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An assessment of dioxins, dibenzofurans and PCBs in the sediments of selected freshwater bodies and estuaries in South Africa

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- my Creator, through whom anything is possible, because "Unless the Lord builds the house, those who build it labour in vain. Unless the Lord watches over the city, the watchman stays awake in vain." (Ps 127:1)

ABSTRACT

An assessment of dioxins, dibenzofurans and PCBs in the sediments of selected freshwater bodies and estuaries in South Africa.

Persistent organic pollutants (POPs) are a threat to the environment and human health because they are ubiquitous, resistant to degradation, can bio-accumulate in organisms and bio-magnify in food chains. They have a detrimental effect on the reproductive, nervous and immunity systems of vertebrates.

An international treaty, the Stockholm Convention on POPs, came into force in 2004 and aims to limit and eventually prohibit any use and unintentional production of POPs. South Africa ratified the Convention in 2002.

Those compounds currently listed by the Stockholm Convention as POPs include chlorinated pesticides such as dichlorodiphenyltrichlorethane (DDT), chlordane and dieldrin, and industry-related compounds such as polychlorinated biphenyls (PCBs) and hexachlorobenzene. Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are also regarded as POPs but - together with some PCBs - they are the unintentional result of anthropogenic activity.

This study focussed on the PCDDs, PCDFs and dioxin-like PCBs in the aquatic environment of South Africa particularly because the water resources in this country are under pressure. Despite the fact that South Africa has the sources of these compounds, little is known about the levels of these three groups of compounds.

The concentration of twelve dioxin-like PCBs, seven PCDDs and ten PCDFs were determined for 22 sites selected on the grounds of their proximity to possible pollution sources. Analytical determinations included gas chromatography/mass spectrometry and a cell-based bio-assay, the H4IIE-*luc* reporter gene assay. Possible sources of the observed pollution were inferred using the following statistical investigative methods: principal component and hierarchical cluster analysis.

Seven of the sites had levels higher than the threshold effect concentration of Canada's sediment quality guidelines of 0.85 ngTEQ kg⁻¹ (Toxic Equivalency Quotient). The other sites had lower levels. The highest concentration, 17.8 ng TEQ kg⁻¹, was measured at a site in the southern Gauteng Province.

Most of the PCDD/F pollution seemed to have come from combustion sources related to human activity, rather than industrial combustion. Most of the dioxin-like PCB pollution seemed to have been from commercial PCB preparations.

Future research would require better characterisation of the sources in order to reduce the formation of these compounds, but also to better understand the exposure and risk scenarios, if humans are to be in close contact with these sources.

Key words: co-planar PCBs; H4IIE-*luc* bio-assay; PCDD/Fs; sediment; South Africa; TEQ

OPSOMMING

'n Onderzoek na dioksiene, dibensofurane en PCBs in die sediment van uitgesoekte varswatermassas en estuariums in Suid-Afrika.

Persisterende organiese besoedelstowwe (POBs) is 'n bedreiging vir die mens en sy omgewing omdat hulle alomteenwoordig is, afbreekprosesse teenstaan, in organismes kan bioakkumuleer en in voedselkettings ophoop. Hulle het 'n nadelige effek op die voorplanting-, senuwee- en immuuniteitsstelsels van vertebrate.

'n Internasionale ooreenkoms, die Stockholmkonvensie vir POBs, het in 2004 van krag geword. Die mikpunte van die Konvensie is om die gebruik en die nuwe-vervaardiging van POBs te beperk en uiteindelik te staak. Suid-Afrika het die Konvensie in 2002 goedgekeur.

Verbindings wat tans deur die Stockholmkonvensie gelys word, sluit gechlloreerde plaagdoders (dichloro-difeniel-trichlooretaan (DDT), chlorodaan en dieldrin) asook industrie-verwante verbindings (poligechlloreerde bifeniële (PCBs) en heksachloro-benseen) in. Poligechlloreerde dibenso-*p*-dioksiene (PCDDs) en poligechlloreerde dibensofurane (PCDFs) word ook as POBs beskou, maar saam met sekere PCBs is hulle nuweprodukte van menslike aktiwiteit.

Hierdie studie het op die PCDDs, PCDFs en dioksien-agtige PCBs in die akwatiese omgewing van Suid-Afrika gefokus omdat die waterbronne onder druk verkeer. Ondanks die feit dat Suid-Afrika bronne van hierdie stowwe het, is baie min oor die vlakke van hierdie drie groepe verbindings bekend.

Die konsentrasies van twaalf dioksien-agtige PCBs, sewe PCDDs en tien PCDFs is by 22 plekke bepaal. Die plekke is, op grond van hulle ligging, naby moontlike bronne gekies. Analitiese metings is deur middel van gaschromatografie/massa spektrofotometrie en 'n selgebaseerde bio-siftingstoets, die H4IIE-*luc*-geenrapporteringstoets, gedoen. Statistiese ondersoekmetodes (hoofkomponent faktoranalise en hiërargiese trosontleding) is aangewend om die moontlike oorsprong vas te stel.

Sewe plekke het vlakke hoër as die drumpel-effek-konsentrasie van $0.85 \text{ ngTEK kg}^{-1}$ (Toksiese Ekwivalentkwosiënt) vir Kanada se sedimentkwaliteitsriglyn, gehad. Die ander plekke se vlakke was laer. Die hoogste konsentrasie, $17.8 \text{ ngTEK kg}^{-1}$, is by 'n plek in die suidelike Gautengprovinsie gemeet.

Verbrandingsprosesse wat met die mens se leefstyl verband hou, en nie industriële verbranding nie, het die meeste tot die PCDD/F-besoedeling bygedra. Die meeste van die PCB-besoedeling is waarskynlik te wyte aan kommersiële bereidings van PCBs.

Toekomstige navorsing behoort op verbeterde karakterisering van die besoedelingsbronne te fokus, sodat die produksie van die stowwe verminder kan word, maar ook vir meer insig van die moontlike blootstelling van die mens in die nabyheid van die bronne.

Trefwoorde: H4IIE-*luc* bio-siftingstoets; ko-planêre PCBs; PCDD/Fs; sediment; Suid-Afrika; TEK

Abbreviations and acronyms

AET	apparent effects threshold
AHH	aryl hydrocarbon hydroxylase
AhR	aryl hydrocarbon receptor
AMAP	Arctic Monitoring and Assessment Programme
AMP	adenosine monophosphate
Arnt	AhR nuclear translocator
ATP	adenosine triphosphate
ASP	African Stockpiles Programme
bHLH	basic helix-loop-helix (proteins)
CAIA	Chemical and Allied Industries Association
CEG	Criteria Expert Group
COP	Conference of Parties
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichlorethane
DEAT	Department of Environmental Affairs and Tourism
DMEM	Dulbecco's Modified Eagle's Medium
DRE	dioxin response elements
d.w.	dry weight
DWAF	Department of Water Affairs and Forestry
EROD	ethoxyresorufin-O-deethylase
EC50	concentration needed to elicit 50% response
EC DG Environment	European Commission Directorate General Environment
EIA	Energy Information Administration
EU	European Union
FBS	foetal bovine serum
GC/MS	gas chromatography and mass spectrometry
GEF	Global Environment Facility
GMP	Global Monitoring Programme
HCA	hierarchical cluster analysis
HCB	hexachlorobenzene
HCH	hexachlorohexane
HLH	helix-loop-helix (proteins)
hsp	heat shock protein
IEAs	International Environmental Agreements
IFCS	Intergovernmental Forum on Chemical Safety
INC	Intergovernmental Negotiating Committee
IOM	Institute of Medicine
IPEN	International POPs Elimination Network
I-TEF	NATO TEFs
IUPAC	International Union of Pure and Applied Chemistry
K_{AW}	air/water partition coefficient
K_{OA}	octanol/air partition coefficient

K_{ow}	octanol/water partition coefficient
LD ₅₀	median lethal dose
LOD	limit of detection
LOQ	limit of quantification
LRT	long range transport
<i>luc</i>	luciferase gene
MEC	minimal effect concentration
MMTV	mouse mammary tumor virus
mRNA	messenger ribonucleic acid
NATO/CCMS	North Atlantic Treaty Organisation Committee on the Challenges of Modern Society
n.d.	non-detect
NIP	National Implementation Plan
NOAA	National Oceanic and Atmospheric Administration
%OC	percentage organic carbon
%OXC	percentage oxidisable carbon
PAHs	polyaromatic hydrocarbons
PBDE	polybrominated diphenyl ether
PBS	phosphate buffered saline
PCA	principal component analysis
PCB(s)	polychlorinated biphenyl(s)
PCDD(s)	polychlorinated dibenzo- <i>p</i> -dioxin(s)
PCDDF(s)	polychlorinated dibenzofuran(s)
PCP	pentachlorophenol
PEC	probable effect concentration
PFOS	perfluorooctane sulphonates
PHAH	polyhalogenated aromatic hydrocarbons
POP(s)	persistent organic pollutant(s)
REP	relative effects potencies
RLUs	relative light units
SADC	Southern African Development Community
SAF	South African Forestry
SQG	sediment quality guidelines
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TCDD-EQ	TCDD equivalency
TEC	threshold effect concentration
TEF	toxic equivalent factor
TEQ	toxic equivalency quotient
TNF	tumour necrosis factor
$t_{1/2}$	half-life time
UN ECE	United Nations Economic Commission for Europe
UNEP	United Nations Environment Programme
USA	United States of America
US EPA	United States Environmental Protection Agency

WHO WRC w.w.	World Health Organisation Water Research Commission wet weight
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TABLE OF CONTENTS

	p
Acknowledgements	i
Abstract	ii
Opsomming	iii
Abbreviations and acronyms	iv
Table of contents	vii
1 INTRODUCTION	1
1.1 Persistent organic pollutants (POPs)	1
1.2 The Stockholm Convention	2
1.3 Deleterious effects of PCDD/Fs and co-planar PCBs	2
1.4 Sources of PCDD/Fs and dioxin-like PCBs	3
1.5 Motivation and aims of this study	4
2 LITERATURE REVIEW	6
2.1 Introduction	6
2.2 The Stockholm Convention	6
2.2.1 History	7
2.2.2 Aims	8
2.3 Characteristics, sources, fate and transport of dioxin-like PCBs and PCDD/Fs	9
2.3.1 Sources and formation	9
2.3.2 Physicochemical characteristics of PCBs	14
2.3.3 Physicochemical characteristics of PCDD/Fs	17
2.4 Environmental fate	18
2.4.1 Long range transport (LRT)	18
2.4.1.1 LRT via atmosphere	18
2.4.1.2 LRT via water	20
2.4.1.3 LRT via migratory animals	21
2.4.2 Fate in environmental compartments	21
2.4.3 In the food chain	23

2.5	Convention of reporting toxicological effects of environmental PCDD/F and PCBs	25
2.6	Environmental levels of PCDD/Fs and PCBs: A global perspective (<i>excluding</i> southern Africa)	27
2.6.1	In air	27
2.6.2	In freshwater	28
2.6.3	In oceans	28
2.6.4	In soils	29
2.6.5	In freshwater sediments	30
2.6.6	In marine sediments	31
2.7	Environmental levels of PCDD/Fs and PCBs: Southern Africa	32
2.8	Health effects of PCDD/Fs and dioxin-like PCBs	35
2.9	Mechanism of dioxin toxicity	38
2.10	Mechanisms for detecting and quantifying PCDD/Fs and dioxin-like PCBs	41
2.10.1	Chemical analysis vs biological analysis	41
2.10.2	The H4IIE bio-assay	42
2.11	In summary	44
3	MATERIAL AND METHODS	46
3.1	Site selection	46
3.2	Sample collection	51
3.3	Sample processing	51
3.3.1	Gas chromatography and mass spectrometry	52
3.3.2	H4IIE- <i>luc</i> reporter gene bio-assay	52
3.3.2.1	Sediment extraction and clean-up	52
3.3.2.2	Bio-assay	53
3.3.3	Particle size composition	55
3.3.4	Determination of organic carbon in the sediments	55
3.4	Statistics	56
3.4.1	Multivariate data analysis of pollutant profiles	58
3.4.1.1	Data transformations and manipulations	58

3.4.1.2	Principal component analysis	59
3.4.1.3	Hierarchical cluster analysis	61
4	RESULTS	63
4.1	Magnitude of pollution	64
4.1.1	Results from chemical analysis	64
4.1.2	Sediment characteristics	68
4.1.3	Results from the H4IIE- <i>luc</i> reporter gene bio-assay	70
4.1.4	Comparison between chemical- and bio-analysis	70
4.2	Nature of pollution	72
4.2.1	Comparison of the northern hemisphere and South African PCDD/F profiles	72
4.2.2	PCDD/F profiles of South African sites only	90
4.2.3	PCB profiles of South African sites	96
4.2.4	PCDD/Fs and PCBs of South African sites	100
4.2.5	Effect of sediment characteristics on PCA	106
5	DISCUSSION	122
5.1	Sediment characteristics and sediment quality guidelines	122
5.2	Comparing South African sediment PCB and PCDD/F levels to others	125
5.2.1	Coastal water bodies	126
5.2.2	Interior water bodies	126
5.3	Evaluation of the H4IIE bio-assay results	130
5.4	Nature of pollution	132
5.4.1	PCDD/F pollution	133
5.4.2	Dioxin-like PCBs	138
5.5	Effect of sediment characteristics	139
5.6	In summary	140
6	CONCLUSION	142
6.1	Levels, risk and sources	142
6.2	Maintaining perspective	144

6.3	The way forward for South Africa	145
6.4	The way forward for this research in South Africa	146
7	REFERENCES	148

1 INTRODUCTION

1.1 Persistent organic pollutants (POPs)

Synthetic or petroleum-derived organic chemicals are major precursors for the production of the many plastic products, drugs, pesticides, and speciality chemicals used for computational and informational equipment. All of these contribute to the high standard of living and health in developed countries. However, many of these chemicals have halogenated aromatic rings in common, have been identified in the environment, and some are now classified as POPs (Vallack, Bakker, Brandt, Broström-Lundén, Brouwer, Bull, Gough, Guardans, Holoubek, Jansson, Koch, Kuylensstierna, Lecloux, Mackay, McCutcheon, Mocarelli & Taalman, 1998).

Environmental problems associated with POPs are related to their physicochemical properties, namely chemical stability and lipophilicity. After their introduction into the environment, most POPs are stable and resistant to chemical- and bio-degradation. The pesticide dichlorodiphenyltrichlorethane (DDT) and its major metabolite dichlorodiphenyl-dichloroethylene (DDE) were among the first POPs identified in environmental samples and, in 1996, polychlorinated biphenyl (PCBs) mixtures were identified in extracts from environmental, wildlife and human samples. POPs that have been produced for various industrial applications include: DDT, PCBs, polychlorinated naphthalenes, terphenyls and diphenyl ethers, polybrominated biphenyls and diphenylethers, organochlorine pesticides such as toxaphene, chlorinated cyclodiene-derived compounds such as dieldrin, endrin and endosulphan; and lindane congeners. Furthermore, the combustion of organic material containing chlorine can also result in the formation of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDDFs) (Safe, 2003).

Because of their chemical characteristics for preferring lipids (in tissues) and organic carbon in soils and sediments (Fiedler, 2003) and their resistance to degradation, these compounds bio-accumulate in organisms and bio-magnify in the food chain (Gobas, 1993). They can also be transported over long distances through air (UNEP, 2003), by ocean currents (Dachs, Bayona, Fowler, Miquel & Albaigés, 1996) and rivers and through migratory animals (Wania, 1998). Birds from the Antarctic and sub-Antarctic (Luke & Johnstone, 1989) and polar bears (Bernhoft, Wiig & Skaare, 1997) were consistently found to contain levels of POPs in their tissues. Soils and sediments are the ultimate sinks for POPs and receive inputs via various pathways including atmospheric deposition as well as from industrial and domestic used water (European Commission DG Environment, 1999).

1.2 The Stockholm Convention

Aware of the fact that persistent organic pollutants pose major and increasing threats to human health and the environment, the governing council of the United Nations Environment Programme (UNEP) requested in May 1995 that an international assessment process be undertaken of an initial list of twelve POPs (aldrin, chlordane, DDT, dieldrin, chlorinated dioxins, endrin, chlorinated furans, hexachlorobenzene (HCB), heptachlor, mirex, PCBs and toxaphene). In June 1996 the Intergovernmental Forum on Chemical Safety (IFCS) concluded that the available information was sufficient to demonstrate the need for international action on the twelve POPs and that a global legally binding instrument was required to reduce the risks to human health and the environment arising from the release of the twelve POPs (Stockholm Convention, 2005). This legally binding instrument now exists in the form of an international convention, namely the Stockholm Convention on Persistent Organic Pollutants that came into force in May 2004. Several countries are parties to the Convention, including South Africa - which ratified the Convention in September 2002 (Stockholm Convention, 2007).

Parties to the Convention are obliged to "reduce or eliminate releases from intentional production and use". South Africa has banned the use, production and import of the pesticides, except for DDT, which is still used for malaria control (Bouwman, 2004). Production of PCBs has been banned since the 1980's, but old transformers with PCB containing oil are still operational and will only be replaced when they break down. For most of the POPs listed in the Stockholm Convention, South Africa has some measures in place to curb or eliminate the release into the environment (Bouwman, 2004). However, a group of chemicals produced unintentionally by industrial and non-industrial combustion and incineration processes - the PCDDs and PCDFs (PCDD/Fs), as well as co-planar PCBs - are mostly still unknown entities in South Africa (Bouwman, Maboeta, Vosloo & Bester, 2002).

1.3 Deleterious effects of PCDD/Fs and co-planar PCBs

The most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), became well known as a contaminant of Agent Orange herbicide used in the Vietnam War (IOM, 2005). In certain laboratory animals and wildlife species, dioxin can cause death following even tiny doses, leading TCDD to be called "the most toxic man-made chemical". Its toxicity varies from species to species. The median lethal dose (LD₅₀) for guinea pigs is ~ 1 µg kg⁻¹ body weight, but ~ 1 000 µg kg⁻¹ body weight for hamsters. The LD₅₀ is not known for man, but from the results of poisoning episodes it is clearly higher than for guinea pigs (Schechter, Birnbaum, Ryan & Constable, 2006). PCDDs were found in Seveso, Italy, after an

industrial explosion in 1976 (Bertazzi & Di Domenico, 2003) and PCDFs and PCBs were involved in rice oil poisoning incidents in Japan in 1968, known as "Yusho" (Masuda, 2003) and an almost identical event in Taiwan in 1979 known as "Yucheng" (Guo, Yu & Hsu, 2003).

The most significant health threat of PCDD/Fs and PCBs are not the occasional poisoning incidents, but rather insidious, chronic exposure to low levels. Because of their cumulative effects, these compounds gradually accumulate in the body, slowly creating a variety of adverse effects. While there is a great deal of species variability in the lethal dose of dioxins, other adverse effects (such as developmental toxicity) occur in similar doses in multiple vertebrate species (Schechter *et al.*, 2006). Schechter *et al.* (2006) list a variety of effects of dioxin in humans and other vertebrates reported in literature: risk factors for cancer, immune deficiency, reproductive and developmental abnormalities, central and peripheral nervous system pathology, endocrine disruption including diabetes and thyroid disorders, decreased pulmonary functions and bronchitis; altered serum testosterone level; eyelid pathology; nausea, vomiting, loss of appetite, liver damage, and more.

One important molecular mechanism of action of PCDD/Fs and co-planar (dioxin-like) PCBs appears to be receptor-based, involving the aryl hydrocarbon receptor (AhR). When entering cells, compounds like dioxins bind with high affinity to the cytosolic AhR protein, which then undergoes a process of activation and moves to the nucleus where the liganded-AhR is bound to dioxin response elements (DRE) on the DNA. This results in the expression of enzymes such as CYP1A1/2. The induction of these enzymes leads to the observed toxic effects (Vallack *et al.*, 1998).

1.4 Sources of PCDD/Fs and dioxin-like PCBs

PCBs were used in transformers, as flame retardants, and as hydraulic fluids until their use and production were banned in the 1970's. They are, however, still present in the environment because of possible leaking from decommissioned equipment not properly disposed of and former spills. They are also, together with PCDD/Fs, produced unintentionally as by-products from a wide variety of industrial-chemical processes such as chemical manufacture and thermal processes such as waste incineration (UNEP, 2004). Examples of PCDD/F and dioxin-like PCB producing processes include: waste incineration (hazardous, municipal, medical and sewage sludge), ferrous and non-ferrous metal production (iron ore sintering, copper, aluminium, lead, zinc and magnesium production), power generation and heating using fossil fuels and biomass, production of mineral products (cement, lime and bricks), transport (diesel and heavy oil-fired engines), waste burning and accidental fires, paper and pulp production, textile production and leather

refining, crematoria, tobacco smoking, landfills and waste dumps, production sites of chlorine and chlorinated organics, and timber manufacture and treatment sites (UNEP, 2005).

South Africa has most of the sources listed in the previous paragraph, but little is known about the levels of these particular compounds in the environmental matrices, biota, and human population.

1.5 Motivation and aims of this study

In a review paper by Lohman, Breivik, Dachs and Muir (2007) on the global fate of POPs, they note that traditionally, PCBs and PCDD/Fs displayed much higher emissions and concentrations in the northern hemisphere, because this hemisphere encompasses most industrialised nations, and accounts for the majority of the earth's population, but major shifts are underway. The southern hemisphere will increasingly experience higher levels of POPs and one of the reasons is the increase of low temperature combustion processes for Asia, Africa and South America where most of biomass burning and forest fires occur. Many currently used pesticides are increasingly used globally, reflecting their major use in agricultural areas in South America, Africa and Asia.

In the light of this and the fact that South Africa is party to the Stockholm Convention and has to commit to its obligations, research on POPs is a necessity. Very little is known about the unintentionally produced PCDD/Fs and dioxin-like PCBs in the South African environment and population. South Africa is a water-stressed country where water planners and managers are faced with increasingly complex issues. Since South Africa's water resources are, in global terms, scarce and extremely limited (DWAF, 2004) and must therefore be managed well and protected, the Water Research Commission (WRC) of South Africa funded a research project in 2000 to determine the levels of the most toxic congeners of the PCDD/Fs and the dioxin-like PCBs (seven PCDDs, ten PCDFs, and twelve dioxin-like PCBs) in the aquatic environment of the country. One of the outcomes of the afore mentioned WRC project is this PhD thesis.

The sites that were selected included freshwater and marine sites: dams, rivers, harbours and river mouths. The sites were selected because of their proximity to industrial areas and therefore expected PCDD/F and dioxin-like PCB pollution.

Although chemical analysis for pesticide POPs is done in South Africa, there is no accredited laboratory that analyses for PCDD/Fs and dioxin-like PCBs. Having this type of analysis done abroad is a very expensive exercise. One of the aims of this research was to evaluate the *H4IIE-luc* reporter gene bio-assay as a useful, less expensive screening

tool, with which semi-quantitative dioxin toxicity of environmental matrices can be determined. Results from this assay are of such a nature that it can be used in risk assessment.

The research hypothesis is that PCDD/Fs and dioxin-like PCBs occur in the aquatic environment of South Africa. Therefore the presence, levels and implications in the natural environment are investigated.

To test this hypothesis the following aims are identified:

- determine the levels of PCDD/Fs and dioxin-like PCBs in the aquatic sediments of 22 different locations in South Africa;
- determine possible risks by comparing the measured levels to international sediment quality guidelines;
- determine the possible sources of the observed dioxin pollution with statistical methods (multivariate analysis); and
- evaluate the applicability of a cell-based bio-assay as a screening tool in conjunction with the standard chemical analysis techniques to determine dioxin toxicity levels.

2 LITERATURE REVIEW

2.1 Introduction

"The most alarming of all man's assaults upon the environment is the contamination of air, earth, rivers, and sea with dangerous and even lethal materials. This pollution is for the most part irrecoverable; the chain of evil it initiates not only in the world that must support life but in living tissues is for the most part irreversible. In this now universal contamination of the environment, chemicals are the sinister and little-recognized partners of radiation in changing the very nature of the world - the very nature of its life." (Carson, 1962)

Rachel Carson is hailed as one of the very first people to give the environment a voice and who widely propagated the disastrous effects that manmade chemicals have on the health of wildlife and humans. A group of these chemicals are called persistent organic pollutants.

In this chapter, the origins of the global treaty to curb a group of particularly detrimental compounds, persistent organic pollutants, are described. South Africa's relation to this treaty and how it influenced this research, are discussed. The physical and chemical properties of the organic pollutants and how these characteristics influence their distribution and toxic effects are also described. The levels found in different environmental matrices from countries around the globe, are presented to give perspective to the levels from South Africa. The processes and sources known to produce persistent organic pollutants are discussed extensively to motivate why research pertaining to these compounds is important for South Africa.

Persistent organic pollutants are compounds of natural or anthropogenic origin that possess a particular combination of physical and chemical properties that enables these compounds to:

- (i) remain intact in the environment for long periods of time because they resist naturally occurring degradation processes;
- (ii) be transported over long distances, by air and/or water, spreading their harmful capabilities to areas where they have not been produced or applied; and
- (iii) bio-accumulate in the higher levels of food chains, exposing humans and wildlife to acute and chronic toxic effects.

2.2 The Stockholm Convention

In recent decades, the risks posed by POPs have become an increasingly urgent concern in many countries, resulting in actions aimed at protecting human health and the

environment. These actions were taken or proposed at national, regional and international levels.

2.2.1 History

The following are some of the more influential regional and global initiatives that were underway before the Chemicals Unit of UNEP started a process in 1997 that resulted in the Stockholm Convention on Persistent Organic Pollutants:

- At a UNEP conference Washington D.C. (last quarter of 1995), the UNEP Global Programme of Action for the Protection of the Marine Environment from land-based activities was agreed to, and POPs were identified as a priority for action under the plan.
- On 24 June 1998, the United Nations Economic Commission for Europe (UN ECE) Convention on Long-Range Transboundary Air Pollution, which included the Aarhus Protocol on POPs, called for action in 16 identified POPs.
- The 1992 Convention on the Protection of the Marine Environment of the Baltic Sea (the Helsinki Convention).
- The 1976 Convention for the Protection of the Mediterranean Sea Against Pollution (Barcelona Convention), as amended in 1995.
- The North America Commission for Environmental Cooperation passed resolution number 95-5 on the Sound Management of Chemicals (1995) and gave immediate priority for Canada, Mexico and the USA to address persistent toxic substances and has resulted in the development and implementation of continental action plans for DDT, chlordane and PCBs and a commitment to develop an action plan on PCDDs, PCDFs and HCB.
- The Canada-USA Great Lakes Water Quality Agreement (1972), including the Binational Toxics Strategy (April 1997), emphasises action on POPs as well as other persistent toxic substances (Buccini, 2003).

The Stockholm Convention is one of several International Environmental Agreements (IEAs), among which there are also the International Climate Change Convention, and the Basel and Rotterdam conventions. It had a rapid negotiation phase that started in Montreal 1998, with the final text negotiated in Sandton, South Africa (December 2000) at the 5th Intergovernmental Negotiating Committee (INC). Those who participated in the negotiations included governments, intergovernmental organisations such as the World Health Organisation (WHO), industry associations such as the Chemical and Allied

Industries Association (CAIA) of South Africa and non-governmental organisations such as Greenpeace, the World Wildlife Fund and the International POPs Elimination Network (IPEN) (Bouwman, 2004).

