5*	*15*	5*	30	5*	190	5*	5*	5*	90	*300*
Croc 1b	2100	5*	5*	5*	*15*	5*	60	5*	280	5*	5*	5*	90	*390*
Drknberg 1	50	5*	5*	2300	*2310*	70	5*	40	1200	5*	170	130	570	*2115*
Drknberg 3	90	5*	5*	920	*930*	210	40	40	990	5*	190	130	460	*1815*

* Where concentrations were below the detection limit, the half-LOD was reported.

Table 5.9. Concentrations (ng/kg, dw) of the 16 US EPA PAHs measured by chemical analysis

	Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
S/L1	141 000	10 000	4 600	93 000	210 000	26 000	220 000	170 000	270 000	110 000	120 000	62 000	30 000	8 700	1 100	14 000
S/L2	63 000	2 600	2 500	7 100	60 000	6 200	100 000	89 000	67 000	77 000	90 000	38 000	69 000	85 000	11 000	90 000
S/L3	26 000	1 100	1 200	3 900	30 000	2 300	33 000	25 000	12 000	14 000	16 000	7 000	11 000	15 000	2 200	15 000
S/L4	180 000	10 000	2 900	13 000	93 000	6 600	53 000	36 000	12 000	18 000	18 000	7 300	10 000	23 000	2 600	26 000
S/L5	70 000	3 200	2 800	5 000	63 000	5 500	91 000	70 000	39 000	56 000	50 000	23 000	41 000	38 000	7 800	37 000
S/L6	180 000	6 500	1 600	15 800	200 000	17 000	250 000	170 000	120 000	170 000	180 000	70 000	78 000	120 000	20 000	120 000
S/L7	140 000	7 300	13 000	23 000	180 000	25 000	240 000	200 000	150 000	140 000	160 000	75 000	140 000	202 000	29 000	190 000
S/L8	1 100 000	37 000	40 000	93 000	700 000	90 000	700 000	500 000	300 000	400 000	320 000	140 000	390 000	250 000	78 000	270 000
S/L9	66 000	2 900	6 000	14 000	120 000	11 000	180 000	150 000	90 000	100 000	120 000	55 000	93 000	120 000	18 000	120 000
S/L10	61 000	2 700	1 400	4 800	46 000	3 200	43 000	31 000	17 000	20 000	30 000	13 000	18 000	34 000	3 800	36 000
S/L12	120 000	4 000	5 300	7 100	150 000	7 600	260 000	230 000	127 000	150 000	130 000	82 000	140 000	170 000	23 000	140 000
S/L13	120 000	3 500	2 200	5 300	56 000	4 300	55 000	40 000	20 000	34 000	40 000	16 000	24 000	36 000	5 600	42 000
CT2	96 000	1 000	25 000	44 000	230 000	29 000	280 000	260 000	180 000	160 000	150 000	77 000	23 000	150 000	24 000	120 000
CT6	13 000	290	900	3 000	14 000	2 500	15 000	20 000	7 200	8 300	10 000	5 100	7 700	10 000	1 100	14 000
CT7	80 000	10 000	21 000	67 000	320 000	50 000	220 000	240 000	280 000	340 000	330 000	130 000	200 000	310 000	42 000	310 000
CT8	22 000	500	1 400	4 200	23 000	4 300	34 000	36 000	18 000	28 000	21 000	9 000	20 000	19 000	3 000	2 200
D14	46 000	2 600	4 600	12 000	51 000	10 000	61 000	84 000	34 000	58 000	60 000	84 000	47 000	59 000	9 700	74 000
RB1	28 000	§	4 900	12 000	110 000	22 000	180 000	160 000	100 000	160 000	140 000	65 000	97 000	120 000	20 000	120 000
RB2	11 000	270	4 600	4 300	38 000	3 200	92 000	80 000	52 000	65 000	87 000	36 000	63 000	68 000	12 000	70 000
RB3	180 000	4 900	14 000	26 000	180 000	19 000	190 000	160 000	97 000	180 000	220 000	83 000	110 000	110 000	21 000	120 000
RB4	66 000	§	3 300	11 000	38 000	6 200	46 000	34 000	22 000	29 000	40 000	15 000	49 000	28 000	4 800	30 000
BF6	44 000	1 800	3 300	8 700	56 000	6 500	91 000	79 000	51 000	63 000	62 000	27 000	51 000	59 000	8 900	57 000
BF7	110 000	5 000	7 100	13 000	130 000	13 000	140 000	130 000	100 000	110 000	130 000	51 000	90 000	120 000	22 000	120 000
BF8	15 000	610	660	2 700	26 000	3 000	58 000	52 000	27 000	44 000	37 000	16 000	31 000	37 000	5 500	39 000
BF9	69 000	1 600	3 000	9 400	52 000	5 200	80 000	71 000	36 000	53 000	73 000	32 000	40 000	73 000	9 200	73 000
BO4	59 000	17 000	5 100	43 000	140 000	13 000	240 000	180 000	100 000	150 000	180 000	81 000	120 000	140 000	28 000	180 000
Lim4	11 000	480	590	2 900	23 000	2 400	48 000	43 000	20 000	23 000	37 000	18 000	37 000	52 000	6 000	49 000
Olif4	93 000	2 500	1 400	6 200	31 000	6 500	33 000	31 000	23 000	20 000	29 000	11 000	20 000	21 000	4 200	29 000
Croc1b	69 000	15 000	17 000	16 000	390 000	44 000	650 000	700 000	730 000	920 000	920 000	520 000	800 000	1 600 000	11 000	1 300 000

§ No peak: Due to interferences on the chromatograms of RB1 and RB4, acenaphthylene (Anaphthy) could not be quantified.

Table 5.10. Concentrations (ng/kg, dw) of certain non-dioxin-like PCBs and PBDEs measured by chemical analysis

	PCBs							**PBDEs**		
	CB-28	**CB-52**	**CB-101**	**CB-118**	**CB-138**	**CB-153**	**CB-180**	**BDE-99**	**BDE-153**	**BDE-183**
S/L1	120	90	1200	890	4100	2200	340	5	2	2
S/L2	80	230	790	1300	5600	4200	120	0.5*	0.5*	1
S/L3	20	110	580	930	4400	4500	410	0.5*	1	2
S/L4	5*	90	990	1500	2200	2100	90	0.5*	0.5*	0.5*
S/L5	5*	40	660	1100	3000	1200	370	0.5*	0.5*	0.5*
S/L6	5*	30	560	1100	1400	3900	210	0.5*	0.5*	0.5*
S/L7	5*	10	680	2200	1400	1900	550	0.5*	0.5*	0.5*
S/L8	5*	10	1300	3500	4200	3700	1300	0.5*	0.5*	0.5*
S/L9	190	590	2900	14000	8200	18000	4700	10	30	20
S/L10	5*	140	560	2800	1200	1200	130	0.5*	0.5*	0.5*
S/L12	160	810	640	2100	5900	3700	1100	0.5*	6	1
CT2	130	320	4700	4300	5800	3400	430	8	0.5*	0.5*
CT6	340	200	4600	7200	8100	2900	1300	10	0.5*	0.5*
CT7	90	450	5800	5800	9200	4900	980	30	50	7
CT8	5*	5*	3900	6100	2200	4900	170	0.5*	0.5*	0.5*
D14	1300	980	8800	7400	6600	17000	4100	60	110	9
RB1	3100	1900	9400	6700	18000	14000	4200	40	60	9
RB2	120	370	8200	7800	9200	12000	2100	10	3	3
RB3	230	690	4400	5100	3900	5500	260	0.5*	0.5*	0.5*
RB4	210	230	6100	6200	5700	3600	1100	0.5*	0.5*	0.5*
BF6	120	230	3200	1700	4200	2900	250	3	5	0.5*
BF7	300	530	2900	1700	3400	3800	30	0.5*	3	0.5*
BF8	260	5*	890	3600	2100	4700	5*	0.5*	2	0.5*
BF9	140	220	530	4900	5100	1300	410	0.5*	2	0.5*
BO4	5*	5*	5*	160	330	410	230	0.5*	0.5*	0.5*
Lim4	5*	5*	5*	240	120	120	90	0.5*	0.5*	0.5*
Olif4	5*	5*	330	980	3000	1100	130	0.5*	6	0.5*
Croc1b	5*	5*	530	690	1400	2600	150	0.5*	3	0.5*
Drknberg1	5*	5*	5*	1800	1300	1900	30	0.5*	0.5*	0.5*
Drknberg3	5*	5*	5*	960	1100	970	70	0.5*	0.5*	0.5*

* Where concentrations were below the detection limit, the half-LOD was reported.

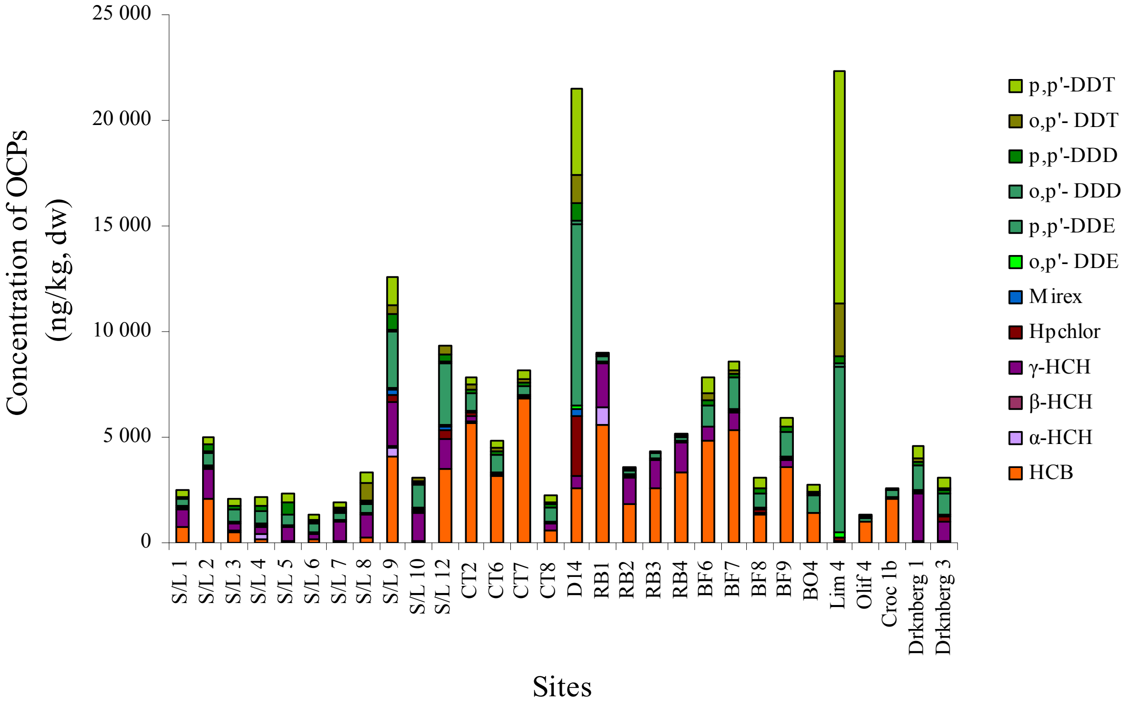


Figure 5.11a. Concentrations (ng/kg, dw) of HCB, HCH, heptachlor, mirex, DDE, DDD and DDT at each site

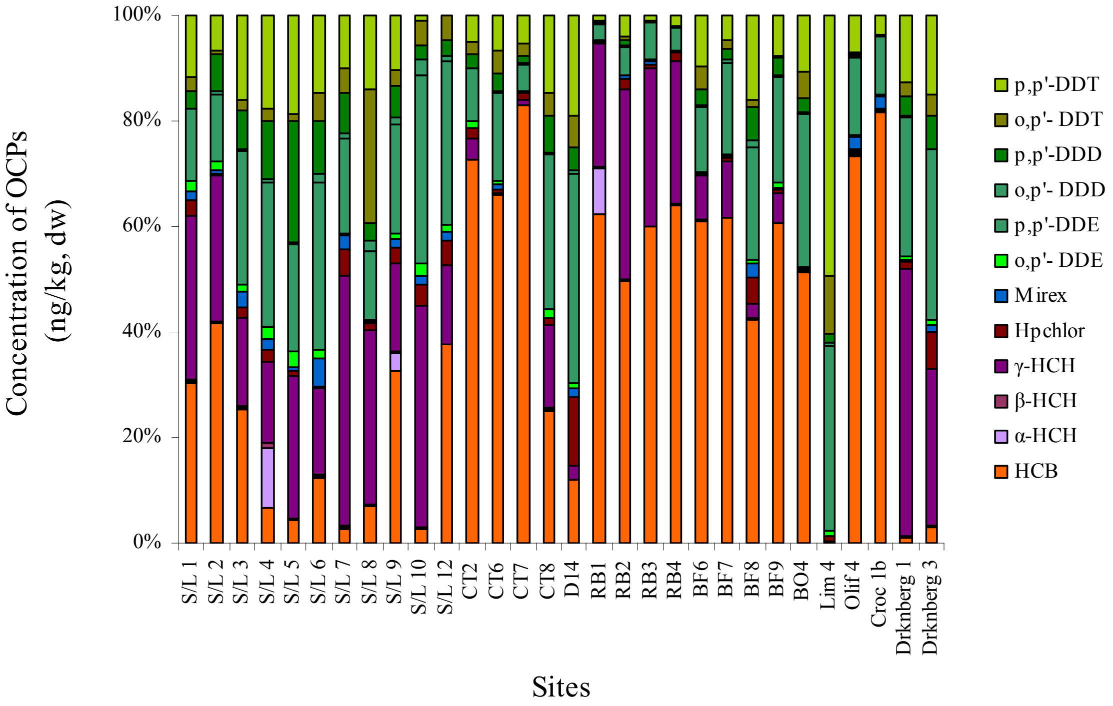


Figure 5.11b. Percentage contribution of HCB, HCH, heptachlor, mirex, DDE, DDD and DDT to the ΣOCPs measured at each site

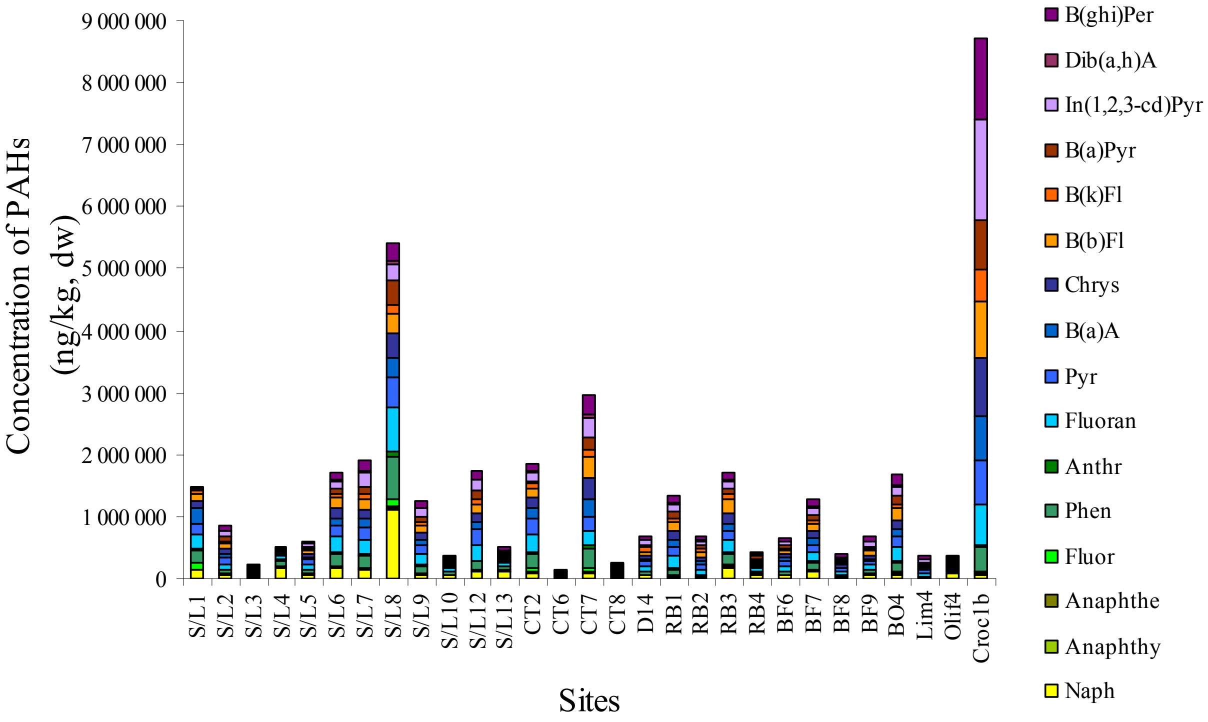


Figure 5.12a. Concentrations (ng/kg, dw) of the 16 US EPA priority PAHs at each site. (Note that acepnahthylene was not included at RB1 and RB4)

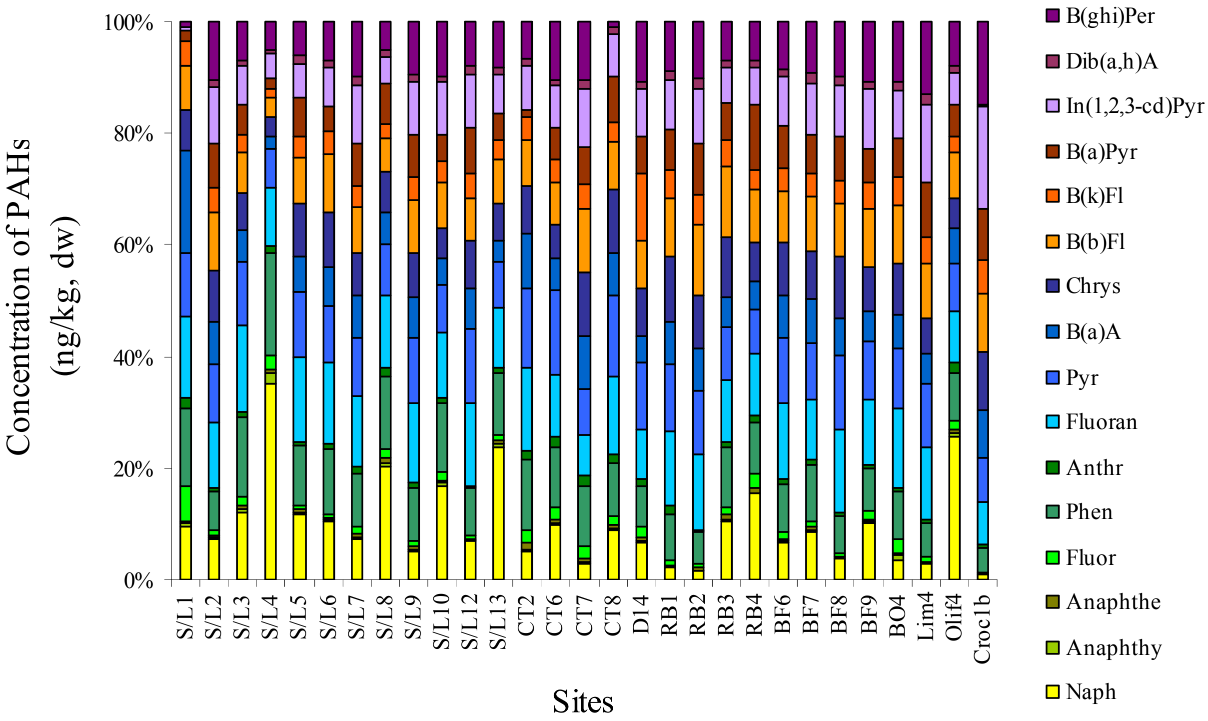


Figure 5.12b. Percentage contribution of the 16 US EPA priority PAHs towards the ΣPAHs measured at each site

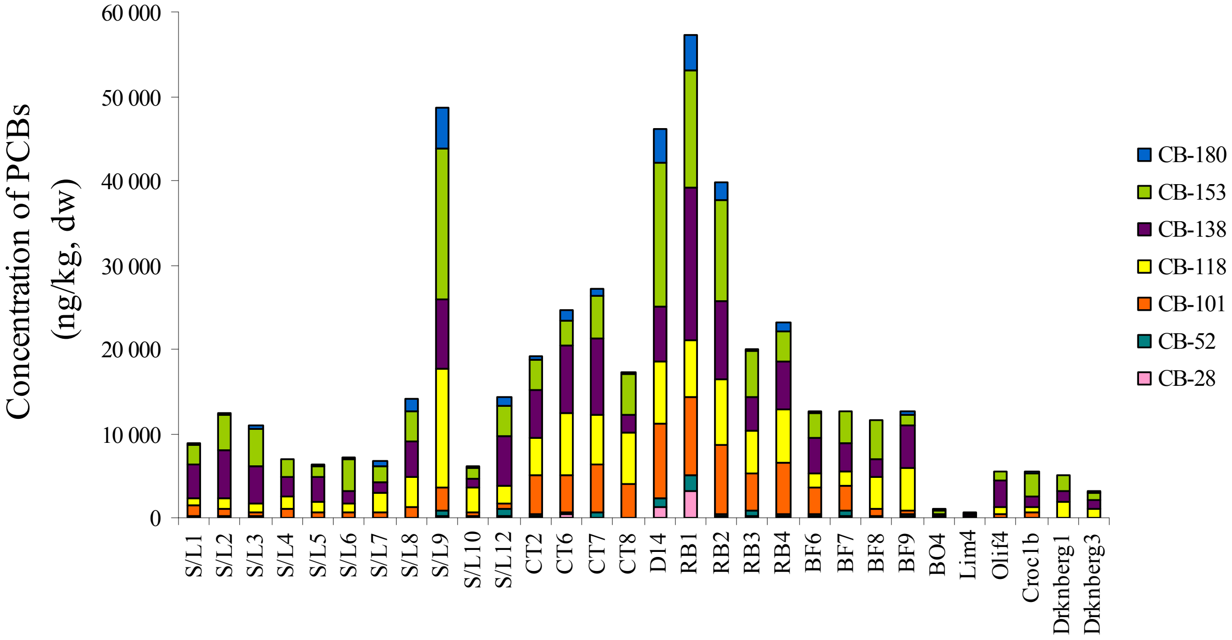


Figure 5.13a. Concentrations (ng/kg, dw) of the seven PCB congeners at each site

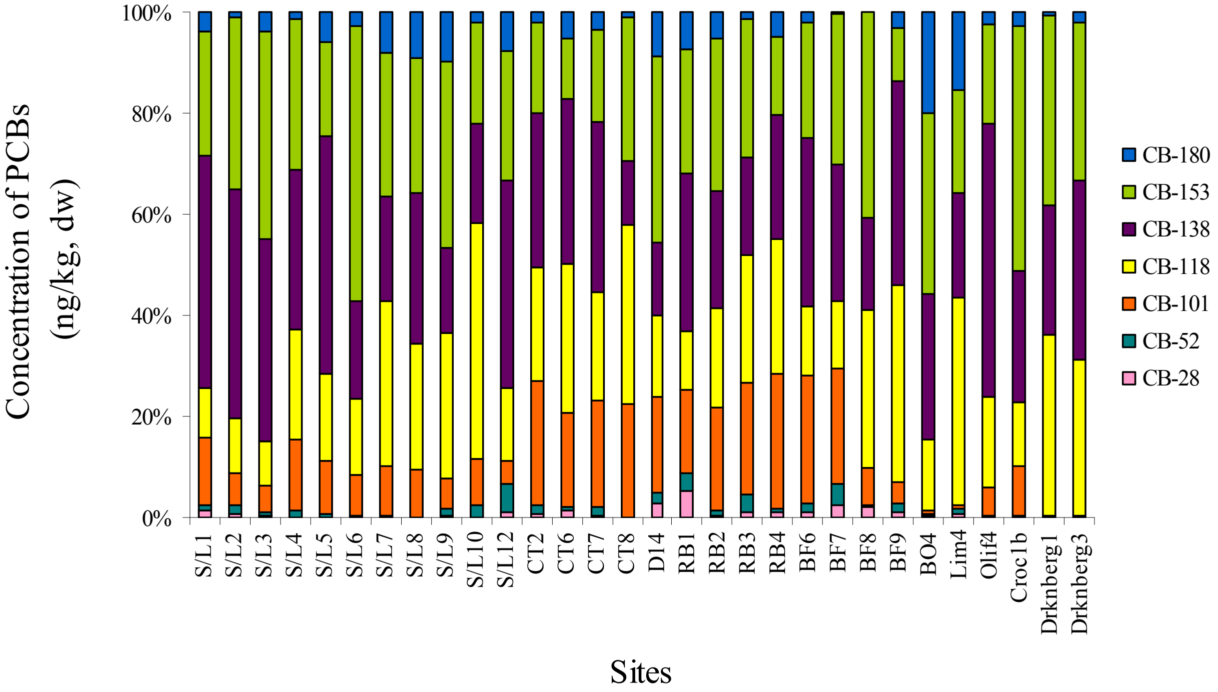


Figure 5.13b. Percentage contribution of the seven PCB congeners to the ΣPCBs measured at each site

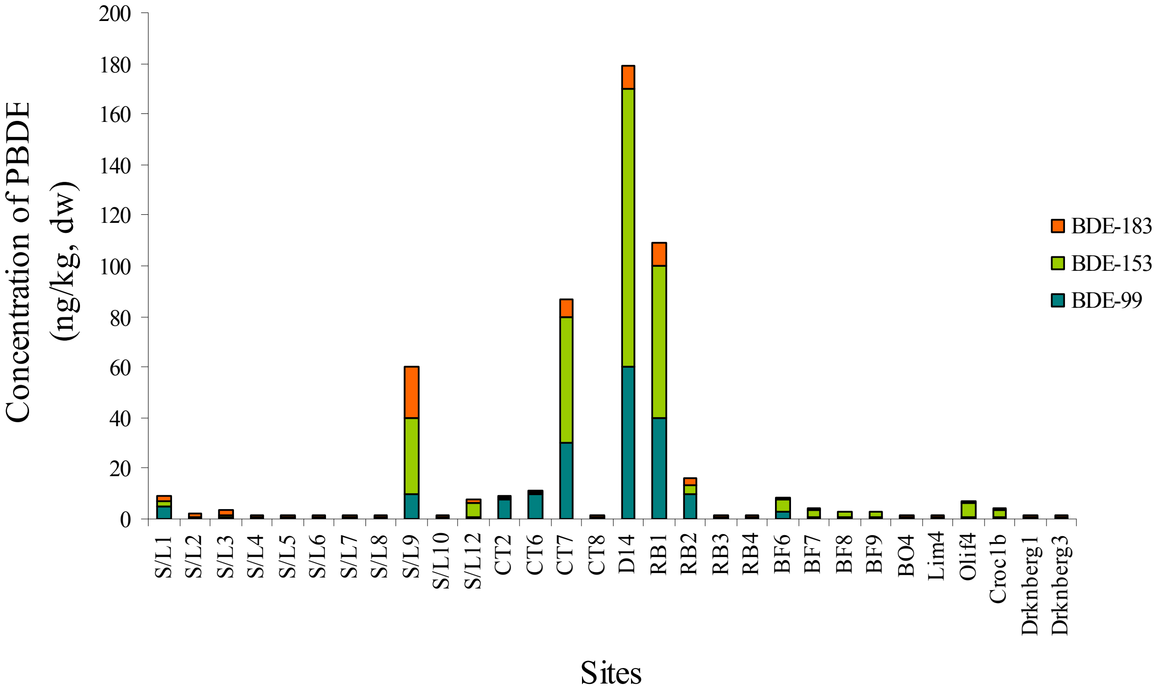


Figure 5.14a. Concentrations (ng/kg, dw) of BDE-183, -153 and -99 towards at each site

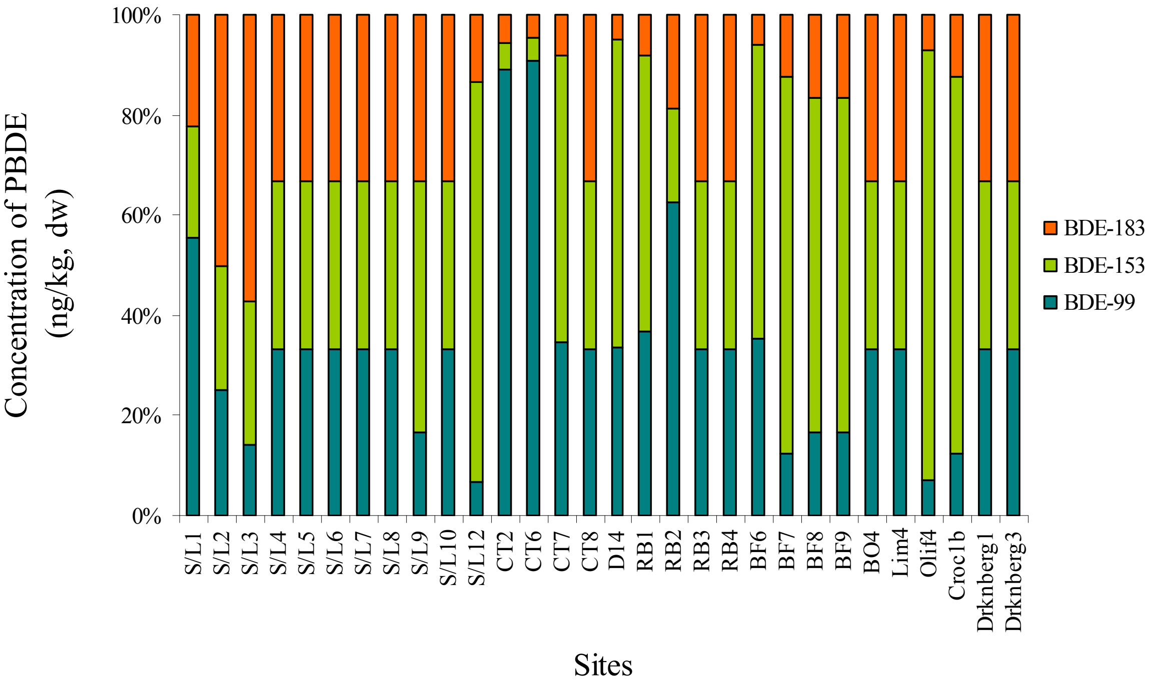


Figure 5.14b. Percentage contribution of BDE-183, -153 and -99 towards the Σ-PBDE measured at each site

5.2.2. Chemical analysis results: Principal component analysis (PCA)

PCA was used to investigate similarities and differences in the sediment pollution profiles of the industrial, semi-industrial, residential and agricultural sites (or combinations thereof). In the industrial areas, industrially-associated compounds such as PAHs (unintentionally produced) and PCBs and PBDEs (intentionally produced) were anticipated, whereas the agricultural areas were expected to be characterised by the predominance of OCPs. In the low-income residential areas (such as the S/L and BO sites), mostly DLCs and PAHs were expected as by-products of domestic and backyard (open) burning used for heating and cooking.

Only chemical analyses data were included in the PCAs, thus DLCs (measured bio-analytically) were not included here. The three sites, S/L 13 and Drknberg 1 and 3, did not participate in the PCAs, because the chemical analysis data for these sites were incomplete. Also, data for the acenaphthylene (PAH) were excluded from the PCAs, since the compound could not be quantified at two of the sites (no 0-values are allowed in PCA).

In this statistical analysis, no carbon normalised data was used, only the measured values, because those values represent the pollutants actually present. The organic carbon content of a sediment sample is but one of the many variables that could and did influence measured concentrations. Variables such as wind direction, distance from point source, amount of pollution, matrice of initial exposure (air vs water), rainfall average and environmental temperatures are but a few that could have influenced the concentration actually determined. There could not be normalised for any of these afore mentioned variables and therefore, normalisation for carbon content was not done either.

Separate PCA’s were run for the following combinations:

- **All compounds:** This included the OCPs (HCB, ΣHCH, mirex, heptachlor and ΣDDT), ΣPAHs, ΣPCBs and ΣPBDEs. The purpose of this PCA was to determine the general distribution of the sites with all of the compounds included to test the hypothesis that industrial sites will associate more with the industrial pollutants (PAHs, PCBs and PBDEs), while the agricultural sites will be characterised by a predominance of OCPs.
- **OCPs only:** Here, the concentrations of HCB and mirex, and the congener or isomer-specific data for HCH and DDT were used in the PCA. The intention was to consider the distribution of the OCPs in more detail.

- **PAHs only:** While the LMM-PAHs are mainly of petrogenic origin, exist in gaseous form, and would deposit farther from their sources of emission, the HMM-PAHs are mainly of pyrogenic origin, associate with particulate matter, and would deposit closer to their sources of emission. Due to their differences in characteristics, it is expected that the various PAH congeners will have distinct distribution profiles. The purpose of this PCA was to establish these differences and to determine the sources of PAHs.
- **PCBs and PBDEs only:** As for the PAHs, differences in the distribution of the heavier, more chlorinated, and lighter, less chlorinated PCBs were expected. Since both PCBs and PBDEs are intentionally produced compounds, they participated in the same PCA.

5.2.2.1. PCA inclucing all compounds

Factor 1 explained 34% and factor 2 24% of the variance in the data. Factor 1 was mainly a contrast between ΣHCH and heptachlor (Hpchlor), with positive loadings, and HCB and ΣPBDEs with negative loadings. Factor 2 distinguished between ΣDDT and heptachlor on the positive axis, and ΣHCH and ΣPCBs on the negative axis (Fig. 5.15).

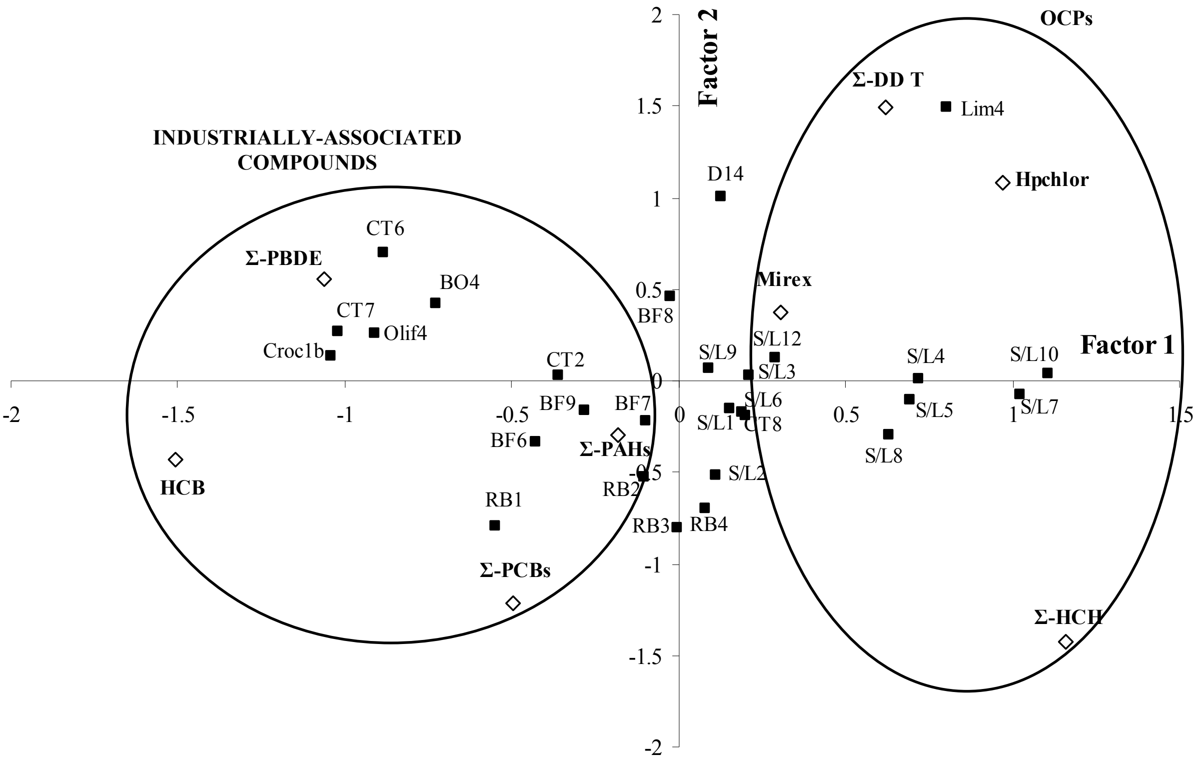


Figure 5.15. PCA biplot of factor 1 and factor 2 of all compounds

The OCPs had a tendency to group in the right quadrants, while the industrially-associated compounds (PCBs, PAHs, PBDEs) tended to group in the left quadrants (Fig. 5.15). HCB grouped with the industrially associated compounds in the left quadrants. Although categorised with the OCPs for the purpose of this study, HCB is presently used for industrial purposes, and were therefore stronger associated with the industrial compounds to the left. This could indicate continued releases of HCB, instead of legacy residues due to previous use as a pesticide.

Sites originating from the same sampling regions clustered together, possibly indicating common sources of pollution (see RB 1 to 4, BF 6 to 9, CT 6 and 7, and the S/L sites; Fig. 5.15).

Lim4 and D14 (the sites with the highest ΣOCP-loads, Fig. 5.11) were characterised by the presence of ΣDDT and heptachlor. Lim4 is situated in the Limpopo Province in an area impacted by farming activities, where DDT is still commonly applied for the control of malaria. The strong association with the compounds were, therefore, anticipated. The high levels of OCPs at D14 were, however, not foreseen, but it could be explained by the fact that the site might receive pesticides associated with up-stream farming activities (mainly sugar cane). The Palmiet-, Aller- and Mbongokazi Rivers, which may be polluted by pesticides from farming activities, converge with the Umgeni River (Fig. 4.6) and might explain the high concentrations of pesticides at D14.

RB1, 3 and 4 associated with ΣHCH and PCBs on the negative axis of factor 2. While strong association between the RB sites and PCBs was to be expected due to the highly industrial nature of the sites, the association with HCH was unexpected. The reason for the relatively high concentrations of HCH at the RB sites is uncertain. It is suspected, but cannot be confirmed, that a form of HCH is or was applied in the wood chipping process to combat stem borers or other wood-related pests.

RB2 was mainly characterised by ΣPAHs, while there was also a close association between ΣPAHs and the industrial sites BF6 and 7, and the low-income residential site, S/L8. PAHs could be produced as by-products of numerous industrial processes, or by open burning, thus, the occurrence of PAHs at these sites were anticipated. Some of the S/L sites were characterised by the insecticide, mirex, while CT7, Croc1b, Olif4 and CT6, associated with ΣPBDEs (Fig. 5.15), flame retardants commonly used in many industrial processes and applied to many synthetic materials.

Sections 5.2.2.2 to 5.2.2.4 will attempt to explain the distribution of each compound or congener, relative to the other compounds or congeners belonging to the same group, namely OCPs, unintentionally produced industrial compounds (PAHs), and intentionally produced industrial compounds (PCBs and PBDEs).

5.2.2.2. PCA with OCPs only

The biplot mainly distinguished between DDE, DDD and DDT in the upper and lower right quadrants, and the HCH congeners in the upper and lower left quadrants (Fig. 5.16). Factor 1 explained 32% of the variance in data with *o,p'*-DDT, *p,p'*-DDD and *pp'*-DDE being the main contributors on the positive end of the axis, and α- and γ-HCH and HCB on the negative end. Factor 2 explained 22% of the variance and was dominated by HCB, β-HCH and *p,p'*-DDT with positive loadings, and γ-HCH and heptachlor with negative loadings (Fig. 5.16). Clustering of sites originating from the same sampling regions was once again evident.

The strong association of Lim4 and D14 with the DDT-congeners shown in Fig 5.15 was confirmed by the second biplot with OCPs only. Lim 4 was largely characterised by the presence of *o,p'*-DDT, whereas D14 was characterised by the predominance of the metabolites, primarily *o,p'*-DDE and *o,p'*-DDD. As stated in Section 5.2.1, the presence of DDD and DDE in samples is generally representative of historic application of DDT, while DDT itself indicates possible recent use. The results of the PCA corresponded with the current situation in South Africa, where DDT application was banned in most parts of the country, except for malaria endemic areas, which include the Limpopo Province (Lim4). The presence of DDT at sites where the substance is not actively applied could be indicative of long-range transport, old stockpiles, or the use of the pesticide dicofol (contaminated by DDT).

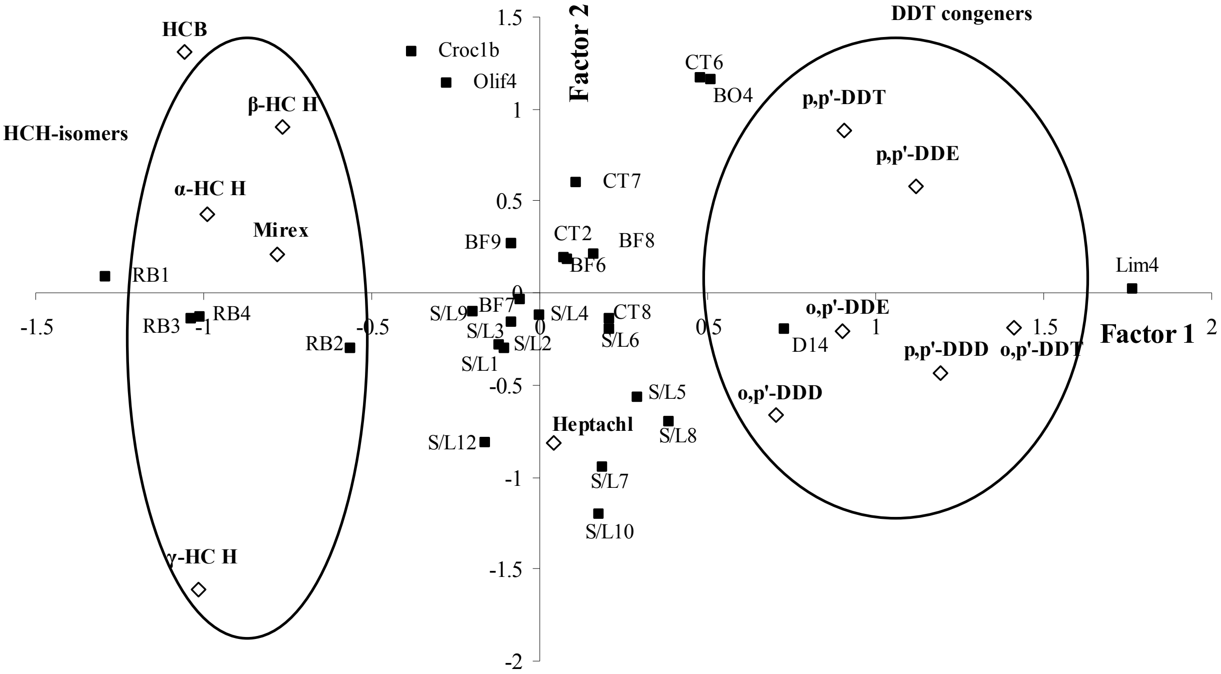


Figure 5.16. PCA biplot of factor 1 and factor 2, with only the OCPs included

RB 1, 3 and 4 were characterised by the presence of α- and γ-HCH, as well as HCB. The association with the two HCH-isomers confirms the observation in Figure 5.15 regarding the strong association between RB 1, 3 and 4 and Σ-HCH. Croc 1b was also characterised by the presence of HCB. Since the agricultural application of HCB was banned in the 1960's, the current sources of HCB should be mainly emissions from metal industries, combustion processes and chemical processes (Bailey, 2001). Since the RB sites and Croc 1b are of a highly industrial nature, it is assumed that the presence of HCBs at these sites is mainly due to current industrial activity, rather than historical applications of HCB in the form of pesticides.

S/L 7, 10 and 12 were associated with heptachlor, which were mostly used for the control of termites, but is now banned (South African Department of Agriculture, 2008). Since heptachlor has a long halflives in sediment, residues of these compound may still remain in the environment due historical use and disposal via accidental spillages, fires and volatilisation from old stockpiles.

5.2.2.3. PCA with PAHs only

The 3-ringed PAH, acenaphthylene, did not participate in the PCA because the concentration of the congener could not be quantified at RB1 and 4, due to interferences on the chromatogram.

For the PAH biplot, 47% of the variance in the data was explained by factor 1, and only 19% by factor 2. The 2-ringed PAH (naphthalene) and 3-ringed PAHs (fluorene and phenanthrene) with positive loadings, and the 6-ringed PAHs [indeno(1,2,3-cd)pyrene and benzo(ghi)perylene] with negative loadings, were the main contributors towards factor 1 (Fig. 5.17). The main contributors to factor 2 were the 4-ringed congeners [benzo(a)anthracene and chrysene] with positive loadings, and the 6-ringed congener [benzo(a,h)anthracene] with a negative loading (Fig. 5.17). The lighter 2- and 3-ringed PAHs grouped in the right half of the biplot, while the heavier 4-, 5- and 6-ringed congeners generally grouped on the left, with the various groups forming distinct clusters (Fig. 5.17).

There was a strong association between S/L 1, 4 and 8 and the lighter PAHs, while Croc 1b, RB2 and Lim4 showed strong associations with the heavier PAHs (Fig. 5.17). The majority of the sites grouped around the origin of the axes, without any other highly significant associations. This was probably because most of the sites had a similar composition of PAH-congeners, with 3- and 4-ringed congeners being the primary contributors to ΣPAHs (Fig. 5.12).

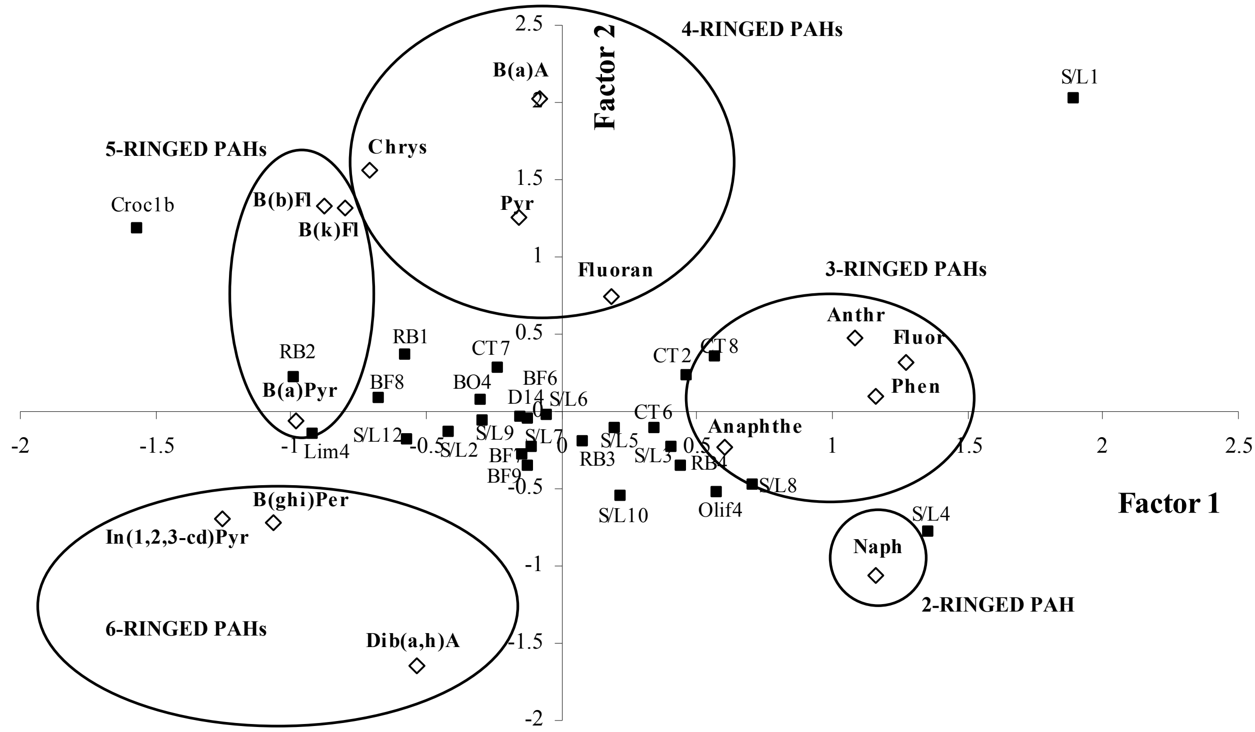


Figure 5.17. PCA biplot of factor 1 and factor 2, with only the PAH-congeners included

Since no sound conclusions on the possible sources and the distribution of PAHs could be made based on the results of the PCA, it was decided to compare concentration ratios of certain PAH-congeners to identify their origins (Pies *et al.*, 2008).