The Convention was adopted on 22 May 2001 at the Conference of Plenipotentiaries on the Stockholm Convention on Persistent Organic Pollutants and it came into force on 24 May 2004. Currently (August 2007), there are 152 signatories and 147 parties to the Convention. Among the developed nations that ratified the Convention are Sweden, Canada, Norway, Japan and Germany (but not the United States of America). Countries from the Southern African Development Community (SADC) which ratified the Convention include Botswana, Lesotho, South Africa and Tanzania. South Africa ratified on 4 September 2002 (Stockholm Convention, 2007).

2.2.2 Aims

There are essentially five objectives or aims to the Stockholm Convention:

- (i) Parties to the Convention are to terminate the release and use of the twelve most toxic POPs, the so called "dirty dozen": aldrin, chlordane, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene, DDT, PCBs, PCDDs and PCDFs (together referred to as PCDD/Fs). The Convention bans and/or limits the production and use of the intentionally produced POPs (aldrin, chlordane, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene, DDT, PCBs), and it aims at reducing releases of the unintentionally produced POPs (PCBs, PCDD/Fs and HCB) which are formed as by-products of combustion and industrial processes (Stockholm Convention, 2005).

DDT may be produced and used only for public health measures, such as for malaria control. Parties that have obtained exemption (South Africa included) must notify the secretariat of any production and use, and have to report on a three-yearly basis. It was because of South Africa's epidemiological information that the country could motivate for the continued use of DDT against strong opposition from some countries during the negotiation stages of the Convention (Bouwman, 2004).

- (ii) The Convention supports the replacement of harmful POPs with safer, cost-effective alternatives. Developed nations are urged to share their knowledge and finances with developing countries that struggle with the transition to more suitable alternatives (Stockholm Convention, 2005).
- (iii) The Stockholm Convention has the continuing aim of identifying additional POPs that need to be reduced and/or eliminated (Stockholm Convention, 2005). In parallel with the INC series of meetings, three meetings of the Criteria Expert Group (CEG) were

held to develop science-based criteria and procedures for the identification of new POPs. A specific body, the POPs Review Committee, was set up to provide the Conference of Parties (COP) with the merits of those chemicals proposed to be added (Bouwman, 2004).

- (iv) Stockpiles and equipment containing POPs must be identified and managed in an environmentally safe manner (Stockholm Convention, 2005).
- (v) Parties to the Convention have to promote awareness with policy and decision-makers, as well as the public; develop and implement appropriate information dissemination programmes; promote and facilitate public participation regarding measures to address POPs and encourage and undertake research on all matters relating to POPs (Bouwman, 2004).

Other obligations of the parties include the development of a National Implementation Plan (NIP) within two years of the Convention's entry into force and the designation of a national focal point. For South Africa, the focal point is the Department of Environmental Affairs and Tourism (DEAT). Parties also have to report to the COP on a regular basis (Bouwman, 2004). Work on the NIP only started in the second half of 2007.

Since South Africa signed and ratified the Stockholm Convention, but has little knowledge of either the current levels of these compounds in the natural environment or the body burdens in humans and different forms of wildlife, this alone is enough reason to undertake research in this field. Exactly how little is known about POPs levels, specifically the unintentionally formed compounds (i.e. the PCBs, and the PCDD/Fs) in South Africa, will be discussed later on in this chapter in section 2.7.

2.3 Characteristics, sources, fate and transport of dioxin-like PCBs and PCDD/Fs

Dioxins and dioxin-like compounds which may have similar effects to dioxins are found in all environmental compartments, are persistent and, being fat-soluble, tend to accumulate in higher animals, including humans. Their resistance to degradation and semi-volatility means that they may be transported over long distances and give rise to trans-national exchanges of pollutants. In addition, dioxins released into the environment many years ago continue to contribute to contemporary exposure (Buckley-Golder, 1999).

2.3.1 Sources and formation

PCBs have been manufactured on purpose since 1929 and it was in the 1960s that their usage increased dramatically until environmental and health concerns resulted in legislative regulation in the 1970s (Tolosa, Readman, Fowler, Villeneuve, Dachs, Bayona & Albaigés, 1997). They are (or have been) used in a wide variety of industrial applications,

for example as dielectric fluids in capacitors and transformers, hydraulic fluids, lubricating oils, plasticisers, additives in pesticides, inks and paints (Erickson, 1997). Although all production of PCBs was banned, PCBs still originate as by-products from a wide variety of chemical processes. Both PCDD/Fs and dioxin-like PCBs are formed unintentionally in industrial-chemical processes such as chemical manufacture, and thermal processes such as waste incineration. The formation of PCDD/Fs has been studied extensively in combustion-related processes and to a lesser extent in non-combustion-related chemical processes. However, the mechanisms and exact formation conditions are not fully resolved. There is far less information regarding the formation of PCBs, but since there are similarities in the structure and occurrence of PCDD/Fs and co-planar PCBs, it is usually assumed that those parameters and factors that favour formation of PCDD/Fs also generate PCBs (UNEP, 2004).

Although PCDD/Fs can be destroyed by thermal processes when incinerated at sufficient temperature with adequate residence time and mixing of combustion gases and waste or fuel, PCDD/Fs can also be formed by thermal processes. Industrial-chemical processes constitute another major formation process of these compounds. For both formation processes carbon, oxygen, hydrogen and chlorine, in elemental, organic or inorganic form, are needed. A prerequisite for the carbon is that it must assume an aromatic structure at some point, whether it was present as a precursor or whether it was generated by a chemical reaction (UNEP, 2004).

During **thermal processes** there are two main pathways by which these compounds can be synthesised: from precursors such as chlorinated phenols or *de novo* from carbonaceous structures in fly ash, activated carbon, soot or smaller molecule products of incomplete combustion. Under conditions of poor combustion, PCDD/Fs can be formed in the burning process itself. During the synthesis, molecules in the same phase (gas or liquid phase) can react with one another, or with molecules from different phases, involving reactions between gas phase molecules and solid surfaces. PCDD/F formation can occur either in poor combustion or in poorly managed post-combustion chambers and air pollution control devices. Combustion techniques vary from the very simple and very poor, such as open burning, to the very complex and greatly improved, such as incineration using the most advanced available techniques. Formation of PCDD/Fs and co-planar PCBs has been reported to range between 200 °C and 650 °C. The range of greatest formation is generally agreed to be between 200 to 450 °C, with a maximum formation at about 300 °C (UNEP, 2004). Typical combustion systems include waste incineration (such as of municipal solid waste, sewage sludge, medical waste and hazardous wastes (McKay,

2002)), burning of various fuels such as coal, wood (Schatzwitz, Brandt, Gafner, Schlump, Buhler, Hasler & Nussbaumer, 1994) and petroleum products (Wikström, Löfvenius, Rapp & Marklund, 1996), other high temperature sources such as cement kilns, and poorly controlled combustion sources such as building fires and burning of any chlorine containing compounds (McKay, 2002).

PCDD/Fs can be formed during various types of **primary and secondary metals operations** (McKay, 2002). Copper, iron, zinc, aluminium, chromium and manganese are known to catalyse PCDD/F formation as well as their chlorination and dechlorination (UNEP, 2004). Examples of industries where this could occur include iron ore sintering, steel production and scrap metal recovery (Beukens, Huang & Stieglitz, 1999). Chlorine must be present in organic, inorganic or elemental form. Its presence in fly ash or in the elemental form in the gas phase may be especially important (UNEP, 2004).

Data by Gullett, Lemieux, Lutes, Winterrowd and Winters (1999) from waste burning experiments under uncontrolled conditions, has shown that the amount of PCDD/Fs generated *does not depend on a single parameter*. High concentrations of PCDD/Fs have been detected when “normal” household waste has been burned in the open. The concentrations increased when the chlorine content, the humidity, or the load of waste increased, or when catalytic metals were present.

In the **chemical formation** process of PCDD/Fs, the synthesis is favoured by one or more of the following conditions: temperatures greater than 150 °C; alkaline conditions; metal catalysts and ultraviolet radiation, or other radical initiators (Hutzinger & Fiedler, 1988). In the manufacture of chlorine-containing chemicals, the manufacturing of the following compounds have been identified as sources of PCDD/Fs with a decreasing probability of generating PCDD/F from top to bottom:

- Chlorinated phenols and their derivatives;
- Chlorinated aromatics and their derivatives;
- Chlorinated aliphatic chemicals, and
- Chlorinated catalysts and inorganic chemicals (UNEP, 2005).

PCDD/Fs can be formed as by-products from the manufacture of chlorine bleached wood pulp (Rappe, Andersson, Bergqvist, Brohede, Hansson, Kjeller, Lindström, Marklund, Nygen, Swanson, Tysklind & Wiberg, 1987) and chlorinated phenols such as pentachlorophenol (PCP) (Oberg, Anderson & Rappe, 1992), phenoxy herbicides (e.g. 2,4,5-trichloro-phenoxy-acetic acid) and chlorinated aliphatic compounds (e.g. ethylene dichloride) (McKay, 2002).

The mechanisms of PCB formation during combustion are assumed to be similar to those of PCDD/F formation (Lemieux, Lee, Ryan & Lutes, 2001). PCBs are also precursors for the formation of PCDFs. PCBs may, furthermore, be formed as unintentional products of industrial processes such as oxychlorination (UNEP, 2004). Non-ortho PCBs in particular (PCB77, 81, 126 and 169 of this study) are reportedly formed during coal combustion and industrial waste incineration, and do not originate solely from commercial PCB mixtures (Boers, De Leer, Gramberg & De Koning, 1994; Kim, Hirai, Kato, Urano & Masunaga, 2004).

PCDD/Fs can be formed by **biological and photochemical processes** such as composting from the action of microorganisms on chlorinated phenolic compounds (Siewers & Schacht, 1994) and during photolysis of highly chlorinated phenol (Tysklind, Fangmark, Marklund, Lindskog, Thaning & Rappe, 1993).

Reservoirs are materials, products, or pieces which contain previously formed PCDD/Fs or PCBs and have the potential for redistribution and circulation of these compounds into the environment. Potential reservoirs include soils, sediments, vegetation and PCP-treated wood (Kjeller & Rappe, 1995). PCDD/Fs have also been discovered in ball clay deposits and their origin might have been a natural occurrence (McKay, 2002).

The United Nations Environment Programme first published the *Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases* in 2003 and a second edition in 2005. This publication is intended to assist countries to establish release inventories of PCDD/Fs at a national or regional level and therefore describes in great detail all possible PCDD/F sources and their possible emissions. Table 2.1 was compiled from the source categories addressed in this toolkit (UNEP, 2005). Inspecting this table, it is clear that South Africa has industries and activities in all ten of the main categories and is therefore contributing to the global load of PCDD/Fs and dioxin-like PCBs, but to an unknown extent. (Explanation of dioxin-like PCB follows in the next section.) Eskom, the main electricity supplier in South Africa, has transformers and capacitors with PCBs, but already has inventories and programmes in place to manage and reduce PCB-contaminated oils and equipment, as well as remediating polluted sites. Sasol, a very large chemical concern, is in the process of screening their own transformer oils for PCBs. Mittal Steel, a major steel producer, embarked on a similar process, and has sent most of its PCB contaminated oils for incineration overseas (Bouwman, 2003).

Table 2.1: Main and sub-categories of all known anthropogenic PCDD/F sources
(compiled from UNEP, 2005).

Source categories	
1 Waste incineration: Municipal solid waste incineration; Hazardous waste incineration; Medical waste incineration; Light-fraction shredded waste incineration; Sewage sludge waste incineration; Waste wood and waste biomass incineration; Destruction of animal carcasses.	2 Ferrous and non-ferrous metal production: Iron ore sintering; Coke production; Iron and steel production and foundries; Copper production; Aluminium production; Lead production; Zinc production; Brass and bronze production; Magnesium production; Other non-ferrous metal production; Shredders; Thermal wire reclamation.
3 Power generation and heating: Fossil fuel power plants; Biomass power plants; Landfill, biogas combustion; Household heating and cooking (biomass); Domestic heating (fossil fuels).	4 Production of mineral products: Cement production; Lime production; Brick production; Glass production; Ceramics production; Asphalt mixing.
5 Transport: 4-Stroke engines; 2-Stroke engines; Diesel engines; Heavy oil fired engines.	6 Uncontrolled combustion processes: Biomass burning; Waste burning and accidental fires.
7 Production and use of chemicals and consumer goods: Pulp and paper production; Chemical industry; Petroleum industry; Textile production; Leather refining.	8 Miscellaneous: Drying of biomass; Crematoria; Smoke houses; Dry cleaning; Tobacco smoking.
9 Disposal: Landfills and waste dumps; Sewage/sewage treatment; Open water dumping; Composting; Waste oil treatment (non-thermal).	10 Identification of potential hot-spots: Production sites of chlorinated organics; Production sites of chlorine; Formulation sites of chlorinated phenols; Application sites of chlorinated phenols; Timber manufacture and treatment sites; PCB-filled transformers and capacitors; Dumps of wastes/residues from categories 1-9; Sites of relevant accidents; Dredging of sediments; Kaolinitic or ball clay sites.

Uncontrolled burning of waste is a problem throughout all of South Africa. Informal waste dumps contribute particularly to the risk. Many of the very poor also use the waste dumps as a resource for food, clothing and combustible materials to burn for cooking. Rubber tyres are also burned to harvest the wire reinforcement for resale (Bouwman, 2003).

2.3.2 Physicochemical characteristics of PCBs

PCBs are a class of chemical compounds in which chlorine atoms replace some or all of the hydrogen atoms on a biphenyl molecule (Figure 2.1). PCBs were manufactured and sold as mixtures with a variety of trade names, including Aroclor, Pyranol, Pyroclor (USA), Phenochlor, Pyralene (France), Clophen, Elaol (Germany), Kanechlor, Santotherm (Japan), Fenchlor, Apirolio (Italy), and Sovol (USSR) (WHO, 2003). In South Africa the brand names also included Askarel, Chlorectol, Elemex and Inerteen (Gray, 2004).

Two different but correlated nomenclature systems are currently used: (i) The International Union of Pure and Applied Chemistry (IUPAC) name identifies the numbered carbons to which chlorines are attached and lists the numbers sequentially, for example the PCB congener with chlorines on carbons 2, 3, 4 and 3' is identified as 233'4. (ii) A second widely used system (and which is used in this thesis) was developed by Ballschmiter and Zell (1980) as a way to simplify reference to specific congeners. It correlates the structural arrangement of the PCB congener in an ascending order of number of chlorine substitutions within each sequential homologue. An unprimed number is considered to be

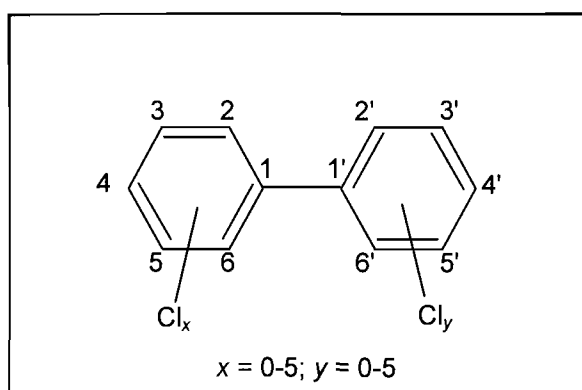


Figure 2.1: Biphenyl molecule with the numbering system. Some or all ten of the hydrogen atoms attached to the numbered carbons are substituted with chlorines (WHO, 2003).

of higher priority than the same number when primed. This results in the congeners being numbered from PCB1 through PCB209. Original typographical errors in the Ballschmiter and Zell (1980) numbering system have subsequently been resolved (WHO, 2003). Table 2.2 shows the relationship between the IUPAC and revised PCB numbering systems. To determine the IUPAC and alternative names of, for example, PCB156, its location on the table must be determined (see grey filled cell in Table 2.2). The chlorinated carbons with the primed numbers are read in the corresponding row on the far left: 3', 4'. The chlorinated carbons with the plain numbers are read at the top of the column of PCB156: 2,3,4,5. The IUPAC name for PCB156 is therefore 2,3,3',4,4',5-hexachlorobiphenyl.

Table 2.2: PCB nomenclature conversion table.

Chlorine positions	None	2	3	4	2 3	2 4	2 5	2 6	3 4	3 5	2 3 4	2 3 5	2 3 6	2 4 5	2 4 6	3 4 5	2 3 4 5	2 3 4 6	2 3 5 6	2 3 4 5 6
2' 3' 4' 5' 6'																				209
2' 3' 5' 6'																			202	208
2' 3' 4' 6'																		197	201	207
2' 3' 4' 5'																	194	196	199	206
3' 4' 5'																169	189	191	193	205
2' 4' 6'															155	168	182	184	188	204
2' 4' 5'														153	154	167	180	183	187	203
2' 3' 6'													136	149	150	164	174	176	179	200
2' 3' 5'												133	135	146	148	162	172	175	178	198
2' 3' 4'											128	130	132	138	140	157	170	171	177	195
3' 5'										80	107	111	113	120	121	127	159	161	165	192
3' 4'									77	79	105	109	110	118	119	126	156	158	163	190
2' 6'								54	71	73	89	94	96	102	104	125	143	145	152	186
2' 5'							52	53	70	72	87	92	95	101	103	124	141	144	151	185
2' 4'						47	49	51	66	68	85	90	91	99	100	123	137	139	147	181
2' 3'					40	42	44	46	56	58	82	83	84	97	98	122	129	131	134	173
4'				15	22	28	31	32	37	39	60	63	64	74	75	81	114	115	117	166
3'			11	13	20	25	26	27	35	36	55	57	59	67	69	78	106	108	112	160
2'		4	6	8	16	17	18	19	33	34	41	43	45	48	50	76	86	88	93	142
None	0	1	2	3	5	7	9	10	12	14	21	23	24	29	30	38	61	62	65	116

The grey scale cell is explained in the text. The twelve most toxic congeners are highlighted in blue

The benzene rings can rotate around the bond connecting them, but the rings are forced towards either the same plane (referred to as planar or co-planar) or perpendicular planes (non-planar) by the electrostatic repulsion of the highly electronegative chlorine atoms. A non-planar orientation is produced by multiple substitutions in the *ortho* positions (2,2',6 and 6'). Some mono-*ortho*-substituted and all non-*ortho*-substituted PCBs are considered to be planar, implying that the rings of some congeners can twist but not turn completely (WHO, 2003).

The twelve most toxic congeners are: PCB77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 of which PCB77, 81, 126 and 169 are co-planar (or non-*ortho* substituted) and PCB105, 114, 118, 123, 156, 157, 167 and 189 are mono-*ortho*-PCBs (Schechter *et al.*, 2006). These are the dioxin-like PCBs produced unintentionally and referred to by Annex C of the Stockholm Convention (UNEP, 2002a).

The dioxin-like PCBs are relatively insoluble in water (Table 2.3) and solubility decreases rapidly in *ortho*-vacant congeners, especially as the *para* positions are filled. PCBs are freely soluble in non-polar organic solvents and biological lipids (high K_{OW} values; Table 2.3). Because of their high chlorination, the dioxin-like PCBs are also relatively non-volatile (cf. Henry's law constants; Table 2.3) (WHO, 2003).

Table 2.3: Physical and chemical properties of some of the co-planar PCBs (Sinkkonen & Paasivirta, 2000^a; Syracuse, 2007^b).

	IUPAC name	Water solubility (mg l ⁻¹ ; 25 °C) ^b	Log K_{OW} ^b	Vapour pressure (mmHg; 25 °C) ^b	Henry's law constant (atm m ³ mol ⁻¹ ; 25 °C) ^b	t _{1/2} in water (months) (7 °C) ^a	t _{1/2} in sediment (months) (7 °C) ^a
PCB81*	3,4,4'-TCB		6.3				
PCB77*	3,3',4,4'-TCB	1.8 x10 ⁻¹	6.0-6.6	4.4x10 ⁻⁷	4.3-9.4x10 ⁻⁵	7x10 ⁻¹	2
PCB126*	3,3',4,4',5-PeCB		7.0			1.4	2
PCB169*	3,3',4,4',5,5'-PeCB	3.6x10 ⁻⁵ -1.2x10 ⁻²	7.4	4.0x10 ⁻⁷	1.5-5.9x10 ⁻⁵	2.8	3.9
PCB105	2,3,3',4,4'-PeCB	3.4x10 ⁻³	7.0	6.5x10 ⁻⁶	8.3x10 ⁻⁴		
PCB114	2,3,4,4',5-PeCB	1.6x10 ⁻²	7.0	5.5x10 ⁻⁶	9.2x10 ⁻⁵		
PCB118	2,3',4,4',5-PeCB	1.3x10 ⁻² (20 °C)	7.1	9.0x10 ⁻⁶	2.9x10 ⁻⁴	1.4	1.4
PCB123	2',3,4,4',5-PeCB		7.0				
PCB156	2,3,3',4,4',5-HxCB	5.3x10 ⁻³	7.6	1.6x10 ⁻⁶	1.4x10 ⁻⁴		
PCB157	2,3,3',4,4',5'-HxCB		7.6				
PCB167	2,3',4,4',5,5'-HxB	2.2x10 ⁻³	7.5	5.8x10 ⁻⁷	6.9x10 ⁻⁵		
PCB189	2,3,3',4,4',5,5'-HpB	7.5x10 ⁻⁴	8.3	1.3x10 ⁻⁷	5.1x10 ⁻⁵		

*non-*ortho*-substituted; t_{1/2} = half-life time

Most PCB congeners, particularly those lacking adjacent unsubstituted positions on the biphenyl rings (e.g. 2,4,5-, 2,3,5- or 2,3,6-substituted on both rings) are extremely

persistent in the environment (UNEP, 2003). They are estimated to have half-lives ($t_{1/2}$) ranging from three weeks to two years in air, and more than six years in aerobic soils and sediments. PCBs also have extremely long half-lives in adult fish; for example, an eight-year study of eels found that the half-life of PCB 153 was more than ten years (UNEP, 2003). The half-lives of selected PCBs for sediment and water are included in Table 2.3. These were determined for Baltic proper environment with an annual average temperature of 7 °C (Sinkkonen & Paasivirta, 2000).

2.3.3 Physicochemical characteristics of PCDD/Fs

The PCDDs and PCDFs are two series of almost planar tricyclic aromatic compounds with very similar chemical properties. The general formulae are given in Figure 2.2 (WHO, 1989). PCDDs have 75 possible positional isomers and PCDFs have 135. Seven PCDDs and ten PCDFs are regarded as the most toxic (UNEP, 2002a).

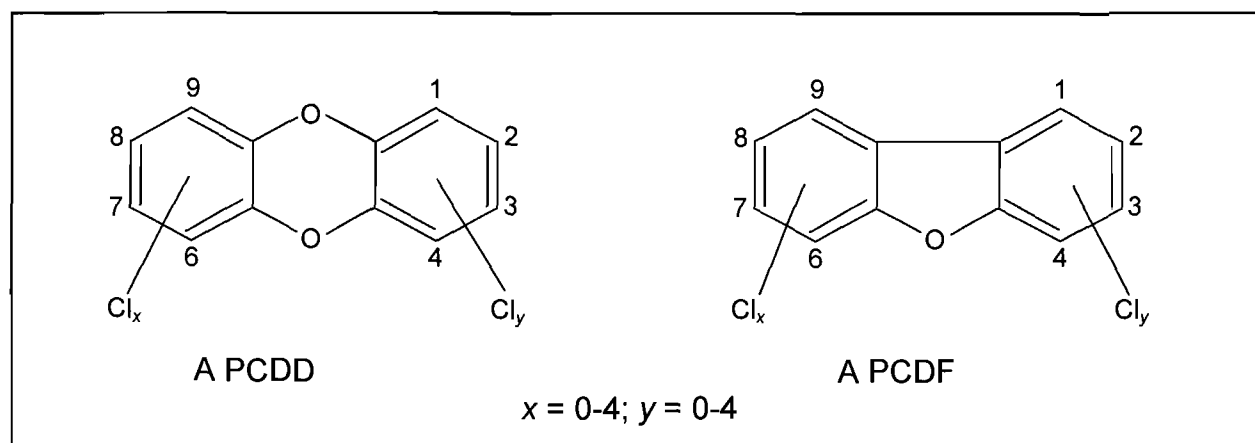


Figure 2.2: The chemical structures of PCDDs and PCDFs

Table 2.4: Physical and chemical properties of some of the PCDD/F isomers (Govers & Krop, 1998^a; Meneses, Schuhmacher & Domingo, 2002^b; Sinkkonen & Paasivirta, 2000^c).

IUPAC name	Water solubility (mg l ⁻¹ ; 25 °C) ^a	Log K _{ow} ^a	Vapour pressure (mmHg; 25 °C) ^a	Henry's law constant (atm m ³ mol ⁻¹ ; 25 °C) ^b	t _{1/2} in water (months) (7 °C) ^c	t _{1/2} in sediment (months) (7 °C) ^c
2,3,7,8-TCDD	1.14x10 ^{-2*}	6.96	8.81x10 ^{-7*}	1.62x10 ⁻³	1x10 ⁻¹	21
1,2,3,7,8-PeCDD	2.77x10 ⁻³	7.5	9.02x10 ⁻⁸	1.48x10 ⁻³	2x10 ⁻¹	23
1,2,3,4,7,8-HxCDD	1.67x10 ^{-3*}	7.79	2.02x10 ^{-8*}	1.45x10 ⁻³	4 x10 ⁻¹	56.2
1,2,3,6,7,8-HxCDD	8.75x10 ⁻⁴	7.98	2.48x10 ⁻⁸	1.45x10 ⁻³	4 x10 ⁻¹	12.9
1,2,3,7,8,9-HxCDD		7.8		8.32x10 ⁻⁴	4 x10 ⁻¹	16.4
1,2,3,4,6,7,8-HpCDD	5.87x10 ^{-4*}	8.2	4.52x10 ^{-9*}	8.32x10 ⁻⁴	7 x10 ⁻¹	21
OCDD	6.50x10 ^{-5*}	8.6	1.40x10 ^{-9*}	5.13x10 ⁻⁴	1.9	30.4
2,3,7,8-TCDF	4.13x10 ^{-2*}	6.46	1.22x10 ^{-6*}	2.69x10 ⁻³	2 x10 ⁻¹	12.9
1,2,3,7,8-PeCDF	1.08x10 ^{-2*}	6.99	4.62x10 ^{-7*}	1.91x10 ⁻³	3 x10 ⁻¹	10.5
2,3,4,7,8-PeCDF	1.15x10 ^{-2*}	7.11	1.46x10 ^{-7*}	2.57x10 ⁻³	0.3	12.5
1,2,3,4,7,8-HxCDF	8.02x10 ^{-4*}	7.53	4.22x10 ^{-8*}	1.91x10 ⁻³	0.7	14
1,2,3,6,7,8-HxCDF	1.97x10 ^{-3*}	7.57	4.42x10 ^{-8*}	1.91x10 ⁻³	0.7	16.4
1,2,3,7,8,9-HxCDF	8.59x10 ⁻⁴	7.76	1.68x10 ⁻⁸	9.55 x10 ⁻⁴	0.7	11.7
2,3,4,6,7,8-HxCDF	1.56x10 ⁻³	7.65	5.69x10 ⁻⁸	1.78x10 ⁻³	0.7	10.5
1,2,3,4,6,7,8-HpCDF	1.63x10 ⁻⁴	7.9	1.06x10 ⁻⁸	1.41x10 ⁻³	1.5	8.2
1,2,3,4,7,8,9-HpCDF	2.58x10 ⁻⁴	8.23	4.96x10 ⁻⁹	1.00x10 ⁻³	1.5	7
OCDF	2.33x10 ^{-4*}	8.78	5.31x10 ^{-9*}	7.760x10 ⁻⁴	4.5	5.6

*Reported by Govers & Krop as experimentally determined values; t_{1/2} = half-life time

PCDD/Fs and PCBs possess low vapour pressures and water solubilities, along with high octan-1-ol/water partition coefficients ($\log K_{OW}$) (Tables 2.3 and 2.4). Suggested half-lives for 2,3,7,8-substituted PCDD/Fs in water and sediment in the Baltic proper are presented in Table 2.4. When the long biological lifetimes of these chemicals are taken into account (human half-lives of up to 27.5 years have been reported for some PCBs (Yakushiji, Watanabe, Kuwabara, Tanaka, Kashimoto, Kunita & Hara, 1984) and the half-life of 2,3,7,8-TCDD in soil is of 10-12 years (UNEP, 2003)), it is unsurprising that PCDD/Fs and PCBs show significant bio-concentration through food chains (Harrard, 1996).