Because of the many possible sources and processes that PAHs may undergo prior to their deposition in soil or sediment, it is difficult to accurately identify their origins (Wilcke, 2007). However, source identification is made possible by comparing the concentration ratios of certain PAHs (Pies *et al.*, 2008). Anthracene/phenanthrene [An/(An + Ph)] and fluoranthene/pyrene [Fl/(Fl + Py)] ratios can be used to determine whether the sources of PAHs are of petrogenic or pyrogenic origin (Budzinski *et al.*, 1997; Gschwend & Hites, 1981; Pies *et al.*, 2008).

Petrogenic processes promote the formation of phenanthrene, which is thermodynamically more stable than anthracene, leading to lowered An/(An + Ph) ratios (<0.1), while the high temperatures during pyrogenic processes facilitate the formation of anthracene, increasing the An/(An + Ph) ratio (>0.1) (Pies *et al.*, 2008). Also, a Fl/(Fl + Py) ratio of less than 0.5 usually indicates petroleum sources, while a ratio of greater than 0.5 indicates combustion (Yunker *et al.*, 2002). However, the petroleum boundary seems to be closer to 0.4 than 0.5, thus ratios between 0.4 and 0.5 are more characteristic of liquid fossil fuels; ratios greater than 0.5 are characteristic of grass, wood or coal combustion (Yunker *et al.*, 2002). The Fl/(Fl+Py) and An/(An+Ph) ratios of each of the sites were calculated and plotted on Figure 5.18.

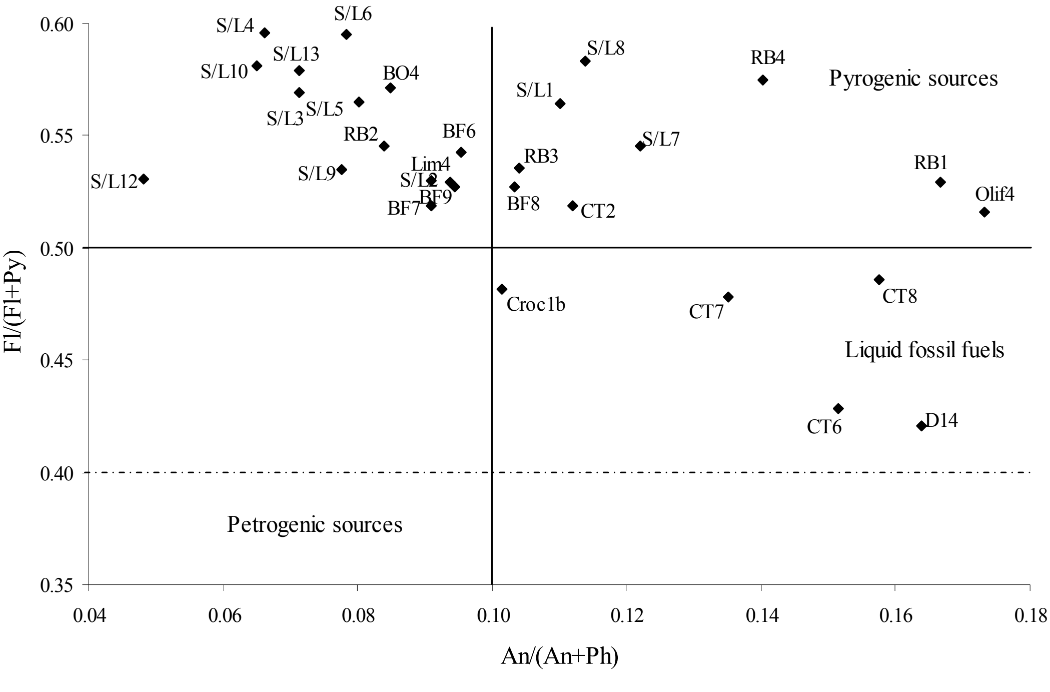


Figure 5.18. Cross-plot with An/(An+Ph) on the x-axis, and Fl/(Fl+Py) on the y-axis, where An/(An+Ph) < 0.1 and Fl/(Fl+Py) < 0.4 indicates petrogenic sources, 0.4 < Fl/(Fl+Py) < 0.5 indicates the use of liquid fossil fuels, and An/(An+Ph) > 0.1 and Fl/(Fl+Py) > 0.5 indicates pyrogenic sources

None of the sites were solely indicative of petrogenic sources (no sites had both An/(An+Ph) ratios of less than 0.1 and Fl/(Fl+Py) ratios of less than 0.4; Fig. 5.18). At five of the sites, the use of liquid fossil fuels was indicated as the main source of PAHs. These sites included Croc1b, located on the premises of a paper mill, CT6, 7 and 8, situated in mixed industrial areas (paper mill, petrochemical plant and other industries), and D14, the Umgeni River mouth, situated down-stream of numerous industries (textile industry, metal producers, building material manufacturers). In addition to liquid fossil fuels used in the above-mentioned industrial processes, vehicle exhaust emissions may also have contributed to the "fossil fuel" origin of PAHs at these sites, since most of the sites are situated in high-traffic areas (Fig. 4.2 to 4.13).

The An/(An+Ph) and Fl/(Fl+Py) ratios of RB1, 3 and 4, Olif4, BF8, SL, 1, 7 and 8, and CT2 ranged between 0.10 and 0.17, and 0.52 and 0.58 respectively, indicating pyrogenic processes as the major source of PAHs(Fig. 5.18). This implies inefficient incineration of organic materials such as wood, coal and oil as possible emission source (Culotta *et al.,* 2006; Masih & Taneja, 2006). Industrial combustion processes during aluminium smelting, wood chip production (RB1, 3 and 4), ferrous and non-ferrous metal production (CT2), paper and pulp manufacturing (Olif4) and coal-based power generation (BF8) were considered as the major contributor towards pyrogenic sources at these sites. On the other hand, domestic combustion (backyard burning or open fires used for cooking and heating) were the most likely sources of pyrogenic PAHs at the low-income residential sites S/L1 and 7. In fact, coal combustion was noted at S/L7 during sampling (Table 4.2).

The PAH origin at the remainder of the sites (S/L2 to 6, 9, 10, 12 and 13, RB2, BO4, BF6 and 7 and 9, and Lim4) were neither classified as entirely pyrogenic nor petrogenic. The An/(An+Ph) ratios were less than 0.1, indicating petrogenic origins, whereas the Fl/(Fl+Py) ratios were larger than 0.5, pointing towards pyrogenic sources (Fig. 5.18). All of these sites, except for RB2 (which was located in an industrial area), were situated in or affected by low-income residential areas or settlements (Table 4.2 to 4.8). It is speculated that the "mixed" origin of the PAHs at these sites are primarily due to open or backyard burning, used for cooking and heating, where various substances may be used to ignite (oil, petrol, diesel, paraffin) and maintain (wood, plastic, charcoal, paper) fires.

5.2.2.4. PCA with PCBs and PBDEs only

Factor 1 explained 32% and factor 2 explained 26% in the variance of data. Factor 1 was largely characterised by the PBDE-congeners (BDE-183 and -153) with positive loadings and the lighter molecular mass, lower chlorinated PCB congeners (CB-101, -52 and -28) with negative loadings. Factor 2, on the other hand, distinguished between the heavier molecular mass, higher chlorinated PCBs (CB-118, -153 and -138) with positive loadings, and lighter chlorinated PCBs (CB-28 and -52) with negative loadings. There was a distinct separation between the PBDEs (lower right quadrant), lighter PCBs (lower left quadrant) and heavier PCBs (upper left quadrant) (Fig. 5.19).

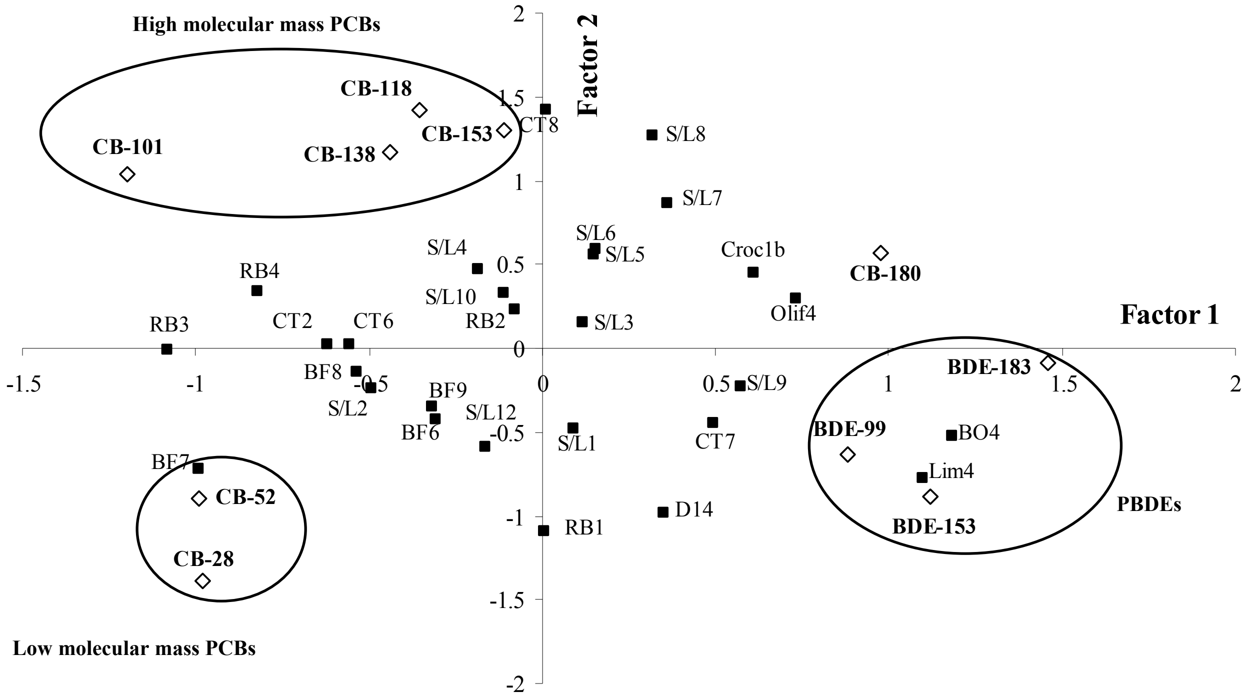


Figure 5.19. PCA biplot of factor 1 and factor 2, with only the PCB- and PBDE-congeners included

BO4 and Lim4 were characterised by the presence of PBDEs, RB 1, 3 and 4, BF 7 and D14 by the presence of the lighter molecular mass PCBs, and CT8 and S/L8 by the heavier PCBs (Figure 5.19). The other sites were scattered around the origin of the axes without any strongly significant correlations between the sites and any PBDE- or PCB-congeners (Fig. 5.19).

BO4 and Lim4 are low-income residential sites, and their positive correlation with the PBDE congeners was unexpected. PBDEs are mainly industrially associated, and the most evident sources of BFRs into the environment are effluents from factories producing BFRs, flame-retarded polymers and other plastic products (Sellström & Jansson, 1995). However, the sources responsible for the formation of polybrominated and mixed brominated/ chlorinated dibenzo-*p*-dioxins and dibenzofurans could also be responsible for the formation of PBDEs

(WHO, 1998). Backyard or open burning of municipal waste, plastics and products containing flame retardants are therefore suspected for the formation of PBDEs at these sites.

PCBs were produced for industrial purposes as insulating materials in electrical equipment, plasticizers (softening materials) in plastic products, hydraulic fluids, adhesives, lubricants, fire retardants and dielectrics in transformers, but their production and use were banned in the 1980s. Currently, they are primarily released into the environment due to historical use, or via accidental spillages, fires and volatilisation from old stockpiles (Koppe & Keys, 2001). The heavier PCB congeners are more abundant in soils and sediments and less abundant in water and the atmosphere, while the lighter congeners are less prominent in soils and sediments, and more abundant in the atmosphere and surface waters (Carey *et al.*, 1998; Henry & De Vito, 2003). This was the case for the South African sites, with CB-28 and -52 present at much lower levels than the heavier PCBs (Fig. 5.13). Both high and low molecular mass PCBs were associated with industrial sites. All of the sites were located in the vicinity or down-stream of mixed industrial areas, which might have contributed to the PCB loads at these sites.

5.3. South Africa's position in the global POPs issue

5.3.1. A comparison to sediment quality guidelines of other countries

Compared to the northern hemisphere, knowledge of the concentrations of organic pollutants in South Africa, especially POPs, is limited. There are currently no guidelines or regulations in place to govern the levels of POPs in the South African environment. One of the strictest sets of quality guidelines, the Candian soil and sediment quality guidelines, were therefore used to assess the levels of POPs for the study area.

The results of this study were normalized to 1% TOC (Table 5.12 a & b) to allow comparison with the quality guidelines, proposed by the Canadian Council of Ministers the Environment (CCME, 2002) and the Ontario Ministry of Environment and Energy (1998) (Table 5.11). Both sets of guidelines were used, because neither of the two had values for the entire set of compounds included in the study.

None of the soil sites (S/L11, 12 and 13) exceeded any soil quality guidelines. Therefore, only the sediment quality guidelines (Table 5.11) are reported from this point onwards. It should also be noted that there are no Canadian sediment quality guidelines available for PBDEs and the compound class was therefore not included in this assessment. Sites exceeding the sediment quality guidelines are printed in bold in Tables 5.12 a and b, and are indicated by the following symbols: * = sites exceeding the interim freshwater sediment quality guidelines (ISQG), ~ = sites exceeding the probable effect level (PEL) and ^ = sites exceeding the lowest effect level (LEL). None of the compounds were present at such concentration that the severe effect level (SEL) was exceeded.

Table 5.11. Sediment quality guidelines (reported in ng/kg, dw) proposed by the CCME§ (2002) and Ontario Ministy of Environment and Energy§§ (1998)

Compound group/class	Compound name	Canadian Sediment Quality Guidelines§		Ontario Sediment Quality Guidelines§§	
		Interim freshwater sediment quality guidelines (ISQG)	Probable Effect Level (PEL)	Lowest Effect Level (LEL)	Severe Effect Level (SEL)
OCPs	HCB			20 000	24 000 000
	γ-HCH (Lindane)	940	1 380	3 000	12 000 000
	Heptachlor epoxide (metabolite of Heptachlor)	600	2 740	5 000	5 000 000
	Mirex			7 000	130 000 000
	DDD (*o,p'*- and *p,p'*-)	3 540	8 510	8 000	6 000 000
	DDE (*o,p'*- and *p,p'*-)	1 420	6 750	5 000	19 000 000
	DDT (*o,p'*- and *p,p'*-)	1 190	4 770	7 000	12 000 000
DLCs	PCDD/Fs and dioxin-like PCBs	0.85 ng TEQ/kg	21.5 ng TEQ/kg		
PAHs	Naphthalene	34 600	391 000		
	Acenaphthylene	5 870	128 000		
	Acenaphthene	6 710	88 900		
	Fluorene	21 200	144 000	190 000	160 000 000
	Phenanthrene	41 900	515 000	560 000	950 000 000
	Anthracene	46 900	245 000	220 000	370 000 000
	Fluoranthene	111 000	2 355 000	750 000	1 020 000 000
	Pyrene	53 000	875 000	490 000	850 000 000
	Benzo(a)anthracene	31 700	385 000	320 000	1 480 000 000
	Chrysene	57 100	862 000	340 000	460 000 000
	Benzo(b)fluoranthene	No guidelines available		No guidelines available	
	Benzo(k)fluoranthene			240 000	1 340 000 000
	Benzo(a)pyrene	31 900	782 000	370 000	1 440 000 000
	Indeno(1,2,3-cd)pyrene			200 000	320 000 000
	Dibenz(a,h)anthracene	622 000	135 000	60 000	130 000 000
	Benzo(ghi)perylene			170 000	320 000 000
PCBs	Total PCBs	34 100	277 000	70 000	530 000 000
PBDEs	Total PBDEs	No guidelines available		No guidelines available	

Table 5.12a. Percentage TOC, and the levels of PAHs measured at each site normalized to 1% TOC and reported in ng/kg, dw to allow comparison to sediment quality guidelines (Table 5.11)

Sites	% TOC	PAHs															
		Naph	Anaphthy	Anaphthe	Fluor	Phen	Anthr	Fluoran	Pyr	B(a)A	Chrys	B(b)Fl	B(k)Fl	B(a)Pyr	In(1,2,3-cd)Pyr	Dib(a,h)A	B(ghi)Per
S/L1	6.63	21 272	1 509	694	14 030	31 681	3 922	33 190	25 647	**40 733***	16 595	18 104	9 354	4 526	1 313	166	2 112
S/L2	4.96	12 693	524	504	1 430	12 089	1 249	20 148	17 932	13 499	15 514	18 133	7 656	13 902	17 126	2 216	18 133
S/L3	5.57	4 665	197	215	700	5 383	413	5 921	4 486	2 153	2 512	2 871	1 256	1 974	2 691	395	2 691
S/L4	7.62	23 620	1 312	381	1 706	12 204	866	6 955	4 724	1 575	2 362	2 362	958	1 312	3 018	341	3 412
S/L5	3.14	22 267	1 018	891	1 591	20 040	1 750	28 947	22 267	12 406	17 814	15 905	7 316	13 042	12 088	2 481	11 770
S/L6	6.30	28 571	1 032	254	2 508	31 745	2 698	39 681	26 983	19 047	26 983	28 571	11 111	12 381	19 047	3 175	19 047
S/L7	7.76	18 032	940	1 674	2 962	23 184	3 220	30 912	25 760	19 320	18 032	20 608	9 660	18 032	26 018	3 735	24 472
S/L8	7.03	**156 482***	5 263	5 690	13 230	**99 579***	12 803	99 579	**71 128***	**42 677***	56 902	45 522	19 916	**55 480***	35 564	11 096	38 409
S/L9	13.64	4 838	213	440	1 026	8 797	806	13 195	10 996	6 598	7 331	8 797	4 032	6 818	8 797	1 320	8 797
S/L10	6.47	9 432	418	216	742	7 113	495	6 649	4 794	2 629	3 093	4 639	2 010	2 783	5 257	588	5 567
S/L12	5.91	20 293	676	896	1 201	25 367	1 285	43 969	38 895	21 477	25 367	21 984	13 867	23 675	28 749	3 890	23 675
S/L13	9.52	12 600	368	231	557	5 880	452	5 775	4 200	2 100	3 570	4 200	1 680	2 520	3 780	588	4 410
CT2	5.64	17 017	177	4 431	7 799	40 769	5 140	49 631	46 086	**31 906***	28 361	26 588	13 649	4 077	26 588	4 254	21 271
CT6	1.32	9 829	219	680	2 268	10 585	1 890	11 341	15 121	5 444	6 275	7 561	3 856	5 822	7 561	832	10 585
CT7	4.75	16 844	2 105	4 421	14 107	**67 374***	10 527	46 320	50 531	**58 953***	**71 585***	69 480	27 371	**42 109***	65 269	8 843	65 269
CT8	1.32	16 606	377	1 057	3 170	17 361	3 246	25 664	27 174	13 587	21 135	15 851	6 793	15 096	14 342	2 264	1 661
D14	3.56	12 928	731	1 293	3 372	14 333	2 810	17 143	23 607	9 555	16 300	16 862	23 607	13 209	16 581	2 726	20 797
RB1	1.88	14 876		2 603	6 376	**58 442***	11 688	95 633	**85 007***	**53 129***	**85 007***	74 381	34 534	**51 535***	63 755	10 626	63 755
RB2	0.71	15 506	381	6 485	6 062	**53 568***	4 511	**129 690***	112 774	**73 303***	**91 629***	122 642	50 748	**88 809***	95 858	16 916	98 677
RB3	1.44	**124 594***	3 392	**9 691***	17 997	**124 594***	13 152	**131 516***	110 750	**67 142***	**124 594***	152 281	57 452	**76 141***	76 141	14 536	83 063
RB4	5.92	11 140		557	1 857	6 414	1 046	7 764	5 739	3 713	4 895	6 751	2 532	8 270	4 726	810	5 064
BF6	1.25	**35 199***	1 440	2 640	6 960	**44 800***	5 200	72 799	**63 200***	**40 800***	50 400	49 600	21 600	**40 800***	47 200	7 120	45 600
BF7	2.05	**53 596***	2 436	3 459	6 334	**63 341***	6 334	68 213	**63 341***	**48 724***	53 596	63 341	24 849	**43 851***	58 468	10 719	58 468
BF8	1.82	8 228	335	362	1 481	14 261	1 646	31 813	28 522	14 810	24 134	20 295	8 776	17 004	20 295	3 017	21 392
BF9	6.71	10 290	239	447	1 402	7 754	775	11 930	10 588	5 368	7 904	10 886	4 772	5 965	10 886	1 372	10 886
BO4	5.65	10 442	3 009	903	7 610	24 778	2 301	42 476	31 857	17 698	26 547	31 857	14 336	21 238	24 778	4 956	31 857
Lim4	3.21	3 431	150	184	905	7 174	749	14 973	13 413	6 239	7 174	11 541	5 615	11 541	16 220	1 872	15 285
Olif4	2.27	**41 031***	1 103	618	2 735	13 677	2 868	14 559	13 677	10 147	8 824	12 795	4 853	8 824	9 265	1 853	12 795
Croc1b	1.49	**46 348***	**10 076***	**11 419***	10 747	**261 968***	29 555	**436 614***	**470 200***	**490 351*~^**	**617 976*^**	617 977	**349 291^**	**53 7371*^**	**1 074 742^**	7 389	**873 227^**

* Levels exceeding ISQG ~Levels exceeding the PEL ^Levels exceeding the LEL None of the sites exceeded the SEL.