2.4 Environmental fate

2.4.1 Long range transport (LRT)

A variety of transport processes distributes PCBs and PCDD/Fs throughout the global environment. Arctic peoples were exposed to dioxin-like POPs even though these pollutants were not released there: the total PCDD/F/non-*ortho* co-planar PCB concentration expressed as toxic equivalency quotient (TEQ) was 50 ng kg⁻¹ lipids of mother's milk in northern Quebec (Ayotte, Dewailly, Bruneau, Careau, & Vézina, 1995). The principle of TEQ is addressed in section 2.5.

2.4.1.1 LRT via atmosphere

Understanding the environmental transport pathways of these compounds provides a link between sources of dioxin-like POPs and exposure to them within a region. It also provides information on the potential of transport from one region to another (UNEP, 2003).

Dioxin-like PCBs and PCDD/Fs are neither very polar substances (which would make them water soluble and primarily a water-borne pollutant), nor are they very volatile (which would make them primarily airborne) and therefore they affect the environment as a whole and are regarded as multimedia pollutants (UNEP, 2003).

LRT can occur by different modes:

- as a vapour, sorbed to suspended particles;
- sorbed to sediment particles in oceans and rivers;
- in tissues of migratory animals; and
- anthropogenic transport in the form of products and waste.

The importance of these transport pathways depends on the specific partitioning characteristics of a compound: the partitioning of PCBs and the lighter PCDD/Fs (lesser chlorinated) readily changes between the gas phase and condensed phase (soil,

vegetation, water) in response to changes in ambient temperature and phase composition (Finizio, Mackay, Bidleman & Harner, 1997). This allows the molecules to travel long distances in repeated cycles of evaporation and deposition (multi-hop LRT). The higher chlorinated PCDD/Fs tend to be less volatile and water insoluble and undergo LRT mostly by being carried sorbed on suspended solids in air and water (single-hop LRT). The boundaries between these two categories are not discrete and a single chemical can move along anywhere on a continuum between these two groups because both the octanol-air (K_{OA}) and air-water (K_{AW}) partition coefficients are temperature-dependent. This means that a chemical may undergo multi-hop LRT at high environmental temperatures, but a single hop at low environmental temperatures (UNEP, 2003).

The transport behaviour of single-hop compounds such as the PCDD/Fs is mainly controlled by the location of its atmospheric source relative to the major atmospheric flow patterns. Atmospheric conditions at the time of release will have a strong impact on their transport behaviour and areas close to the sources are generally affected more strongly than those further away. Single-hop LRT is restricted to conditions that favour rapid horizontal, but limited vertical air movement and no precipitation. Once deposited, these chemicals will only move if the particles to which they sorb are remobilised as a result of a storm run-off or dust storms (UNEP, 2003).

The LRT behaviour of multi-hop substances such as tetrachlorobiphenyl (PCB77 & 81) is facilitated by the ease of their transfer between the atmosphere and the earth's surface. Chemicals which change from a gaseous state to a condensed state within the environmentally relevant temperature range will undergo air-surface exchange (hop) more often and are most likely to travel far. Because cold temperatures favour deposition over evaporation and warm temperatures favour evaporation over deposition, hopping is enhanced by diurnal (Hornbuckle & Eisenreich, 1996; Lee, Hung, Mackay & Jones, 1998) and seasonal temperature changes (Hoff, Muir & Grift, 1992; Wania, Haugen, Lei & Mackay, 1998).

Since PCBs and PCDD/Fs undergo different air-surface exchanges, mixtures of these compounds tend to shift in their relative composition with distance from source or along latitudinal and altitudinal gradients. The less volatile constituents are found closer to the source while the more volatile compounds tend to travel farther (UNEP, 2003). Total tetrachlorobiphenyls:octachlorobiphenyls decreased with increasing north latitude in a study on PCBs in Canada's midlatitude and arctic lake sediments (Muir, Omelchencko, Grift, Savoie, Lockhart, Wilkinson & Brunskill, 1996).

Chemicals undergoing multi-hop transport have a higher potential for LRT than single-hop chemicals. The partitioning properties of the intermediate PCBs and the lighter PCDD/Fs enable them to undergo LRT, provided that the environmental conditions are such that their degradation is slow. The heavier PCDD/Fs have a comparatively small potential for atmospheric LRT.

It can be argued that a chemical with a small LRT is likely to achieve higher concentrations close to sources and this is more likely to cause effects, but chemicals with high LRT will potentially affect a larger area. This implies that any associated effects or threats would not be contained and may affect human populations and ecosystems over very large areas (Stockholm Convention, 2005). (And although the larger distribution area would imply a greater dilution factor, the dilution effect might be erased over a period of time, because these compounds are persistent and capable of bio-accumulation.) Therefore, through LRT via the atmosphere, some PCBs and PCDD/Fs might cause risk and exposure to humans and wildlife in areas far removed from their sources, justifying international regulations of such chemicals (Stockholm Convention, 2005).

2.4.1.2 LRT via water

Transport by oceans will be addressed before riverine transport is discussed.

LRT by **ocean** is limited for dioxin-like PCBs and PCDD/Fs because these compounds are not water soluble. Less water soluble compounds ($\log K_{OW} > 5$) will sorb effectively to suspended organic solids and therefore have only a limited residence time near the surface ocean because of gravitational settling (Dachs, Bayona, Ittekkot & Albaigés, 1999). The extent of gravitational settling of POPs is dependent on marine biological productivity and is thus likely highest in coastal and shelf areas, and marine regions of nutrient upwelling. Deposition from the surface ocean has been estimated to be highest in mid to high latitudes (Dachs, Lohmann, Ockenden, Méjanelle, Eisenreich & Jones, 2002).

If the degradation half-lives of POPs in air are only a few days, it is still sufficient for LRT to take place. For oceanic LRT, compounds have to survive in water for several months to years. The rate of POPs degradation in ocean water is dependent on temperature in the case of hydrolytic reactions (Ngabe, Bidleman & Falconer, 1993), the presence and activity of microorganisms capable of metabolising a chemical in the case of microbial reactions (Harner, Jantunen, Bidleman, Barrie, Kylin, Strachan & MacDonald, 2000) and the intensity of sunlight in the case of aqueous phase photo-oxidation. This suggests that degradation is slower in high latitudes (darker and colder) and faster in warm, sunny and biologically active seas (UNEP, 2003).

Transport of PCBs and PCDD/Fs in **rivers** is dependent on the transport of colloidal or suspended sediment matter to which these compounds sorb. The load of suspended solids and colloidal organic matter in rivers, in turn, depends on the hydrological regime and drainage basin characteristics. Organic pollutants transported via rivers will eventually contaminate coastal sediments. Beyond the zone of influence of these discharges, concentrations drop rapidly, reflecting the enhanced sedimentation processes which take place at the freshwater/sea water interface. More than three quarters of the terrestrial sediments are trapped on the continental shelf and only the finest particles are transported by currents to deep sea sediments (UNEP, 2003). The continental shelf will thus likely constitute the final resting place of many POPs delivered by rivers to the oceans (Jönsson, Gustafsson, Axelman & Sundberg, 2003).

2.4.1.3 LRT via migratory animals

Migratory animals, such as birds, often contain high levels of dioxin-like compounds because of the bio-accumulative properties of the compounds and also because of the high trophic status of the organisms (predatory birds). As the organisms migrate, they transport the compounds within and between regions. The gross transport rate of POPs with migratory organisms is usually smaller than, but can under some circumstances be of a similar order of magnitude as the fluxes in the abiotic media air and water (Wania, 1998). The subsistence hunter or high trophic level predator may take up more dioxin-like compounds from a migratory bird or marine mammal than by consuming non-migratory animals. Since animal migrations occur generally in a north-south direction rather than a zonal direction, migratory birds might expose South African predators to POPs. This possibility has not been investigated yet, but seems to be likely considering the number of birds arriving in sub-Saharan Africa from the Palaearctic that is estimated to amount to 3 750 million, about one million of which are waterbirds (Moreau, 1972). Approximately 58 species of the Palaearctic migratory birds migrate to the Cape provinces of South Africa (Curry-Lindahl, 1981). More than 5×10^9 birds of 200 species migrate between Europe and Africa annually (Elphick, 1995).

Migratory animals themselves may be particularly vulnerable to POPs that bio-accumulate in lipid tissues and are released upon mobilisation of lipids during migration (UNEP, 2003).

2.4.2 Fate in environmental compartments

As South Africa has the industrial and other sources of these toxic chemicals (Table 2.1), and due to its LRT potential (preceding sections) and toxic characteristics (sections 2.8 and 2.9), it is important to know the behaviour of these compounds in the environment. Dioxin-like pollutants are ubiquitous in the environment and have the potential to bio-accumulate

and bio-magnify from one trophic level to the next. They are modified by various processes over time (see section 2.4.3): biochemical transformation because of enzyme activity inside vertebrate bodies; aerobic and anaerobic microbial changes, and breakdown due to ultra-violet radiation.

PCDD/Fs and PCBs are multimedia pollutants, and once released into the environment are distributed between environmental compartments (Buckley-Golder, 1999). Their low solubility in water (highly lipid soluble) and low vapour pressure lead to high partition coefficients into abiotic and biotic particles (Eisenreich, Capel, Robbins & Bourbonniere, 1989). High K_{OW} -values generally result in greater bio-accumulation. The organic carbon-water partition coefficient ($\text{Log } K_{OC}$) values for 2,3,7,8-TCDD are between 6.4 and 7.6 (Fiedler, 2003). The more toxic mono- and non-*ortho*-PCBs are associated to a greater degree with particles in ambient air, and are consequently more likely to be removed by precipitation and dry deposition (Falconer & Bidleman, 1994).

The most likely breakdown of POPs chemicals in the atmosphere is with the hydroxyl radical (OH) (Kwok, Atkinson & Arey, 1995), but also NO_3 and O_3 (Atkinson, 1991). The concentration of OH radicals varies with season, time of day, altitude and latitude. Highest OH radical concentrations, that is, fastest degradation and thus reduced atmospheric LRT, occur in low latitudes, at high altitudes, during daytime and in summer. The reaction of the OH radical is temperature-dependent: the higher the temperature, the faster the reaction time. In the sub-tropical atmosphere, daytime depletions of PCB concentrations could be explained by efficient reaction with OH radicals (Mandalakis, Berresheim & Stephanou, 2003).

Czuczwa and Hites (1986) observed that the PCDD/F congener profile of combustion sources (municipal waste incineration from various European countries and chemical waste incineration) showed, on average, an almost flat congener profile (i.e. equal amounts of all congeners) while sediments from the Great Lakes showed a shift toward the OCDD congener. The air particulates from urban air had the same congener profile as the lake sediments, which indicated that no degradation has taken place in the water column. The authors concluded that the variety of PCDD/F congener distributions emitted by several combustion systems have all been transformed in the atmosphere and not in the water or sediment. The lower chlorinated PCDD/Fs are removed from the atmosphere by the gas-phase removal processes and the higher chlorinated PCDD/Fs are removed by particle-phase removal processes such as wet and dry deposition (Brubaker & Hites, 1997).

In countries at mid-latitudes, such as South Africa, there is a constant fluctuation of conditions favourable for evaporation/degradation and deposition/persistence. These

fluctuations occur on a diurnal and seasonal scale, and as a result of the nature of mid-latitudinal circulation also on a time-scale in between. The LRT of lighter PCB congeners - which are limited by efficient degradation - is higher in winter, whereas chemicals whose LRT is limited by efficient deposition, such as the heavier PCBs or the PCDD/Fs, are higher in summer (UNEP, 2003)

2.4.3 In the food chain

Because of their chemical characteristics and very low solubility, these compounds accumulate in most soil types, with very little water leaching and negligible degradation of the 2,3,7,8-substituted PCDD/F congeners. (This finding, however, has not been substantiated for South African conditions.) They adsorb quickly to organic matter and so accumulate in sediments. They then accumulate in aquatic fauna as a result of the ingestion of contaminated organic matter (Fiedler, 2003). The concentration of PCDD/F and dioxin-like PCB in fish tissue is found to bio-magnify in the food web as a progressive ingestion of contaminated prey (Buckley-Golder, 1999).

In the terrestrial food chain (air→grass→cattle→milk/meat→man) these compounds are deposited on plant surfaces via wet deposition, via dry deposition of chemicals bound to atmospheric particles, or via diffusive transport of gaseous chemicals in the air to plant surfaces. Dry particle-bound deposition is mainly responsible for the uptake of the higher chlorinated compounds (six and more chlorines) while dry gaseous deposition plays the dominant role in the accumulation of the lower chlorinated compounds (Fiedler, 2003). The accumulation of the gas-phase concentrations by plant surfaces is affected by temperature, which influences gas-particle partitioning and air-leaf exchange (Paterson, Mackay, Bacci & Calamari, 1991; Paterson, Mackay & McFarlane, 1994). Grazing animals are exposed to dioxins by ingesting contaminated pasture crops.

Once absorbed by the gastrointestinal tract, the compounds are transported - mainly by binding to lipoproteins in the blood - to different tissues and organs in the body. Bio-accumulation in mammals is observed not only in adipose tissue or blubber, but also in the liver, bone marrow and brain tissue (Carey, Cook, Giesy, Hudson, Muir, Owens & Solomon, 1998).

In all species, chlorinated aromatic compounds accumulate in tissues in proportion to the lipid percentage. Exceptions are the 2,3,7,8-substituted PCDD/Fs, which also accumulate strongly in the livers of mammals and birds. This greater liver retention has, in part, been attributed to the presence of inducible binding proteins for these compounds, such as cytochrome P4501A2 (Poland, Teitelbaum & Glover, 1989). PCBs and PCDD/Fs are non-polar compounds that cannot readily be excreted or transformed to polar excretable

compounds without the introduction of a polar functional group through metabolism – or via lactation. None of these compounds are metabolised at a significant rate by invertebrates, and therefore they bio-magnify through the diet in invertebrate food chains (Gobas, 1993; Thomann, 1989).

PCBs that are easily metabolised by *meta*- and *para*-epoxidation and possess 2,5 or 2,3,6 chlorine substitution on the same ring, tend to form persistent methyl sulphone metabolites through a complex process involving the substitution of glutathione at the 3 or 4 positions, excretion of the mercapturic acid, cleavage by gut microflora C-S lyase, resorption, methylation, and hepatic oxidation to 3- and 4-methyl sulphone PCB (Oppenhuizen & Sijm, 1990). Many of these compounds are resistant to further metabolism and are sufficiently hydrophobic that they are not excreted.

PCDD/Fs that do not possess chlorines at all four 2,3,7 and 8 positions, do not bio-accumulate in vertebrates (except cetaceans). Cetaceans appear to be able to metabolise (or excrete) 2,3,7,8-substituted PCDD/F and accumulate only the non-2,3,7,8-substituted congeners (Norstrom, Simon & Muir, 1990). Clearance rates of 2,3,7,8-substituted PCDD/Fs are low enough (except in cetaceans) that bio-magnification occurs in food chains, in the order of 3- to 10- fold per trophic level (Endicott & Cook, 1994).

PCBs bio-accumulate in invertebrates and most fish with little metabolic alteration of the compounds. Thus, excretion of less chlorinated compounds may occur across the gills in fish, but generally PCBs are bio-accumulated by fish with little change in patterns (Norstrom, 1988). Vertebrates of a higher order than fish, including reptiles, birds, and mammals (except marine mammals), possess hepatic cytochrome P450 mono-oxygenase enzyme systems that are efficient at degrading certain PCB congeners. C4502B enzymes are responsible for metabolism of PCB congeners that are not substituted by chlorine at one or more *meta*- and *para*-positions. Thus, PCB congeners with at least one unsubstituted *meta*- and *para*-positions are not bio-magnified in higher animals. As for PCDD/F, cetaceans have a metabolic capability different from other mammals (Tanabe, Watanabe, Kan & Tatsukawa, 1988). They do not degrade *meta*- and *para*-unsubstituted PCB congeners to any extent.

Although PCBs and PCDD/Fs sources are mostly land-based, many receptors are aquatic (fish/marine mammals). Human exposure to dioxin-like chemicals is also often mediated through the aquatic food chain. Populations relying heavily on aquatic organisms for nutrition tend to be most at risk with respect to POPs (UNEP, 2003).

In aquatic systems, dioxin-like chemicals can be removed through adsorption to or partitioning into abiotic settling particles and organisms with subsequent sedimentation.

They accumulate in aquatic fauna as a result of the ingestion of contaminated organic matter (Eisenreich *et al.*, 1989; Fiedler, 2003). The sediment acts as an important sink, but through resuspension and mixing by aquatic organisms, the surface sediment can also act as a secondary source for these contaminants, increasing the residence time of PCBs in the ecosystem (Eisenreich *et al.*, 1989). Sediment can receive inputs via different pathways such as atmospheric deposition, industrial and domestic effluents, stormwater, and spills (Fiedler, 2003).

Because of their hydrophobicity, halogenated organic compounds such as PCBs tend to transfer to, and accumulate at environmental interfaces and partition, for example, into anaerobic habitats where reductive cleavage of the carbon-halogen bonds may be an important environmental biotransformation process (Alder, Häggblom, Oppenheimer & Young, 1993; Kim & Rhee, 2001). Aerobic breakdown of PCBs is also known (Abramowicz, Brennan, Van Dort & Gallagher, 1993). The combination of these biotransformation processes can therefore alter PCB congener profiles (Kannan, Maruya & Tanabe, 1997), as well as the profiles for PCDD/Fs.

2.5 Convention of reporting toxicological effects of environmental PCDD/F and PCBs

When establishing the toxicological effects of the dioxins present in environmental matrices, it is common and accepted practice to estimate a so-called "toxic equivalent" value based on the comparative toxicity of each dioxin congener/isomer present in the matrix. Because 2,3,7,8-TCDD is the most toxic congener, the equivalence values of the toxic PCDD/Fs and co-planar PCBs are proportionally related to it. Equivalence values are a factor of unity and by convention 2,3,7,8-TCDD is assigned a toxicity rating of 1.0 (called a toxic equivalent factor or TEF). The other dioxin-like congeners are assigned lower TEFs comparable to their toxicity, relative to that of 2,3,7,8-TCDD. The toxicity of any mixture of PCDD/Fs and dioxin-like PCBs relative to 2,3,7,8-TCDD can be expressed by multiplying the concentrations of the individual congeners by their respective TEFs. The resulting products for each congener are called toxic equivalent quotients (TEQs), with units identical to that in which the concentrations of the individual congeners are expressed. An example of the written expression of TEQ, would be 243 ngTEQ kg⁻¹. The TEQ of the mixture is determined by the sum of the individual TEQs (McKay, 2002) and can be used to conduct comprehensive dioxin-equivalent risk assessment for humans and the ecosystem (Yao, Masunaga, Takada & Nakanishi, 2002).

A number of toxicity rating schemes have been developed by different countries, but the scheme that has been internationally adopted is that of the North Atlantic Treaty

Organisation Committee on the Challenges of Modern Society (NATO/CCMS). This is known as I-TEF (Table 2.5) (Ahlborg, Brouwer, Fingerhut, Jacobson, Jacobson, Kennedy, Kettrup, Koeman, Poiger, Rappe, Safe, Seegal, Tuomisto & Vandenberg, 1992). The I-TEF scheme was revised by the WHO to also include values for PCBs (Van den Berg, Birnbaum, Bosveld, Brunstrom, Cook, Feeley, Giesy, Hanberg, Hasegawa, Kennedy, Kubiak, Larsen, Van Leeuwen, Liem, Nolt, Peterson, Poellinger, Safe, Schrenk, Tillit, Tysklind, Younes, Waern & Zacharewski, 1998). The WHO-TEF recognises different toxicities in different animal groups and has values for human/mammals, fish and birds (Table 2.5). The I-TEF does not make this distinction. Recently the WHO re-evaluated their values for mammals/humans (Table 2.5) (Van den Berg, Birnbaum, Denison, De Vito, Farland, Feeley, Fiedler, Hakansson, Hanberg, Haws, Rose, Safe, Schrenk, Tohyama, Tritscher, Tuomisto, Tysklind, Walker & Peterson, 2006).

Table 2.5: I-TEF, WHO-TEF and revised WHO-TEF values for the different PCDD/F and PCB congeners.

	I-TEFs	WHO-TEF ₁₉₉₈			WHO-TEF ₂₀₀₅
		Fish	Birds	Humans/ Mammals	Humans/ Mammals
PCDDs					
2,3,7,8-TCDD	1	1	1	1	1
1,2,3,7,8-PeCDD	0.5	1	1	1	1
1,2,3,4,7,8-HxCDD	0.1	0.5	0.05	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.01	0.01	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.01	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.001	<0.001	0.01	0.01
OCDD	0.001			0.0001	0.0003
PCDFs					
2,3,7,8-TCDF	0.1	0.05	1	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.05	0.1	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.5	1	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1	0.1	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1	0.1	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1	0.1	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1	0.1	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01	0.01	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01	0.01	0.01	0.01
OCDF	0.001	0.0001	0.0001	0.0001	0.0003
PCBs					
3,3',4,4'-TCB				0.0001	0.0001
3,4,4',5'-TCB				0.0001	0.0003
3,3',4,4',5'-PeCB				0.1	0.1
3,3',4,4',5,5'-HxCB				0.01	0.03
2,3,3',4,4'-PeCB				0.0001	0.00003
2,3,4,4',5'-PeCB				0.0005	0.00003
2,3',4,4',5'-PeCB				0.0001	0.00003
2',3,4,4',5'-PeCB				0.0001	0.00003
2,3,3',4,4',5'-HxCB				0.0005	0.00003
2,3,3',4,4',5'-HxCB				0.0005	0.00003
2,3',4,4',5,5'-HxCB				0.00001	0.00003
2,3,3',4,4',5,5'-HpCB				0.0001	0.00003

All available scientific data was evaluated to derive consensus on TEF values. Studies including *in vitro* binding of a dioxin-like compound to the aryl hydrocarbon receptor (AhR) in vertebrate cells and *in vitro* and *in vivo* toxicity studies were considered for determining the toxicity of each congener compared to 2,3,7,8-TCDD (Van den Berg *et al.*, 1998).

2.6 Environmental levels of PCDD/Fs and PCBs: A global perspective (excluding southern Africa).

There has been an extensive body of publications on the levels of persistent toxic substances in the environment over the past four decades, but the majority of them from the northern hemisphere. Detailed review papers and reports by institutions such as the United States of America's Environmental Protection Agency (US EPA), UNEP, the WHO and the Arctic Monitoring and Assessment Programme (AMAP) are proof of this. The levels presented in this section for the different environmental compartments are not comprehensive, but is meant as a sample of values reported in the literature for the northern hemisphere from studies and reports from about the last 20 years. Reference to a few southern hemisphere countries is made as well. Since levels of POPs were reported as either I-TEQ, WHO-TEQ or concentrations, this section was not summarised in a table.

Soils and sediments proved to be acting as reservoirs for these compounds and concentrations from these matrices were generally higher than in the others (air and water). Some of the references referred to in section 2.6 did not distinguish between different congeners, but reported concentrations for the entire PCDD/F or PCB group. Where the concentrations for the individual congeners were presented, a TEQ-value was calculated from the published concentrations. Since no I-TEF values exist for PCBs, the latest WHO-TEF values, i.e. for 2005, were used. For the PCDD/F congeners of which the concentrations were reported before 1998, the I-TEF values of 1998 were used to facilitate comparison to literature which reported I-TEQ values (cf. Table 2.5).

2.6.1 In air

Data on dioxins and furans in air has been reported in the industrialised world since the 80's. Ambient concentrations show a high variability from urban to rural and from contaminated to uncontaminated sites. Typical concentrations in Europe and Mediterranean regions range from <1 to 14 800 fgTEQ m⁻³, in rural and contaminated areas respectively. In Asia, reported values range from 7 and 1 486 fgTEQ m⁻³. In South America, reported values range between 3 and 394 pgTEQ m⁻³ (in airborne particulate matter) (UNEP, 2003). No such data could be found for sub-Saharan Africa.

2.6.2 In freshwater

Freshwater environments basically include rivers, dams, wetlands and lakes. Pollutant loads are highly influenced by the respective hydrological regimes and if sampling is not adequately performed, data is hardly representative. From the collected data, it appears as if PCB concentrations in freshwater environments are low - usually below detection limits - which can be explained by their low water solubility. However, concentrations of PCBs in Arctic lake and river water in Canada are in the range of 0.15 to 4.83 $\mu\text{g m}^{-3}$, exceeding the US EPA Great Lakes Water Quality guideline for protection of aquatic life of 17 ng m^{-3} (AMAP, 1998). Practically no data has been reported for PCDD/Fs in water across the globe, or their levels are below detection limits. An exception to this is the reported data from the Republic of Korea, where the ranges are 0.001 to 1.061 ngTEQ m^{-3} (UNEP, 2002b). In 2000, the Japan national dioxin survey average level in water (rivers, lakes and coastal sea water) in 2 116 sites was reported to be 0.31 ngTEQ m^{-3} with a range of 0.012 to 48 ngTEQ m^{-3} . The levels in 83 sites exceeded the water quality standard (1 ngTEQ m^{-3}) of Japan (UNEP, 2002b). The 1996 studies of several New Zealand rivers revealed no PCDD/Fs in the water samples (the limit of detection (LOD) was 2 ng m^{-3} for 2,3,7,8-TCDD) (UNEP, 2002c).

2.6.3 In oceans

Predictably, the highest concentrations of PCBs were reported in urban and industrial coastal wastewaters as well as in river discharges. Accordingly, decreasing concentration gradients have been found in transects offshore from these sources (UNEP, 2003). Concentrations of PCBs in filtered ocean water are usually reported to be in the low ng m^{-3} range and this makes reliable quantification difficult. A large survey conducted by Iwata, Tanabe, Sakai and Tatsukawa (1993) showed a rather uniform distribution of PCBs in surface waters of the Atlantic, Pacific and Indian Oceans, with relatively higher levels in the temperate northern hemisphere. Levels in the North Pacific varied between 9.1 and 63 ng m^{-3} , and for the Bay of Bengal and the Arabian Sea, levels were between 13 and 46 ng m^{-3} . Particular studies have been performed in the European and Mediterranean Seas. In general, the concentrations of PCBs for all the investigated areas in the Mediterranean Sea were similar, except in the Ligurian Sea where concentrations were higher: PCB concentrations in the suspended particulate matter from coastal and open Western Mediterranean waters were 5 to 35 ng m^{-3} , and for the Ligurian Sea 100 to 17 700 ng m^{-3} , in 1990 (Tolosa *et al.*, 1997).