Table 5.12b. Percentage TOC, and the levels of OCPs, DLCs and PCBs measured at each site normalized to 1% TOC and reported in ng/kg, dw to allow comparison to sediment quality guidelines (Table 5.11)

Sites	%TOC	OCPs							DLCs	PCBs
		HCB	γ-HCH (Lindane)	Hpchlor	Mirex	ΣDDD	ΣDDE	ΣDDT	Total DLCs (bio-assay)	Total PCBs
S/L1	6.63	115	118	11	6	13	59	54	**1.14***	1 349
S/L2	4.96	423	282	4	6	77	147	75	0.20	2 482
S/L3	5.57	97	65	7	11	30	102	68	0.17	1 965
S/L4	7.62	18	43	7	5	33	84	56	0.13	915
S/L5	3.14	32	204	6	6	177	175	150	0.31	2 028
S/L6	6.30	25	33	1	11	24	68	41	0.15	1 144
S/L7	7.76	6	116	12	6	21	45	36	0.81	869
S/L8	7.03	33	156	7	1	24	63	186	0.62	1 994
S/L9	13.64	301	154	26	17	66	199	123	**6.35***	3 561
S/L10	6.47	12	201	19	8	28	181	26	**2.32***	933
S/L12	5.91	592	237	73	25	64	509	72	**2.34***	2 437
CT2	5.64	1 010	57	25	4	38	154	103	**2.86***	3 382
CT6	1.32	2 419	4	30	30	132	643	401	**9.33***	18 629
CT7	4.75	1 432	17	19	2	28	95	131	**2.51***	5 731
CT8	1.32	423	264	23	4	125	521	317	**8.67***	13 043
D14	3.56	731	157	**787***	93	287	**2 470***	**1 518***	**8.12***	12 978
RB1	1.88	2 975	**1 116***	21	11	13	157	58	**54.72*~**	30 443
RB2	0.71	2 537	**1 833*~**	113	28	63	275	240	**39.49*~**	**56 091***
RB3	1.44	1 800	900	21	21	7	211	38	**12.75***	13 899
RB4	5.92	557	236	14	3	2	38	18	**1.26***	3 906
BF6	1.25	3 840	536	8	16	200	792	888	**35.46*~**	10 080
BF7	2.05	2 582	434	29	19	102	745	268	**22.01*~**	6 168
BF8	1.82	713	49	82	44	132	373	291	**26.03*~**	6 341
BF9	6.71	537	51	3	4	31	186	72	**1.54***	1 879
BO4	5.65	248	1	1	1	13	141	76	**6.38***	203
Lim4	3.21	19	9	53	2	162	**2 502***	**4 211***	**7.16***	182
Olif4	2.27	424	2	2	13	4	86	42	**14.70***	2 449
Croc 1b	1.49	1 411	3	3	40	7	191	64	**5.39***	3 614
Drknberg1	1.41	36	**1 634*~**	50	4	124	881	497	0.69	3 584
Drknberg3	1.55	58	595	136	26	126	667	382	0.63	2 016

*Levels exceeding ISQG ~Levels exceeding the PEL

^Levels exceeding the LEL None of the sites exceeded the SEL

The levels of HCB, mirex and DDD measured at the South African sites did not exceed any of the sediment quality guidelines (Table 5.12b). The concentration of γ-HCH was above the proposed ISQG at RB1, RB2 and Drknberg1. At RB2 and Drknberg1, the concentrations of γ-HCH exceeded the PEL (the level above which adverse effects are expected to occur on a frequent basis) as well (Table 5.12b). Since there are no Canadian sediment quality guidelines available for heptachlor, the ISQG of the metabolite, heptachlor epoxide, was used (Table 5.11). D14 was the only site with a heptachlor level above the recommended ISQG of 600 ng/kg, dw. The concentrations of ΣDDE and ΣDDT at D14 and Lim4 exceeded the ISQG, but were below the PEL (Table 5.12b).

Of the industrially-associated compounds, the ISQG for DLCs were exceeded at 21 of the 30 sites. Of this 21 sites, five (RB1, RB2, BF6, BF7 and BF8) had DLC concentrations of above the PEL as well (Table 5.12b). It should be taken into account that the biological data of DLCs was used for this assessment. Although a positive correlation between biological and chemical results generally exists, biological results may be an order of magnitude higher (Carbonnelle *et al.*, 2004; Nieuwoudt *et al.*, 2009; Vanderperren *et al.*, 2004; Van Wouwe *et al.*, 2004) and might therefore be an over-estimation of DLC levels.

The concentrations of the PAH congeners were above the sediment quality guidelines at eleven of the sites. The ISQGs of some PAH congeners were exceeded at S/L1, S/L8, CT2, CT7, RB1, RB2, RB3, BF6, BF7 and Olif4, while some of the congeners' ISQGs, PELs and LELs were exceeded at Croc1b (Table 5.12a).

The following sites were identified as "priority" areas from this study for further investigation due to the high concentrations of certain compounds or the amount of compounds exceeding the environmental quality guidelines:

- ○ **S/L1 and 8 (Soweto/Lenasia wetland)**, where the ISQGs of some PAH congeners were exceeded;
- ○ **D14 (Umgeni mouth – possibly receiving effluent from various industries up-stream**, where the levels of heptachlor, DDE, DDT and DLCs were all above the ISQGs;
- ○ **RB1 and RB2 (Richards Bay industrial sites):** At RB1 the ISQGs of several PAH-congeners and γ-HCH were exceeded, while the ISQG and PEL of DLCs were exceeded. At RB2, PAHs and PCBs were present at such concentrations that the ISQGs of these compounds were exceeded, and for γ-HCH and DLCs the PELs were exceeded as well;
- ○ **BF6, BF7 and BF8 (Bloemfontein industrial and low-income residential sites):** The concentrations of some of the PAH-congeners at BF6 and BF7 where higher than the proposed ISQG, and the DLC concentrations at all three of the sites exceeded the ISQGs and the PELs;
- ○ **Lim4 (Low-income residential site in the Limpopo Province – a malaria-endemic area):** The ISQGs of DDE, DDT and DLCs were exceeded at Lim4;
- ○ **Croc1b (On the premises of a paper mill in Mpumalanga):** This site had the largest amount of PAH-congeners exceeding the ISQGs, PELs and LELs. The concentration of DLCs at this site was above the ISQG.

5.3.2. A comparison to the levels found elsewhere in the world

Table 5.13 compares the minimum and maximum concentrations of pollutants measured during the current study to previous South African studies and studies done elsewhere in the world. When considering the levels of organic pollutants and POPs measured at the 30 South African sites, the levels were generally lower than, or within the same range as that measured in other countries. The maximum concentrations of certain pollutants measured at a few of the sites were, however, higher than that measured in some of the other countries (Table 5.13). The study focussed mainly on sediment sites, since only one soil sample was chemically analysed for OCPs, PCBs and PBDEs, and only three soil sites for DLCs and PAHs.

The maximum level of HCB measured during the current study was higher than that measured during the previous South African study, but lower than the maximum levels measured in China, Germany and Australia (Table 5.13). The maximum level of ΣHCH (measured at RB1) was also higher than that measured during the previous study. It exceeded the levels measured near an old pesticide factory, residue store, residential and reference areas in Spain, but it was one to two orders of magnitude lower than samples collected in the vicinity of a chemical plant at Ya-Er Lake and the Haihe and Dagu Draingage Rivers in China (Wu *et al.*, 1997; Yang *et al.*, 2005) (Table 5.13).

This was the first analysis for heptachlor and mirex in South African soils and sediments done by our research group. In both matrices, the maximum concentrations of heptachlor were much lower than the levels measured in soil and sediments of other countries such as Mexico, Australia and the USA (Table 5.13). On the other hand, the concentrations of mirex were relatively high, with the maximum concentration in sediment reaching 330 ng/kg, dw and soil reaching 150 ng/kg, dw. This was less than the concentrations measured in Brazil, but higher than the concentrations measured in Korea, India and the Czech Republic (Table 5.13).

Compared to the levels of ΣDDT found in other countries, the maximum concentration of ΣDDT in South African sediment were relatively high, with concentrations exceeding the levels measured in China, the USA and Russia. The maximum concentration measured during the current study was also an order of magnitude higher than that measured in the previous South African study (Table 5.13). This is mainly due to the site located in the Limpopo Province (Lim4) where DDT is still sprayed for the control of malaria. Levels of ΣDDT measured in Canadian sediments in a study area which included urban, suburban and rural sites were an order of magnitude higher than the maximum level measured during this study (Wong *et al.*, 2009) (Table 5.13).

Weighing the concentrations of DLCs measured with the bio-assay against the levels of DLCs measured elsewhere in the world (either by chemical or bio-analysis), the concentration found in South African soils and sediments were relatively low. The concentrations of DLCs measured in the USA, Spain, Norway, Sweden and the Netherlands were one to three orders of magnitude higher than the maximum concentration measured during the current study (Table 5.13). This was the same case for ΣPBDEs, with the levels of ΣPBDEs measured in Italy, the Netherlands, Portugal, Sweden, Norway and the United Kingdom, being two to three orders of magnitude higher than the concentrations measured in South African soils and sediments (Table 5.13).

At 8 992 000 ng/kg, dw, the maximum level of ΣPAHs measured during this study was high when compared to the levels measured in Germany, Canada and the previous South African study, but it was lower than the levels measured in an industrial area, impacted on by coal mining in Germany. The maximum concentration of ΣPCBs exceeded the concentrations measured in Yugoslavia and China, but was less than the concentrations measured in Romania (Table 5.13).

Considering the levels of the above-mentioned pollutants, the concentrations measured in South African soil and sediment were generally intermediate when compared to other countries, with the exception of a few sites (listed in Seciton 5.3.1) exceeding the pollutant levels measured elsewhere in the world. Combined with probably much lower overall emissions from a smaller industrial base in South Africa when compared with China and other industrialised countries, another probable major reasons for the lower levels of these pollutants found in South African matrices is the country's climate, characterised by high temperatures, low precipitation, and long summers, different from the more moderate conditions in Europe, North-America and elsewhere where most of the POPs research has been conducted.

Table 5.13. Levels of organic pollutants and POPs measured in soil and sediment from the current and previous South African studies and other countries

Chemical species	Site description	Sediments (ng/kg, dw)		Soil (ng/kg, dw)		Reference
		Min	Max	Min	Max	
HCB						
Current study (South Africa)		**<50**	**6 800**	**3 500***		
Previous study (South Africa)	Central South Africa. Sampling areas included industrial, agricultural and residential sites.	30	320	530	15 000	Quinn *et al*., 2009
China	Ya-Er Lake area in the vicinity of a chemical plant.	31 500 000	57 100 000	35 400 000	37 700 000	Wu *et al*., 1997
Germany	Upper Rhine River	ND	60 000			Breitung *et al*., 2008
Australia	Northern Australia – sites along the east coast of Queensland	<500	28 000			Müller *et al*., 1999
ΣHCH[a]						
Current study		**15**	**2 900**	**1 410***		
Previous study (South Africa)	Central South Africa.	200	1 700	320	1 860	Quinn *et al*., 2009
China	Ya-Er Lake area in the vicinity of a chemical plant.	1 460	18 260	980	4 660	Wu *et al*., 1997
Spain	Sampling areas included area near an old pesticide factory, residue store, residential and reference areas.	ND	ND	4.31	80 693	Concha-Graña *et al*., 2006
China	Haihe and Dagu Drainage River (industrial and urban areas)	1880	141 030			Yang *et al*., 2005
Heptachlor						
Current study		**ND**	**2 800**	**430***		
Mexico	North-western Mexico: coastal lagoons and agricultural drains	1 160	49 000			González-Farias *et al*., 2002
Australia	Port Jackson, Sydney	ND	24 400			Birch & Taylor, 2000
USA	Corn belt agricultural area			ND	56 000	Aigner *et al*., 1998
Mexico	Natural soil form south-eastern region of Argentina			740	4 420	Miglioranza *et al*., 2003
Mirex						
Current study		**ND**	**330**	**150***		
India	West Bengal - Hugli estuary	ND	<100			Guzzella *et al*., 2005
Korea	Han River	ND				Kim *et al*., 2009
Czech Republic	Along the Czech border with Poland, Germany and Slovakia			ND	<48	Shegunova *et al*., 2007
Brazil	Northeastern part of São Paulo State			ND	2 780	Rissato *et al*., 2006

Table 5.13. *Continued*

Chemical species	Site description	Sediments (ng/kg, dw)		Soil (ng/kg, dw)		Reference
		Min	**Max**	**Min**	**Max**	
ΣDDT^{b}						
Current study		**300**	**22 000**	**3820***		
Previous study (South Africa)	Central South Africa.	270	4 620	480	6 700	Quinn *et al*., 2009
Canada	Sampling areas included urban, suburban and rural sites.	200	472 000	1 000	18 000	Wong *et al*., 2009
China	Mainly industrial, but sites include agricultural and suburban areas.			56 000	1 335 000	Gong *et al*., 2004
China	Yangtze Estuary and Hangzhou Bay	<60	6 040			Yang *et al*., 2005
USA	San Francisco Bay	<100	9 000			Pereira *et al*., 1994
Russia	Lake Baikal	14	2 700			Iwata *et al*., 1994
ΣPCDD/Fs and dioxin-like PCBs						
Current study	**(Bio-assay results - ng TCDD-EQ/kg)**	**ND**	**103**	**ND**	**14**	
Previous study (South Africa)	Central South Africa.	0.12	32	0.34	20	Nieuwoudt *et al*., 2009
USA	South Mississippi: rural area.	12.7	615	0.08	22.6	Rappe *et al*., 1997
Spain	Montcada, Barcelona: near a municipal solid waste incinerator.			0.15	29.27	Domingo *et al*., 1999
Norway	Grenlandsfjords: industrial area.	25 000	730 000			Ishaq *et al*., 2009
Sweden	Background and industrial sites.	<1 - 200	1 700	<1	11 000	Fiedler *et al*., 1999; Yoon *et al*., 2004
The Netherlands	Background and industrial sites.	1 – 10	4 000	2.2 – 16	98 000	Fiedler *et al*., 1999; Yoon *et al*., 2004
ΣPAHs^{c}						
Current study (South Africa)		**134 690**	**8 992 000**	**517 900**	**1 877 000**	
Previous study (South Africa)	Central South Africa.	44 000	2 799 000	201 000	38 846 000	Quinn *et al*., 2009
Canada	Sampling areas included urban, suburban and rural sites.	42 000	3 300 000	58 000	3 200 000	Wong *et al*., 2009
Italy	Marsala, Stagnone coastal lagoon: industrial area.	72 000	18 381 000			Culotta *et al*., 2006
Germany	Mossel and Saar Rivers: Impacted by industries and coal mining.	12 200	31 200			Pies *et al*., 2008
Slovakia	Industrial area in the vicinity of an aluminium plant.			40 000	200 000	Wilcke *et al*., 1996

Table 5.13. *Continued*

Chemical species	Site description	Sediments (ng/kg, dw)		Soil (ng/kg, dw)		Reference
		Min	Max	Min	Max	
<u>ΣPBDEs</u>						
Current study[g]		**1.5**	**179**	**7.5***		
Italy[d]	Northern Italy: Lake Maggiore	60	27 000			Mariani *et al*., 2008
Netherlands[e]	Background and industrial sites.	4 600	527 600			De Boer *et al*., 2003
Portugal[e]	Major river basins.	500	21 000			Lacorte *et al*., 2003
Sweden	Reference soils from agricultural research stations.			29	95	Sellström *et al.*, 1998
United Kingdom and Norway[f]	Latitudinal transect which includes both countries: rural woodlands.			65	12 000	Hassanin *et al.*, 2004
United Kingdom	Rural area.			1 000	12 000	
<u>ΣPCBs</u>						
Current study		**585**	**57 300**	**14 410***		
Previous study (South Africa)	Central South Africa. Sampling areas included industrial, agricultural and residential sites.	460	8 550	1 480	38 320	Quinn *et al*., 2009
Yugoslavia	Residential, recreational and industrial areas.	ND	320	ND	410	Skrbic *et al*., 2007
Romania	Sampling areas included forested zones, waste-disposal sites and sediments from the Bahlui River.	24 000	158 000	8 000	1 132 000	Dragan *et al*., 2006
China	Urban lakes in Wuhan, Central China.	900	46 140			Yang *et al*., 2009

[a] Sum of alpha-, beta- and gamma-HCH.

[b] Sum of *o,p*'-DDE, *p,p*'-DDE, *o,p*'-DDD, *p,p*'-DDD, *o,p*'-DDT and *p,p*'-DDT.

[c] The 16 US EPA PAHs.

[d] Sum of BDE-28, -47, -100, -99, -154, -153, -183 and -209.

[e] Sum of BDE-47, -99, -153 and -209.

[f] Sum of BDE-47, -99, -100, -153 and -154.

[g] Sum of BDE-99, -153, and -183. BDE-28, -47 and -209 were present at levels below the LOD.

ND = Not detected (below the detection limit).

*Only one **soil** sample was chemically analysed for OCPs, PCBs and PBDEs. No min or max values.

5.4. Assessing effects on human health

According to the National Cancer Registry of South Africa, the background rates of cancer in the country are one in four for males and one in five for females (CANSA 2009, Mqoqi *et al.*, 2004). This means that background numbers of cancer would be approximately 250 000 in 1 000 000 for males and 200 000 in 1 000 000 for females. On average, childhood cancers (ages 0 – 6) comprised 1% of all female and 2% of all male cancers reported in 1998 and 1999 (Mqoqi *et al.*, 2004), bringing the background rates for cancer in children to 2 000 in 1 000 000 for girls and 5 000 in 1 000 000 for boys. The majority of the pollutants investigated during this study is classified as *likely*, *probable* or *possible* carcinogens, and may contribute to additional cancer cases in the country. The provisional cancer risk (CR) associated with these compounds were therefore calculated. Table 5.14 explains the classification system for carcinogens used by some international organisations.

Table 5.14. Classification system for carcinogens used by various international organisations

International Agency for Research on Cancer (IARC)	Globally Harmonized System (GHS)	US National Toxicology Program (NTP)	American Conference of Governmental Industrial Hygienists (ACGIH)	European Union (EU)
Group 1 Definitely carcinogenic to humans.	**Category 1A** Known or presumed to have carcinogenic potential for humans (based on human evidence).	**Known** Known to be a human carcinogen.	**Group A1** Confirmed human carcinogen.	**Category 1** Substances known to be carcinogenic to humans.
Group 2A Probably carcinogenic to humans.	**Category 1B** Known or presumed to have carcinogenic potential for humans (based on animal evidence).	**Reasonably suspected** Reasonably anticipated to be a human carcinogen.	**Group A2** Suspected human carcinogen.	**Category 2** Substances that should be regarded as if they are carcinogenic to humans.
Group 2B Possibly carcinogenic to humans.	**Category 2** Suspected human carcinogens.		**Group A3** Confirmed animal carcinogen with unknown relevance to humans.	**Category 3** Substances which cause concern for humans.
Group 3 Not classifiable as to its carcinogenicity to humans.			**Group A4** Not classifiable as a human carcinogen.	
Group 4 Probably not carcinogenic			**Group A5** Not suspected as a human carcinogen.	

Animal studies have shown an increase in lung, thyroid, liver and spleen tumours upon exposure to HCB. The compound is an expected human carcinogen, yet the effects of HCB on humans are not fully understood (ATSDR, 2002a; Edwards *et al*., 1991). Studies done on HCH are inconclusive and contradictory, but the majority of studies suggest that HCH-isomers, especially lindane, are "reasonably anticipated to be human carcinogens" (ATSDR, 2007a; Smith, 1991). There is insufficient evidence available to assess the potential of heptachlor to cause cancer in humans, but mirex is classified as a Group 2B possible human carcinogen by the US EPA (UNEP, 2002). Positive correlations between DDT exposure and some cancers, especially pancreatic cancers have also been found (ATSDR, 2002b). DLCs and PCBs are classified as "likely human carcinogens", while the most toxic congener, 2,3,7,8-TCDD, is identified as a "known human carcinogen" by the US EPA (US EPA, 2008b). Of the PAH congeners, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracne and indeno(1,2,3-cd)pyrene are classified as Group B2 probable human carcinogens (US EPA, 2002). The health effects related to PBDEs are insufficiently documented. Deca-BDE are thought to be "possibly carcinogenic to humans", while tetra-, penta- and octa-BDE have been classified as non-carcinogenic (Hellström, 2000).

The CR associated with exposure to these substances was determined for all sites. CR was estimated for physical contact with contaminated sediment. People may come into regular physical contact with contaminated sediment while fishing, swimming, bathing or collecting water for domestic purposes. Although ingestion and inhalation are regarded as relevant routes of exposure for soil, they were not considered as relevant routes of exposure for sediment, and were therefore not included in the human health risk assessment.

The provisional CR associated with dermal absorption of the above-mentioned pollutants was estimated according to equations and values proposed by the US EPA (2004) and the Canadian Environmental Assessment Services Safe Environments Programme (2004). Since there are no equations available for dermal contact with contaminated sediment, the equations proposed for soil were used. Calculations may therefore be an over- or under estimation of the actual cancer risk associated with sediment. CR was calculated for adults and children. The CR associated with exposure to mirex and PBDEs was not calculated, since insufficient information on these compounds' skin absorbsion factors (AF_{skin}) and slope factors were available. Since the concentrations of these compounds were generally relatively low, the CR associated with dermal contact with mirex and PBDEs was expected to be low.

To estimate CR, the dermal absorbed dose (DAD) of each compound was first calculated (equation 1), and then multiplied by the compound's dermal cancer risk slope factor (equation 2).

Equation 1:

$$\text{DAD (mg/kg/day)} = \frac{(C_s \text{ x } SA_H \text{ x } SL_H) \text{ x } AF_{skin} \text{ x } EF \text{ x } D_1 \text{ x } D_2 \text{ x } D_3}{BM \text{ x } LE}$$

Equation 2:

$$\text{Cancer risk (CR)} = \text{DAD x Dermal cancer slope factor}$$

The explanations of the abbreviations and values used in the equations are given in Table 5.15. The dermal absorption factors (AFskin) and dermal cancer slope factors for the compounds included in the assessment are listed in Table 5.16.

Table 5.15. Explanation of the abbreviations and values used to determine the CR (Canadian Environmental Assessment Services Safe Environments Programme, 2004; US EPA, 2004)

Abbreviation	**Explanation**	**Value**		**Unit**
		Adults	**Children**	
C_s	Concentration of the carcinogenic substance in sediment	Site specific - See Table 5.17		mg/kg
SA_H	Skin surface area exposed/available for contact*	6610	3660	cm^2
SL_H	Soil loading to exposed skin	$1 \text{ x } 10^{-7}$	$1 \text{ x } 10^{-7}$	Kg/cm^2-event
AF_{skin}	Dermal absorption factor	Pollutant specific - See Table 5.16		
EF	Exposure frequency	1	1	event/day
D_1	Days per week exposed	7	7	Days
D_2	Weeks per year exposed	52	52	Weeks
D_3	Total years exposed to site	30	6	Years
BM	Body mass	70	15	Kg
LE	Life expectancy	70	70	Years
DAD	The mean dose of PAHs which adults or children are exposed to per day.	Site specific - See Table 5.17		mg/kg-day
Dermal cancer risk slope factor	The upper-bound (95% confidence limit) estimate of a chemicals probability of causing cancer over a 70 year lifetime.	Pollutant specific - See Table 5.16		$(mg/kg\text{-}day)^{-1}$
CR	Number of additional cancer cases due to exposure to compounds.	Site specific - See Table 5.17		

*Sum for hands and upper and lower legs.

Table 5.16. Dermal absorption factors (AFskin) and dermal cancer slope factors for the compounds included in the cancer risk assessment

	HCB	HCH	Heptachlor	Mirex	DDT	DLCs	PAHs	PCBs	PBDEs
AFskin	0.13	0.04	0.2	_	0.2	0.03	0.2	0.14	_
Dermal cancer slope factor $(mg/kg\text{-}day)^{-1}$	1.8	1.18	4.5	_	0.34	1.56×10^5	7.3	2	_

_ Insufficient information available and therefore not included in the CR calculations.