Antarctic data for surface seawater showed concentrations varying from 35 to 69 ng m^{-3} total PCBs (Tanabe, Hidaka & Tatsukawa, 1983).

Information on dioxin levels in seawater is scarce, but a few point source emissions have been studied, such as those associated with the Frierfjorden (North Sea). Dioxin concentrations seem ten to twenty times higher in samples from the northern North Sea than in samples from the Barents Sea (UNEP, 2003). In both the river and coastal sea waters of Japan, the sum of dioxins and furans reported by the Ministry of the Environment in 1997 gave a mean concentration of $0.37 \text{ pgTEQ m}^{-3}$. Data from local governments at 21 sites ranged from non-detect (n.d.) to 0.4 pgTEQ m^{-3} , with an average concentration of $0.061 \text{ pgTEQ m}^{-3}$ (UNEP, 2002b). In general, dioxin levels in coastal water were one order of magnitude lower than those in rivers (UNEP, 2003).

2.6.4 In soils

A global survey was conducted by a European Group (GLOBAL SOC PROJECT) and a wide range of levels were reported (UNEP, 2003). Higher concentrations were detected in areas where PCB use was intense and where PCBs had been disposed of: In Eastern Europe, several sites reached levels of 53 mg kg^{-1} (Kocan, Petrik, Jursa, Chavancova & Drobna, 2001). In Panama (Central America), PCBs were detected in several studies around transformer sites in levels up to 185 mg kg^{-1} (UNEP, 2003). Background soil PCB concentrations were strongly influenced by proximity to source region and soil organic matter content.

More than 80% of the estimated PCB burden remains in the "global source region" of the northern hemisphere temperate latitudes (30 to 60° N), or in the organic matter rich soils just north of that. The lowest and highest background PCB concentrations (26 and $97\,000 \text{ pg g}^{-1}$ dry weight (d.w.)) were found in samples from Greenland and mainland Europe (France, Germany and Poland), respectively, in surface soils (Meijer, Ockenden, Sweetman, Breivik, Grimalt & Jones, 2003).

Sewage sludge is monitored for PCBs in Europe where they are widely used in agriculture. The European Union (EU) established a maximum concentration of 0.8 mg kg^{-1} of PCBs. The values found in 50 wastewater plants in the east of France were between 0.04 and 1.13 mg kg^{-1} , and 0.5 mg kg^{-1} in the region of Paris. The mean value in sludges disposed through agriculture was of 0.19 mg kg^{-1} (UNEP, 2002d).

In Europe, a number of intensive surveys on PCDD/F levels have been carried out. Within the EU Member States, a large database for PCDD/F concentrations in soil is available (Fiedler, Buckley-Golder, Coleman, King & Petersen, 1999). As shown in Table 2.6, concentrations range from several hundred to around $100\,000 \text{ ng I-TEQ kg}^{-1} \text{ d.w.}$ at contaminated locations. The highest concentrations were found in Finland at sites contaminated with wood preservatives, and in the Netherlands close to a scrap car and

Table 2.6: Summary of PCDD/F concentrations in soils from EU Member States. Concentrations in ngTEQ kg⁻¹ d.m. (Fiedler *et al.*, 1999).

	Forest	Pasture	Arable	Rural	Contaminated sites	Others
Austria	0.01-64	1.6-14			332	
Belgium				2.1-2.7		2.7-8.9
Finland					85 000	
Germany	10-30	0.004-30	0.03-25	1	30 000	0.1-42
Greece					1 144	2-45
Ireland	4.8	0.8-13				0.15-8.6
Italy		0.1-43	1.9-3.1			0.057-0.12
Luxembourg	6.0			1.4		1.8-20
The Netherlands				2.2-17	98 000	2-55
Spain				0.1-8.4		0.63-8.4
Sweden				0.11	11 446	
United Kingdom				0.78-20	1 585	0.78-87

scrap wire incinerator.

The US EPA's Dioxin reassessment document estimate mean TEQ values for background urban and rural soils in the USA to be 13.4 and 4.1 ng I-TEQ kg⁻¹ of soil, respectively (US EPA, 2003).

2.6.5 In freshwater sediments

PCBs have been detected in Arctic lake sediments. Concentrations of a total of 12 congeners were in the range of 2 000 to 40 000 ng kg⁻¹ d.w., with a significant latitudinal decline of penta- to octachlorobiphenyls, with increasing north latitude (Muir *et al.*, 1996). Reported sediment data from the South American continent is biased by contaminated sites in Argentina, the Río Santiago (998 000 ng kg⁻¹) and Brazil, 580 000 ng kg⁻¹. Excluding these sites, the general PCB mean decrease from 58 000±149 000 to 9 100±7 700 ng kg⁻¹ is lower than the Canadian guideline for protection of aquatic life (34 100 ng kg⁻¹) (UNEP, 2002e). On the North American continent, relatively low concentrations for dioxin-like PCBs were reported for a variety of lakes and the average TEQ was 0.49 ng kg⁻¹ WHO₂₀₀₅-TEQ (Cleverly, Monetti, Phillips, Cramer, Hert, McCarthy, O'Rourke, Stanley & Winters, 1996) and 0.81 ng kg⁻¹ WHO₂₀₀₅-TEQ for Lake Ontario (Oliver & Niimi, 1988). Lake Michigan sediments collected at Waukegan, Illinois, had 2 001 ng kg⁻¹ WHO₂₀₀₅-TEQ (Huckins, Schwartz, Petty & Smith, 1988). The Sheboygan River in Eastern Wisconsin had a 410 ng kg⁻¹ WHO₂₀₀₅-TEQ (Sonzogni, Maack, Gibson & Lawrence, 1991). (All WHO₂₀₀₅-values were calculated from concentrations reported in the respective papers.)

Vartiainen, Mannio, Korhonen, Kinnunen and Strandman (1997) determined PCDD/F profiles in three lakes in northern Finland and found low TEQ concentrations ranging from 1.4 to 4.2 ng kg⁻¹ d.w. in surface slices of sediments. Evans, Bourbonniere, Muir, Lockhart, Wilkinson and Billeck (1996) determined PCDD/Fs in two cores from the Great Slave Lake, as well as in sediment surface grab samples from the same lake, and found TEQs in

surface slices ranging from < 0.01 to $\approx 0.2 \text{ ng kg}^{-1}$. Other literature surveyed for the AMAP 1998 reported a variation of 0.07 to $68.4 \text{ ngTEQ kg}^{-1}$ for Arctic freshwater sediments. Marvin, Stern, Reiner, MacPherson, Kolic, Braekevelt and Painter (2002) conducted a survey in Lake Ontario (Canada) to assess spatial and temporal trends in sediment PCDD/F contamination. The analysis showed a mean of 91 ngTEQ kg^{-1} , but four sites had concentrations in excess of $200 \text{ ngTEQ kg}^{-1}$.

PCDD/F levels in the sediment of Italy's Po River were reported to be between 121 and 814 , and from 1.3 to $13 \text{ ng kg}^{-1} \text{ d.w.}$, respectively (Fattore, Viganò, Mariani, Guzzi, Benfenati & Fanelli, 2002). Concentrations of PCDD/Fs in sediments of the Housatonic River in the United States of America ranged from 160 to $5\,400 \text{ ng kg}^{-1} \text{ d.w.}$, except in one sample that contained a concentration of $82\,000 \text{ ng kg}^{-1} \text{ d.w.}$ (Eitzer, 1993). Total concentrations of PCDD/Fs in sediments from rivers in the United Kingdom ranged from 446 to $9\,310 \text{ ng kg}^{-1} \text{ d.w.}$ (Rose, McKay & Ambigde, 1994), and in river sediments from Japan from 377 to $15\,792 \text{ ng kg}^{-1} \text{ d.w.}$ (Sakurai, Kim, Suzuki & Nakanishi, 1996). Concentrations of PCDD/Fs in sediments from New Zealand ranged between 0.081 and $2.71 \text{ ng I-TEQ kg}^{-1}$ (UNEP, 2002c). In general, levels of PCDD/Fs in river sediments sampled were higher in industrial and urban areas (UNEP, 2003).

2.6.6 In marine sediments

Because 80% of the terrestrial sediments are trapped on the continental shelf and only the finest particles are transported by currents to deep sea basins, research attention has been mainly focused on coastal sediments (UNEP, 2003). In the AMAP 1998 report, sediment levels for Arctic seas were low: < 500 to $9\,300 \text{ ng kg}^{-1} \text{ d.w.}$. Most sites had concentrations less than $1000 \text{ ng kg}^{-1} \text{ d.w.}$ and were from nearshore areas in Norway, Russia, Greenland, the North American shelves of the Bering/Chukchi Seas, and the Beaufort Sea/Mackenzie River Delta area. A mapping of PCBs in nearly 100 sediment samples collected from different areas of the Western Mediterranean basin revealed the widespread occurrence of these pollutants in the region (Tolosa, Bayona, & Albaigés, 1995; Tolosa *et al.*, 1997). Point sources were identified near sewage outfalls from highly industrialised and populated cities: $229\,000 \text{ ng kg}^{-1}$ for nine PCB congeners. Other substantially PCB-contaminated sediments arose from freshwater discharges like the Rhône and Ebro rivers: $34\,000 \text{ ng kg}^{-1}$ for nine PCB congeners. Deep basin levels were between $2\,000$ and $6\,000 \text{ ng kg}^{-1} \text{ d.w.}$ In the east coast of India, PCBs were found at levels from not detected to $1\,400 \text{ ng kg}^{-1}$ in sediments from river mouths, and from not detected to $1\,090 \text{ ng kg}^{-1}$ in the coastal region (Sarkar & Everaarts, 1998).

In the Pacific Islands region, measured PCBs in sediments ranged from not detected to several hundred thousands of ng kg^{-1} in heavily polluted sites. In New Zealand, the South-East Asia and the South Pacific Region, the sum of 25 PCB congeners was in the range of 120 to 8 800 ng kg^{-1} d.w. for sediment samples. Kennicutt, McDonald, Sericano, Boothe, Oliver, Safe, Presley, Liu, Wolfe, Wade, Crockett and Bockus (1995) analysed sediments from Winter Quarters Bay near the McMurdo Station, and from Arthur Harbour on the Antarctic Peninsula. They reported total PCB concentrations ranging from 250 000 to 4 300 000 ng kg^{-1} and 2 800 to 4 200 ng kg^{-1} respectively. Concentrations decreased rapidly further away from sources of contamination.

The greatest PCDD/F concentrations observed in sediments of the Rhine and Humber Rivers along the estuarine and coastal North Sea ranged between 1 846 and 10 557 ng g^{-1} d.w. (Evers, Klammer, Laane & Govers, 1993). Sediment from the inner Stockholm archipelago had 86.8 ng I-TEQ kg^{-1} PCDD/F (Broman, Nat, Zebuhr & Lexen, 1989). Contaminated sites in the sediments of New Jersey measured concentrations of 21 000 ng kg^{-1} for 2,3,7,8-TCDD (Bopp, Gross, Tong, Simpson, Monson, Deck, & Moser, 1991). The I-TEQ values for the sediments from a selection of North American harbours showed a high contamination of PCDD/Fs: Black Rock Harbour: 1 780 ng I-TEQ kg^{-1} ; Providence River: 1 602 ng I-TEQ kg^{-1} ; New Bedford Harbour: 103.3 to 2 555.9 ng I-TEQ kg^{-1} and Eagle Harbour: 147.6 to 890.3 ng I-TEQ kg^{-1} (Norwood, Hackett, Powell, Butterworth, Williamson & Naumann, 1989). I-TEQ values varied between 580.2 to 3 666.7 ng kg^{-1} for sediments from Hamburg harbour (Gotz & Schumacher, 1990) and Oehme, Mane, Brevik and Knutzen (1989) determined the concentration of PCDD/Fs for a fjord in Norway of which the I-TEQ was 86 118.2 ng kg^{-1} . The I-TEQ for the sediments from Masan Bay in Korea was in the range of 1 to 76 ng kg^{-1} d.w. (Im, Kannan, Matsuda, Giesy & Wakimoto, 2002).

2.7 Environmental levels of PCDD/Fs and PCBs: Southern Africa

Very little is known about some of the compounds included in the Stockholm Convention for southern Africa. An initiative published in 2002 by UNEP, the Regionally Based Assessment of Persistent Toxic Substances, it was revealed that in the previous 30 years only 48 publications usable for the assessment appeared on persistent toxic substances for sub-Saharan Africa in scientific literature (Bouwman *et al.*, 2002). The countries for which publications appeared, include Botswana, Namibia, South Africa and Zimbabwe. In these 48 publications, reference was made to 15 different compounds (or groups of compounds). Most of the 48 publications referred to DDT (Figure 2.3). Other compounds reported more than once include dieldrin, HCB, mercaptothion and PCBs. Some of the compounds appearing in Figure 2.3, such as nonylphenol, mercaptothion and atrazine, are not

currently listed as a concern of the Stockholm Convention. (In this literature survey, no distinction was made between PCBs in general and those that are dioxin-like.)

Although South Africa is a party to the Stockholm Convention (Bouwman, 2004), the country has limited legislation regarding these chemicals. POPs pesticides are covered as pesticides under Act 36 of 1947: Fertilizers, farm feeds, agricultural remedies and stock remedies Act, and most of them were withdrawn in the 80's and 90's. Chlordane may still be used for protection of constructions against termites, and DDT is only used by the government for malaria control (Bouwman, 2003). The only policy mentioning dioxins occurs under the Waste Management Policy, in process 39: Waste Incineration Processes. According to this policy PCDD/F should not exceed 80 ng m^{-3} in air emissions in a period of 6 to 16 hours, or $0.2 \text{ ng I-TEQ m}^{-3}$ (SA, 2007).

Scientific literature for POP compounds in South Africa has mainly focussed on pesticide POPs, and specifically DDT and its metabolites. DDT has been detected in birds (Davies &

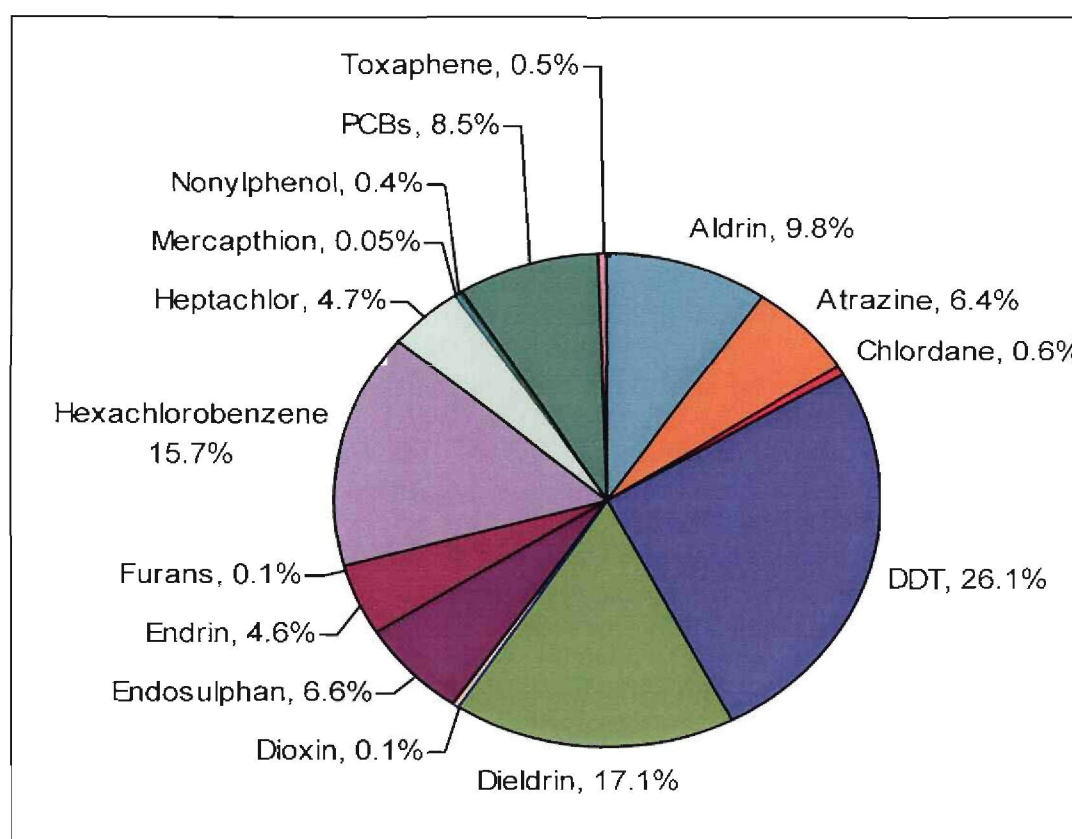


Figure 2.3: The percentage of scientific literature on persistent toxic substances in Southern Africa that appeared in the 30 years prior to 2002 in 48 papers (Bouwman *et al.*, 2002).

Randall, 1989; Evans & Bouwman, 2000; Lötter, & Bouwman, 2001; Macdonald, Brooks & Gardner, 1985), other terrestrial and marine wildlife (De Kock, Best, Cockcroft & Bosma, 1994; Gardner, 1984) as well as in humans (Bouwman, Becker & Schutte, 1994; Bouwman, Cooppan, Botha & Becker, 1991). Other POPs pesticides include dieldrin, toxaphene, endosulphan and hexachlorobenzene (Brooks & Gardner, 1980; De Kock *et al.*,

1994; Little, Crowe & Peall, 1997; Schulz, 2001; Van Wyk, Bouwman, Van der Bank, Verdoorn & Hofmann, 2001; Vetter, Weichbrodt, Schultz & Oelschläger, 1999; Wiktelius & Edwards, 1997).

Some papers reported on PCBs, but focussed on the group as a whole. No distinctions were made between the different congeners, except for one (Vetter *et al.*, 1999) where the authors reported on the levels of eight PCB congeners in the blubber of seals. They reported a mean of $104 \mu\text{g kg}^{-1}$. Other authors who reported PCBs in marine animals were Cockcroft, De Kock, Lord and Ross (1989) who reported PCBs (Aroclor 1260) for bottlenose dolphins off the coast of KwaZulu-Natal, varying between $8.4 \mu\text{g PCB g}^{-1}$ blubber for the south coast animals, $13 \mu\text{g PCB g}^{-1}$ blubber for the central coast animals and $20 \mu\text{g PCB g}^{-1}$ blubber for the north coast animals. The same authors reported a mean PCB of $4.04 \mu\text{g g}^{-1}$ blubber wet weight (w.w.) for common dolphins caught during a "sardine run" (Cockcroft, De Kock, Ross & Lord, 1990). The PCB load in the blubber of seals and small whales caught in the period 1977 to 1987 varied between 0.01 to $15.51 \mu\text{g g}^{-1}$ w.w. (De Kock *et al.*, 1994). Total PCBs were also determined for fish species from the Isipingo Estuary, KwaZulu-Natal. Concentrations varied between 5.7 to $869 \mu\text{g kg}^{-1}$ w.w. with a mean of $96.5 \mu\text{g kg}^{-1}$ w.w. across all species (Grobler, Badenhorst & Kempster, 1996). The eggs of coastal birds also had PCB residues. Concentrations varied between 0.05 to $0.89 \mu\text{g g}^{-1}$ w.w. (De Kock & Randall, 1984) and the PCB concentrations in African Marsh Harrier eggs varied between 0.2 to $2.24 \mu\text{g g}^{-1}$ w.w. (De Kock, 1988).

A single paper reported on PCBs measured for sediments of water bodies from the interior of the country: both the Voëlvlei Dam (in the Western Cape): 0.06 mg kg^{-1} d.w. and the Hartbeespoort Dam (in the North-West Province): 0.32 mg kg^{-1} d.w. (Greichus, Greichus, Amman, Call, Hamman & Pott, 1977). Grobler (1994) also checked for the presence of PCBs (Aroclor 1254 and 1260) in the sediment, water and fish of the Olifants River in Mpumalanga, but levels were below the detection limit. In another study on the same river, fish from one site showed detectable levels of PCBs: $41.0 \mu\text{g kg}^{-1}$ w.w. (Bouwman, 2003).

At the onset of this study (2001) the only publication on PCDD/F levels in South Africa was published by Schecter, Startin, Rose, Wright, Parker, Woods and Hansen (1990) and focussed on the levels in human breast milk: for six black rural South Africans and 18 white urban South Africans the mean PCDD levels were 279.8 and 355.6 ng kg^{-1} milk fat, respectively. The levels for the PCDFs were 20.4 and 23.1 ng kg^{-1} milk fat, respectively. The corresponding TEQ for the PCDDs was 8.3 ng kg^{-1} milk fat and for the PCDFs it was 12.6 ng kg^{-1} milk fat. Since 2001, another publication on specifically PCDD/Fs and dioxin-like PCBs levels, found on a platinum mine, appeared early 2007 (Jordaan, Pieters, Quinn,

Giesy, Jones, Murphy & Bouwman, 2007). These authors reported that slag from the smelter contributed the most (45 ng TEQ kg⁻¹ d.w.) to the TEQ of the tested matrices. The other matrices included dump site soil, tailings dam soil and wood chips.

Thus, when South Africa ratified the Stockholm Convention, the country committed to control and stop the intentional and unintentional release of POPs chemicals, but at the onset of this study, little was known about dioxin-like PCB levels, and nothing about PCDD/F concentrations in the environment.

2.8 Health effects of PCDD/Fs and dioxin-like PCBs

Since the identification of 2,3,7,8-TCDD as a chloracneagen, more than 5 000 publications have discussed its biological and toxicological properties (US EPA, 2004a). Animal studies first characterised the health effects of varying doses and combinations of dioxins (Martinez *et al.*, 2003).

As mentioned in the Introduction chapter, dioxin can cause death following even tiny doses in certain species, leading 2,3,7,8-TCDD to be called "the most toxic man-made chemical." The LD₅₀ for guinea pigs is ~ 1 µg kg⁻¹ body weight, and from the results of poisoning episodes the LD₅₀ for humans is clearly higher than for guinea pigs (Schechter *et al.*, 2006). Instead of reacting directly with cellular macromolecules, it is believed that the toxicity associated with TCDD is achieved through disruption of signalling pathways or production of a secondary toxicant capable of reacting with macromolecules like DNA. Most of the effects of TCDD require activation of the AhR, which results in transcriptional activation or the repression of a diverse array of genes (Mandal, 2005).

To summarise some of the effects that TCCD and related compounds have on different animal species, Table 2.7 (adapted from US EPA, 2004a) is included. Clinical manifestations of dioxins in humans and other vertebrates include:

- Being a risk factor for human cancer (Fingerhut, Halperin, Marlow, Piacitelli, Honchar, Sweeney, Greife, Dill, Steenland & Suruda, 1991; US EPA, 2004a). There is adequate evidence that 2,3,7,8-TCDD is a carcinogen in laboratory animals, based on long-term bio-assays conducted on both sexes of several strains of rats and mice, hamsters, and fish (US EPA, 2004a). TCDD is mutagenic *in vivo*, but is not directly genotoxic: even though genetic mutation can occur as a result of TCDD exposure, TCDD is not metabolised into a DNA-binding compound (Mandal, 2005);
- Immune deficiency (Weisglas-Kuperus, Patandin, Berbers, Sas, Mulder, Sauer & Hooijkaas, 2000). Suggestions of immunological disturbances also have been

Table 2.7: Effects of TCDD and related compounds in different animal species (US EPA, 2004a)

Effect	Human	Monkey	Guinea pig	Rat	Mouse	Hamster	Cow	Rabbit	Chicken	Fish	Avian wildlife	Marine mammals	Mink
Enzyme induction	+	+	+	+	+	+		+	+	+	+	+	+
Acute lethality	0	+	+	+	+	+	+	+	+	+	+	+	+
Wasting syndrome	+	+	+	+	+	+	+	+		+	+	+	+
Teratogenesis/fetal toxicity, mortality	+/-	+	+	+	+	+		+	+	+	+	+	+
Endocrine effects	+/-	+		+	+					+	+	+	+
Immunotoxicity	+/-	+	+	+	+	+	+		+	+		+	
Carcinogenicity	+/-			+	+	+				+			
Neurotoxicity	+	+		+	+				+				
Chloracne/genic effects	+	+			+		+	+		+			
Porphyria	+	0	0	+	+	0			+				
Hepatotoxicity	+	+	+/-	+	+	+/-	+	+	+	+	+	+	+
Edema		+	0	0	+	+			+	+			
Testicular atrophy		+	+	+	+								
Bone marrow hypoplasia		+	+		+/-				+				
Teeth development	+	+		+									

+: observed
 +/-: observed to limited extent, or +/- results
 0: not observed
 blank cells: no data

observed in a small group of exposed workers (Tonn, Esser, Schneider, Steinmann-Steiner-Haldenstatt, & Gleichmann, 1996) and in perinatally exposed children (Ten Tusscher, Steerenberg, Van Loveren, Vos, Von dem Borne, Westra, Van der Slikke, Olie, Pluim & Koppe, 2003). The primary antibody response to the T-cell-dependent antigen, sheep red blood cells, is the most sensitive immunological response that is consistently suppressed in mice exposed to TCDD and related compounds. Results of studies that have compared the effects of acute exposure to individual PCDD/Fs and PCB congeners which have different binding affinities for the AhR, provided evidence that certain congeners are also immunosuppressive (US EPA, 2004a). In addition to immune suppression, TCDD promoted inflammatory responses. This effect may result from an upregulation of the production of inflammatory cytokines such as interleukin-1 and tumour necrosis factor (TNF- α) (Kerkvliet, 1995);

- Reproductive and developmental abnormalities (Guo *et al.*, 2003). In the rice oil contamination incidents at Yusho in Japan and Yu-Cheng in Taiwan, all four manifestations of developmental toxicity (reduced viability, structural alterations, growth retardation, and functional alterations) were observed to some degree. The Yusho and Yu-Cheng incidents resulted in increased perinatal mortality and low birth weight in infants born to women who had been exposed (US EPA, 2004a). The primary effects of TCDD on female reproduction in animals appear to be decreased fertility, inability to maintain pregnancy for the full gestational period, and, in the rat, decreased litter size. In some studies of rats and primates, signs of ovarian dysfunction such as anovulation and suppression of the oestrous cycle have been reported (Allen, Barsotti, Lambrecht, & Miller, 1979; Barsotti, Abrahamson & Allen, 1979; Kociba, Keeler, Park & Gehring, 1976; Li, Johnson & Rozman, 1995a,b). In male animals, dioxin compounds cause decreased testis and accessory sex organ weights, abnormal testicular morphology, decreased spermatogenesis, and reduced fertility when given to adult animals in doses sufficient to reduce feed intake and/or body weight (US EPA, 2004a);
- Central and peripheral nervous system pathology (Guo *et al.*, 2003). It is suggested that dioxins exhibit endocrine-disrupting actions on the gonadal and thyroid hormone axes, as well as "neural-disrupting action" on neural transmission and neural network formation (Kakeyama & Tohyama, 2003);
- Endocrine disruption, including diabetes (Longnecker & Michalek, 2000) and thyroid disorders (Pavuk, Schechter, Akhtar & Michalek, 2003). An inverse correlation between TCDD levels and prolactin in 2,4,5,-T herbicide sprayers was also demonstrated (Johnson, Shorter, Bestervelt, Patterson, Needham, Piper, Lucier & Nolan, 2001). The

risk of abnormally low testosterone was two to four times higher in exposed workers who had serum 2,3,7,8-TCDD levels above 20 000 ng kg⁻¹ compared with unexposed referents (Egeland, Sweeney, Fingerhut, Wille, Schnorr & Halperin, 1994);

- Eyelid pathology, including meibomian gland (modified sebaceous glands) hypersecretion and hyperpigmented conjunctivae (Masuda, 2003);
- Gum pigmentation (Masuda, 2003);
- Nausea; vomiting; loss of appetite; skin rashes; including, rarely, chloracne (a severe acne-like condition that develops within months of first exposure to high levels of dioxin and related compounds); hypertrichosis; liver damage; elevated serum cholesterol and triglycerides (Kimbrough, Carter, Liddle, Cline & Phillips, 1977).