The DAD and CR calculated for each of the sites are summarized in Tables 5.17 to 5.20. CR is expressed as *x in 1 000 000*. The sites that exceeded the negligible CR of 1 in 1 000 000 proposed by the US EPA are indicated in bold.

Table 5.17. Concentration of carcinogenic compounds (Cs), and the calculated DAD and CR (expressed as *x in 1 000 000*) calculated for **adults** for the OCPs (HCB, ΣHCH, heptachlor and DDT)

Sites	HCB			ΣHCH			Heptachlor			ΣDDT		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
S/L1	0.00076	1.0188E-06	**1.8**	0.00079	3.25847E-07	0.38	0.00007	1.44362E-07	0.65	0.000835	1.72204E-06	0.6
S/L2	0.0021	2.8151E-06	**5.1**	0.00141	5.81574E-07	0.68	0.00002	4.12464E-08	0.18	0.00148	3.05223E-06	**1.03**
S/L3	0.00054	7.2387E-07	**1.3**	0.00037	1.52612E-07	0.18	0.00004	8.24928E-08	0.37	0.001115	2.29949E-06	0.78
S/L4	0.00014	1.8767E-07	0.3	0.0006	2.47478E-07	0.29	0.00005	1.03116E-07	0.46	0.00132	2.72226E-06	0.92
S/L5	0.0001	1.3405E-07	0.24	0.00065	2.68102E-07	0.31	0.00002	4.12464E-08	0.18	0.001575	3.24815E-06	**1.1**
S/L6	0.00016	2.1448E-07	0.38	0.00022	9.07421E-08	0.11	0.000005	1.03116E-08	0.05	0.00084	1.73235E-06	0.6
S/L7	0.00005	6.7025E-08	0.12	0.00091	3.75342E-07	0.44	0.00009	1.85609E-07	0.83	0.00079	1.62923E-06	0.6
S/L8	0.00023	3.0832E-07	0.55	0.00111	4.57835E-07	0.54	0.00005	1.03116E-07	0.46	0.001925	3.96997E-06	**1.3**
S/L9	0.0041	5.4961E-06	**9.9**	0.00256	1.05591E-06	**1.24**	0.00036	7.42435E-07	3.34	0.0053	1.09303E-05	**3.7**
S/L10	0.00008	1.0724E-07	0.19	0.00131	5.40328E-07	0.64	0.00012	2.47478E-07	**1.11**	0.00152	3.13473E-06	**1.1**
S/L12	0.0035	4.6918E-06	**8.4**	0.00141	5.81574E-07	0.68	0.00043	8.86798E-07	**3.9**	0.003815	7.86775E-06	**2.7**
CT2	0.0057	7.6409E-06	**13**	0.00033	1.36113E-07	0.16	0.00014	2.88725E-07	**1.3**	0.001665	3.43376E-06	**1.2**
CT6	0.0032	4.2896E-06	**7.7**	0.000015	6.18696E-09	0.007	0.00004	8.24928E-08	0.37	0.001555	3.20691E-06	**1.1**
CT7	0.0068	9.1155E-06	**16.4**	0.00009	3.71218E-08	0.04	0.00009	1.85609E-07	0.84	0.001205	2.4851E-06	0.84
CT8	0.00056	7.5068E-07	**1.35**	0.00036	1.48487E-07	0.17	0.00003	6.18696E-08	0.28	0.001275	2.62946E-06	0.89
D14	0.0026	3.4853E-06	**6.3**	0.00057	2.35104E-07	0.27	0.0028	5.7745E-06	**26**	0.01521	3.13679E-05	**11**
RB1	0.0056	7.5068E-06	**13**	0.002905	1.19821E-06	**1.41**	0.00004	8.24928E-08	0.37	0.00043	8.86798E-07	0.3
RB2	0.0018	2.4129E-06	**4.3**	0.00131	5.40328E-07	0.64	0.00008	1.64986E-07	0.74	0.00041	8.45551E-07	0.3
RB3	0.0026	3.4853E-06	**6.3**	0.00131	5.40328E-07	0.64	0.00003	6.18696E-08	0.28	0.00037	7.63058E-07	0.25
RB4	0.0033	4.4237E-06	**7.9**	0.00141	5.81574E-07	0.68	0.00008	1.64986E-07	0.74	0.00034	7.01189E-07	0.23
BF6	0.0048	6.4344E-06	**11.5**	0.00068	2.80476E-07	0.33	0.00001	2.06232E-08	0.09	0.00235	4.84645E-06	**1.64**
BF7	0.0053	7.1047E-06	**12.7**	0.0009	3.71218E-07	0.44	0.00006	1.23739E-07	0.55	0.00229	4.72271E-06	**1.6**
BF8	0.0013	1.7427E-06	**3.13**	0.0001	4.12464E-08	0.048	0.00015	3.09348E-07	**1.39**	0.00145	2.99036E-06	**1.01**
BF9	0.0036	4.8258E-06	**8.7**	0.00035	1.44362E-07	0.17	0.00002	4.12464E-08	0.18	0.00194	4.0009E-06	**1.36**
BO4	0.0014	1.8767E-06	**3.4**	0.000015	6.18696E-09	0.007	0.000005	1.03116E-08	0.05	0.0013	2.68102E-06	0.91

Table 5.17. *Continued*

Sites	HCB			ΣHCH			Heptachlor			ΣDDT		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
Lim4	0.00006	8.043E-08	0.14	0.00004	1.64986E-08	0.019	0.00017	3.50594E-07	**1.6**	0.02204	4.54535E-05	**15**
Olif4	0.00096	1.2869E-06	**2.32**	0.000015	6.18696E-09	0.007	0.000005	1.03116E-08	0.05	0.0003	6.18696E-07	0.21
Croc1b	0.0021	2.8151E-06	**5.1**	0.000015	6.18696E-09	0.007	0.000005	1.03116E-08	0.05	0.00039	8.04305E-07	0.27
Drknberg1	0.00005	6.7025E-08	0.12	0.00231	9.52792E-07	**1.12**	0.00007	1.44362E-07	0.64	0.002115	4.36181E-06	**1.48**
Drknberg3	0.00009	1.2065E-07	0.21	0.00093	3.83592E-07	0.45	0.00021	4.33087E-07	**1.94**	0.001815	3.74311E-06	**1.27**

Table 5.18. Concentration of carcinogenic compounds (Cs), and the calculated DAD and CR (expressed as *x in 1 000 000*) calculated for **adults** for DLCs, carcinogenic PAHs and PCBs

Sites	DLCs			Σcarcinogenic-PAHs			ΣPCBs		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
S/L1	0.0000076	2.35104E-09	**366**	0.6018	0.001241104	**9100**	0.00894	1.2906E-05	**26**
S/L2	9.7E-07	3.00068E-10	**47**	0.437	0.000901234	**6600**	0.01232	1.77854E-05	**36**
S/L3	9.7E-07	3.00068E-10	**47**	0.0772	0.000159211	**1200**	0.01095	1.58077E-05	**32**
S/L4	9.7E-07	3.00068E-10	**47**	0.0909	0.000187465	**1400**	0.00697	1.00621E-05	**20**
S/L5	9.7E-07	3.00068E-10	**47**	0.2548	0.000525479	**3800**	0.00637	9.19588E-06	**18**
S/L6	9.7E-07	3.00068E-10	**47**	0.758	0.001563239	**11 000**	0.0072	1.03941E-05	**21**
S/L7	0.0000063	1.94889E-09	**304**	0.896	0.001847839	**13 000**	0.00674	9.73003E-06	**19**
S/L8	0.0000043	1.3302E-09	**206**	1.878	0.003873037	**28 000**	0.01401	2.02252E-05	**40**
S/L9	0.000087	2.69133E-08	**4200**	0.596	0.001229143	**9000**	0.04858	7.01313E-05	**140**
S/L10	0.000015	4.64022E-09	**720**	0.1358	0.000280063	**200**	0.00603	8.70505E-06	**17**
S/L12	0.000014	4.33087E-09	**670**	0.822	0.001695227	**12 000**	0.01441	2.08026E-05	**42**
S/L13	9.7E-07	3.00068E-10	**47**	0.1756	0.000362143	**2600**	¤	¤	
CT2	0.000016	4.94957E-09	**770**	0.764	0.001575612	**11 000**	0.01908	2.75443E-05	**55**
CT6	0.000012	3.71218E-09	**580**	0.0494	0.000101879	**740**	0.02464	3.55709E-05	**71**
CT7	0.000012	3.71218E-09	**580**	1.632	0.003365706	**2500**	0.02722	3.92954E-05	**79**
CT8	0.000011	3.40283E-09	**530**	0.118	0.000243354	**1800**	0.01727	2.49314E-05	**50**
D14	0.000029	8.97109E-09	**1400**	0.3517	0.000725318	**530**	0.04618	6.66666E-05	**130**
RB1	0.000103	3.18628E-08	**4900**	0.702	0.001447749	**11 000**	0.0573	8.27197E-05	**160**
RB2	0.000028	8.66174E-09	**1300**	0.383	0.000789869	**5800**	0.03979	5.74418E-05	**110**
RB3	0.000018	5.56826E-09	**870**	0.821	0.001693165	**12 000**	0.02008	2.8988E-05	**58**
RB4	0.0000075	2.32011E-09	**360**	0.1878	0.000387304	**2800**	0.02314	3.34055E-05	**67**
BF6	0.000044	1.36113E-08	**2100**	0.3219	0.000663861	**4800**	0.0126	1.81897E-05	**36**
BF7	0.000045	1.39207E-08	**2200**	0.623	0.001284825	**9400**	0.01266	1.82763E-05	**37**
BF8	0.000047	1.45394E-08	**2200**	0.1975	0.000407308	**2800**	0.01155	1.66739E-05	**33**
BF9	0.00001	3.09348E-09	**480**	0.3162	0.000652106	**4800**	0.0126	1.81897E-05	**36**
BO4	0.000036	1.11365E-08	**1700**	0.799	0.001647794	**12 000**	0.00113	1.6313E-06	**3.3**

Table 5.18. *Continued*

Sites	DLCs			Σcarcinogenic-PAHs			ΣPCBs		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
Lim4	0.000023	7.115E-09	**1100**	0.193	0.000398028	**2900**	0.00057	8.22866E-07	**1.6**
Olif4	0.000033	1.02085E-08	**1600**	0.1282	0.000264389	**1900**	0.00554	7.99768E-06	**16**
Croc1b	0.000008	2.47478E-09	**370**	5.501	0.011344822	**83 000**	0.00537	7.75226E-06	**16**
Drknberg1	9.7E-07	3.00068E-10	**47**	¤	¤	¤	0.00503	7.26143E-06	**14**
Drknberg3	9.7E-07	3.00068E-10	**47**	¤	¤	¤	0.0031	4.47523E-06	**8.9**

¤The CR associated with PAHs at Drknberg 1 and 3, and with PCBs at S/L13 could not be calculated. Due to the lack in sample quantities, Drknberg1 and 3 were not analysed for PAHs and S/L13 was not analysed for PCBs.

Table 5.19. Concentration of carcinogenic compounds (Cs), and the calculated DAD and CR (expressed as *x in 1 000 000*) calculated for **children** for the OCPs (HCB, ΣHCH, heptachlor and DDT)

Sites	HCB			ΣHCH			Heptachlor			ΣDDT		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
S/L1	0.00076	5.265E-07	0.9	0.00079	1.68395E-07	0.2	0.00007	7.46054E-08	0.34	0.000835	8.89936E-07	0.3
S/L2	0.0021	1.4548E-06	**2.6**	0.00141	3.00553E-07	0.35	0.00002	2.13158E-08	0.09	0.00148	1.57737E-06	0.54
S/L3	0.00054	3.7409E-07	0.7	0.00037	7.88686E-08	0.09	0.00004	4.26317E-08	0.2	0.001115	1.18836E-06	0.4
S/L4	0.00014	9.6987E-08	0.2	0.0006	1.27895E-07	0.15	0.00005	5.32896E-08	0.24	0.00132	1.40685E-06	0.5
S/L5	0.0001	6.9276E-08	0.1	0.00065	1.38553E-07	0.16	0.00002	2.13158E-08	0.09	0.001575	1.67862E-06	0.6
S/L6	0.00016	1.1084E-07	0.2	0.00022	4.68948E-08	0.05	0.000005	5.32896E-09	0.02	0.00084	8.95265E-07	0.3
S/L7	0.00005	3.4638E-08	0.06	0.00091	1.93974E-07	0.22	0.00009	9.59213E-08	0.43	0.00079	8.41976E-07	0.29
S/L8	0.00023	1.5934E-07	0.3	0.00111	2.36606E-07	0.28	0.00005	5.32896E-08	0.23	0.001925	2.05165E-06	0.7
S/L9	0.0041	2.8403E-06	**5.1**	0.00256	5.45686E-07	0.64	0.00036	3.83685E-07	**1.7**	0.0053	5.6487E-06	**1.9**
S/L10	0.00008	5.5421E-08	0.09	0.00131	2.79238E-07	0.33	0.00012	1.27895E-07	0.57	0.00152	1.62E-06	0.55
S/L12	0.0035	2.4247E-06	**4.4**	0.00141	3.00553E-07	0.35	0.00043	4.58291E-07	**2**	0.003815	4.066E-06	**1.38**
CT2	0.0057	3.9488E-06	**7.1**	0.00033	7.03423E-08	0.08	0.00014	1.49211E-07	0.67	0.001665	1.77454E-06	0.6
CT6	0.0032	2.2168E-06	**4**	0.000015	3.19738E-09	0.003	0.00004	4.26317E-08	0.19	0.001555	1.65731E-06	0.56
CT7	0.0068	4.7108E-06	**8.5**	0.00009	1.91843E-08	0.02	0.00009	9.59213E-08	0.43	0.001205	1.28428E-06	0.44
CT8	0.00056	3.8795E-07	0.7	0.00036	7.6737E-08	0.09	0.00003	3.19738E-08	0.14	0.001275	1.35888E-06	0.46
D14	0.0026	1.8012E-06	3.2	0.00057	1.215E-07	0.14	0.0028	2.98422E-06	**13**	0.01521	1.62107E-05	**5.5**
RB1	0.0056	3.8795E-06	**6.9**	0.002905	6.19225E-07	0.73	0.00004	4.26317E-08	0.19	0.00043	4.58291E-07	0.15
RB2	0.0018	1.247E-06	**2.2**	0.00131	2.79238E-07	0.33	0.00008	8.52634E-08	0.38	0.00041	4.36975E-07	0.15
RB3	0.0026	1.8012E-06	**3.2**	0.00131	2.79238E-07	0.33	0.00003	3.19738E-08	0.14	0.00037	3.94343E-07	0.13
RB4	0.0033	2.2861E-06	**4.1**	0.00141	3.00553E-07	0.35	0.00008	8.52634E-08	0.38	0.00034	3.62369E-07	0.12
BF6	0.0048	3.3253E-06	**5.9**	0.00068	1.44948E-07	0.17	0.00001	1.06579E-08	0.05	0.00235	2.50461E-06	0.85
BF7	0.0053	3.6717E-06	**6.6**	0.0009	1.91843E-07	0.23	0.00006	6.39475E-08	0.29	0.00229	2.44066E-06	0.83
BF8	0.0013	9.0059E-07	**1.6**	0.0001	2.13158E-08	0.03	0.00015	1.59869E-07	0.72	0.00145	1.5454E-06	0.53
BF9	0.0036	2.494E-06	**4.5**	0.00035	7.46054E-08	0.08	0.00002	2.13158E-08	0.09	0.00194	2.06764E-06	0.7
BO4	0.0014	9.6987E-07	**1.7**	0.000015	3.19738E-09	0.004	0.000005	5.32896E-09	0.023	0.0013	1.38553E-06	0.47

Table 5.19. *Continued*

Sites	HCB			ΣHCH			Heptachlor			ΣDDT		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
Lim4	0.00006	4.1566E-08	0.07	0.00004	8.52634E-09	0.01	0.00017	1.81185E-07	0.82	0.02204	2.34901E-05	**7.9**
Olif4	0.00096	6.6505E-07	**1.2**	0.000015	3.19738E-09	0.004	0.000005	5.32896E-09	0.024	0.0003	3.19738E-07	0.11
Croc1b	0.0021	1.4548E-06	**2.6**	0.000015	3.19738E-09	0.004	0.000005	5.32896E-09	0.024	0.00039	4.15659E-07	0.14
Drknberg1	0.00005	3.4638E-08	0.06	0.00231	4.92396E-07	0.58	0.00007	7.46054E-08	0.34	0.002115	2.25415E-06	0.77
Drknberg3	0.00009	6.2349E-08	0.11	0.00093	1.98237E-07	0.23	0.00021	2.23816E-07	**1**	0.001815	1.93441E-06	0.66

Table 5.20. Concentration of carcinogenic compounds (Cs), and the calculated DAD and CR (expressed as *x in 1 000 000*) calculated for **children** for DLCs, carcinogenic PAHs and PCBs

Sites	DLCs			Σcarcinogenic-PAHs			ΣPCBs		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
S/L1	0.0000076	1.215E-09	**190**	0.6018	0.000641394	**4700**	0.00894	6.66973E-06	**13**
S/L2	9.7E-07	1.55073E-10	**24**	0.437	0.000465751	**3400**	0.01232	9.19139E-06	**18**
S/L3	9.7E-07	1.55073E-10	**24**	0.0772	8.22791E-05	**600**	0.01095	8.1693E-06	**16**
S/L4	9.7E-07	1.55073E-10	**24**	0.0909	9.68805E-05	**710**	0.00697	5.2E-06	**10**
S/L5	9.7E-07	1.55073E-10	**24**	0.2548	0.000271564	**2000**	0.00637	4.75237E-06	**9.5**
S/L6	9.7E-07	1.55073E-10	**24**	0.758	0.00080787	**5900**	0.0072	5.37159E-06	**10**
S/L7	0.0000063	1.00717E-09	**160**	0.896	0.00095495	**7000**	0.00674	5.02841E-06	**10**
S/L8	0.0000043	6.87436E-10	**100**	1.878	0.002001557	**15 000**	0.01401	1.04522E-05	**21**
S/L9	0.000087	1.39086E-08	**2200**	0.596	0.000635212	**4600**	0.04858	3.62433E-05	**72**
S/L10	0.000015	2.39803E-09	**370**	0.1358	0.000144735	**1000**	0.00603	4.49871E-06	**89**
S/L12	0.000014	2.23816E-09	**350**	0.822	0.000876081	**6400**	0.01441	1.07506E-05	**21**
S/L13	9.7E-07	1.55073E-10	**24**	0.1756	0.000187153	**1400**	¤	¤	¤
CT2	0.000016	2.5579E-09	**400**	0.764	0.000814265	**5900**	0.01908	1.42347E-05	**28**
CT6	0.000012	1.91843E-09	**300**	0.0494	5.26501E-05	**380**	0.02464	1.83828E-05	**37**
CT7	0.000012	1.91843E-09	**300**	1.632	0.001739373	**13 000**	0.02722	2.03076E-05	**41**
CT8	0.000011	1.75856E-09	**270**	0.118	0.000125763	**920**	0.01727	1.28844E-05	**26**
D14	0.000029	4.6362E-09	**720**	0.3517	0.000374839	**2700**	0.04618	3.44528E-05	**69**
RB1	0.000103	1.64665E-08	**2600**	0.702	0.000748186	**5500**	0.0573	4.27489E-05	**85**
RB2	0.000028	4.47633E-09	**700**	0.383	0.000408198	**3000**	0.03979	2.96855E-05	**59**
RB3	0.000018	2.87764E-09	**450**	0.821	0.000875015	**6400**	0.02008	1.49808E-05	**30**
RB4	0.0000075	1.19902E-09	**190**	0.1878	0.000200156	**1500**	0.02314	1.72637E-05	**34**
BF6	0.000044	7.03423E-09	**1100**	0.3219	0.000343078	**2500**	0.0126	9.40029E-06	**19**
BF7	0.000045	7.1941E-09	**1100**	0.623	0.000663988	**4800**	0.01266	9.44505E-06	**19**
BF8	0.000047	7.51383E-09	**1200**	0.1975	0.000210494	**1500**	0.01155	8.61693E-06	**17**
BF9	0.00001	1.59869E-09	**250**	0.3162	0.000337003	**2500**	0.0126	9.40029E-06	**19**

Table 5.20. *Continued.*

Sites	DLCs			Σcarcinogenic-PAHs			ΣPCBs		
	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk	Cs (mg/kg)	DAD (mg/kg-day)	Cancer Risk
BO4	0.000036	5.75528E-09	**900**	0.799	0.000851568	**6200**	0.00113	8.43041E-07	**1.7**
Lim4	0.000023	3.67698E-09	**570**	0.193	0.000205698	**1500**	0.00057	4.25251E-07	0.9
Olif4	0.000033	5.27567E-09	**820**	0.1282	0.000136635	**990**	0.00554	4.13314E-06	**8.3**
Croc1b	0.000008	1.27895E-09	**200**	5.501	0.005862922	**43 000**	0.00537	4.00631E-06	**8**
Drknberg1	9.7E-07	1.55073E-10	**24**	¤	¤	¤	0.00503	3.75265E-06	**7.5**
Drknberg3	9.7E-07	1.55073E-10	**24**	¤	¤	¤	0.0031	2.31277E-06	**4.6**

¤The CR associated with PAHs at Drknberg 1 and 3, and with PCBs at S/L13 could not be calculated. Due to the lack in sample quantities, Drknberg1 and 3 were not analysed for PAHs and S/L13 was not analysed for PCBs.

The dermal CR associated with exposure to PAHs, DLCs and PCBs were high when compared to the cancer risk associated with exposure to the OCPs (Table 5.17 to 5.20). In the case of PAHs and PCBs, it was due to high concentrations of the compounds present at the sites, but in the case of DLCs (were low concentrations were generally present), the high dermal cancer slope factor was responsible for the high CR.

The CR for dermal exposure to OCPs were generally below the negligible CR of 1 in 1 000 000 and rarely exceeded 10 in 1 000 000 (except for HCB at a three of the sites). On the other hand, the CR for dermal exposure to DLCs, PAHs and PCBs, ranged between 47 and 4900 in 1 000 000, 200 and 83 000 in 1 000 000, and 3.3 and 160 in 1 000 000 respectively for adults, and between 24 and 2600 in 1 000 000, 380 and 43 000 in 1 000 000, 0.9 to 89 in 1 000 000 respectively for children (Table 5.18 and 5.20).

The CR calculated for adults was higher than for children, due to a lower body mass (BM) and shorter exposure duration (D_3) of children, compared to adults (CR α 1/BM, and CR α D_3).

The sites where the highest potential CRs were calculated include:

- **Soweto/Lenasia wetland sites**, S/L1, 6 to 9, and 12;
- **Cape Town industrial sites**, CT2 and 7;
- **Umgeni River mouth in Durban**, D14;
- **Richards Bay industrial sites**, RB1, 2 and 3;
- **Bloemfontein sites**, BF6, 7 and 8, and BO4 in **Botshabelo**;
- **The international rivers,** Olif4 and Croc1b.

It should, however, be noted that these calculations are based on extrapolations and assumptions (exposure probability, frequency and duration), and could be over- or under estimations of the potential CR. The calculations were done according to the US EPA's formulae and proposed values, which is based on first world scenarios, and not ideal to apply for a developing country, such as South Africa. The calculations are, however, useful to identify and flag the sites where the highest potential CRs were calculated. To obtain clarity more samples need to be analysed and contact patterns established.

6

Conclusions and Recommendations

6.1. Conclusions

This is the fist South African study of its kind that makes use of a tiered approach (wherby the presence of POPs was first identified using a screening test) to evaluate the levels of POPs in environmental matrices and as such makes a very important contribution to the development of future environmental POPs monitoring programmes in the country.

All of the compounds tested for were found in South African sediments. The levels of DLCs in soils and sediments were generally low, with only 23 of the 96 sites eliciting quantifiable responses when screened with the H4IIE-*luc* bio-assay. Of the 23 sites, 77% was of industrial or semi-industrial origin, 15% was industrial-residential combinations, 6% was high-density low-income residential areas and 2% was residential-agricultural combinations. TOC content, seasonal and meteorological conditions (high temperatures, low precipitation and long summers), photodegradation, sedimentation shifts, effects of dilution, and degradation by micro-organisms were identified as the possible causes for the low levels of DLCs in the South African environment. A loss in cell viability caused by the cytotoxicity of some of the samples could also have contributed to reduced RLUs.

Chemical analyses results indicated that PAHs were the most abundant of all the groups of compounds investigated, and were present at the highest levels of all the compounds analysed. OCPs and PCBs were present in intermediate concentrations, while PBDEs were the least abundant and present in the lowest concentrations. Aldrin and chlordene were not detected at any of the sites, whereas nonachlor, chlordane and oxychlordane were present at only a few of the sites in minor concentrations. HCB, HCH and DDT were the predominant OCPs, while heptachlor and mirex were present in lower concentrations. This might be ascribed to the fact that HCB is still produced for industrial applications, and HCH and DDT are presently applied as pesticides in some parts of the country, while the use of heptachlor and mirex has been banned.

In general, 4-ringed PAHs were the most abundant, followed by 5-ringed congeners and finally either by 3- or 6-ringed congeners. Two-ringed PAHs were the least abundant. HMM-PAHs are less susceptable to biodegradation and loss from soil or sediment, which may explain the high prevalence of these congeners. CB-153, -138, -118 and -101 were the major PCB congeners in South African sediments. The lighter PCBs were less abundant, because they are rapidly biodegraded in the environment. PBDEs were generally present at low concentrations, with BDE-153 being the predominant PBDE.

PCA distinguished between two main groups of chemicals – OCPs and industrially associated compounds. Separate PCAs for each group of compounds illustrated strong associations between:

- Lim 4 and *o,p'*-DDT indicating current use of the pesticide,
- D14 and the metabolites, DDE and DDD, indicating historic use of DDT,
- Some S/L sites and heptachlor,
- RB1, 3 and 4 and HCH and HCB,
- BO4, Lim4 and PBDEs,
- RB 1, 3 and 4, BF7, D14 and the lighter PCBs,
- CT8, S/L8 and the heavier PCBs,
- S/L 1, 4 and 8 and the lighter PAHs and
- Croc1b, RB2, Lim4 and the heavier PAHs.

Additionally, An(An+Ph) and Fl(Fl+Py) ratios indicated that the PAHs at the majority of the sites were of pyrogenic origin, or neither solely pyrogenic or petrogenic (“mixed origin”).

The normalized concentrations (1% TOC) of the compounds of interest at a few of the sites exceeded the Canadian sediment quality quidelines. The concentrations of OCPs and PCBs were generally below the proposed sediment quality guidelines, while the concentrations of PAHs and DLCs at many of the sites exceeded the guidelines.

The concentration of pollutants measured in South African soils and sediments were intermediate when compared to the levels measured in some European, Asian and Scandinavian countries, with the exception of a few sites where exceptionally high levels of certain compounds were measured.

For this study, it was estimated that the dermal absorption of OCPs due to sediment contact, could result in an incremental increase in cancer risk. On the other hand, dermal exposure to PCB-, DLC- and PAH-contaminated sediment may lead to severe increases in cancer cases. These results are based on extrapolations and assumptions, as discussed earlier, and more samples need to be analysed and sediment contact patterns established, before sound conclusions can be reached.

6.2. Recommendations

- Further investigation is recommended into the levels of certain POPs and organic pollutants at the following sites:
    - **D14 (Umgeni mouth – possibly receiving effluent from various industries up-stream)**, where the levels of heptachlor, DDE, DDT and DLCs were all above the ISQGs;
    - **RB1 and RB2 (Richards Bay industrial sites):** At RB1 the ISQGs of several PAH-congeners and γ-HCH were exceeded, while the ISQG and PEL of DLCs were exceeded. At RB2, PAHs and PCBs were present at such concentrations that the ISQGs of these compounds were exceeded, and for γ-HCH and DLCs the PELs were exceeded as well;
    - **BF6, BF7 and BF8 (Bloemfontein industrial and low-income residential sites):** The concentrations of some of the PAH-congeners at BF6 and BF7 where higher than the proposed ISQG, and the DLC concentrations at all three of the sites exceeded the ISQGs and the PELs;
    - **Lim4 (Low-income residential site in the Limpopo Province – a malaria-endemic area):** The ISQGs of DDE, DDT and DLCs were exceeded at Lim4. Lim4 was identified as the only site where South Africa's contribution of POPs into a neighbouring country was substantial. This site should therefore be treated as a high priority.
    - **Croc1b (On the premises of a paper mill in Mpumalanga):** This site had the largest amount of PAH-congeners exceeding the ISQGs, PELs and LELs. The concentration of DLCs at this site was above the ISQG. The sites

situated down-stream of Croc1b and closer to the borders of neighbouring countries had less significant concentrations of organic pollutants.

- Further investigations are also recommended for the following sites where the highest potential CRs (associated with dermal contact with soil) were calculated:

  - **Soweto/Lenasia wetland sites**, S/L1, 6 to 9, and 12;
  - **Cape Town industrial sites**, CT2 and 7;
  - **Umgeni River mouth in Durban**, D14;
  - **Richards Bay industrial sites**, RB1, 2 and 3;
  - **Bloemfontein sites**, BF6, 7 and 8, and BO4 in **Botshabelo**;
  - **The international rivers,** Olif4 and Croc1b.

- It is recommended that other cell-based bio-assays are performed to determine if sediments are capable of eliciting estrogenic and androgenic responses. MVLN- and MDA bio-assays, where stably transfected human breast cancer cell lines are employed, are suggested.

- It is suggested that sediment samples should in the future be analysed by HRGC/HRMS for the presence of DLCs as well, to compare biologically and chemically derived results, if sufficient funds are available.

- It is recommended that the results from this study are used to guide and inform the development of appropriate policy and regulatory measures that will support the implementation of the requirements of the SC POPs to contribute to the protection of water resources and water-linked ecosystems with regard to POPs.

- It is proposed that the hydrology of the sites, and the physical chemistry and particle size of sediments are considered in future similar studies.

7

Bibliography

Aigner, J.E., Leone, D.A. & Falconer, L.R. 1998. Concentrations and enantiomeric ratios of organochlorine pesticides in soils from the U.S. corn belt. *Environmental Science & Technology* 32: 1162 – 1168.

Alaee, M., Arias, P., Sjödin, A. & Berman, A. 2003. An overview of commercially used brominated flame retardants, their applications, their use patterns in different countries/regions and possible modes of release. *Environment International* 29: 683 – 689.

Alcock, R.E., Sweetman, A.J., Anderson, D.R., Fisher, R., Jennings, RA. & Jones, K.C. 2002. Using PCDD/F congener patterns to determine the source of elevated TEQ concentrations in cows milk: a case study. *Chemosphere* 46: 383 - 391.

Allchin, C.R., Law, R.J. & Morris, S. 1999. Polybrominated diphenylethers in sediments and biota downstream of potential sources in the UK. *Environmental Pollution* 105: 197 – 207.

Andreu, V. & Picó, Y. 2004. Determination of pesticides and their degradation products in soil: Critical review and comparison of methods. *Trends in Analytical Chemistry* 23 (10 – 11): 772 – 789.

Ashworth, P.J. & Ferguson, R.I. 1989. Size-selective entrainment of bed load in gravel bed streams. *Water Resources Research* 25 (4): 627 – 634.

ATSDR. Agency for Toxic Substances and Disease Registry. 1995. Toxicological profile for mirex and chlordecone. [Web:] http://www.atsdr.cdc.gov/toxprofiles/tp66.pdf. Accessed on 7 December 2009.

ATSDR. Agency for Toxic Substances and Disease Registry. 2002a. Hexachlorobenzene. [Web:] http://www.atsdr.cdc.gov/tfacts90.pdf. Accessed on 1 December 2009.

ATSDR. Agency for Toxic Substances and Disease Registry. 2002b. Toxicological Profile for DDT, DDE, and DDD. U.S. Department of Health and Human Services: Public Health Service. [Web:] http://www.atsdr.cdc.gov/toxprofiles/tp35.pdf. Accessed on 21 September 2009.

ATSDR. Agency for Toxic Substances and Disease Registry. 2007a. Hexachlorocyclohexane: Toxicological profile. [Web:] http://www.atsdr.cdc.gov/tfacts43.pdf. Accessed on 2 December 2009.

ATSDR. Agency for Toxic Substances and Disease Registry. 2007b. Heptachlor and heptachlor epoxide: Toxicological profile. [Web:] http://www.atsdr.cdc.gov/tfacts12.pdf. Accessed on 4 December 2009.

Augustijn-Beckers, P.W.M., Hornsby, A.G. & Wauchope, R.D. 1994. Pesticide properties database for environmental decisionmaking II. *Reviews in Environmental Comtamination & Toxicology* 137: 1 – 82.

Bailey, R.E. 2001. Global hexachlorobenzene emissions. *Chemosphere* 43: 167 – 182.

Beall, M.L., Jr. 1976. Persistence of aerially applied hexachlorobenzene on grass and soil. *Journal of Environmental Quality* 5 (4): 367 – 369.

Beard, J. 2006. DDT and human health. *Science of the Total Environment* 335: 78 – 89.

Beard, J., Marshall, S., Jong, K., Newton, R., Triplett-McBride, T. Humphries, B. & Bronks, R. 2000. 1,1,1-Tirchloro-2,2-bis(p-chlorophenyl)-ethane (DDT) and reduced bone mineral density. *Archives of Environmental Health* 55: 177 – 180.

Behnisch, P.A., Hosoe, K. & Sakai, S-I. 2001. Bioanalytical screening methods for dioxins and dioxin-like compounds – a review of bioassay/biomarker technology. *Environment International* 27: 413 – 439.

Bengtson Nash, S.M., Poulsen, A.H., Kawaguchib, S., Vetter, W. & Schlabach, M. 2008. Persistent organohalogen contaminant burdens in Antarctic krill (*Euphausia superba)* from the eastern Antarctic sector: a baseline study. *Science of the Total Environment* 407: 304 – 314.

Bergman, Å. & Ulrika, O. 2001. Polybrominated flame retardants – a threat? *Organohalogen compounds* 50: 13 – 20.

Besselink, H.T., Schripper,C., Klamer, H., Leonards, P., Verhaar, H., Felzel, E., Murk, A.J., Thain, J., Hosoe, K., Schoeters, G., Legler, J. & Brouwer, B. 2004. Intra- and interlaboratory calibration of the DR Calux bioassay for the analysis of dioxins & dioxin-like chemicals in sediments. *Environmental Toxicology and Chemistry* 23: 2781 – 2189.

Bhatt, P., Kumar, M.S. & Chakrabarti, T. 2009. Fate and degradation of POP – Hexachlorocyclohexane. *Critical Reviews in Environmental Science & Technology* 39 (8): 655 – 695.

Bidleman, T. 1988. Atmospheric processes: Wet and dry deposition of organic compounds are controlled by their vapor-particle partitioning. *Environmental Science and Technology* 22 (4): 361 – 367.

Binning, K. & Baird, D. 2001. Survey of heavy metals in the sediments of the Swartkops River Estuary, Port Elizabeth, South Africa. *Water SA* 27 (4): 461 -466.

Birch, G.F. & Taylor, S.E. 2000. Distribution and possible sources of organochlorine residues in sediments of a large urban estuary, Port Jackson, Sydney. *Australian Journal of Earth Science* 47 (4): 749 – 756.

Birnbaum, L.S. 1995. Developmental effects of dioxins. *Environmetal Health Perspectives*: 103 (7): 89 – 94.

Blaha, L., Hecker, M., Murphy, M., Jones, P.D., Newsted, J. & Giesy, J.P. 2004. Procedure for determination of cell viability/cytotoxicity using the MTT bioassay. *Aquatic Toxicology Laboratory, Michigan State University.*

Boffetta, P., Jourenkova, N. & Gustavsson, P. 1997. Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. *Cancer Cause Control* 8: 444 – 472.

Bornman, R., de Jager, C., Worku, Z., Farias, P. & Reif, S. 2010. DDT and urogenital malformations in newborn boys in a malarial area. *British Journal of Urology International* 106 (3): 405 – 411.

Bouwman, H. 2004. South Africa and the Stockholm Convention on Persistent Organic Pollutants. *South African Journal of Science* 100: 323 – 328.

Bouwman, H., Becker, P.J., Cooppan, R.M. & Reinecke, A.J. 1992. Transfer of DDT used in malaria control to infants via breast milk. *Bulletin of the World Health Organization* 70 (2): 241 – 250.

Bouwman, H., Sereda, B. & Meinhardt, H.M. 2006. Simultaneous presence of DDT and pyerthroid residues in human breast milk from a malaria endemic area in South Africa. *Environmental Pollution* 144: 902 – 917.

Breitung, V., Vollmer, S. & Lehmann, M. 2008. HCB-contaminated sediments in the Upper Rhine – a case study while developing a sediment management plan. [Web:] http://www.sednet.org/download/conference2008/42%20%20Breitung_Vera_%5B1%5D.pdf. Accessed on 4 December 2009.

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Klasson-Wehler, E., Bergman, A. & Visser, T.J. 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: Mechanisms and possible consequences for animal and human health. *Toxicology & Industrial Health*: 14 (1 – 2): 59 – 84.

Budzinski, H., Jones, I., Bellocq, J., Piérard, C. & Garrigues, P. 1997. Evaluation of sediment contamination by polycyclic aromatic hydrocarbons in the Gironde estuary. *Marine Chemistry* 58: 85 - 97.

Canadian Environmental Assessment Services Safe Environments Programme. 2004. Federal Contaminated Site Risk Assessment in Canada. Part I: Guidance on human health preliminary quantitative risk assessment (PQRA). ISBN 0-662-38244-7.

CANSA (Cancer Association of South Africa). 2009. What is cancer, its causes, and the warning signs? [Web:] http://www.canso.org.za. Accessed on 13 July 2009.

Carbonnelle, S., Van Loco, J., Van Overmeire, I., Windal, I., Van Wouwe, N., Van Leeuwen, S. & Goeyens, L. 2004. Importance of REP values when comparing the CALUX bioassay results with chemoanalyses results. Example with spiked vegetable oils. *Talanta* 63: 1255 – 1259.

Carey, J., Cook, P., Giesy, J., Hodson, P., Muir, D., Owens, W. & Solomon, K. 1998. Ecotoxicological risk assessment of the chlorinated organic chemicals. Pensacola: SETAC Press. 375 p.

Carlson, D.A., Konyha, K.D. & Wheeler, W.D.B. 1976. Mirex in the environment: Its degradation to Kepone and related compounds. *Science* 194 (4268): 939 – 941.

Casida, J.E. & Quistad, G.B. 1998. Golden age of insecticide research: Past, present, or future? *Annual Reviews in Entomology* 43: 1 – 16.

CCME. Canadian Council of Ministers of the Environment. 2002. Canadian sediment quality guidelines for the protection of aquatic life. [Web:] http://www.doeal.gov/laso/SWEIS/OtherDocuments/ 328%20envi%20canada%202002.pdf. Accessed on 12 January 2009.

Chan, K.Y., Bowman, A. & Oates, A. 2001. Oxidizible organic carbon fractions and soil quality changes in an oxic paleustalf under different pasture leys. *Soil Science* 166 (1): 61 – 67.

Chen, B., Xuan, X., Zhu, L., Wang, J., Gao, Y., Yang, K., Shen, X., & Lou, B. 2004. Distributions of polycyclic aromatic hydrocarbons in surface waters, sediments and soils of Hangzhou City, China. *Water Research* 38: 3558-3568.

Chen, Y.C. & Hsu, C.C. 1994. Effects of prenatal exposure to PCBs on the neurological function of children: A neuropsychological and neurophysiological study. *Developmental Medicine & Child Neurology* 36: 312 – 320.

Chowdhury, A.R., Gautam, A.K. & Venkatakrishna-Bhatt, H. 1990. DDT (2,2,bis(p-chlorophnyl) 1,1,1,-trichloroethane) induced structural changes in adrenal glands of rats. *Bulletin of Environmental Contamination and Toxicology* 45: 193 – 196.

Chutter, F.M. 1998. Research on the rapid biological assessment of water quality impacts in streams and rivers. Water Research Commission. Pretoria. WRC Report no. 422/1/98.

Clark, D.R., Spann, J.W. & Bunk, C.M. 1990. Dicofol (Kelthane ®)- induced eggshell thinning in captive American Kestrels. *Environmental Toxicology and Chemistry* 9: 1063 – 1069.

Coetzee, M. & Hunt, R.H. 1998. Malaria at its southern-most fringe in Africa. *Research and Reviews in Parasitology* 58: 175 – 179.

Concha-Graña, E., Turnes-Carou, M.I., Lorenzo-Muniategui, S., López-Mahía, P., Prada-Rodríguez, D. & Fernández- Fernández, E. 2006. Evaluation of HCH isomers and metabolites in soils, leachates, river water and sediments of a highly contaminated area. *Chemosphere* 64: 588 – 595.

Corsolini, S., Kannan, K., Imagawa, T., Focardi, S. & Giesy, J.P. 2002. Polychloronaphthalenes and other dioxin-like compounds in Arctic and Antarctic marine food webs. *Environmental Science & Technology* 36 (16): 3490 – 3496.

Coulson, F. 1985. Reconsideration of the dilemma of DDT for the establishment of an acceptable daily intake. *Regulatory Toxicology & Pharmacology* 5: 332 – 383.

Culotta, L., De Stefano, C., Gianguzza, A., Mannino, M.R. & Orecchio, S. 2006. The PAH composition of surface sediments from Stagnone coastal lagoon, Marsala (Italy). *Marine Chemistry* 99: 117 - 127.

Darnerud, P.O., Eriksen, G.S., Jóhannesson, T., Larsen, P.B. & Viluksela, M. 2001. Polybrominated diphenyl ethers: Occurrence, dietary exposure, and toxicology. *Environmental Health Perspectives* 109 (1): 49 – 68.

Davies, B. & Day, J. 1998. Vanishing waters. Cape Town: University of Cape Town Press. pp 3 – 15.

DEAT. Department of Environmental Affairs and Tourism. 1999. National State of the Environment Report – South Africa: Freshwater Systems and Resources. [Web:] http://www.ngo.grida.no/soesa/nsoer/issues/water/. Accessed on 27 August 2009.

DEAT. Department of Environmental Affairs and Tourism. 2005. South African National Profile 2002 – 2005: A comprehensive assessment of the national infrastructure relating to the legal administrative and technical aspects of chemicals management in South Africa. Chemical and hazardous waste management. Department of Environmental Affairs and Tourism, Siyaya Publishing, Hatfield, Pretoria, South Africa.

De Boer, J., Wester, P.G., Van der Horst, A. & Leonards, P.E.G. 2003. Polybrominated diphenyl ethers in influents, suspended particulate matter, sediments, sewage treatment plant and effluents and biota from the Netherlands. *Environmental Pollution* 122: 63 – 74.

Denison, M.S., Rogers, W.J., Fair, M., Ziccardi, M., Clark, G., Murk, A.J. & Brouwer, A. 1996. Application of the CALUX bioassay system for the detection of dioxin-like chemicals (Ah-receptor ligands) in whole serum samples and in extracts from commercial and consumer products. *Organohalogen Compounds* 27: 280 - 284.

De Wit, C.A. 2002. An overview of brominated flame retardants in the environment. *Chemosphere* 46: 583 – 624.

Domingo, J.L., Schuhmacher, M., Granero, S., Llobet, J.M. & de Kok, H.A.M. 1999. PCDD/F levels in the vicinity of an old municipal solid waste incinerator: temporal variation in soils. *Archives of Environmental Contamination and Toxicology* 36 (4): 377 – 383.

Dragan, D., Cucu-Man, S., Dirtu, A.C., Mocanu, R., Vaeck, L.V. & Covaci, A. 2006. Occurrence of organochlorine pesticides and polychlorinated biphenyls in soils and sediments from Eastern Romania. *International Journal of Environmental and Analytical Chemistry* 86 (11): 833 – 842.

Du Preez, L.H., Jansen van Rensburg, P.J., Jooste, A.M., Carr, J.A., Giesy, J.P., Gross, T.S., Kendall, R.J., Smith, E.E., Van Der Kraak, G. & Solomon, K.R. 2005. Seasonal exposures to triazine and other pesticides in surface waters in the western Highveld corn-production region in South Africa. *Environmental Pollution* 135 (1): 131 – 141.

DWAF. Department of Water Affairs and Forestry. 1986. Management of the water resources of the Republic of South Africa. Department of Water Affairs. Pretoria: Government Printers. p 2.30 – 2.41.

ECHA. European Chemicals Agency. 2008. Hexabromocyclododecane: SVHC Support Document. [Web:] http://echa.europa.eu/doc/candidate_list/svhc_supdoc_hbccd_publication.pdf. Accessed on 5 October 2009.

Ecobichon, D.J. 1991. Toxic effects of pesticides. (*In* Amdur, M.O, Doull, J. & Klaassen, C.D. eds. Casarett and Doull's Toxicology. 4th ed. New York: Pergamon Press. p. 6 – 61.)

Edwards, I.R., Ferry, D.G. & Temple, W.A. 1991. Fungicides and related compounds. (*In* Hayes, W.J., Jr. & Laws, E.R., Jr., eds. Handbook of Pesticide Toxicology. New York: Academic Press. p. 6 – 8.)

Eickhoff, C.V., He, S-X, Gobas, F.A.P.C. & Law, F.C.P. 2003. Determination of polycyclic aromatic hydrocarbons in Dungeness crabs (*Cancer Magister*) near an aluminium smelter in Kitimat Arm, Brittish Columbia, Canada. *Environmental Toxicology & Chemistry* 22 (1): 50 – 58.

Eisen, H.J., Hannah, R.R., Legraverend, C., Okey, A.B. & Nebert, D.W. 1983. The Ah-receptor: controlling factor in the induction of drug-metabolizing enzymes by certain chemical carcinogens and other environmental pollutants. (*In* Litwack, G., *ed.* Biochemical actions of hormones, Vol. X. New York: Academic Press. p 227 – 258.)

Elferink, C.J. 2003. Aryl hydrocarbon receptor-mediated cell cycle control. *Progress in Cell Cycle Research* 5: 261 – 267.

Ellis, S.M. & Steyn, H.S. 2003. Practical significance (effect sizes) versus or in combination with statistical significance (p-values). *Management Dynamics* 12 (4):51 - 53.

Eriksson, P., Viber, H., Fischer, C., Wallin, M. & Fredriksson, A. 2002. A comparison on developmental neurotoxic effects of hexabromocyclododecane, 2,2',4,4',5,5'-hexabromodihenylether (PBDE 153) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153). *Organohalogen Compounds* 57: 389 – 392.