A recent, deliberate poisoning with TCDD was that of the then Ukrainian presidential candidate Victor Yushchenko, who presented with chloracne (Schechter *et al.*, 2006).

The most unusual aspect of the toxic action of dioxin is that it affects many organs. Its effects vary greatly, depending on the type of cell, tissue, age, sex, species, and the timing and duration of exposure. Even within the same cell line, its toxicity can vary greatly, depending on the culture conditions and stage of growth. Exposure to TCDD thus results in numerous pathophysiological abnormalities.

2.9 Mechanism of dioxin toxicity

There is a great deal of evidence which indicates that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and other dioxin-like compounds, including the co-planar PCBs, act via the intracellular protein called AhR, which is a ligand-dependent transcription factor that functions in partnership with a second protein (known as the AhR nuclear translocator, or Arnt) to alter gene expression (Figure 2.4). Receptor binding may also result in the release of cytoplasmic proteins that, in turn, alter the expression or activity of cell-regulatory proteins.

An understanding of the mechanism of the AhR will give insight into the principles of the H4IIE bio-assay that was used in this study. The AhR has several features in common with a class of transcription factors known as basic helix-loop-helix (bHLH) proteins. The basic region mediates DNA binding, whereas the helix-loop-helix domain is necessary for dimerisation with other proteins. Two other receptor regions function in dimerisation: PAS-A and PAS-B. These are known as PAS regions because of their sequence homology with **Per** (a *Drosophila* circadian rhythm protein), **Arnt** (another protein that contributes to dioxin responsiveness and referred to in the previous paragraph) and **Sim** (a regulatory protein that participates in *Drosophila* central nervous system development (Huang, Edery & Rosbash, 1993).

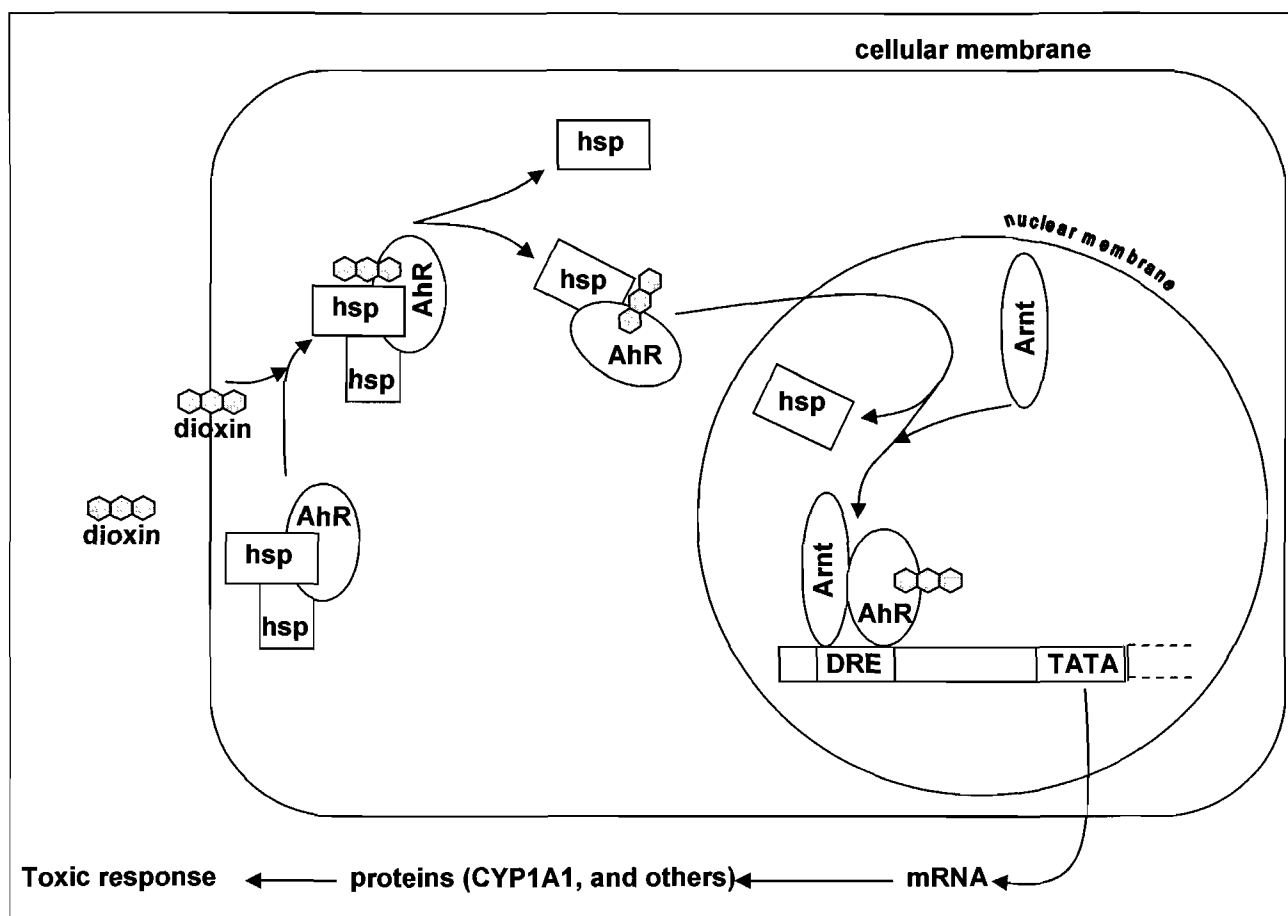


Figure 2.4: A schematic representation of the cellular mechanism of AhR action (adapted from US EPA, 2004).

hsp = heat shock protein; AhR = aryl hydrocarbon receptor; Arnt = AhR nuclear translocator; DRE = dioxin response element; TATA = initiation sequence for gene to be transcribed

In the absence of a ligand, the PAS-B region associates with one heat shock protein 90 (hsp 90) molecule, permitting binding of a second hsp 90 to the HLH region (cf. Figure 2.4) (Fukanaga, Probst, Reisz-Porszasz & Hankinson, 1995). Also associated to this complex is a small protein (p23) and an immunophilin-like protein (XAP2) (Petrulis, Hord & Perdew, 2000). TCDD and like compounds interact with a ligand-binding pocket near the PAS-B region, and one of the hsp 90 molecules is released from the AhR. The unliganded AhR resides in the cytoplasm, but when bound to a dioxin-type molecule, the liganded receptor moves to the inside of the nucleus (Figure 2.4). Inside of the nucleus, the AhR-ligand-hsp 90 complex releases the second and last hsp and interacts with the nuclear protein, Arnt, to form a heteromeric DNA-binding protein complex that can activate gene transcription. Arnt is also a bHLH family member, but the AhR is the only ligand-activated member (Mandal, 2005). The Arnt is also known as hypoxia inducible factor-1 β (Hankinson, 1995). The AhR-ligand-Arnt heterodimer binds to a DRE on the DNA to activate the expression of a dioxin-responsive reporter gene further downstream. The core sequence of the DRE is 5'-TNGCGGTG-3' (Denison, Fisher & Whitlock, 1988). In Figure 2.4, the start of the reporter gene is represented with the nucleotide sequence TATA (US EPA, 2003).

Much of the current understanding of the mechanism of dioxin action is based on analyses of the induction of particular enzyme activities by TCDD. Examples of these are the cytochrome P450A1 drug-metabolising enzymes *CYP1A1*, *CYP1A2*, and *CYP1B1*, which have elicited some of the most sensitive responses observed in a variety of different animal species, including humans, and it occurs at body burdens as low as 3 to 8 ngTCDD kg⁻¹ body mass in animals.

Aryl hydrocarbon hydroxylase (AHH) is one of the enzymes that reflects the action of the *CYP1A1* enzyme, and catalyses oxygenation of polycyclic aromatic substrates as the initial step in their metabolic processing to water-soluble derivatives (Conney, 1982).

TCDD-elicited activation of the AhR has been shown to mediate altered transcription of other genes too, including several oncogenes and those encoding growth factors, receptors and hormones. The transcription of some genes may be directly regulated by the activated AhR as for the cytochrome P450 enzymes, but other alterations in gene expression may be secondary to the initial biochemical events directly regulated transcriptionally by the AhR (US EPA, 2004a). There appears to be considerable cross-regulation of the AhR with other signalling proteins, including hypoxia inducible factor, estrogen receptors, and the retinoic acid and thyroid hormone receptors (Caruso, Laird & Batist, 1999; Chan, Yao, Gu, & Bradfield, 1999; Kumar, Tarpey & Perdew, 1999; Romkes, Piskorska-Pliszczynska & Safe, 1987; Romkes & Safe, 1988; Ryan, Sunahara, Lucier, Birnbaum & Nelson, 1989; Pavuk *et al.*, 2003). Some of the changes may occur by post-transcriptional processes such as messenger ribonucleic acid (mRNA) stabilisation (Gaido, Maness, Leonard & Greenlee, 1992) or altered protein phosphorylation (Matsumura, 1994). Therefore, TCDD's adverse effects appear likely to reflect alterations in gene expression or protein activity that occur at an inappropriate time and/or for an inappropriate length of time (US EPA, 2004a).

Evidence indicates that the AhR evolved prior to the introduction of halogenated aromatic hydrocarbons into the environment (Czuczwa, McVeety & Hites, 1984; Hahn, Karchner, Shapiro & Perera, 1997). It might have evolved as part of a substrate inducible system designed to metabolise and/or activate dietary lipophylic substances. The level and activity of the AhR appear to be regulated by changes in cell differentiation stages, growth factors, cell activation, diurnal cycle and exposure to receptor agonists (Crawford, Holsapple & Kaminski, 1997; Hayashi, Okabe-Kado, Honma & Kawajiri, 1995; Liu, Phillips & Matsumura, 1996; Pollenz, Sullivan, Holmes, Necela & Peterson 1996; Richardson, Santostefano & Birnbaum, 1998). Naturally occurring high-affinity ligands for the receptor exist in the environment, particularly in plants (Bjeldanes, Kim, Grose, Bartholomew &

Bradfield, 1991). Other endogenous ligands could include tryptophan derivatives (Helferich & Denison, 1991), carotenoids (Gradelet, Leclerc, Siess & Astorg, 1996), arachidonic acid metabolites (Schaldach, Riby & Bjeldanes, 1999) and tetrapyrroles or their derivatives (Sinal & Bend, 1997).

The best evidence for a normal physiological function of the AhR comes from studies with AhR-deficient animals. These mice exhibit altered hepatic growth and development, immune system abnormalities, development of vasculature, adverse reproductive outcomes, and reproductive tissue development (Benedict, Lin, Loeffler, Peterson & Flaws, 2000; Lahvis, Lindell, Thomas, McCuskey, Murphy, Glover, Bentz, Southard & Bradfield, 2000; Robles, Morita, Mann, Perez, Yang, Matikainen, Sherr & Tilly, 2000).

2.10 Mechanisms for detecting and quantifying PCDD/Fs and dioxin-like PCBs

2.10.1 Chemical analysis vs biological analysis

High-resolution gas chromatography and mass spectrometry (GC/MS) are used for the detection and quantification of dioxin-like compounds, and are considered as standard techniques to reliably quantify even minute concentrations of these compounds. However, quantitative instrumental analysis of complex mixtures of these chemicals is complicated and time-consuming. In addition, instrumental analysis provides little information about the additive, synergistic, or antagonistic interactions produced by the chemical mixtures (Song, Xu, Jiang, Lam, O'Toole, Giesy & Jiang, 2007). Thus, chemical analysis can underestimate the potential risks posed by these chemicals (Hilscherova, Machala, Kannan, Blankenship & Giesy, 2000).

In recent years, a variety of *in vitro* assays using cultured cells have been developed as an alternative to the use of animals in assessing the risk to fish and wildlife posed by these dioxin-like compounds, which may also reflect the risk to humans (Song *et al.*, 2007). Mechanism-based *in vitro* bio-assays have several attributes that make them a useful complement to routine quantitative chemical analysis. Firstly, *in vitro* bio-assays can detect unknowns for which there are no established analytical methods or standards. Secondly, *in vitro* bio-assays target only those chemicals that mediate a biological response through a defined mechanism of action. As a result, analyses are focused on biologically relevant compounds such as PCDD/Fs and PCBs (Koh, Khim, Villeneuve, Kannan & Giesy, 2006b). The *in vitro* cell bio-assays enable the estimation of total biological activity of all compounds that act through the same mode of action present in extracts of any environmental media (Hilscherova *et al.*, 2000). They can describe the differences in toxicity among chemicals or how these chemicals exert their effects as a mixture (Whyte,

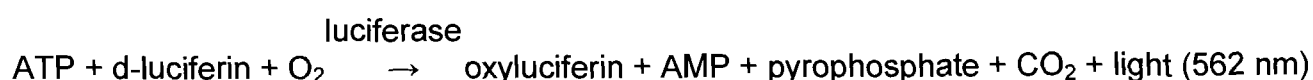
Schmitt & Tillitt, 2004). Finally, bio-assays integrate the response of complex chemical mixtures, providing an indication of the potential biological potency and/or relevance of an entire mixture (Hilscherova *et al.*, 2000; Koh *et al.*, 2006b). Because bio-assays can be used to indicate the presence and potential effects of contaminants, they have the potential to be used for ecological risk assessment (Citterio, Aina & Labra, 2002).

However, unlike instrumental analytical methods, bio-assays generally cannot identify the chemical(s) causing the response, nor can they precisely quantify the concentrations of chemical(s) present, as bio-assay response is a function of concentration, potency, and interactions. Thus, *in vitro* bio-assays serve as a useful complement, although not a substitute, for chemical analysis (Koh *et al.*, 2006b).

2.10.2 The H4IIE bio-assay

Benedict, Gielen, Owens, Niwa and Nebert (1973) reported that an ideal cell line for examining the potency of polyhalogenated aromatic hydrocarbons (PHAH) including PCDD/Fs and dioxin-like PCBs, was the H4IIE rat hepatoma cell line, because it has excellent growth properties and low basal AHH activity. Relative effects potencies (REP) for different compounds can be derived by comparing the activity of AHH or ethoxyresorufin-O-deethylase (EROD), both of which are catalytic measures of CYP1A1 induction, to the activity in 2,3,7,8-TCDD exposed cells (Whyte *et al.*, 2004). As 2,3,7,8-TCDD is the most toxic congener of the PCDD/Fs and dioxin-like PCBs, it is used as the reference compound in the assay (Besselink, Schipper, Klammer, Leonards, Verhaar, Felzel, Murk, Thain, Hosoe, Schoeters, Legler & Brouwer, 2004; Yoo, Khim & Giesy, 2006). Papers that published REPs for the different congeners were included in the literature studied to determine TEF values (Van den Berg *et al.*, 1998; Van den Berg *et al.*, 2006).

The particular version of the H4IIE bio-assay employed in this study uses a genetically modified cell line. The H4IIE cells were transfected with the pGudLuc1.1 plasmid by electroporation. The pGudLuc1.1 vector contains the firefly, *Photinus pyralis*, luciferase gene (*luc*) under control of the mouse mammary tumor virus (MMTV) viral promoter, and PHAH responsiveness is conferred by four DRE sequences located immediately upstream of the promoter and gene (Aarts, Denison, Cox, Schalk, Garrison, Tullis, De Haan & Brouwer, 1995). In practice, this means that the genetically modified cells would produce the luciferase gene in the presence of PHAHs and when the substrate, luciferin, is added to the cell's substrate, light is produced as a result of the ensuing adenosine triphosphate (ATP) activity:



(AMP: adenosine monophosphate) (Gould & Subramani, 1988). The quantity of light produced is directly proportional to the amount of PHAH.

Results obtained by the H4IIE-bio-assay relate to the total amount of bio-activity present in the environmental matrix; while this does not necessarily reflect the toxicity, it provides information on the potential for gene induction. Although AhR-dependant gene induction is a prerequisite for AhR-dependant toxicity, additional analysis by instrumental, *in vivo* animal studies - or both - must be conducted before any definitive conclusions regarding the toxicity of a sample or extract can be made. Accordingly, accurate exposure and risk assessment analysis would also require information on the bio-availability of the target pollutant(s), which might be obtained through the use, for instance, of mild extraction techniques (Nording, Denison, Baston, Persson, Spinnel & Haglund, 2007).

Indirect bio-assay approaches (of which the H4IIE-bio-assay is an example) are based on the assumption that the sample being analysed responds as if it were a dilution (or more concentrated form) of the reference compound (i.e., sample concentration response is parallel to the curve generated by the reference compound and maximum efficacy of sample and reference compound are equal) (Finney, 1971). This means that an estimate of equally effective doses of 2,3,7,8-TCDD and sample is determined, such that the inverse ratio of their equally effective doses describes the potency of the sample relative to 2,3,7,8-TCDD. The indirect assay therefore assumes that doses of 2,3,7,8-TCDD and sample that yield some selected magnitude of response can be defined as equally effective doses (Finney, 1971).

$$\text{REP} = \text{dose TCDD}_i / \text{dose sample}_i$$

where *i* equals a defined magnitude of response. An example would be the EC50 - the concentration needed to elicit a 50% response. The EC50 is widely regarded in toxicology as the standard point estimate for describing the potency of a chemical to elicit a specified response in an organism or population. In an ideal world, the dose-response curve of 2,3,7,8-TCDD would be parallel to the dose-response curve of the environmental sample, so that REPs based on a ratio of EC20s, EC50s and EC80s would give the same value, but this is usually not the case for environmental samples (Villeneuve, Blankenship & Giesy, 2000). Nor can it be assumed that the complex mixtures from the environment will exhibit equal efficacy to the 2,3,7,8-TCDD. The combination of compounds in a complex mixture may affect other aspects of cell function that interact with the AhR-pathway to modulate the magnitude and/or rate of change in response relative to dose (Villeneuve *et al.*, 2000).

These two problems: (i) non-parallelism between the reference dose-response curve and sample dose-response curve, and (ii) unequal efficacy, have to be addressed during the experimental procedure so that the assay results can be valid and useful. To address the phenomenon of non-parallelism between the dose-response curves, multiple point estimates must be calculated and reported, e.g. REP₂₀₋₈₀. As long as the sample and standard dose-response curves have the same efficacy and have been modelled over the entire range of effective response, the REP range generated provides a quantitative estimate of relative potency that is valuable for comparing among samples, as well as establishing TEFs or REPs, conducting mass balance analyses, and guiding risk assessments (Villeneuve *et al.*, 2000).

Equal efficacy can be tested empirically by examining whether the maximum responses observed for the samples and 2,3,7,8-TCDD were approximately equal. This requires that the maximal response of the sample is known. For practical purposes, the upper plateau of the S-shaped dose-response curve is regarded as the maximal response. If the maximal response of the sample is less than 20% of 2,3,7,8-TCDD's maximum response, equal efficacy cannot be assumed and must be reported as such. If possible, the sample should be tested at greater concentrations. If the maximum response for the sample was greater than 20% of the maximum of 2,3,7,8-TCDD, the REP₂₀₋₈₀ can be calculated and reported (Villeneuve *et al.*, 2000).

Converting REP values into TCDD equivalent (TCDD-EQ) values requires back-calculation based on the volume of the extract assayed and the degree of concentration during the extraction procedure (Koh *et al.*, 2006b).

2.11 In summary

A significant body of literature has been published on POPs, its levels, its sources and its effects on the environment. Due to their chemical characteristics they persist in the environment, bio-accumulate in food chains and can be transported over long distances. They are ubiquitous and have been found in all environmental matrices. POPs and similar compounds are globally recognised as a threat to the environment; the Stockholm Convention on Persistent Organic Pollutants is proof of that. South Africa has also signed and ratified this Convention. Under the Stockholm Convention, South Africa has certain obligations, among which is to limit and/stop the release of these chemicals into the environment. Almost all of the pesticide POPs are either banned or have restricted use and application - such as the spraying of DDT by the government for malaria control. Three groups of POPs, however, have not been addressed adequately. One of these groups is the PCBs and, although PCB production is banned, South Africa still has old

transformers in operation that are only replaced when they fail. This is not the only source of PCBs. PCBs are still produced unintentionally as by-products of combustion processes. Two other groups of POPs are also produced in the same manner, namely PCDDs and PCDFs. South Africa has most of the anthropogenic activities recognised in UNEP's Toolkit as sources of PCDD/Fs and the dioxin-like PCBs. Although some information was known on other organic compounds, almost nothing was known about specifically PCDD/F and dioxin-like PCB levels in its environment by the time South Africa ratified the Convention.

In the light of this lack of knowledge, the WRC of South Africa funded research in 2000 that also led to this PhD thesis, to determine the levels of PCDD/Fs and dioxin-like PCBs specifically in the aquatic environment of South Africa, since water is one of this country's limited resources. Water has to be safe not only for human consumption, but also for aquatic organisms, because as a consumer at the end of the food chain, humans are mainly exposed to PCDD/Fs and dioxin-like PCBs through their food. PCDD/Fs and dioxin-like PCBs have been shown to cause damage to the reproductive, neurological and immune systems of wildlife and humans and some congeners are known carcinogens.

In a first survey of its kind in South Africa, sediment samples were collected at, or near, industrial areas of the country. Sediment was selected because of its ability to act as a sink for these compounds. These sediment samples were analysed by GC/MS, but because analysis of this kind is extremely expensive - and there is no accredited laboratory in South Africa that analyses for PCDD/Fs - a bio-assay, the H4IIE-*luc* reporter gene assay, was evaluated in parallel to GC/MS analysis, to serve as screening tool. The bio-assay also has the capability to yield results that are more than just qualitative. Because of the semi-quantitative nature of TCDD-EQs, it can be used in limited risk assessment, provided all the conditions for the assay have been met. The bio-assay results would give an indication of the toxic effect the mixture of dioxin-like compounds would have on benthic vertebrate organisms exposed to them. These TEQs can be compared to international sediment quality guidelines established to classify the degree of harm the dioxin levels might pose to humans and other invertebrates. The assay is also reputed to be as sensitive as gc/ms, detecting dioxin levels as low as ng kg^{-1} . Chemical analysis however, distinguishes between the individual congeners, giving a concentration for each. The congener profile can be helpful in determining the pollution source. Together with the chemical analysis, the bio-assay are powerful tools to predict the toxicity of PCDD/Fs and dioxin-like PCBs on vertebrate organisms, without exposing and sacrificing wildlife.

3 MATERIALS AND METHODS

3.1 Site selection

As POPs converge in particular parts of the environment such as in estuaries, lakes and mountain regions (UNEP, 2003) due to factors such as climate, air flow and ocean currents, surface coverage (land/water ratio, vegetation type and topography) and the characteristics of the hydrological cycle (type, frequency, seasonality and intensity), the aquatic sites close to industrial areas were expected to be hot-spots and were selected for this study.

Table 3.1 A list of the 22 aquatic sites in South Africa selected for this study, and their coordinates

	Name	Closest town/farm/resort	Date of collection	Coordinates	
				S	E
1	Orange River (mouth)	Alexander Bay	31/03/2002	28°31.900'	16°36.338'
2	Saldanha Bay harbour	Saldanha Bay	01/04/2002	33°00.010'	17°59.805'
3	Berg River	Hermon, Wellington, Paarl	02/04/2002	33°26.011'	18°57.374'
4	Theewaterskloof Dam	Villiersdorp	02/04/2002	34°01.616'	19°15.795'
5	Groot River (mouth)	Nature's Valley	04/04/2002	33°58.799'	23°33.913'
6	Swartkops Estuary	Port Elizabeth	06/04/2002	33°52.030'	25°36.400'
7	Vaal River (before confluence with Orange River)	Douglas	07/04/2002	29°03.132'	23°45.594'
8	Buffalo River	Dundee	21/04/2002	28°14.777'	30°30.599'
9	Mooi River	Rosetta	22/04/2002	29°18.315'	29°58.561'
10	Umlazi River (mouth)	Durban	23/04/2002	29°57.800'	30°58.210'
11	Umgeni River (mouth)	Durban	24/04/2002	29°48.480'	31°01.969'
12	Richard's Bay (harbour)	Richard's Bay	25/04/2002	28°47.496'	32°01.705'
13	Thulazihleka Pan	Richard's Bay	25/04/2002	28°46.889'	32°02.430'
14	Vaal Dam	Leboya Bay	19/06/2002	26°48.566'	28°08.607'
15	Riet Spruit	Vanderbijl Park	19/06/2002	26°44.205'	27°42.673'
16	Riet Spruit (channel)	Louisrus	19/06/2002	26°39.825'	27°47.185'
17	Loch Vaal	Vanderbijl Park	03/07/2002	26°45.039'	27°42.026'
18	Crocodile River	Nelspruit	08/07/2002	25°29.238'	31°09.535'
19	Olifants River	Phalaborwa	09/07/2002	24°03.085'	31°43.801'
20	Loskop Dam	Grobiersdal	10/07/2002	25°25.088'	29°21.689'
21	Hartbeespoort Dam	Oberon	10/07/2002	25°44.250'	27°52.931'
22	Modderfontein Spruit	Modderfontein	11/07/2002	26°04.468'	28°08.167'

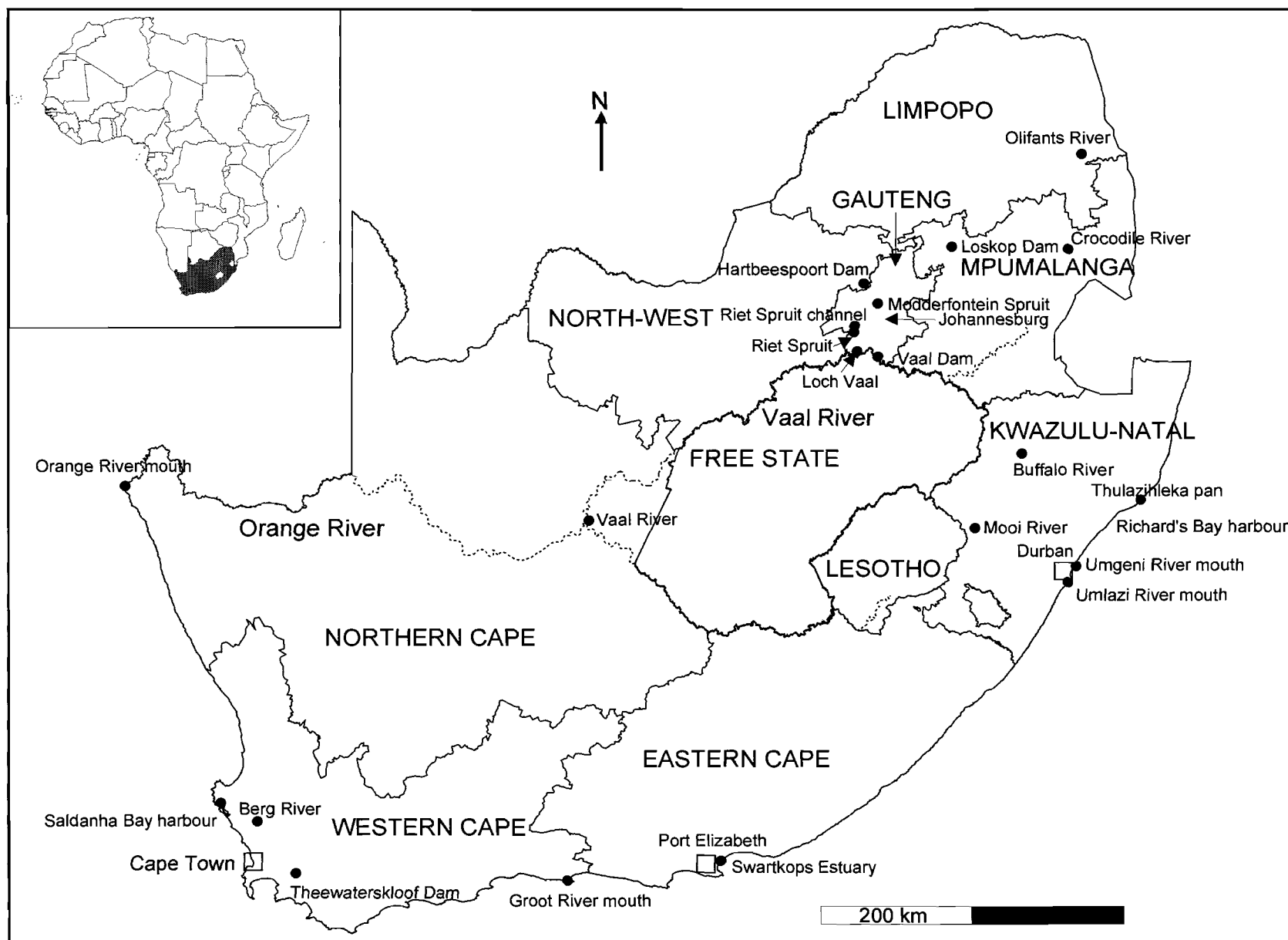


Figure 3.1: The locations where samples were taken for this study. The coordinates are listed in Table 3.1.

The site locations are presented in Table 3.1 and their locations within South Africa are indicated in Figure 3.1.

Sites that were selected because of their proximity to industrialised areas included Loch Vaal, Riet Spruit channel and Riet Spruit, and were meant to represent the Vaal Triangle. The Vaal Triangle is the triangle formed between Vereeniging, Vanderbijlpark (in the Gauteng Province) and Sasolburg (in the Free State) and through which the Vaal River, the second largest river in South Africa, flows. The Vaal Triangle is highly industrialised, and both steel processing plants and an oil-from-coal refinery with associated petro-chemical industries are located within this area. The Riet Spruit channel is a man-made canal lined with concrete, probably linking the steel manufacturing plant with Riet Spruit. The Riet Spruit feeds into Loch Vaal. The Vaal Dam, with a surface area of 320 km² (DWAF, 2007) and approximately 70 km upstream from Loch Vaal, was included in this investigation to act as a possible reference site to the downstream Vaal and Orange Rivers. Apart from the Vaal River, the Vaal Dam also receives clean water from the Lesotho Highlands Water Project via the Wilge River (DWAF, 2007). The main use of water from this dam is for domestic and industrial purposes (Burger, 1999).

Two sites downstream of the Vaal Triangle were selected in order to investigate the fate of the expected pollution from the Vaal Triangle. For this purpose, the Vaal River was sampled once more, just before its confluence with the Orange River (Vaal River site), 800 km downstream of Loch Vaal. The Orange River was sampled at the Atlantic shoreline, close to the border town of Alexander Bay. Sampling at the Orange River mouth would provide insight into the possible dilution of the pollution that was introduced into the Orange River by the Vaal River. It would also shed light on long-range riverine transport of these pollutants. It would, furthermore, be possible to establish whether additional sources of dioxin-like pollution were present along the lower Orange River. The Orange River system drains almost the entire highland plateau - 48% of the total surface area of the country (Burger, 1999). The lower Orange River (which is the section referred to after confluence with the Vaal River) is irrigated for maize, peanuts, cotton and dates, and pesticide use may contribute to dioxin levels in the river. The last 100 km of the river, approximately, is also extensively mined for diamonds. Although diamond mining as such is not listed in UNEP's Toolkit (UNEP, 2005) as a possible dioxin source. Excavating the river bed for diamonds could release trapped dioxin-like compounds, similarly as dredging would – which is listed in category 10 of the Toolkit (Table 2.1). Diesel operated equipment, such as water pumps and human activity, such as waste burning might inadvertently add to the levels because diamond mining takes place inside the river and on its banks.

In the Northern Cape Province, the Orange River forms the border with Namibia, which is another good reason for determining the levels of pollution. Countries sharing an aquatic system have to use and manage the shared waters responsibly.

The Modderfontein Spruit was sampled because it drains an industrial area called Modderfontein. It is situated northeast of Johannesburg, between Sandton, Midrand, Kempton Park and Edenvale and is known for its explosives and fertiliser manufacturers. Other chemicals industries are also located here: insecticides, paints, varnishes, cyanide and sulphuric acid (Anon., 2007).

Two sites were selected to represent another industrial city, Durban, on the south coast of Kwa-Zulu Natal. The Umgeni and Umlazi Rivers were sampled at the mouths where they enter the Indian Ocean. Both rivers drain industrial areas of Durban, and the Umlazi River traverses the Umlazi residential area, which is mostly a low-income urban area. The site chosen in the Umlazi River is next to a paper mill. The Swartkops Estuary, Port Elizabeth, in the Eastern Cape was added to the list of industrial sites. Automobile assembly is the chief industry of this area, but shoe manufacturing, metal and timber processing and electrical engineering are also present. The Swartkops Estuary drains industrial as well as low-income urban areas (similar to the Umlazi River).

The Hartbeespoort Dam was sampled because the dam reservoir receives water from a 4 100 km² catchment that includes Johannesburg, via the Jukskei and Hennops Rivers that flow into the Crocodile River (West). Roughly 90% of the yearly inflow is derived from the Crocodile River. Waste water treatment discharges into the Jukskei River (Boshoff, 2005). The dam has a storage capacity of $212 \times 10^6 \text{ m}^3$ and supplies water for domestic use (Burger, 1999).

The Olifants River in the Mpumalanga and Limpopo provinces was sampled at two locations: in the Loskop Dam (Mpumalanga) and at Phalaborwa (Limpopo) just before it enters the Kruger National Park. The Loskop Dam is mainly used for irrigation and has a storage capacity of $360 \times 10^6 \text{ m}^3$ (Burger, 1999). The Olifants River drains a part of Mpumalanga known for its coal mining and coal-powered electricity generation. South Africa is the world's sixth largest coal producer, and Mpumalanga accounts for 83% of South African coal production. In 2002, South Africa used 90% of its coal resources for generating electricity (EIA, 2005) and coal combustion is a known source of dioxins (UNEP, 2005). The Olifants River crosses into Moçambique when it leaves the Kruger National Park. Thus, another shared river for which South Africa has to take responsibility of in the spirit of good neighbourliness. The Buffalo River in Kwa-Zulu Natal is another

river that was selected because of its suspected exposure to coal combustion. Approximately 1% of the country's coal production comes from this province (EIA, 2005).

The Crocodile River (East) in Mpumalanga (not to be confused with its namesake in Gauteng (Crocodile West) which joins the Molopo River after exiting the Hartbeespoort Dam) was sampled at Nelspruit. This is the third river in the study that is trans-boundary. Upstream of this site, the Ngodwana paper mill is located, and effluent from this site might have contributed to dioxin pollution. Paper and pulp mills are known dioxin sources (UNEP, 2005). In 2002, South Africa produced 2.18 million tonnes of pulp, accounting for 1.2% of the world's production of 182 million tonnes, making it the 14th biggest pulp producer in the world (SAF, 2004). The Nelspruit area is also known for its citrus and sub-tropical fruit production, and pesticide spraying might further have added to dioxin pollution.

The Groot River in the Western Cape was sampled because it drains plantation forests and the associated wood preservation facilities - also a known dioxin source (UNEP, 2005). The Theewaterskloof Dam, the main water source for Cape Town, has a storage capacity of 480 250 m³ and a perimeter of 82 km. This dam was included because it is flanked on its north-west banks by plantation forests and because Grabouw, renowned for its apple orchards and subsequent pesticide spraying, is situated to the south of the dam.

The Berg River (Western Cape) was sampled where it runs through vineyards (near the towns of Wellington and the Paarl), and may also have been exposed to pesticide spraying.

Richard's Bay is another coastal city included in the study. It is situated in northern Kwa-Zulu Natal, and most of South Africa's export coal is dispatched through the Richard's Bay harbour (Mbendi, 2007). The city is also known for its aluminium smelting plants, a paper mill, manufacturing of heavy industrial and construction vehicles and equipment, wood chipping plants and production of chemical fertilisers. The harbour was sampled, and a freshwater pan - the Thulazihleka Pan - was sampled too. This pan is bordered by a fertiliser plant.

The Saldanha Bay harbour was added to the list because one of the major employers of this area is a steel mill, producing hot rolled steel coil. Most of the iron ore mined at Sishen (800 km to the interior of the country) is also shipped from this harbour.

A reference site, the Mooi River in the Kwa-Zulu Natal Midlands, was selected because it is far removed from industrial regions, and drains the highlands of the Drakensberg.

3.2 Sample collection

Sediment samples were collected following standardised protocols (US EPA, 2004b). All equipment that came into contact with the sediment samples were made of glass or stainless steel and was pre-cleaned as follows: all equipment and containers were washed with warm water and phosphate-free soap, rinsed in tap water, followed with de-ionised water (18 MΩ), acetone (Burdick & Jackson), and hexane (Burdick & Jackson). Both the acetone and hexane were of the quality used in high pressure liquid chromatography. The acetone removed all polar contaminants and the hexane all non-polar contaminants.

Equal volumes (120 ml) of individual sediment samples from five different locations at a particular site were pooled to obtain a composite sample. Sediment from the biological-active surface layer, i.e. 0 to 5 cm, was collected with a brass grab sampler. The composite sample was stirred vigorously before the sediment was transferred into pre-cleaned glass jars. Each sample was covered with cleaned aluminium foil to prevent possible contamination of the sample by the lining of the screw top of the jars. The jars were labelled appropriately, kept in darkness, and transported to the laboratory at the North-West University (Potchefstroom) at 4 °C. These precautions were taken to prevent the samples from degrading. The samples were stored at -20 °C until further processing. Sampling equipment was cleaned between consecutive sampling sites to prevent potential cross-contamination.

All the rivers, dams and harbours were sampled from the banks and shores, i.e. where the water bodies were accessible to humans on foot. This limited how far from the shore/bank deep water bodies could be sampled. Where applicable, slow flowing stretches of the rivers were chosen and where the bottom was visible, sampling was focussed on pockets of silt and fine sediment. Sampling took place from April to middle July 2002 (Table 3.1) which is the period after the rainy season for most part of the country. Due the limited scope of this survey, temporal variation was not accounted for.

3.3 Sample processing

A variety of analyses was performed on the sediment samples: (i) determination of the PCDD/F and dioxin-like PCB concentrations by high resolution gas chromatography and high resolution mass spectrometry in a German laboratory; (ii) determination of the TCDD-EQ of the sediment with the aid of the H4IIE-*luc* reporter gene bio-assay in a USA laboratory; (iii) determination of the particle size composition, and (iv) organic carbon content by South African laboratories.

3.3.1 Gas chromatography and mass spectrometry

The sediments were freeze dried before a 30 g sample from every site was sent to an internationally accredited laboratory in Germany: The Bayreuth Institute of Environmental Research, Ökometric GmbH, is accredited according to the European standard, EN 45001. This laboratory used the accelerated solvent extraction technique to prepare extracts from the dried sediment samples. This means that the sediment samples were extracted under high pressure (13 982.85 kPa) and at high temperatures (180 °C). They used toluene as the organic solvent. The concentrations of the 12 dioxin-like PCB congeners (Table 2.3) and 17 PCDD/F congeners (Table 2.4) were determined by high resolution gas chromatography and mass spectrometry. The limit of quantification (LOQ) of each sample was determined at the 5:1 signal:noise ratio level. The recovery of the internal standards was mostly between 80 and 110% for all samples according to the Ökometric report. Their report contained concentrations only and no gas chromatograms and mass spectra were included, nor did they report any of the gc/ms conditions.

Total concentrations of TEQs based on this instrumental analysis were calculated as the sum of TEQs from individual compounds, assuming additive responses to chemicals in the mixture. The TEQs were obtained by multiplying each concentration by the corresponding TEF value, using the WHO₂₀₀₅-TEF value for humans and mammals (Table 2.5):

$$\text{TEQ} = \sum C_i \times \text{TEF}_i$$

C_i indicates the concentration of each congener and TEF_i is the TEF for that congener.

3.3.2 H4IIE-*luc* reporter gene bio-assay

The application of the H4IIE-*luc* cells in the bio-assay for measuring the TCDD-EQ for the sediment samples was learned and applied at the Aquatic Toxicology Laboratory of the Michigan State University, East-Lansing, USA, under the guidance of Prof. John P. Giesy.

However, extracts had to be prepared from the sediment samples to be exposed onto the cells. The sediment extraction procedure that was used at the US laboratory is therefore explained first, before the experimental procedure of the assay is described.

3.3.2.1 Sediment extraction and clean-up

Small twigs and stones were removed from the freeze dried samples by hand. The samples were ground with a mortar and pestle to increase surface area for extraction. To obtain homogenous samples, they were sieved, using a brass sieve with a mesh size of 0.5 mm.

Twenty grams of each sample was mixed with anhydrous sodium sulphate (Merck, uniVAR) of equal volume to remove any remnants of water. The anhydrous sodium sulphate was pre-cleaned with dichloromethane (Burdick & Jackson) overnight in a Soxhlet apparatus, before it was oven-dried and stored in a clean container. The sediment-sodium sulphate mixture was extracted with 400 ml high purity toluene (Burdick & Jackson) in a Soxhlet apparatus for 20 hours (Hilscherova, Kannan, Kang, Holoubek, Machala, Masunaga, Nakanishi & Giesy, 2001). Toluene was the elected solvent to facilitate comparison of assay results to that of the GC/MS. The extracts were concentrated to almost dryness on a rotary evaporator before being transferred into test tubes with hexane. This change in organic solvent had to be done, because toluene is cytotoxic and would have killed the cells used in the bio-assay (Hilscherova, Kannan, Nakata, Hanari, Yamashita, Bradley, McCabe, Taylor & Giesy, 2003). The hexane solution was evaporated to 1 ml under a gentle stream of nitrogen gas (Hilscherova *et al.*, 2001).

To remove poly-aromatic hydrocarbons (PAHs), such as naphthalenes and pyrenes, that may have interfered with the bio-assay because they too are AhR ligands, the samples were treated with sulphuric acid (Whyte *et al.*, 2004). The extracts were diluted to 10 ml with hexane and 10 ml concentrated sulphuric acid (98%, Merck) added. This was repeated three times. Every time the mixture was shaken gently and the separation funnel vented before the mixture was left for 1 hour for the phases to separate. The bottom, acid containing phase, was discarded. The acid residuals were removed from the hexane phase by washing with 10 ml de-ionised water (Yoo *et al.*, 2006). The hexane-sample-containing phase was filtered through a small volume of anhydrous sodium sulphate to remove any remnants of water. The samples were concentrated to 1 ml under a nitrogen gas stream and stored at 4 °C in amber gas chromatograph vials until commencement of the assay.

Since the acid treatment is a generally accepted technique to destroy less stable aromatic hydrocarbons no additional verification was done to determine how many other than dioxin-like AhR-ligands, survived the acid treatment. It is therefore possible that a small number of AhR ligands might have survived the acid treatment.

3.3.2.2 Bio-assay

The cells were maintained at 37 °C in humidified 5% CO₂ / 95% air. The culture media was Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich) containing L-glutamine, 1 000 mg l⁻¹ glucose without phenol red and sodium bicarbonate, and supplemented with 10% foetal bovine serum (FBS; Gibco). DMEM without phenol red was used because

phenol red might have created a false positive response from the cells in the assay. Sodium bicarbonate had to be added to the prepared solution and the pH was adjusted to 7.4. However, the culture medium used during the assay was modified to contain hormone stripped FBS, also to prevent the hormones from eliciting a false positive response from the cells.

The entire assay takes five days. On day one, 96-well microplates (white walls and optically clear bottoms) were seeded with 50 000 cells mL^{-1} (adapted from Giesy, Jude, Tillitt, Gale, Meadows, Zajieck, Peterman, Verbrugge, Sanderson, Schwartz & Tuchman, 1997) in 250 μL of culture media. Only the inner 60 wells were seeded. The wells on the perimeter were filled with phosphate buffered saline (PBS). This was done to create an evenly humidified micro climate around each cell-containing well. After a 24 hour incubation period, i.e. on the second day, cells were dosed with sample extracts or TCDD standards. Each well was dosed with 2.5 μL . A minimum of three solvent control (hexane) and three blank wells were analysed with each 96-well plate, and all samples were tested in triplicate. Sample dose response curves consisted of six concentrations prepared by serial dilution (Koh, Khim, Kannan, Villeneuve, Senthilkumar & Giesy, 2004). Full dose-responses were achieved for the standard and the concentration range of the TCDD reference exposures was: $4.69 \times 10^{-5} \text{ mg } \mu\text{L}^{-1}$, $1.88 \times 10^{-4} \text{ mg } \mu\text{L}^{-1}$, $7.5 \times 10^{-4} \text{ mg } \mu\text{L}^{-1}$, $3 \times 10^{-3} \text{ mg } \mu\text{L}^{-1}$, $1.2 \times 10^{-2} \text{ mg } \mu\text{L}^{-1}$, $7.8 \times 10^{-2} \text{ mg } \mu\text{L}^{-1}$. The plates were incubated for another 72 hours. On the fifth day, the luciferase assay was conducted.

The plates were inspected microscopically and each well scored for viability. Culture media were removed and the wells were washed with PBS. PBS containing Mg^{2+} and Ca^{2+} was added to each well, after which the reagent from the LucLite® kit (PerkinElmer) was added. These additional Mg^{2+} - and Ca^{2+} -ions were added to preclude them as a possible rate-limiting factor during the light-producing reaction (cf. section 2.10.2). After a 10 minute incubation period, the light emissions of the wells were registered as relative light units (RLUs) using a luminometer (Microplate Fluorescence Reader Flx 800, Bio-Tek Instruments).

Sample responses were reported as percentage maximal induction to 2,3,7,8-TCDD ($\% \text{TCDD}_{\text{max}}$) and plotted as a function of $\log \mu\text{L}$ sample. The RLU from the solvent control should have been subtracted first. This was not done, because the levels of elicitation were so low that subtraction would have rendered all dose-response curves worthless. This means that the extracts used for the cells had very low concentrations of dioxin-like compounds.

Due to unequal slopes and efficacies (maximal induction) of the responses, multiple point estimates (see section 2.10.2) of the TCDD-EQs were used to characterise the responses and slopes (Hilscherova *et al.*, 2001). Regression equations were derived for the linear portion of each dose-response curve. Effective concentrations to elicit 20%, 50% and 80% were determined from the sample dose response curves. The same was done for the reference TCDD's dose-response curves, and relative effects potencies (REP_{20-80}) were derived as the ratio between the EC_{20-80} of the reference and the EC_{20-80} of the sample. Conversion to pg TCDD-EQs was back-calculated based on the volume of extract assayed (2.5 μ l) and the degree of concentration during the extraction procedure (i.e. 20 g sediment concentrated into 1 ml of extract) (Koh *et al.*, 2006b).

3.3.3 Particle size composition

The particle size distribution of the sediment samples was determined by a local laboratory: Viridus Technologies (Pty) Ltd., Potchefstroom. This laboratory participated in the following quality control schemes:

- Agricultural Laboratory Association of Southern Africa, and
- International Soil-Analytical Exchange (ISE), Wageningen, the Netherlands.

The texture of a soil is extremely important in the sorption process. If a soil is made up mostly of clay and organic matter, a significant amount of sorption will take place. Clay, especially intermixed with organic particles, by far absorbs the most out of the three main soil textures (clay, silt and sand) because of its small particle size, high surface area, and high surface charge (Carey *et al.*, 1998), and this must be taken into consideration when comparing results from the different sites. It was assumed that determining the grain size characteristics would suffice to address possible variation of congener distribution between sites, even though determining the specific surface area ($m^2 g^{-1}$) of the sediment would have been a more accurate description of such an influence.

3.3.4 Determination of organic carbon in the sediments

By measuring the percentage organic carbon (%OC) in sediments and soils, TEQ can be expressed as normalised TEQ, i.e. what the TEQ would have been if the entire sediment sample consisted of only organic carbon. Normalised TEQ allows the comparison between sites without considering the characteristics of each site (Froese, Verbrugge, Ankley, Niemi, Larsen & Giesy, 1998). The Walkley-Black method was used to determine the organic carbon content. During this method, available oxidisable organic carbon is oxidised by dichromate ions ($Cr_2O_7^{2-}$) and the concentration determined by a titration. The determination of the organic carbon content of the sediment samples were also performed

by Viridus Technologies, but since then, the technique has been established in our own laboratory.

1.0 g of air-dried sediment sample was added into an Erlenmeyer flask and swirled gently for 30 s after 10 ml of $0.167 \text{ mol dm}^{-3}$ potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (Merck, Pro Analys) and 30 ml of concentrated (98%) sulphuric acid was added. It was left to stand for 30 min. After adding 10 ml of 85% orthophosphoric acid (H_3PO_4) (Merck, univLAB) to eliminate interferences from the ferric ion, 10 drops of the ferroin indicator solution, barium diphenylamine indicator (BDH, Indicators TM) were added. This solution was titrated with 0.5 mol dm^{-3} ferrous ammonium sulphate solution ($\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$), until the colour changed from green to blue. The same process was applied to the blank solution. The percent oxidisable carbon (%OXC) was calculated by applying the following equation:

$$\% \text{OXC} = (B - S) \times (M \text{ of } \text{Fe}^{2+}) \times 100 \times 12/w \times 4000 \times 1.4$$

where

B = volume (ml) of the Fe(II) solution used for the titration of the blank solution;

S = volume (ml) of the sample solution used in the titration;

w = mass of the sample

12/4000 = the corresponding number of milli-equivalents of carbon contained in 1 g of sample

1.4 = the correction factor (Jeong, Kim, Joo, Kim & So, 2001; Schumacher, 2002; Walkley, 1947).

Since the Walkley-Black method estimates only the oxidisable organic carbon, rather than the total organic carbon, the total organic carbon (%OC) was calculated by applying the following equation (Sánchez-Monedero, Roig, Martínez-Pardo, Cegarra & Paredes, 1996):

$$\% \text{OC} = 1.23 \times \% \text{OXC} + 0.35$$

3.4 Statistics

All statistical calculations were done in conjunction with and advice from the Statistical Consultancy Service at the North-West University (Potchefstroom).

Throughout the Results chapter, a small *p*-value (i.e. smaller than 0.05) was considered as sufficient evidence that the result was statistically significant. However, statistical significance does not necessarily imply that the result is important in practice and therefore effect sizes were calculated to determine their practical significance. Practical significance can be understood as a large enough difference to have an effect in practice. For correlations, R^2 was used as determinant of effect size where R^2 is the square of Pearson's correlation coefficient. Thus,

- if $|R^2| \leq 0.13$, the correlation was non-significant;
- if $0.13 < |R^2| \leq 0.3$, the correlation was significant; and
- if $0.3 < |R^2| \leq 0.5$ the correlation was practically important or statistically practical significant (Ellis & Steyn, 2003).

When the means of two groups were compared, as in the Student's t-test (cf. Table 4.12), effect size was calculated using the following formula (Ellis & Steyn, 2003; Steyn, 2006):

$$d = |\bar{x}_1 - \bar{x}_2| / s_{max}$$

where: d = Cohen's effect size;

$\bar{x}_1$ and $\bar{x}_2$ = averages of the two groups compared; and

s_{max} = the largest standard deviation of the two groups.

Interpretation of the effect size was done according to the guidelines proposed by Cohen (1988):

- small effect: $d \leq 0.2$
- medium effect: $0.2 \leq d \leq 0.8$
- large effect: $d \geq 0.8$.

Data with $d \geq 0.8$ was considered practically significant, since it was the result of a difference having a large effect (Ellis & Steyn, 2003).

The motivation for the performance of the multivariate analysis, specifically principal component analysis (PCA) and hierarchical cluster analysis (HCA), on the chemical data was to attempt answering the following questions:

- How do the PCDD/F patterns of pollution found in the investigated South African sites compare to northern hemisphere patterns?
- Can the pollution sources responsible for observed PCDD/F pollution patterns in the South African sites be inferred from these patterns?
- How does the PCB congener pattern at the South African sites fit into the pattern observed for the PCDD/F congeners?
- Do any of the measured sediment characteristics influence the PCDD/F and PCB pollution patterns?

Hypothesis H_0 or H_1 were not provided because this study was not a random sample of sediment sites, but a subjective selection of sites.

3.4.1 Multivariate data analysis of pollutant profiles

3.4.1.1 Data transformations and manipulation

In order to minimise the effects associated with either orders of magnitude differences in concentrations across samples, and to incorporate samples measured in different units (e.g. pollutant levels in pg g^{-1} d.w. from soil and ng m^{-3} from air samples in the same statistical analysis), individual concentrations were expressed as a proportion of the sum of all other pollutant concentrations. Many authors followed this approach: Alcock, Sweetman, Anderson, Fisher, Jennings and Jones (2002); Masunaga, Yao, Ogura, Sakurai and Nakanishi (2003) and Wenning, Paustenbach, Harris and Bedbury, (1993). This reflects the relative size of component parts, and the resulting proportions are known as the pollutant "profile" or "fingerprint" of a sample. Such data is often referred to as "compositional" and the feature is known as "closure" or the "unit-sum constraint".

In a recent paper, Howel (2007) motivated for the use of log-ratio transformations on compositional data prior to using standard multivariate analysis rather than the compositional data. (Compositional data are data where the variables for each element of the study population aggregates to one.) This 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 also to differ for different subsets of elements. Since compositional data attempts to describe the relative magnitudes of pollutants, the ratios of proportions should remain the same, however many pollutants there are in a composition. The absence of this property for compositional data renders the standard statistical methods invalid for compositional data. Aitchison (1986) proved that this could be rectified when dealing with either of the following two functions of log ratios of proportions rather than with the proportions (compositional data). If d pollutants are measured in n samples so that p_{ij} is the proportion of pollutant i ($i = 1, 2, \dots, d$) in sample j ($j = 1, 2, \dots, n$), then the two most common choices of sets of log-ratios are:

- The $d-1$ log-ratios obtained by dividing the proportions of each pollutant by the proportions of one of the pollutants $\text{xlog}(p_{ij}/p_{xj})$.
- The d log-ratios obtained by dividing each proportion by the geometric mean across the sample $\text{log}(p_{ij}/g(p_j))$ where $g(p_j) = (p_{1j}p_{2j}\dots p_{dj})^{1/d}$. These are called "centred log-ratios" (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 (second bullet's equation), rather than the covariance or correlation matrix of the proportions.

All the chemical analysis data reported in this thesis was therefore treated as described above, and centred log-ratios were determined before PCAs were performed on the covariance matrix.