Euro Chlor. 2002. Hexachlorobenzene: Euro Chlor risk assessment for the marine environment OSPARCOM region – North Sea. [Web:] http://www.eurochlor.org/upload/documents/document80.pdf. Accessed on 1 December 2009.

Fenster, L., Eskenazi, B., Anderson, M., Bradman, A., Harley, K. & Hernandez, H. 2006. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. *Environmental Health Perspectives* 114: 597 – 602.

Fiedler, H. 2003. Dioxins and furans (PCDD/PCDF). (*In* Hutzinger, O., *ed*. The Handbook of Environmental Chemistry, Vol. 3. Berlin: Springer. p 123 – 201.)

Fiedler, H., Buckley-Golder, D., Coleman, P., King, K. & Petersen, A. 1999. Compilation of EU dioxin exposure and health data: environmental levels. *Organohalogen Compounds* 43: 141 – 144.

Fiedler, H., Lau, C., Kjeller, L.-O. & Rappe, C. 1996. Patterns and sources of polychlorinated dibenzo-p-dioxins and dibenzofurans found in soil and sediment samples in Southern Mississippi. *Chemosphere* 32 (3): 421 – 432.

Fischer, L.J., Seegal, R.F., Geney, P.E., Pessah, I.N. & Kodavanti, P.R. 1998. Symposium overview: toxicity of non-coplanar PCBs. *Toxicological Sciences* 41: 49 – 61.

Ford, W.M. & Hill, E.P. 1991. Organochlorine pesticides in soil, sediments and aquatic animals in the upper Steele bayou watershed of Mississippi. *Archives of Environmental Contamination* 20: 161 – 167.

Fuerst, P., Meemken, H.A., Krueger, C. & Groebel, W. 1987. Polychlorinated dibenzodioxins and dibenzofurans in human milk samples from West Germany. *Chemosphere* 16 (8/9): 1983 – 1988.

Gao, H.J., Jiang, X., Wang, F., Wang, D.Z. & Bian, Y.R. 2005. Residual levels and bioaccumulation of chlorinated persistent organic pollutants (POPs) in vegetables from suburb of Nanjing, People's Republic of China. *Bulletin of Environmental Contamination and Toxicology* 74 (4): 673 – 680.

Gao, J.P., Maguhn, J., Spitzauer, P. & Kettrup, A. 1998. Sorption of pesticides in the sediment of the Teufelsweiher pond (Southern Gemany).I: Equilibirum assessments, effect of organic carbon content and pH. *Water Research* 32: 1662 - 1672.

Garcia-Pichel, F. & Bebout, B.M. 1996. Penetration of ultraviolet radiation into shallow water sediments: high exposure for photosynthetic communities. *Marine Ecology Progress Series* 131: 257 – 262.

Giesy, J.P., Jude, D.J., Tillit, D.E., Gale, R.W., Meadowns, J.C., Zajieck, J.L., Peterman, P.H., Verbrugge, D.A., Sanderson, J.T., Schwartz, T.R. & Tuchman, M.L. 1997. Polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls and 2,3,7,8-tetrachloro dibenzo-p-dioxin equivalents in fishes from Saginaw Bay, Michigan. *Environmental Toxicology and Chemistry* 16 (4): 713 – 724.

Gladen, B.C., Shkiryak-Nyzhnyk, Z.A., Chyslovska, N., Zadorozhnaja, T.D. & Little, R.E. 2003. Persistent organochlorine compounds and birth weight. *Annual Epidemiology* 13: 151 – 157.

Gong, X., Qi, S., Wang, Y., Julia, E.B. & Lv, C. 2007. Historical contamination and sources of organochlorine pesticides in sediment cores from Quanzhou Bay, Southeast China. *Marine Pollution Bulletin* 54: 1434 – 1440.

Gong, Z.M., Tao, S., Xu, F.L., Dawson, R., Liu, W.X., Cui, Y.H., Cao, J., Wang, X.J., Shen, W.R., Zhang, W.J., Qing, B.P. & Sun, R. 2004. Level and distribution of DDT in surface soils from Tianjin, China. *Chemosphere* 54 (8): 1247 – 1253.

González-Farias, F., Cisneros Estrada X., Fuentes Ruis, C., Diaz González, G. & Botello, A.V. 2002. Pesticides distribution in sediments of tropical coastal lagoon adjacent to an irrigation district in northwest Mexico. *Environmental Technology* 23 (11): 1247 – 1256.

Gosselin, R.E., Smith, R.P. & Hodge, H.C. 1984. Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams & Wilkins.

Gravelet-Blondin, L.R., Barclay, S.J., Carliell, C.M. & Buckley, C.A. 1997. Management of water resources in South Africa with respect to the textile industry. *Water Science and Technology* 36 (2 – 3): 303 – 310.

Greichus, Y.A., Greichus, A., Amman, B.D., Call, D.J., Hamman, D.C.D. & Pott, R.M. 1977. Insecticides, polychlorinated biphenyls and metals in African lake ecosystems. 1. Hartbeespoort Dam, Transvaal and Voëlvlei Dam, Cape Province, Republic of South Africa. *Archives of Environmental Contamination & Toxicology* 6: 371 – 383.

Grobler, D.F., Badenhorst, J.E. & Kempster, P.L. 1996. PCBs, chlorinated hydrocarbon pesticides and chlorophenols in the Isipingo Estuary, Natal, Republic of South Africa. *Marine Pollution Bulletin* 32 (7): 572 – 575.

Grochowalski, A. & Maślanka, A. 2003. Comparison of ASE and Soxhlet- apparatus extraction in determination of polychlorinated dibenzodioxins and benzofurans. *Organohalogen Compounds* 60 - 65: 118 – 123.

Gschwend, P.M. & Hites, R.A. 1981. Fluxes of polycyclic aromatic hydrocarbons to marine and lacustrine sediments in the northeastern United States. *Geochimica et Cosmochimica Acta* 45: 2359 - 2367.

Guzzella, L., Roscioli, C., Viganò, L., Saha, M., Sarkar, S.K. & Battacharya, A. 2005. Evaluation of the concentration of HCH, DDT, HCB, PCB and PAH in sediments along the lower stretch of Hugli estuary, West Bengal, northeast India. *Environment International* 31: 523 – 534.

Halden, R.U., Halden, B.G. & Dwyer, D.F. 1999. Removal of dibenzofuran, dibenzo-p-dioxin, and 2-chlorodibenso-p-dioxin from soils inoculated with *Sphingomonas* sp. Strain RW1. *Applied and Environmental Microbiology* 65 (5): 2246 – 2249.

Hassanin, A., Breivik, K., Meijer, S.N., Steinnes, E., Thomas, G.O. & Jones, K.C. 2004. PBDEs in European background soils: levels and factors controlling their distribution. *Environmental Science & Technology* 38 (8): 2345 – 2350.

Helleday, T., Tuominen, K.L., Bergman, A. & Jenssen, D. 1999. Brominated flame retardants induce intragenic recombination in mammalian cells. *Mutation Research* 439: 137 – 147.

Hellström, T. 2000. Brominated flame retardants (PBDE and PBB) in sludge – a problem? Report No M. 113. The Swedish Water and Wastewater Association. [Web:] http://www.biosolids.org/docs/23481.pdf. Accessed on 1 October 2009.

Henry, T.R. & De Vito, M.J. 2003. Non-dioxin-like PCBs: Effects and consideration in ecological risk assessment. Office of Research and Development of the US EPA. Cincinnati, OH. NCEA-C-1340.

Hilscherova, K., Kannan, K., Kong, Y-S., Holoubek, I., Machala, M., Masunaga, S., Nakanishi, J. & Giesy, J.P. 2001. Characterization of dioxin-like activity of sediments from the Czech river basin. *Environmental Toxicology & Chemistry* 20 (12): 2768 – 2777.

Hilscherova, K., Kannan, K., Nakata, H., Hanari, N., Yamashita, N., Bradley, P.W., McCabe, J.M., Taylor, A.B. & Giesy, J.P. 2003. Polychlorinated dibenzo-p-dioxin and dibenzofuran concentration profiles in sediments and flood-plain soils of the Tittabawassee River, Michigan. *Environmental Science and Technology* 37 (3): 468 – 474.

Hilscherova, K., Machala, M., Kannan, K., Blankenship, A.L. & Giesy, J.P. 2000. Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Environmental Science & Pollution Research* 7 (3): 159 – 171.

Hölscher, K., Maulshagen, A., Shirkhan, H., Lieck, G. & Behnisch, P.A. 2004. Automated rapid analysis for dioxins and PCBs in food, feedingstuff and environmental matrices. *Organohalogen compounds* 66: 117 – 125.

Hooper, K., Petreas, M.X., She, J., Visita, P., Winkler, J., McKinney, M., Mok, M., Sy, F., Garcha, J., Gill, M., Stephens, R.D., Semenova, G., Sharmanov, T. & Chuvakova, T. 1997. Analysis of breast milk to assess exposure to chlorinated contaminants in Kazakstan: PCBs and organochlorine pesticides in southern Kazakstan. *Environmental Health Perspectives* 105 (11): 1250 – 1254.

Howel, D. 2007. Multivariate data analysis of pollutant profiles: PCB levels across Europe. *Chemosphere* 67: 1300 - 1307.

Hung, C., Gong, C., Chen, H., Hsieh, H., Santschi, P.H., Wade, T.L. & Sericano, J.L. 2007. Relationships between pesticides and organic carbon fractions in sediment of the Danshui River estuary and adjacent areas in Taiwan. *Environmental Pollution* 148: 546 – 554.

Hunziker, R.W., Gonsior, S., MacGregor, J.A., Desjardins, D., Ariano, J. & Friederich, U. 2004. Fate and effect of hexabromocyclodecane in the environment. *Organohalogen Compounds* 66: 2300 – 2305.

INCHEM. International Programme on Chemical Safety. 2001. Hexachlorocyclohexane (mixed isomers). [Web:] http://www.inchem.org/documents/pims/chemical/pim257.htm. Accessed on 2 December 2009.

Institute for Futures Research. 2009. The state of water in South Africa – are we heading for a crisis? Natural Environment 7 (1). [Web:] http://www.anthonyturton.com/admin/my_documents/my_files/A59_Nat_Env_Apr_2009.pdf. Accessed on 11 August 2009.

Ishaq, R, Persson, N.J., Zebühr, Y. & Broman, D. 2009. PCNs, PCDD/Fs and non-ortho PCBs in water and bottom sediments from industrialized Norwegian Grelandsfjords. *Environmental Science & Technology* 43: 3442 – 3447.

Isosaari, P. 2004. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran contamination of sediments and photochemical decontamination of soils. Finland: University of Kuopio. (Dissertation – PhD). 95 p.

Iwata, H., Tanabe, S., Aramoto, M., Sakai, N. & Tatsukawa, R. 1994. Geographical distribution of persistent organochlorines in air, water and sediment from Asia and Oceania, and their implications for global redistribution from lower latitudes. *Environmental Pollution* 85: 15 – 33.

Jackson, V.A., Paulse, A.N., Bester, A.A., Neethling, J.H., Khan, S. & Khan, W. 2009. Bioremediation of metal contamination in the Plankenburg River, Western Cape, South Africa. *International Biodeterioration & Biodegradation* 63 (5): 559 – 568.

Jarrel, J. & Gocmen, A. 2000. A review of human and sub-human primate toxicity of hexachlorobenzene. *Pure Applied Chemistry* 72: 1015 – 1021.

Jarrel, J., Gocmen, A., Foster, W., Brant, R., Chan, S., & Sevcik, M. 1998. Evaluation of reproductive outcomes in women inadvertently exposed to hexachlorobenzene in southeastern Turkey in the 1950s. *Reproductive Toxicology* 12: 469 – 476.

Kannan, K., Hilscherová, K., Imagawa, T., Yamashita, N., Williams, L.L, & Giesy, J.P. 2001. *Environmental Science and Technology* 35: 441 – 447.

Karickhoff, W.S., Brown, D. & Scott, T.A. 1979. Sorption of hydrophobic pollutants on natural sediments. *Water Research* 3: 241 - 248

Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheenan, D.M., Sinks, T. & Tilson, H.A. 1996. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: A report of the US EPA sponsored workshop. *Environmental Health Perspectives* 104 (4): 715 – 740.

Kayama, F., Horiguchi, H., Jin, Y., Kunisue, T., Tanabe, S., Fukatsu, H., Yamazaki, N., Nakamura, M., Yabushita, H., Fujino, J. & Clark, G. 2003. Dioxin and organochlorine pesticide concentrations of breast milk in China and Japan. *Organohalogen Compounds* 60 – 65: 1 – 4.

Kenaga, E.E. 1980. Predicted bioconcentration factors and soil sorption coefficients of pesticides and other chemicals. *Ecotoxicology & Environmental Safety* 4: 26 – 38.

Khanjani, N. & Sim, M.R. 2006. Reproductive outcomes of maternal contamination with cyclodiene insecticides, hexachlorobenzene and beta-benzene hexachloride. *Science of the Total Environment* 368: 557 – 564.

Khim, J.S., Kannan, K., Villeneuve, D.L., Koh, C.H. & Giesy, J.P. 1999. Characterization and distribution of trace organic contaminants in sediment from Masan Bay, Korea. 1. Instrumental analysis. *Environmental Science and Technology* 33: 4199 – 4205.

Kidd, H. & James, D.R. 1991. The Agrochemicals Handbook. 3rd ed. Cambridge: Royal Society of Chemistry Information Services. p 6 – 8.

Kim, K-S., Lee, S.C., Kim, K-H., Shim, W.J., Hong, S.H., Choi, K.H., Yoon, J.H. & Kim, J-G. 2009. Survey of organochlorine pesticides, PCDD/Fs, dioxin-like PCBs and HCB in sediments from the Han River, Korea. *Chemosphere* 75: 580 – 587.

Kim, M. & O'Keefe, P.W. 2000. Photodegradation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in aqueous solution and in organic solvents. *Chemosphere* 41 (6): 793 – 800.

Knutzen, J., Bjerkeng, B., Naes, K., Schlabach, M., 2003. Polychlorinated dibenzofurans/dibenzo-p-dioxins (PCDF/PCDDs) and other dioxin-like substances in marine organisms from the Grenland fjords, S. Norway, 1975– 2001: Present contamination levels, trends and species specific accumulation of PCDF/PCDD congeners. *Chemosphere* 52: 745 – 760.

Kochany, J. & Maguire, R.J. 1994. Abiotic transformations of polynuclear aromatic hydrocarbons and polynuclear aromatic nitrogen heterocycles in aquatic environments. *Science of the Total Environment* 144: 17 – 31.

Koh, C-H., Khim, J.S., Villeneuve, D.L., Kannan, K. & Giesy, J.P. 2006. Characterization of trace organic contaminants in marine sediment from Yeongil Bay, Korea: 2. Dioxin-like and estrogenic activities. *Environmental Pollution* 142: 48 - 57.

Koh, C-H, Khim, J.S., Villeneuve, D., Kannan, K., Johnson, B.G. & Giesy, J.P. 2005. Instrumental and bioanalytical measures of dioxin-like and estrogenic compounds and activities associated with sediment from the Korean coast. *Ecotoxicology and Environmental Safety* 61: 366 – 379.

Koppe, J.G. & Keys, J. 2001. PCBs and the precautionary principle. (*In* Harremoes, P., Gee, D., MacGarvin, M., Stirling, A., Keys, J., Wynne, B. & Guedes Vaz, S., *eds*. Late lessons from early warnings: The precautionary principle 1896-2000. Environment Issue Report no. 22. Copenhagen: European Environment Agency. p. 64 – 72.)

Koppe, J.G., Pluim, H.J., Olie, K. & van Wijnen, J. 1991. Breastmilk, dioxins and the possible effects on the health of newborn infants. *Science of the Total Environment* 106 (1): 33 – 41.

Krümmel, E.M., Macdonald, R.W., Kimpe,L.E., Gregory-Eaves, I., Demers, M.J., Smol, P., Finney, B. & Blais, J.M. 2003. Aquatic ecology: Delivery of pollutants by spawning salmon. *Nature* 425: 255 – 256.

Kumar, M., Lakshmi, C.V., & Khanna, S. 2008. Biodegradation and bioremediation of endosulfan contaminated soil. *Bioresource Technology* 99: 3116 – 3122.

Lacorte, S., Guillamón, M., Martinez, E., Viana, P. & Barceló, D. 2003. Occurrence and specific congener profile of 40 polybrominated diphenyl ethers in river and coastal sediments from Portugal. *Environmental Science & Technology* 37: 892 – 898.

Lee, K., Tanabe, S. & Koh, C.H. 2001. Distribution of organochlorine pesticides in sediments from Kyeonggi bay and nearby areas, Korea. *Environmental Pollution* 114: 207 – 213.

Lohman, K. & Signeur, S. 2001. Atmospheric fate and transport of dioxins: local impacts. *Chemposphere* 45 (2): 161 – 171.

Lohmann, R., Green, N.J.L. and Jones, K.C. 1999. Detailed studies of the factors controlling atmospheric PCDD/F concentrations. *Environmental Science & Technology* 33: 4440 – 4447.

Lundstedt, S. 2003. Analysis of PAHs and their transformation products in contaminated soil and remedial processes. Umeå: Umeå University, Sweden. (Thesis – PhD.). 55 p.

Mackay, D., Shiu, W.Y. & Ma, K.C. 1992. Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals: Polynuclear aromatic hydrocarbonsk polychlorinated dioxins and dibenzofurans. 2nd ed. Michigan: Lewis Publishers.

Mai, T.A., Doan, T.V., Tarradellas, J., De Alencastro, L.F. & Grandjean, D. 2007. Dioxin contamination in soils of Southern Vietnam. *Chemosphere* 67: 1802–1807.

Malisch, R. & Baum, F. 2007. PCDD/Fs, dioxin-like PCBs and marker PCBs in eggs of peregrine falcons from Germany. *Chemosphere* 67 (9): S1 – S15.

Maltby, E. 1991. Wetland management goals: wise use and conservation. *Landscape and Urban Planning* 20: 9 – 18.

Manoli, E. & Samara, C. 1999. Occurrence and mass balance of polycyclic aromatic hydrocarbons in the Thessaloniki sewage plant. *Journal of Environmental. Quality* 28: 176 - 187.

Mariani, G., Canuti, E., Castro-Jiménez, J., Christoph, E.H., Eisenreich, S.J., Hanke, G., Skejo, H. & Umlauf, G. 2008. Atmospheric input of POPs into Lake Maggoire (Northern Italy): PBDE concentrations and profile in air, precipitation, settling material and sediments. *Chemosphere* 73: S114 – S121.

Masih, A. & Taneja, A. 2006. Polycyclic aromatic hydrocarbons (PAHs) concentrations and related carcinogenic potencies in soil at a semi-arid region of India. *Chemosphere* 65: 449 - 456.

Mastrangelo, G., Fadda, E. & Marzia, V. 1996. Polycyclic aromatic hydrocarbons and cancer in man. *Environmental Health Perspectives* 104: 1166 – 1170.

Masunaga, S., Yao, Y., Ogura, I., Sakurai, T. & Nakanishi, J. 2003. Source and behavior analysis of dioxins based on congener-specific information and their application to Tokyo Bay basin. *Chemosphere* 53: 315 - 324.

Matsumura, F. 1985. Toxicology of Insecticides. 2nd ed. New York: Plenum Press. p. 6-14.

McCant, D.D., Inouye, L.S. & McFarland, V.A. 1999. A One-Step ASE™ Extraction Method for TCDD TEQ Determination. *Environmental Contamination and Toxicology* 63: 282 – 288.

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

McFarland, V.A., McCant, D.D. & Inouye, L.S. 1998. Guidance for performance of the H4IIE dioxin screening assay, Technical Note DOER – C1, Vicksburg, MS.

Meneses, M., Schumacher, M. & Domingo, J.L. 2003. Health risk assessment of emissions of dioxins and furans from a municipal waste incinerator: Comparison with other emission sources. *Environment International* 30: 481 – 489.

Miglioranza, K.S.B., Aizpun de Moreno, J.E. & Moreno, V.J. 2003. Dynamics of organochlorine pesticides in soils form a south-eastern region of Argentina. *Environmental Toxicology and Chemistry* 22 (4): 712 – 717.

Moon, H-B., Lee, S-J., Choi, H-G., & Ok, G. 2005. Atmospheric deposition of polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF) in urban and suburban areas of Korea. *Chemosphere* 58: 1525 – 1534.

Moore, R.W., Potter, C.L., Theobald, H.M., Robinson, J.A. & Peterson, R.E. 1985. Androgenic deficiency in male rats treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Toxicological Applications in Pharmacology* 79: 99 – 110.

Morillo, E., Romero, A.S., Maqueda, C., Madrid, L., Ajmone-Marsan, F., Grcman, H., Davidson, C.M., Hursthouse, A.S. & Villaverde, J. 2007. Soil pollution by PAHs in urban soils: a comparison of three European cities. *Journal of Environmental Monitoring* 9: 1001 – 1008.

Mqoqi, N., Kellet, P., Sitas, F. & Jula, M. 2002. Incidence of histologically diagnosed cancer in South Africa 1998–1999. National Cancer Registry of South Africa. National Health Laboratory Service, Johannesburg, South Africa. [Web:] http://www.cansa.org.za/unique/cansa/files/stats98.pdf. Accessed on 8 February 2009.

Müller, J.F., Haynes, D., McLachlan, M., Böhme, F., Will, S., Shaw, G.R., Mortimer, M., Sadler, R. & Connell, D.W. 1999. PCDDs, PCDFs, PCBs and HCB in marine and esturarine sediments from Queensland, Australia. *Chemosphere* 39 (10): 1707 – 1721.

Nadal, M., Schuhmacher, M. & Domingo, J.L. 2004. Levels of PAHs in soil and vegetation samples from Tarragona Country, Spain. *Environmental Pollution* 132: 1 - 11.

Negri, E. & Vecchia, C. 2001. Epidemiology and prevention of bladder cancer. *European Journal of Cancer Prevention* 10: 7 – 14.

Nel, A., Krause, M. & Khelawanlall, N. 2002. A guide for the control of plant pests. Thecnical advice (Act No. 36/1947), Edition 39. Directorate – Agricultural Production Inputs. Department of Agriculture, Republic of South Africa. Ockenden.

New York State Department of Environmental Conservation. 2004. Does burning trash make it disappear? [Web:] http://www.dec.ny.gov/chemical/32065.html. Accessed on 2 September 2009.

Nieuwoudt, C. 2006. The determination of dioxin-like POPs in sediments and fish of the Vaal Triangle region, Gauteng, South Africa. Potchefstroom: North-West University, South Africa. (Dissertation – M. Env. Sci.). 121 p.

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

Ogutu, C.B.A. 2007. Water demand management options for Cape Metropolitan area – South Africa. Pretoria: Tshwane University of Technology, South Africa. (Dissertation – M. Tech.). 147 p.

Oh, H., Livingston, R., Smith, K. & Abrishamian-Garcia, L. 2004. Comparative Study of the Time Dependancy of Cell Death Assays. *Aptosis Methods in Pharmacology and Toxicology: Approaches to measurement and quantification* 11: 53 – 62.

Ongley, E.D., Yuzyk, T.R. & Krishnapppan, B.G. 1990. Vertical and lateral distribution of fine grained particles in prairie and cordilleran rivers: sampling implications for water quality programs. *Water Research* 24 (3): 303 – 312.

Ontario Ministry of Environment & Energy. 1998. Guidance on site specific risk assessment for use at contaminated sites. Ontoriao Ministry of Environment & Energy: Standards Development Branch. [Web:] http://www.ene.gov.on.ca/../326701e.pdf. Accessed on 2 February 2010.