Wenning *et al.* (1993) substituted the limit of detection for all the non-detects, but for the South African data, half the detection limit was substituted. According to Helsel (2005), there are a number of ways to treat non-detects. Using the detection limit would have presented inflated mean levels rather than more realistic values. Helsel (2005) showed that using half the detection limit yields approximately the same descriptive statistics as the maximum likelihood and Kaplan-Meier estimates. In the South African data, a large amount of data was below the detection limit - so that neither the maximum likelihood nor Kaplan-Meier estimates could be determined; thus half the detection limit was substituted for data below the detection limit.

3.4.1.2 Principal component analysis

PCA was performed on the PCDD/F and dioxin-like PCB congener data for the 22 South African sites as well as PCDD/F congener data from literature (Wenning *et al.*, 1993) to which the South African data was compared. A similar comprehensive paper on dioxin-like PCBs could not be found in literature. A compilation of published dioxin-like PCB data and subsequent PCA thereof was not within the scope of this study.

PCA is a widely used statistical method (also used as a chemometric method) to search for similarities and differences in congener patterns in a wide range of environmental media and then infer source links. PCA, in effect, reduces several variables to a few underlying descriptive dimensions that can be used to explain patterns within a set of observed values (Alcock *et al.*, 2002). These underlying descriptive dimensions are uncorrelated linear combinations of the original variables that explain decreasing amounts of the total variation (i.e. the first linear combination or component explains the maximum percentage of variance, and so on) (Howel, 2007).

The importance of each congener in determining the positions of individual sediment samples and clusters of samples along the principal components axes are partially explained by the principal components loadings plot. The isomers with the highest contributions to the differences observed between samples and among clusters are located furthest from the origin of the axes. The greater the distance along a given principal

component axis, the more an isomer contributes to the variation accounted for along that axis. A negative or positive position along the axis indicates whether a congener is negatively or positively correlated with the principal component. A sediment sample or cluster positioned along a positive principal component axis contains low concentrations of congeners with negative loadings, and higher concentrations of isomers with positive loadings. A sediment sample or cluster positioned along a negative principal components axis contains the opposite relationship. As a result, the position of sediment samples or clusters indicates the differences in the composition of the congeners (Wenning, Harris, Unga, Paustenbach & Bedbury, 1992).

South African environmental pollution profiles were compared to northern hemisphere source data profiles. The northern hemisphere source data were derived from Wenning *et al.* (1993) based on 137 samples from 28 different publications. The South African data were therefore included in the Wenning data set and their locations on a biplot relative to the Wenning data were examined. (For the sake of brevity, this data will be referred to as "Wenning data" from here onwards.) The pollution sources of the Wenning samples were known for all 137 samples and included graphite electrode sludge, chimney soot from oil central heating, residues in Aroclor® and Kanechlor®, amongst others (Table 3.2).

Table 3.2: A list of the northern hemisphere PCDD/F pollution sources referred to by Wenning *et al.* (1993)

Sample types
Pyrolysis of polychlorinated biphenyls & polyvinylchloride
Residue in <i>ortho(o)</i> -chloranil
Graphite electrode sludge
Municipal water treatment plant sludge
Chlorinated tap water
Residue in commercial detergents
Residues in Aroclor® & Kanechlor® products
Chimney soot from oil central heating
Chimney soot from coal ovens
Municipal sewage sludge
Municipal sewage waste incinerator fly ash
Residue in pentachlorophenol
Residue in sodium (Na) pentachlorophenol
Residue in trichlorobenzene

Pyrolysis of polyvinylchloride
Soil from former 2,4,5-trichlorophenoxy acetic acid (2,4,5- T) plant
Soil contaminated with graphite sludge
Soil from chlorine production plant
Soil beneath waste electrical equipment incineration
Semi-rural British soils
Soils from scrap wire & car incineration sites
Ambient air near municipal sewage waste incinerator
Air from automobile traffic tunnel
Urban air from Hamburg, Germany
Urban air from Bridgeport, USA

PCA on the covariance matrix of the centred log-ratios was performed with the software package SPSS for Windows 15.0 (SPSS Inc., 2006) and all variables (congeners) were considered equally important. To improve the separation of variables contributing to the identified factors, the variety of rotations offered by the software package was investigated. This included "varimax", "biquartimax", "quartimax" and "equamax". The "varimax raw" rotation offered the best separation and was also used by other authors (Jones, Newsted, Henningsen, Slocomb & Giesy, 2005; Sakurai, Suzuki, Masunaga & Nakanishi, 1998). Using the same type of rotation for investigating the data facilitates comparison of datasets of different authors.

The factor loadings of the congeners contributing to a particular component (or factor) were represented together with the factor scores of the same factors as biplots in the Results chapter.

PCA was performed on the Wenning data, the South African data, and the Wenning-SA combination data, using only PCDD/F congener data, the South African data, using only PCBs, and on both congener groups. To determine the possible influence of the sediment characteristics on the groupings of the South African sites, they were included in a final PCA, together with the congener data.

3.4.1.3 Hierarchical cluster analysis

HCA were performed on the centred log-ratios of proportions with STATISTICA for Windows 7 (StatSoft, Inc., 2006). Of the variety of amalgamations (linkage rules) offered by the software package, Ward's method performed the best and the Euclidean distance

measure was used because the centred log-ratios are continuous data. HCA was used as another method to elucidate with which Wenning sites the South African sites would associate. An advantage of HCA is that the association is presented in a one-dimensional space, while PCA is a multi-dimensional space which makes interpreting more difficult. In a HCA, the sites with a strong association (i.e. those that have a similar pollution profile) are grouped into the same cluster (Hong, Yonlong, Dawson, Yajuan & Tieyu, 2005; Wenning *et al.*, 1992).

The South African data was further considered on its own to learn the influence, if any, that the PCB congener data would have on the distribution of the South African sites in the PCA biplots as well as the clusters formed in a HCA. Centred log-ratios of all the sediment characteristics were included in PCAs performed on the PCDD/F and PCB congeners separately before including all congeners with the sediment characteristics in a PCA.

4 RESULTS

The approach followed in the Results chapter was to present the data related to the magnitude of pollution caused by the PCDD/Fs and PCBs first, followed by the nature and profile of pollution.

- The concentration of each congener at each site as determined by GC/MS, as well as the corresponding TEQ was reported, and compared with the bio-assay TCDD-EQs.
- The effect of the organic carbon content of the sediment on the TEQ was graphically represented.
- The TEQ due to PCBs was also compared to the TEQ due to the PCDD/Fs.
- The results of chemometric analysis by PCA were also reported on and this was aided by HCA. Using the PCA and HCA, an attempt was made to compare the South African sites' congener profile data to that of the northern hemisphere to learn of possible sources of pollution.
- The PCB profile and the PCDD/F profile for the South African sites were also investigated by PCA to compare the type of pollution at the different sites.
- The possible influence that the sediment characteristics might have had on the groups into which the sites were arranged, was also investigated by PCA.

The abbreviations for sites and congeners are summarised in Table 4.1, and a copy of this table is also available on a separate, folded page at the back of the Discussion chapter. This page can be kept unfolded for quick referencing when reading through the Results and Discussion chapters. Throughout this chapter the abbreviations for the site names are used to improve legibility and prevent cumbersome sentences.

Table 4.1: Abbreviation list for sites and congeners referred to in the Wenning and SA data.

Abbreviations for samples	Site or type of sample from Wenning <i>et al.</i> (1993)	Abbreviations for SA sites	SA site names
A	Soil from former 2,4,5-T plant	Berg	Berg River
AIR	Ambient air near municipal sewage waste incinerator	Bfalo	Buffalo River
ASH	Municipal sewage waste incinerator fly ash	Croc	Crocodile River
B	Soil contaminated with graphite sludge	Grt	Groot River
C	Soil from chlorine production plant	HrtbDam	Hartbeespoort Dam
CL	Chimney soot from coal ovens	LochV	Loch Vaal
D	Soil beneath waste electrical equipment incineration	LskpD	Loskop Dam
DT	Residue in commercial detergents	Modder	Modderfontein Spruit
E	Semi-rural British soils	Mooi	Mooi River
GE	Graphite electrode sludge	Olifant	Olifants River
H	Soils from scrap wire and car incineration sites	OrangeM	Orange River mouth
NPCP	Residue in Na-pentachlorophenol	RchrdBy	Richard's Bay
OC	Residue in o-chloranil	RietSpr	Riet Spruit

OL	Chimney soot from oil central heating	RtsprCh	Riet Spruit channel
P	Pyrolysis of polychlorinated biphenyls and polyvinylchloride	SldnhBy	Saldanha Bay
PC	Residue in pentachlorophenol	SwkpEst	Swartkops Estuary
PCB	Residues in Aroclor® & Kanechlor® products	Thlzhlk	Thulazihleka Pan
PV	Pyrolysis of polyvinylchloride	TH20Dam	Theewaterskloof Dam
SS	Municipal sewage sludge	Umgeni	Umgeni River
TB	Residue in trichlorobenzene	Umlazi	Umlazi River
TT	Air from automobile traffic tunnel	VaalDam	Vaal Dam
UA1-8	Urban air from Hamburg, Germany	VaalRiv	Vaal River
UA9-15	Urban air from Bridgeport, USA		
WT1-3	Municipal water treatment plant sludge		
WT4	Chlorinated tap water		

Name of congener	Abbreviation
2,3,7,8-TCDD	TeD
1,2,3,7,8-PeCDD	PeD
1,2,3,4,7,8-HxCDD	HxD1
1,2,3,6,7,8-HxCDD	HxD2
1,2,3,7,8,9-HxCDD	HxD3
1,2,3,4,6,7,8-HpCDD	HpD
OCDD	OD
2,3,7,8-TCDF	TeF
1,2,3,7,8-PeCDF	PeF1
2,3,4,7,8-PeCDF	PeF2
1,2,3,4,7,8-HxCDF	HxF1
1,2,3,6,7,8-HxCDF	HxF2
1,2,3,7,8,9-HxCDF	HxF3
2,3,4,6,7,8-HxCDF	HxF4
1,2,3,4,6,7,8-HpCDF	HpF1
1,2,3,4,7,8,9-HpCDF	HpF2
OCDF	OF
3,4,4',5'-TCB	*PCB81
3,3',4,4'-TCB	*PCB77
3,3',4,4',5'-PeCB	*PCB126
3,3',4,4',5,5'-PeCB	*PCB169
2,3,3',4,4'-PeCB	PCB105
2,3,4,4',5'-PeCB	PCB114
2,3',4,4',5'-PeCB	PCB118
2',3,4,4',5'-PeCB	PCB123
2,3,3',4,4',5'-HxCB	PCB156
2,3,3',4,4',5'-HxCB	PCB157
2,3',4,4',5,5'-HxCB	PCB167
2,3,3',4,4',5,5'-HxCB	PCB189

* non-ortho substituted PCBs

4.1 Magnitude of pollution

4.1.1 Results from chemical analysis

The individual PCB and PCDD/F congener concentrations are listed separately in Tables 4.2a and 4.2b, and the total PCB and PCDD/F concentrations for each site, together with the total TEQ per site, are presented in Table 4.3a.

Table 4.2a: PCB congener concentrations for each of the SA sites.

Sites	Concentration of PCB congeners (ng kg ⁻¹ d.w.)												
	PCB 77*	PCB 81*	PCB 126*	PCB 169*	PCB 105*	PCB 114*	PCB 118*	PCB 123*	PCB 156*	PCB 157*	PCB 167*	PCB 189*	ΣPCB
OrangeM	3.0	0.1	0.1	0.1	5	1	14	1	1	1	1	1	28.3
SldnhBy	1.0	0.1	0.1	0.1	2	1	9	1	1	1	1	1	18.3
Berg	6.3	0.1	0.1	0.1	10	1	24	1	4	1	2	1	50.6
TH20Dam	3.2	0.1	0.1	0.1	4	1	13	1	4	1	2	1	30.5
Grt	5.0	0.1	0.1	0.1	5	1	18	1	2	1	1	1	35.3
SwkpEst	44.5	1.3	4.9	0.5	174	8	418	16	68	14	34	11	794.2
VaalRiv	6.4	0.2	0.2	0.1	9	1	20	1	3	1	2	1	44.9
Bfalo	2.7	0.1	0.1	0.1	4	1	10	1	1	1	1	1	22.3
Mooi	3.6	0.1	0.1	0.1	7	1	17	1	3	1	1	1	36.6
Umlazi	32.5	0.6	2.0	0.2	139	10	339	13	61	11	24	5	637.4
Umgenei	52.7	1.8	2.4	0.1	129	10	266	7	47	9	20	6	549.9
RchrdBy	3.0	0.1	0.1	0.1	4	1	10	1	1	1	1	1	22.8
Thlzhlk	16.0	0.6	0.2	0.2	22	2	44	2	7	1	3	1	97.7
VaalDam	2.3	0.1	0.1	0.1	3	1	10	1	1	1	1	1	21.8
RietSpr	78.5	1.6	2.3	0.1	123	12	227	9	44	5	19	5	526.5
RtsprCh	2500	104	62.1	7.1	6650	584	10600	590	2540	162	839	298	24936.2
LochV	205	2.3	4.6	0.3	360	33.4	584	22.3	101	10.6	39.4	11.5	1374.4
Croc	20	0.2	14.8	2	53.7	4	337	62.7	339	15.2	153	100	1101.6
Olifant	4.9	0.1	0.1	0.1	8.1	1	20.1	1	2.5	1	1.2	1	41.1
LskpD	2.3	0.1	0.1	0.1	3.7	1	12.6	1	1	1	1	1	24.9
HrtbDam	19.4	0.3	3.4	0.4	152	7.4	417	23.6	102	15.2	55.8	17.9	814.4
Modder	72.7	2	13.2	1.4	296	17.7	689	33.7	221	24.4	98.1	35.8	1505.0
Total	3085.0	116.0	111.2	13.5	8163.2	699.5	14097.8	791.7	3554.3	278.8	1300.7	503.0	

grey scale < limit of quantification

*non-ortho-substituted PCBs; ♦mono-ortho-substituted PCBs

Table 4.2b: PCDD/F congener concentrations for each of the SA sites.

Sites	Concentration of PCDD/F congeners (ng kg ⁻¹ d.w.)																	ΣPCDD/F
	TeD	PeD	HxD1	HxD2	HxD3	HpD	OD	TeF	PeF1	PeF2	HxF1	HxF2	HxF3	HxF4	HpF1	HpF2	OF	
OrangeM	0.05	0.05	0.05	0.15	0.08	0.26	0.87	0.05	0.05	0.05	0.2	0.16	0.05	0.09	0.76	0.15	0.73	3.8
SldnhBy	0.05	0.05	0.07	0.2	0.07	0.25	0.61	0.05	0.1	0.14	0.16	0.18	0.05	0.11	0.31	0.15	0.5	3.1
Berg	0.05	0.05	0.05	0.14	0.08	1.99	14.6	0.06	0.07	0.07	0.19	0.18	0.05	0.12	1.01	0.15	1.41	20.3
TH2ODam	0.05	0.05	0.05	0.35	0.36	2.2	16.6	0.07	0.05	0.07	0.1	0.17	0.11	0.07	0.63	0.15	0.92	22.0
Grt	0.05	0.05	0.05	0.07	0.08	0.92	4.87	0.07	0.05	0.1	0.07	0.07	0.05	0.05	0.43	0.15	0.67	7.8
SwkpEst	0.06	0.33	0.49	1.78	1.02	30.9	184	0.57	0.45	0.46	0.52	0.58	0.05	0.49	5.47	0.17	9.86	237.2
VaalRiv	0.05	0.05	0.05	0.12	0.2	0.75	5.06	0.06	0.07	0.06	0.07	0.08	0.06	0.05	0.29	0.15	0.5	7.7
Bfalo	0.05	0.05	0.05	0.07	0.05	0.57	3.73	0.05	0.07	0.13	0.1	0.09	0.05	0.05	0.37	0.15	0.65	6.3
Mooi	0.05	0.05	0.12	0.31	0.17	1.54	8.6	0.05	0.09	0.13	0.17	0.2	0.12	0.13	0.51	0.15	0.83	13.2
Umlazi	0.05	0.12	0.24	0.75	0.45	15.9	123	0.32	0.19	0.37	0.44	0.5	0.11	0.39	4.09	0.15	8.8	155.9
Umgeni	0.06	0.14	0.21	0.95	0.49	18.7	148	0.42	0.34	0.62	0.6	0.58	0.21	0.57	5.32	0.21	11.5	188.9
RchrdBy	0.05	0.05	0.05	0.18	0.06	0.33	1.45	0.05	0.06	0.11	0.13	0.15	0.05	0.11	0.29	0.15	0.5	3.8
Thlzhlk	0.05	0.09	0.05	0.22	0.23	1.82	14.3	0.62	0.27	0.23	0.22	0.31	0.12	0.13	0.71	0.15	0.95	20.5
VaalDam	0.05	0.05	0.06	0.18	0.08	0.31	1.29	0.05	0.07	0.08	0.14	0.14	0.05	0.11	0.36	0.15	0.5	3.7
RietSpr	0.08	0.11	0.12	0.63	0.29	8.36	61	0.57	0.27	0.42	0.43	0.48	0.21	0.35	2.29	0.15	3.79	79.6
RtsprCh	1.02	0.94	1	6.39	2.45	104	906	13.6	6.72	7.57	7.39	7.06	0.81	5.28	26.1	2.17	38.4	1136.9
LochV	0.09	0.23	0.34	1.71	1.02	26.2	191	1.83	0.9	1.15	1.31	1.48	0.7	1.07	7.24	0.52	10.7	247.5
Croc	0.05	0.14	0.31	0.66	0.45	15.8	218	0.15	0.08	0.16	0.33	0.35	0.19	0.18	6.6	0.15	7.19	250.8
Olifant	0.05	0.05	0.05	0.16	0.15	0.57	3.12	0.06	0.11	0.07	0.09	0.13	0.07	0.07	0.31	0.15	0.5	5.7
LskpD	0.05	0.05	0.05	0.14	0.1	0.2	2.29	0.05	0.05	0.05	0.07	0.08	0.09	0.05	0.19	0.15	0.5	4.2
HrtbDam	0.05	0.05	0.22	0.36	0.2	6.51	47.7	0.29	0.15	0.3	0.28	0.26	0.06	0.23	1.72	0.15	2.62	61.2
Modder	0.14	0.73	0.69	3.13	2.03	40.2	421	3.16	1.62	2.05	3.03	3.26	0.7	1.9	18.4	1.06	32.6	535.7
Total	2.25	3.48	4.37	18.65	10.11	278.3	2377	22.2	11.83	14.39	16.04	16.49	3.96	11.6	83.4	6.68	134.6	

grey scale < limit of quantification

The four PCB congeners with the highest concentrations amongst the 22 sites were PCB118, PCB105, PCB156 and PCB77 in decreasing order (Table 4.2a). The PCDD/F congeners with the highest concentrations were the octa and hepta chlorinated dioxins and the octa chlorinated furan, in decreasing order (Table 4.2b). The total PCB concentrations were, without exception, higher than the total PCDD/F concentrations (Table 4.3a). The RtsprCh site had the highest Σ PCDD/F ($1\,136.9\text{ ng kg}^{-1}\text{ d.w.}$) and Σ PCB ($24\,936.2\text{ ng kg}^{-1}\text{ d.w.}$) concentrations which resulted in the highest TEQ ($17.8\text{ ng TEQ kg}^{-1}\text{ d.w.}$) of the 22 sites investigated (Table 4.3a). The site with the second highest Σ PCB concentration was the Modder site ($1\,505.0\text{ ng kg}^{-1}\text{ d.w.}$) and its Σ PCDD/F load was also the second highest at $535.7\text{ ng kg}^{-1}\text{ d.w.}$ The site with the lowest TEQ was the LskpD site at $0.2\text{ ng kg}^{-1}\text{ d.w.}$ (Table 4.3a) and not Mooi in the KwaZulu-Natal midlands with a TEQ of $0.308\text{ ng kg}^{-1}\text{ d.w.}$, the site selected as the reference site. There were twelve other sites with TEQ values equal to or smaller than $0.308\text{ ng kg}^{-1}\text{ d.w.}$ These were OrangeM, SldnhBy, Berg, TH20Dam, Grt, VaalRiv, Bfalo, RchrdBy, VaalDam, Olifant, LskpD and the already mentioned Mooi. These sites had nine or more of the possible 29 congeners of which the concentrations were below the quantification limit.

In Figure 4.1, the TEQ-contributions by the PCBs are presented separately from the TEQ-

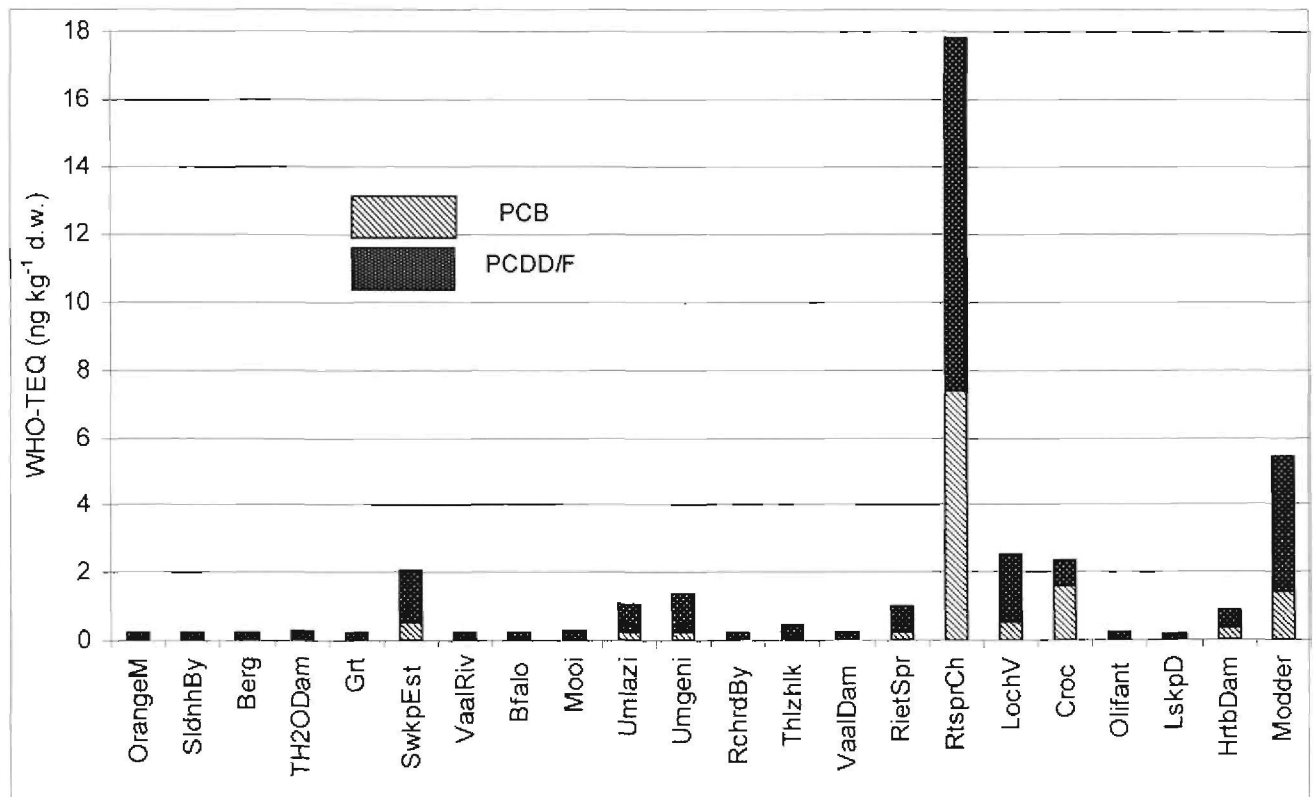


Figure 4.1: A summary of the TEQ values calculated from the chemical analysis. WHO-TEQs were calculated using WHO₂₀₀₅-TEFs for mammals (Van den Berg *et al.*, 2006, Table 2.5).

contributions of the PCDD/Fs. The TEQ contribution of the PCDD/Fs was significantly ($p = 0.00043$, Wilcoxon matched pairs test) higher than the PCB dependent TEQ. The Croc

site was the only exception where the PCB TEQ contribution was larger than from PCDD/F TEQ (Table 4.3a).

4.1.2 Sediment characteristics

By measuring the percentage total organic carbon (%OC) (Table 4.3a) in sediments and soils, TEQ can be expressed as normalised TEQ, as was explained in section 3.3.4 of the Materials and Methods chapter. Normalised TEQ allows for the comparison of pollution

Table 4.3a: The Σ PCB and Σ PCDD/F congener concentrations, with the corresponding WHO-TEQs for each site.

Sites	%OC	Total concentrations (ng kg ⁻¹ d.w.)		WHO ₂₀₀₅ -TEQ (LOQ included) (ng kg ⁻¹ d.w.)			Normalised TEQ (ng kg ⁻¹ d.w.)
		Σ PCB	Σ PCDD/F	PCB	PCDD/F	Total	
OrangeM	0.42	28.3	3.8	0.01	0.21	0.23	53.27
SldnhBy	0.35 [#]	18.3	3.1	0.01	0.24	0.26	72.88
Berg	0.35 [#]	50.6	20.3	0.02	0.25	0.26	74.68
TH2ODam	1.35	30.5	22	0.01	0.29	0.30	22.26
Grt	2.39	35.3	7.8	0.01	0.2	0.21	8.93
SwkpEst	1.75	794.2	237.2	0.53	1.52	2.05	116.84
VaalRiv	1.84	44.9	7.7	0.03	0.20	0.23	12.38
Bfalo	0.55	22.3	6.3	0.01	0.20	0.22	39.90
Mooi	2.71	36.6	13.2	0.01	0.29	0.31	11.35
Umlazi	1.53	637.4	155.9	0.23	0.85	1.08	70.23
Umgeni	1.19	549.9	188.9	0.26	1.09	1.35	114.04
RchrdBy	0.56	22.8	3.8	0.01	0.22	0.24	42.03
Thlzhlk	6.29	97.7	20.5	0.03	0.44	0.47	7.45
VaalDam	0.35 [#]	21.8	3.7	0.01	0.22	0.23	65.62
RietSpr	2.59	526.5	79.6	0.26	0.76	1.01	39.18
RtsprCh	9.26	24 936.2	1 136.9	7.37	10.44	17.81	192.42
LochV	3.01	1 374.4	247.5	0.53	2.04	2.56	85.25
Croc	1.84	1 101.6	250.8	1.57	0.8	2.37	128.89
Olifant	1.44	41.1	5.7	0.02	0.21	0.23	15.80
LskpD	0.57	24.9	4.2	0.01	0.19	0.20	34.94
HrtbDam	0.90	814.4	61.2	0.38	0.48	0.86	95.31
Modder	3.40	1 505.0	535.7	1.41	4.06	5.47	160.82

*WHO-TEQs were calculated using WHO₂₀₀₅-TEFs for mammals (Van den Berg *et al.*, 2006, Table 2.5)

Walkley-Black results: 0% oxidisable organic carbon

Table 4.3b: The correlation (R) between %OC and congener levels, and between %OC and other sediment characteristics.