Pereira, W.E., Hostettler, F.D., Cashman, J.R. & Nishioka, R.S. 1994. Occurrence and distribution of organochlorine compounds in sediment and lovers of striped bass (*Morone saxatilis*) from the San Francisco Bay-delta estuary. *Marine Pollution Bulletin* 28 (7): 434 – 441.

Pies, C., Hoffmann, B., Petrowsky, J., Yang, Y., Ternes, T.A. & Hofmann, T. 2008. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. *Chemosphere* 72: 1594 – 1601.

Pocar, P., Fisher, B., Klonish, T. & Haunbach-Klonish, S. 2005. Molecular interactions of the aryl hydrocarbon receptor and its biological and toxicological relevance to reproduction. *Reproduction* 129: 379 – 389.

Pretorius, P.J., Linder, P.W., Slabbert, J.L. & Wade, P.W. 2001. The chemical behaviour of heavy metals and their bioavailability and toxicity to organisms: implications for environmental quality criteria. *South African Journal of Science* 97: 431 – 434.

Qui, X., Zhu, T., Yoa, B., Hu, J. & Hu, S. 2005. Contribution of Dicofol to the current DDT pollution in China. *Environmental Science and Technology* 39: 4385 – 4390.

Quinn, L.P., Pieters, R., Nieuwoudt, C., Borgen, A.R., Kylin, H. & Bouwman, H. 2009. Distribution profiles of selected organic pollutants in soils and sediments of industrial, residential and agricultural areas of South Africa. *Journal of Environmental Monitoring* 11: 1647 – 1657.

Rappe, C., Andersson, R., Bonner, M., Cooper, K., Fiedler, H., Howell, F., Kulp, S.E. & Lau, C. 1997. PCDDs and PCDFs in soil and river sediment samples from a rural area in the United States of America. *Chemosphere* 34 (5 - 7): 1297 – 1314.

Rissato, S.R., Galhiane, M.S., Ximenes, V.F., de Andrade, R.M.B., Talamoni, J.L.B., Libânio, M., de Almeida, M.V., Apon, B.M. & Cavalari, A.A. 2006. Organochlorine pesticides and polychlorinated biphenyls in soil and water samples in the Northeastern part of São Paulo State, Brazil. *Chemosphere* 65: 1949 – 1958.

Ritter, L., Solomon, K.R. & Forget, J. 2005. Persistent organic pollutants: An assessment report on DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene, mirex, toxaphene, PCBs, dioxins and furans. Report for the International Programme on Chemical Safety (IPCS) within the framework of the Inter-Organization Programme for the Sound Management of Chemicals (IOMC). 44 p.

Rogan, W.J. & Gladen, B.C. 1992. Neurotoxicology of PCBs and related compounds. *Neurotoxicolgy* 13: 27 – 36.

Safe, S.H. 1995. Modulation of gene expression and endocrine response pathways by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds. *Pharmacological Therapy* 67 (2): 247 – 281.

Sagiv, S.K., Tolbert, P.E., Altshul, L.M. & Korrick, S.A. 2007. Organochlorine exposures during pregnancy and infant size at birth. *Epidemiology* 18: 120 – 129.

Sala, M., Ribas-Fitó, N., Cardo, E., de Muga, M.E., Marco, E., Marzón, C., Verdú, A., Grimalt, J.O. & Sunyer, J. 2001. Levels of hexachlorobenzene and other organochlorine compounds in cord blood: exposure across placenta. *Chemosphere* 43 (4 – 7): 895 – 901.

Sánchez-Monedero, M.A., Roig, A., Martínez-Pardo, C., Cegarra, J. & Paredes, C. 1996. A microanalysis method for determining total organic carbon in extracts of humic substances. Relationships between total organic carbon and oxidable carbon. *Bioresource Technology* 57: 291 – 295.

Schade, G. & Heinzow, B. 1998. Organochlorine pesticides and polychlorinated biphenyls in human milk of mothers living in northern Germany: current extent of contamination, time trend from 1986 to 1997 and factors that influence the levels of contamination. *Science of the Total Environment* 215: 31 – 39.

Schantz, S.L., Moshtaghian, J. & Ness, D.K. 1995. Spatial learning deficits in adult rats exposed to ortho-substituted PCB congeners during gestation and lactation. *Fundamental Applied Toxicology* 26: 117 – 126.

Schecter, A., Birnbaum, L., Ryan, J.J. & Constable, J.D. 2006. Dioxins: An overview. *Environmental Research* 101: 419 – 428.

Schecter, A., Piskac, A.L., Grosheva, E.I., Matorova, N.I., Ryan, J.J., Fürst, P., Päpke, O., Adibi, J., Pavuk, M., Silver, A. & Ghaffar, S. 2002. Levels of dioxins and dibenzofurans in breast milk of women residing in two cities in the Irkutsk region of Russian Siberia compared with American levels. *Chemosphere* 47 (2): 157 – 164.

Schramm, K-W., Klimm, C., Hofmaier, A. & Kettrup, A. 2001. Comparison of dioxin-like response: *in vitro* and chemical analysis of emissions and materials. *Chemosphere* 62: 551 – 587.

Schumacher, B.A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments. Las Vegas, ERASC.

Scott, L.L.F., Staskal, D.F., Williams, E.S., Luksemburg, W.J., Urban, J.D., Nguyen, L.M., Haws, L.C., Birnbaum, L.S., Paustenbach, D.J. & Harris, M.A. 2009. Levels of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in southern Mississippi catfish and estimation of potential health risks. *Chemosphere* 74 (7): 1002 – 1010.

SCPOPs. Stockholm Convention on Persistent Organic Pollutants. 2009. Chemicals review process and chemicals under review. [Web:] http://chm.pops.int. Accessed on 12 August 2009.

Sellström, U. & Jansson, B. 1995. Analysis of tetrabromobisphenol A in a product and environmental samples. *Chemosphere* 31: 3085 – 3092.

Sellström, U., Kierkegaard, A., de Wit, C. & Jansson, B. 1998. Polybrominated diphenyl ethers and hexabromocyclododecane in sediment and fish from a Swedish river. *Environmental Toxicology & Chemistry* 17: 1065 – 1072.

Shain, W., Bush, B. & Seegal, R. 1991. Neurotoxicity of polychlorinated biphenyl congeners: Structure-activity relationships of individual congeners. *Toxicology & Applied Pharmacology* 141: 448 – 455.

Sharp, B.L. & Le Sueur, D. 1996. Malaria in South Africa – the past, the present and the selected implications for the future. *South African Medical Journal* 86: 89.

Shegunova, P., Klánová, J. & Holoubek, I. 2007. Residues of organochlorinated pesticides in soils from the Czech Republic. *Environmental Pollution* 146: 257 – 261.

Shimizu, Y., Sato, K., Kim, S., Suzuki, T., Takamatu, M., Nakamura, M., Yabushita, H., Yamamoto, T. & Murata, H. 2003. Behaviour of dioxins and the relevance to soil characteristics in a River Basin of Lake Biva, Japan. *Organohologen Compounds* 61: 377 – 380.

Simpson, C.D., Wilcock, R.J., Smith, T.J., Wilkins, A.L. & Langdon, A.G. 1995. Determination of octanol-water partition coefficients for the major components of technical chlordane. *Bulletin of Environmental Contamination and Toxicology* 55: 149 – 153.

Sinkkonen, S. & Paasivirta, J. 2000. Degradation half-life times of PCDDs, PCDFs and PCBs for environmental fate modelling. *Chemosphere* 40: 934 – 949.

Sittig, M. 1980. Chlordecone & Mirex. (*In* Sittig, M., *ed.* Pesticide Manufacturing and Materials Control Encyclopedia. Noyes Data Corporation. p. 171 – 173.)

Skrbic, B., Cvejanov, J. & Durisic-Mladenovic, N. 2007. Organochlorine pesticides and polychlorinated biphenyls in surface soils of Novi Sad and bank Sediment of the Danube River. *Journal of Environmental Science & Health B* 42 (3): 311 – 319.

Smith, A.G. 1991. Chlorinated hydrocarbon insecticides. (*In* Hayes, W.J., Jr. & Laws, E.R., Jr., *eds*. Handbook of Pesticide Toxicology. New York, Academic Press. p. 6 – 8.)

Smith, J.A., Witkowski, P.J. & Fusillo, T.V. 1988. Manmade organic compounds in the surface waters of the United States - A review of current understanding. U.S. Geological Survey Circular 1007, 92 p.

South African Department of Agriculture. 2008. Banned and restricted substances in the Republic of South Africa. [Web:] http://na.agric.za/act36/Banned%20and%20restricted.htm. Accessed on 8 December 2009.

South African Info. 2009. South African Economy. [Web:] http://www.southafrica.info/business/economy/. Accessed on 1 July 2009.

South African Weather Service. 2009. [Web:] http://www.weathersa.co.za. Meteorological data requested on 10 July 2009. .

Statistics South Africa (StatsSA). 2008. Mid-year population estimates: 2008. Statistical release P0302. [Web:] http://www.statssa.gov.za/publications/P0302/P03022008.pdf. Accessed on 1 July 2009.

Stegeman, J.J., Brouwer, M., Di Guilio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M. & Van Veld, P.A. 1992. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. (*In* Huggett, R.J., Kimerle, R.A., Mehrle, P.M. & Bergman, H.L., *eds.* Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. Florida: Lewis Publishers. p. 235 – 336.)

Storelli, M.M. & Marcotrigiano, G.O. 2003. Levels and congener pattern of polychlorinated biphenyls in the blubber of the Mediterranean bottlenose dolphins *Tursiops truncatus*. *Environment International* 28 (7): 559 – 565.

Suarez, M.P, Rifai, H.S., Palachek, R., Dean, K. & Koenig, L. 2006. Distribution of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in suspended sediments, dissolved phase and bottom sediment in the Houston Ship Channel. *Chemosphere* 62: 417 – 429.

Syracuse. 2007. Syracuse Research Corporation PhysProp database. [Web:] http://www.syrres.com/esc/databases.htm. Accessed on 19 April 2007.

Tanabe, S. 1988. PCB problems in the future: Foresight from current knowledge. *Environmental Pollution* 50: 5 – 28.

Thomsen, V., Schatzlein, D. & Mercuro, D. 2003. Limits of detection in spectroscopy. *Spectroscopy* 18 (12): 112 – 114.

Tysklind, M., Fängmark, I., Marklund, S., Lindskog, A., Thaning, L. & Rappe, C. 1993. Atmospheric transport and transformation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Environmental Science & Technology* 27: 2190 – 2197.

UNEP. United Nations Environment Programme. 2001. Stockholm Convention on Persistent Organic Pollutants. Geneva, Switzerland.

UNEP. United Nations Environment Programme. 2002. GPA Clearing-House Mechanism: Mirex. [Web:] http://www.chem.unep.ch/gpa_trial/17mirex.htm. Accessed on 7 December 2009.

UNEP. United Nations Environment Programme. 2005a. Ridding the world of POPs: A guide to the Stockholm Convention on Persistent Organic Pollutants. Geneva, Switzerland.

UNEP. United Nations Environment Programme. 2005b. Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases. 2nd Ed. Geneva, Switzerland.

US EPA. United States Environmental Protection Agency. 1975. DDT: A review of scientific and economic aspects of the decision to ban its use as a pesticide. Office of Pesticide Programs. Washington, DC. EPA-540/1-75-022.

US EPA. United States Environmental Protection Agency. 1986. Test Methods for Evaluating Solid Waste – Physical/chemical Methods. Method 3660. Office of Solid Waste and Emergency Response. Washington, DC. EPA-SW-846.

US EPA. United States Environmental Protection Agency. 1993. Provisional guidance for quantitative risk assessment of PAH. US Environmental Protection Agency. Washington, DC. EPA/600/R_93/089.

US EPA. United States Environmental Protection Agency. 1994. Method 1613: Tetra-through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. Revision B. Office of Water: Engineering and Analysis Division. Washington, DC. EPA-821-B-94-005.

US EPA. United States Environmental Protection Agency. 1996a. Test methods for evaluating solid waste. Method 3600 C: Cleanup procedures. Revision 3. Office of Solid Waste and Emergency Response. Washington, DC. EPA-SW-846.

US EPA. U.S. Environmental Protection Agency. 1996b. Test Methods for Evaluating Solid Waste. Method 3620 B: Florisil cleanup. Revision 2. Office of Solid Waste and Emergency Response. Washington, DC. EPA/SW-846.

US EPA. United States Environmental Protection Agency. 1999. Method 1568: Chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS. Revision A. Office of Water: Engineering and Analysis Division. Washington, DC. EPA-821-R-00-002.

US EPA. United States Environmental Protection Agency. 2002. Polycyclic Organic Matter. [Web:] http://www.epa.gov/ttn/atw/hlthef/polycycl.htm. Accessed on 13 January 2009.

US EPA. United States Environmental Protection Agency. 2004. Risk assessment guidance for superfund Volume I: Human Health Evaluation Manual (Part E, Supplemental guidance for dermal risk assessment). Office of Superfund Remediation and Technology Innovation. Washington, DC. EPA/540/R/99/005.

US EPA. United States Environmental Protection Agency. 2008a. Persistent Bioaccumulative and Toxic (PBT) Chemical Program: Dioxins and furans. [Web:] http://www.epa.gov/pbt/pubs/dioxins.htm. Accessed on 15 September 2009.

US EPA. United States Environmental Protection Agency. 2008b. Method for toxic equivalents (TEQs) determinations for dioxin-like chemical activity with the CALUX® bioassay. Method 4435. Revision 0. Office of Solid Waste and Emergency Response. Washington, DC. EPA-SW-846.

Van Aardt, W.J. & Erdmann, R. 2004. Heavy metals (Cd, Pb, Cu, Zn) in mudfish and sediments from three hard-water dams of the Mooi River catchment, South Africa. *Water SA* 30 (2): 211 – 215.

Vanderperren, H., Van Wouwe, N., Behets, S., Windal, I., Van Overmeire, I. & Fontaine, A. 2004. TEQ-value determinations of animal feed; emphasis on the CALUX bioassay validation. *Talanta* 63: 1277 – 1280.

Van Ert, M. & Sullivan, J.B. 1992. Organochlorine pesticides. (*In* Sullivan, J.B. & Krieger, G.R., *eds*. Hazardous Materials Toxicology, Clinical Principles of Environmental Health. Baltimore, MD: Williams & Wilkins.)

Van Wouwe, N., Windal, I., Vanderperren, H., Eppe, G., Xhrouet, C., Massart, A-C., Debacker, N., Sasse, A., Baeyens, W., De Pauw, E., Sartor, F., Van Oyen, H. & Goeyens, L. 2004. Validation of the CALUX bioassay for PCDD/F analyses in human blood plasma and comparison with GC–HRMS. *Talanta* 63: 1147 – 1157.

Verschueren, K. 1983. Mirex & Kepone. (*In* Verschrueren, K. *ed.* Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Company. p. 787 – 789.)

Villeneuve, D.L., Blankenship, A.L. & Giesy, J.P. 2000. Derivation and application of relative potency estimates based on in vitro bioassay results. *Environmental Toxicology & Chemistry* 19 (11): 2835 – 2843.

Villeneuve, D.L., Richter, C.A., Blankenship, A.L. & Giesy, J.P. 1999. Rainbow trout cell bioassay-derived relative potencies for halogenated aromatic hydrocarbons: comparison and sensitivity analysis. *Environmental Toxicology and Chemistry* 18 (5): 879 – 888.

Vistica, D.T., Skehan, P., Scudiero, D., Monks, A., Pittman, A. & Boyd, M.R. 1991. Tetrazolium-based assays for cellular viability – a critical-examination of selected parameters affecting formazan production. *Cancer Research* 51: 2515 - 2520.

Vondráček, J., Machala, M., Minsková, K., Blácha, L., Murk, A.J., Kozubík, A., Hovmanova, J., Hilscherová, K., Ulrich, R., Ciganek, M., Neča, J., Švrčková D. & Holoubek, I. 2001. Monitoring river sediments contaminated predominantly with polyaromatic hydrocarbons by chemical and *in vitro* bioassay techniques. *Environmental Toxicology and Chemistry* (20)7: 1499 - 1506.

Vosloo, R. & Bouwman, H. 2005. Survey of certain persistent organic pollutants in major South African waters. WRC Report no. 1213/1/05. Report to the Water Research Commission, South Africa.

Wania, F. & MacKay, D. 1993. Global fractions and cold condensation of low volatility organochlorine compounds in polar regions. *Ambio* 22: 10 – 18.

Watanabe, I. & Sakai, S. 2003. Environmental release and behaviour of brominated flame retardants. *Environment International* 29: 665 – 682.

Watanabe, I. & Tatsukawa, R. 1990. Anthropogenic brominated aromatics in the Japanese environment. Proceedings of the Workshops on Brominated Flame Retardants. Stockholm, Sweden: National Chemicals Inspectorate (KEMI): 63 – 71.

Wauchope, R.D., Buttler, T.M., Hornsby, A.G., Augustijn Beckers, P.W.M. & Burt, J.P. 1992. Pesticide properties database for environmental decision making. *Reviews in Environmental Contamination & Toxicology* 123: 1 – 157.

Weissenfels, W.D., Klewer, H.J. & Langhoff, J. 1992. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles – influence on biodegradability and biotoxicity. *Applied Microbiological Biotechnology* 36: 689 – 696.

Wenning, R.J., Harris, M.A., Ungs, M.J., Paustenbach, D.J. & Bedburry, H. 1992. Chemometric comparisons of polychlorinated dibenzo-p-dioxin and dibenzofuran residues in surficial sediments form Newark Bay, New Jersey and other industrialized waterways. *Archives of Environmental Contamination and Toxicology* 22: 397 - 413.

Wevers, M., De Fré, R. & Desmedt, M. 2004. Effect of backyard burning on dioxin depositon and air concentrations. *Chemosphere* 54: 1351 – 1356.

WHO. World Health Organization. 1979. DDT and its derivatives: Environmental Health Criteria. Vol. 9. Geneva, Switzeraland.

WHO. World Health Organization. 1997. Derivation of toxic equivalency factors (TEFs) for dioxin-like compounds in humans and wildlife. *Organohalogen Compounds* 34: 237 – 240.

WHO. World Health Organization. 1998. Environmental Health Criteria 205. Polybroninated dibenzo-*p*-dioxins and furans. International Programme on Chemical Safety, World Health Organization. Geneva, Switzerland.

WHO. World Health Organization. 2006. Concise International Chemical Assessment Document 70: Heptachlor. International Programme on Chemical Safety, World Health

Organization. Geneva, Switzerland. ISBN 9241530707. [Web:] http://www.who.int/ipcs/publications/cicad/cicad70.pdf. Accessed on 4 December 2009.

Whylie, P., Albaiges, J., Barra, R., Bouwman, H., Dyke, P., Wania, F. & Wong, M. 2003. Regionally based assessment of persistent toxic substances: Global report. UNEP Chemicals/GEF. Geneva, Switzerland.

Whyte, J.J., Schmidt, C.J. & Tillit, D.E. 2004. The H4IIE bio-assay as an indicator of dioxin-like chemicals in wildlife and the environment. *Critical reviews in Toxicology* 34 (1): 1 – 83.

Wilcke, W. 2007. Global patterns of polycyclic aromatic hydrocarbons (PAHs) in soil. *Geoderma* 141: 157 - 166.

Wilcke, W., Zech W. & Kobza, J. 1996. PAH-pools in soils along a PAH-deposition gradient. *Environmental Pollution* 92 (3): 307 – 313.

Wild, S.R. & Jones, K.C. 1995. Polynuclear aromatic hydrocarbons in the United-Kingdom environment – a preliminary source inventory and budget. *Environmental Pollution* 88: 91 – 108.

Willet, K.L., Ulrich, E.M. & Hites, R.A. 1998. Differential toxicity and environmental fates of hexachlorocyclohexane isomers. *Environmental Science & Technology* 32: 2197 – 2207.

Wilson, S.C. & Jones, K.C. 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs) – a review. *Environmental Pollution* 81: 229 – 249.

Wisconsin Department of Natural Resources, 2003. Consensus-based Sediment Quality Guidelines: Recommendations for Use and Application. Contaminated Sediment Standing Team. Washington, DC. WT-732 2003.

Witkowski, P.J., Smith, J.A., Fusillo, T.V. & Chiou, C.T. 1987. A review of surface-water sediment fraction interactions with persistent manmade organic compounds. U.S. Geological Survey, Denver, CO, p. 39.

Wittich, R-M. 2004. Degradation of dioxin-like compounds by microorganisms. *Applied Microbiology and Biotechnology* 49 (5): 489 – 499.

Wong, F., Robson, M., Diamond, M.L., Harrad, S. & Truong, J. 2009. Concentrations and chiral signatures of POPs in soils and sediments: A comparative urban versus rural study in Canada and UK. *Chemosphere* 74: 404 – 411.

Woodwell, G., Craig, P. & Johnson, H. 1971. DDT in the biosphere: Where does it go? *Science* 174: 1101 – 1107.

World Bank. 2009. Africa: Data and Statistics. [Web:] http://www.worldbank.org. Accessed on 1 July 2009.

Wu, W.Z., Schramm, K.-W., Henkelmann, B., Xu, Y., Yediler, A. & Kettrup, A. 1997. PCDD/Fs, PCBs, HCHs and HCB in sediments and soil of Ya-Er Lake area in China: Results on residual levels and correlation to the organic carbon and the particle size. *Chemosphere* 34 (1): 191 – 202.

Yang, R., Jiang, G., Zhou, Q, Yuan, C & Shi, J. 2005. Occurrence and distribution of organochlorine pesticides (HCH and DDT) in sediments collected from East China Sea. *Environment International* 31 (6): 799 – 804.

Yang, R., Lv, A., Shi, J. & Jiang, G. 2005. The levels and distribution of organochlorine pesticides (OCPs) in sediments from the Haihe River, China. *Chemosphere* 61 (3): 347 – 354.

Yang, Z., Shen, Z., Gao, F., Tang, Z., Niu, J. & He, Y. 2009. Polychlorinated biphenyls in urban lake sediments from Wuhan, Central China: Occurrence, composition and sedimentary record. *Journal of Environmental Quality* 38: 1441 – 1448.

Yoo, H., Khim, J.S. & Giesy, J.P. 2006. Receptor-mediated in vitro bioassay for chraracterization of Ah-R-active compounds and activities in sediment from Korea. *Chemosphere* 62: 1261 – 1271.

Yoon, J., Choi, K., Kim, S., Kim, E., Kim, E., Jeon, S-H. & Na, J-G. 2004. Concentration levels of endocrine disrupting chemicals in environmental media of Korea. *Organohalogen Compounds* 66: 1436 – 1440.

Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D. & Sylvestre, S. 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Organic Geochemistry* 33: 489 - 515.

Zegers, B.N., Lewis, W.E., Booij, K., Smittenberg, R.H., Boer, W., De Boer, J. & Boon, J.P. 2003. Levels of polybrominated diphenyl ether flame retardants in sediment cores from western Europe. *Environmental Science & Technology* 37: 3803 – 3807.

Zeng, E., Yu, C. & Tran, K. 1999. In situ measurements of chlorinated hydrocarbons in the water column off the Palos Verdes Peninsula, California. *Environmental Science & Technology* 33: 392 – 398.

Zhang, Y. & Tao, S. 2009. Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmospheric Environment* 43: 812 - 819.

Zhu, Y., Liu, H., Xi, Z., Cheng, H. & Xu, X. 2005. Organochlorine pesticides (DDTs and HCHs) in soils from the outskirts of Beijing, China. *Chemosphere* 60: 770 – 778.