%OC Mud*	Σ PCDD/Fs	Σ PCBs	Mud*
	0.76	0.78	0.67
	0.43	0.42 [#]	

*Mud was calculated by summing %silt and %clay (Koh, Khim, Villeneuve, Kannan & Giesy, 2006a)

All correlations were statistically significant at the 5% level except the correlation marked with

levels between sites without considering the sediment characteristics of each site (Froese *et al.*, 1998; Koh, Khim, Villeneuve, Kannan, Johnson & Giesy, 2005). The last column in Table 4.3a contains the total TEQ normalised for the organic carbon content of the sediment samples. Three sites (VaalDam, SldnhBy and Berg) had no reported oxidisable

organic carbon as determined by the Walkley-Black method, but when the total organic carbon was calculated, the values changed (cf. section 3.3.4 in the Materials and Methods chapter) and normalised TEQ values could be calculated. Normalisation of non-polar organic compounds to %OC content is valid only if the %OC content in the sediments is greater than 0.2%. At %OC concentrations less than 0.2%, other factors that influence partitioning to the sediment pore waters (e.g. particle size and sorption to non-organic mineral fractions) become relatively more important (Di Toro, Zarba, Hansen, Berry, Swartz, Cowan, Pavlou, Allen, Thomas & Paquin, 1991). For the sake of comprehensiveness, the normalised TEQ was calculated for all of the sites (Table 4.3a).

According to the normalised data, the RtsprCh had the highest normalised TEQ (192.42 ng kg⁻¹ d.w.), followed by the Modder (160.82 ng kg⁻¹ d.w.). The lowest normalised TEQ was calculated for the Thlzhlk (7.45 ng kg⁻¹ d.w.). When the TEQ was correlated with the %OC, the correlation was positive. In Figure 4.2 a practically significant relationship of $R^2 = 0.63$ ($p < 0.01$) indicated that the higher the carbon content, the higher the WHO₂₀₀₅-TEQ. Two of the sites (Thlzhlk and the RtsprCh) could be considered as outliers that might

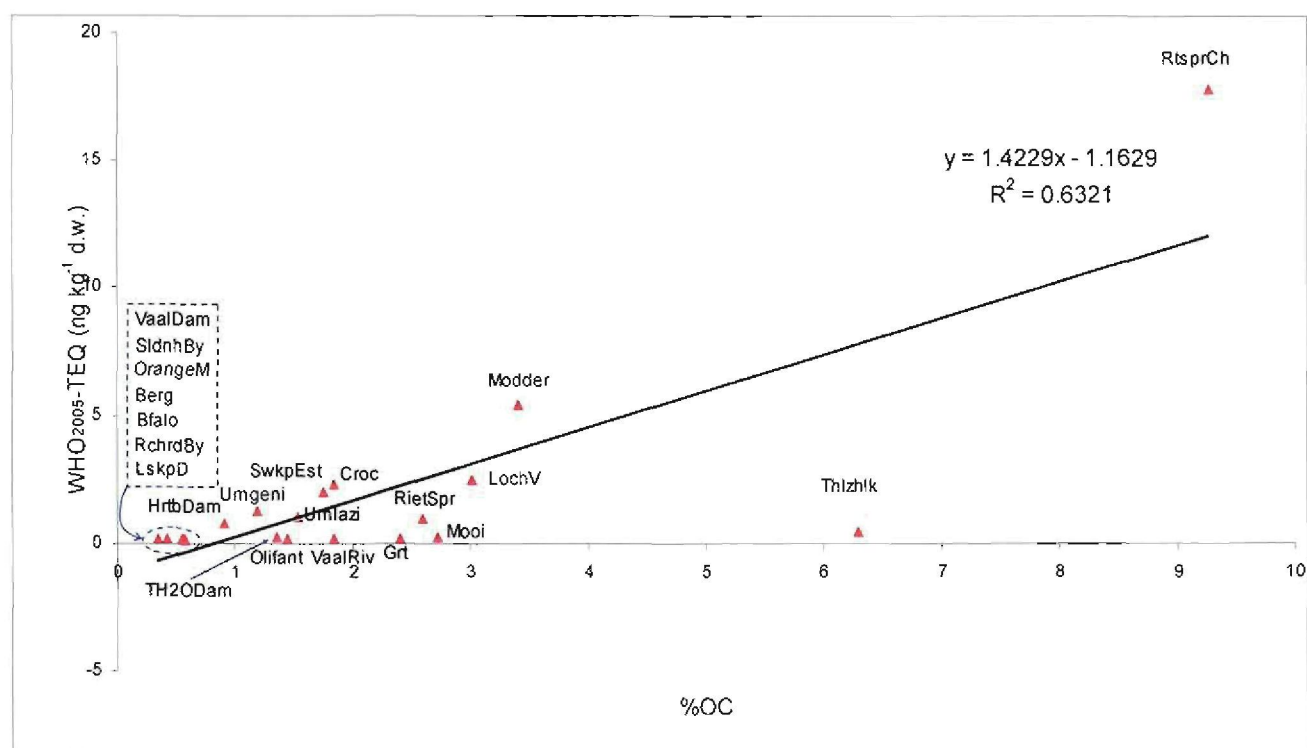


Figure 4.2: The relationship between organic carbon content and the WHO-TEQ of each sample

have influenced this relationship. When they were removed from the data, the relationship was still practically significant ($R^2 = 0.42$; $p < 0.01$) (Ellis & Steyn, 2003; section 3.4).

A similar correlation between %OC and the Σ PCDD/F concentration and Σ PCB concentration respectively was observed (Table 4.3b). Mud (%silt + %clay) also correlated significantly with Σ PCDD/F ($R^2 = 0.183$; $p < 0.05$), but not with Σ PCB ($R^2 = 0.178$; $p > 0.5$).

From the results presented in Table 4.3a and Figure 4.2 it is clear that the higher the %OC, the greater the concentrations of both groups of compounds were. It therefore seems as if there is a relation between sediment characteristics and the magnitude of the pollutant load. In a later section, this aspect will be addressed (see section 4.2.5).

4.1.3 Results from the H4IIE-*luc* reporter gene bio-assay

This section of the research was unsatisfactory: Of the 22 sites, successful bio-assay data was obtained for only nine. The other samples were sacrificed during the training on the bio-assay technique without learning their TCDD-EQ values. Many of the samples also showed maximal response less than the required 20% of 2,3,7,8-TCDD's maximum response and could not be used (see section 2.10.2). Fortunately, those sites for which bio-assay data was obtained included the RtsprCh, LochV, the Croc and Modder, which were some of the sites that had higher (when compared to the others) WHO₂₀₀₅-TEQ values (Figure 4.1). The assay results from the nine sites are presented in Table 4.4. Extracts of only four of the sites elicited a measurable response from the cells, i.e. a response $\geq 20\%$ compared to the maximum response elicited by the reference chemical 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. These sites were the LochV, the LskpD, the HrtbDam and the Modder. The TCDD-EQ₂₀ values of the other five sites were estimated with an extrapolated linear relationship (cf. section 3.3.2.2).

Table 4.4: The TCDD-EQ values as determined by the H4IIE-reporter gene bio-assay, and the corresponding TEQ as determined by the chemical analysis (cf. Table 4.3a).

Site name	Maximum response (%)	TCDD-EQ ₂₀ (ng kg ⁻¹ d.w.)	TCDD-EQ ₅₀ (ng kg ⁻¹ d.w.)	GC/MS TEQ (ng kg ⁻¹ d.w.)
RchrdBy	<10	2.33×10^{-8} *		2.35×10^{-1}
Thlzhlk	<10	8.31×10^{-6} *		4.69×10^{-1}
RietSpr	16.2	1.02×10^{-2} *		1.01
RtsprCh	15.5	5.17×10^{-3} *		1.78×10
LochV	35.2	1.72×10^{-1}	6.50×10^{-2} *	2.56
Croc	14.1	2.59×10^{-3} *		2.37
LskpD	26.1	2.32×10^{-2}	5.71×10^{-3} *	2.0×10^{-1}
HrtbDam	41.3	7.39×10^{-2}	4.82×10^{-2} *	8.61×10^{-1}
Modder	50.8	2.01×10^{-1}	1.2×10^{-1}	5.47

*extrapolated

The sample from the Modder was the only one that elicited a response that reached the 50% of the TCDD_{max} and the corresponding TCDD-EQ₅₀ was 1.2×10^{-1} ng kg⁻¹ d.w. Other sites for which a measurable TCDD-EQ was found only reached a response higher than the 20% TCDD_{max}, but not 50% (Table 4.4). For these sites the TCDD-EQ₅₀ values were extrapolated (cf. 3.3.2.2).

4.1.4 Comparison between chemical- and bio-analysis

Comparing the measured and extrapolated TCDD-EQ₂₀ values of the bio-assay with the corresponding TEQ values as chemically determined, showed no significant correlation

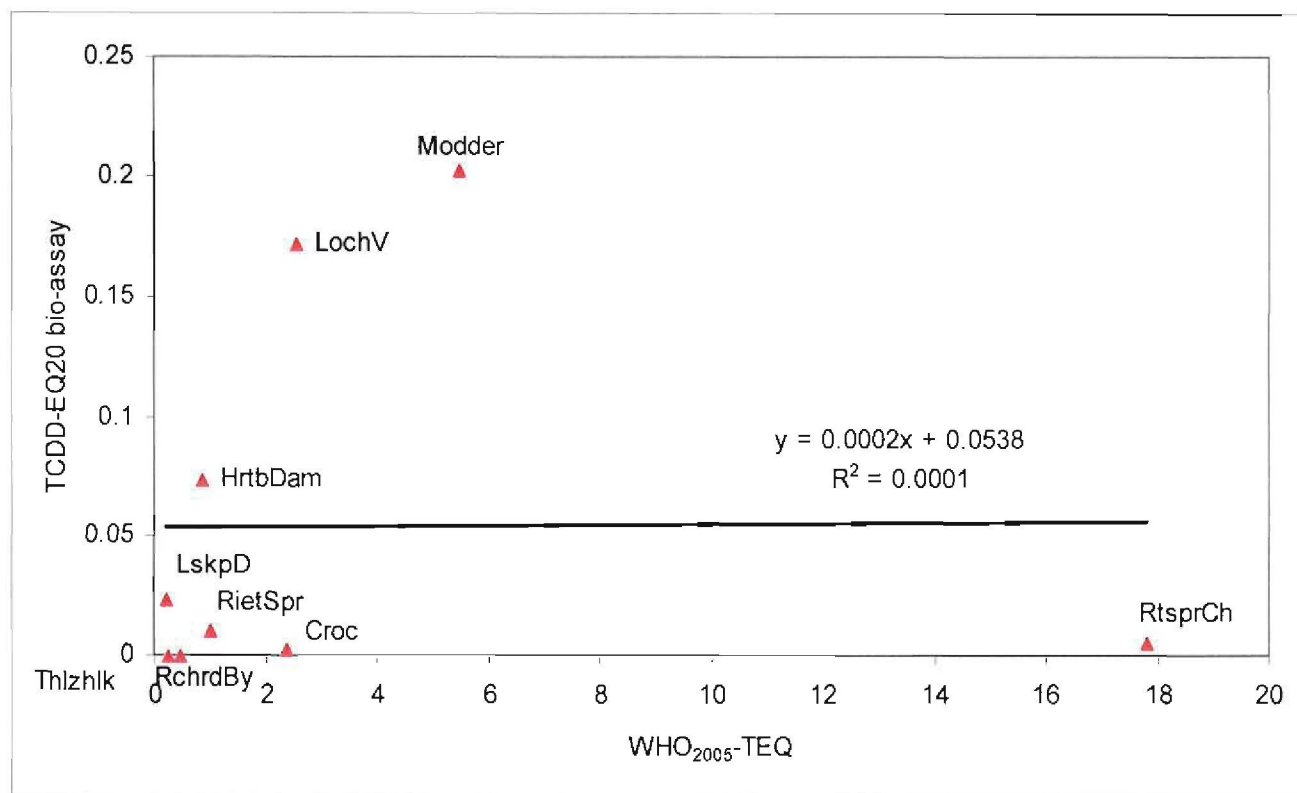


Figure 4.3a: The correlation between the TCDD-EQ20 values as determined by the bio-assay and the TEQ values as calculated from the GC/MS results.

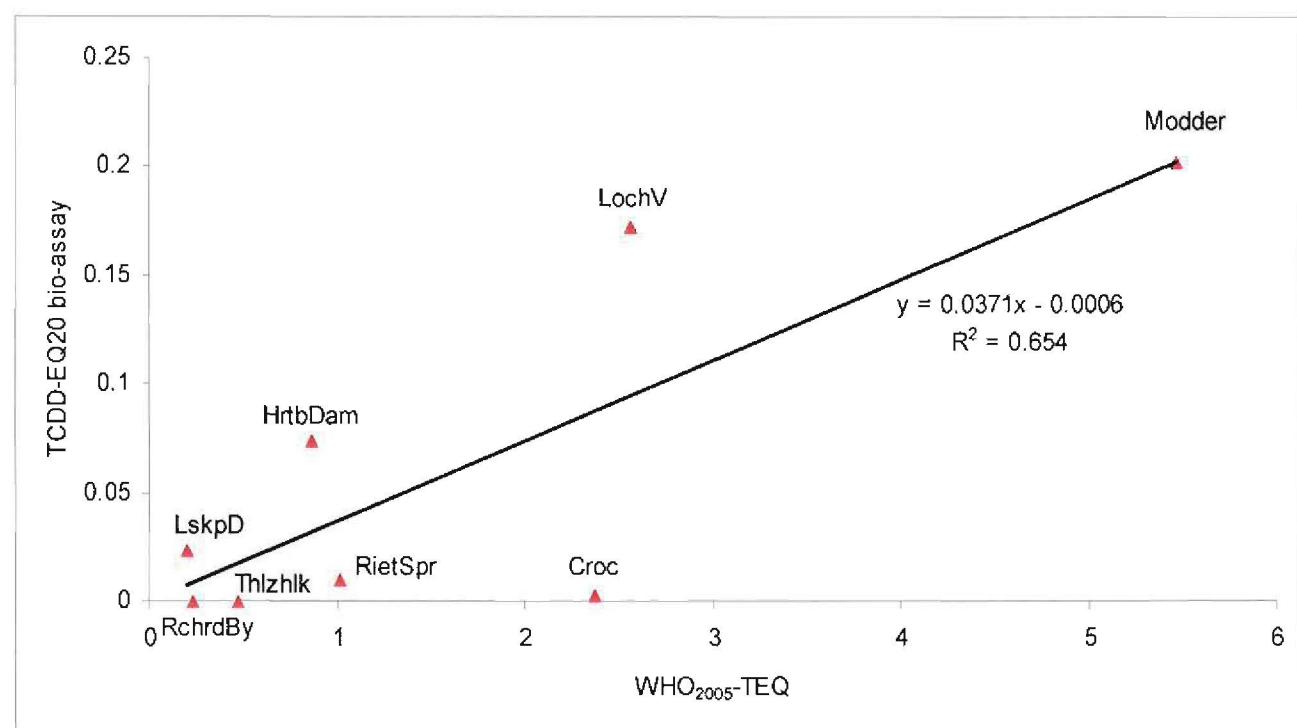


Figure 4.3b: The correlation between the TCDD-EQ20 values and the GC/MS TEQ values without the RtsprCh site.

($R^2 = 0.0001$; $p > 0.05$) (Figure 4.3a) for the nine measured sites. The RtsprCh site could be considered as an outlier - when it was removed, the correlation improved to $R^2 = 0.654$ ($p = 0.015$) (Figure 4.3b).

It seems as if there was a statistically significant correlation between the bio-assay and the chemical analysis results, notwithstanding the small sample size, the outliers, and the low TCDD-EQ values for many sites. Differences between the two analytical techniques are to be expected and this phenomenon will be further explored in the Discussion chapter.

4.2 Nature of pollution

The nature of the pollution, i.e. sources and associated congener profiles, was investigated with multivariate analysis as was described in the Materials and Methods chapter (see section 3.4.1).

4.2.1 Comparison of the northern hemisphere and South African PCDD/F profiles

In an attempt to learn what type of pollution source(s) was/were responsible for the observed PCDD/F congener profile at the South African sites, a series of PCAs was done. The South African data was combined with a dataset from the northern hemisphere (Wenning *et al.* 1993; Table 3.2). The PCA results of the Wenning data, the combined data set (Wenning data & South African data), and the South African data set alone, will be reported on in this order. For the South African data, half the detection limit was substituted for all those concentrations below the detection limits in Tables 4.2a and b, before the centred log-ratios were determined for PCA.

Wenning *et al.* (1993) reported on only three factors that cumulatively explained 72% of the variance. A PCA was repeated on their data with two adjustments to their methods:

(i) They normalised congener concentrations as a percentage of the combined sum of the seventeen PCDD/F congeners and then range-transformed the data to fix the variance between zero and one in the data matrix. In this study, the centred log-ratios of the proportions of the congener concentrations were determined - as was explained in section 3.4.1.1.

(ii) Wenning *et al.* (1993) apparently did not perform any rotations during their PCA. In the current study, the varimax raw rotation was selected because it gave a clearer separation of the factors. After these adjustments, the three factors of the Wenning data explained 71% of the total variance (Table 4.5). The significance of the factors was based on the Kaiser-Guttman criterion, i.e. components with eigenvalues greater than 1 were extracted (McCune & Grace, 2002), where eigenvalues originated from the decomposition of the co-variance matrix.

Factor 1 explained 33.7% of the variance in the data. This factor was a contrast between the medium chlorinated furans (PeF1, PeF2, TeF, HxF2, HxF4 and HxF1) and the three highly chlorinated PCDD/Fs (OD, HpD and OF). Only two of the possible seven dioxin

congeners contributed to factor 1 (Table 4.5). Factor 2 was about the contrast between three lower chlorinated dioxins (TeD, PeD and HxD1) and the two hepta chlorinated furans

Table 4.5: The varimax-rotated factor loadings of the PCDD/F congeners contributing to the three factors explaining 71% of the variance in the Wenning data. The congeners are arranged with factor loadings in decreasing order.

Factor 1		Factor 2		Factor 3	
Congener	Factor loading	Congener	Factor loading	Congener	Factor loading
PeF1	0.933	TeD	0.929	HxF3	0.565
PeF2	0.904	PeD	0.681	HxF1	0.541
TeF	0.855	HxD1	0.640	HpF2	0.526
HxF2	0.762	HxD3		OF	
HxF4	0.561	HxD2		HpF1	
HxF1	0.509	TeF		HxF2	
HxF3		PeF2		TeD	
PeD		PeF1		PeF2	
TeD		HpD		PeF1	
HpF2		HxF3		TeF	
HxD1		OD		HxF4	
HxD3		HxF4		PeD	
HpF1		HxF2		HxD1	
HxD2		HxF1		OD	
OF	-0.647	OF		HpD	-0.589
HpD	-0.762	HpF1	-0.605	HxD2	-0.680
OD	-0.831	HpF2	-0.613	HxD3	-0.740
%Total variance	33.7		18.7		18.6
Cumulative %	33.7		52.4		71

Only factor loadings $\geq |0.5|$ are reported.

(HpF1 and HpF2). This factor explained 18.7% of the variance. Factor 3 showed a contrast between positively loaded furan and negatively loaded dioxin congeners. In this case, all isomers concerned were either hepta or hexa chlorinated (cf. last column, Table 4.5). This third factor explained 18.6% of the variance in the data. All of the isomers contributed at least once to any of the factors; two of them (HxF1 and HpF2) contributed to two factors (Table 4.5).

Factor scores (for the sites or samples types) were plotted with the factor loadings (of the congeners) on biplots. ("Sites" refers to the South African data, and "samples types" refers to the Wenning data.) On such a biplot, sites with similar congener profiles are expected to group together, and congeners contributing the most to a factor appear the farthest from the origin of the graph. Most of Wenning's sample types arranged around the origin (Figures 4.4, 4.5 and 4.6). The origin represents the mean concentration of all samples (Škrbić & Đurišić-Mladenović, 2007). Sites that were expected to have similar congener profiles did group together in all three biplots.

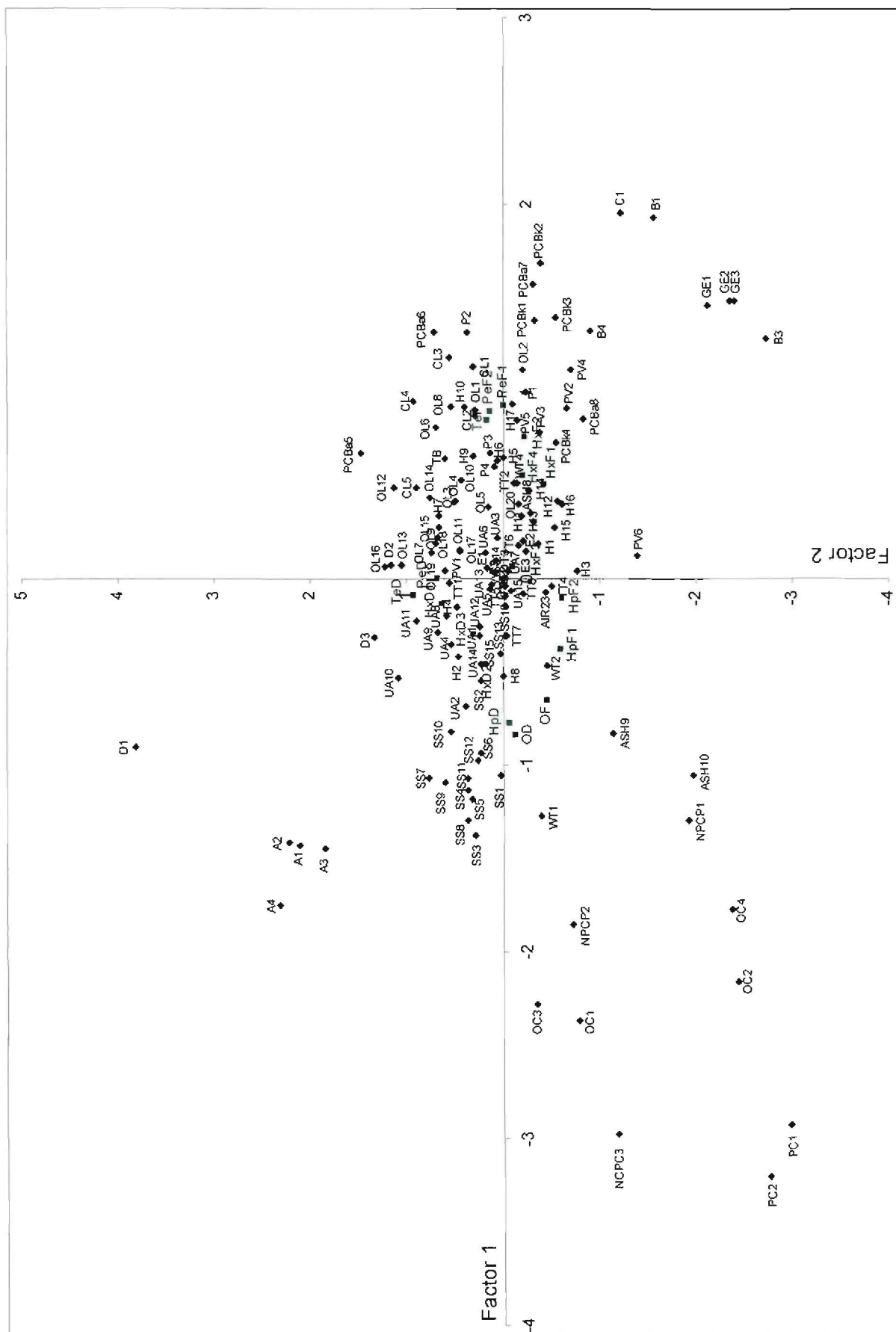


Figure 4.4: A PCA biplot of factors 1 and 2 of the Wenning data. ♦ = sites/type of sample ■ = PCDD/F congeners

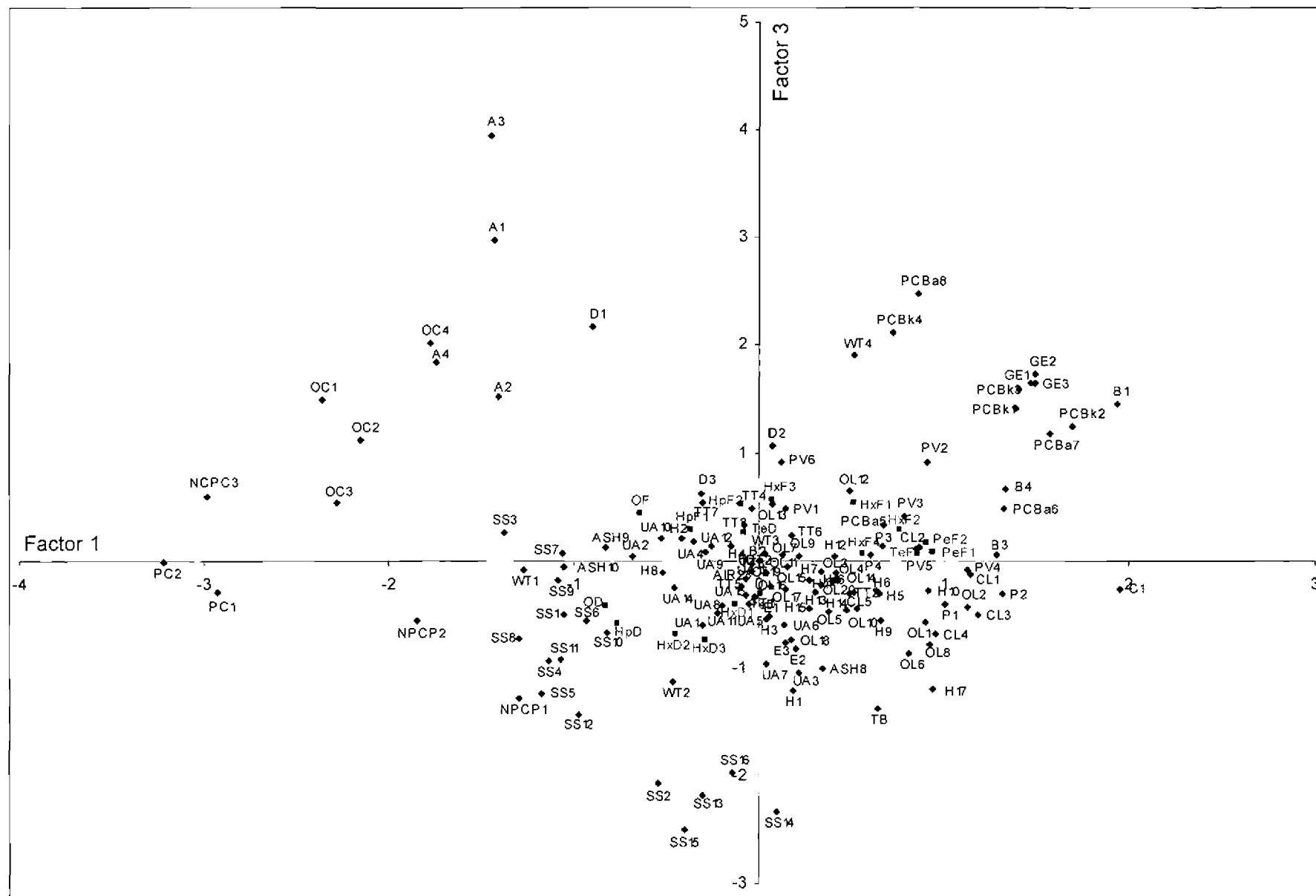


Figure 4.5: A PCA biplot of factors 1 and 3 of the Wenning data. ◆ = sites/type of sample ■ = PCDD/F congeners

Figure 4.6: A PCA biplot of factors 2 and 3 of the Wenning data. ♦ = sites/type of sample ▪ = PCDD/F congeners

Sample types A1, A2, A3 and A4 (soils from a former 2,4,5-T plant) were in close proximity to each other as were most of the SS (municipal sewage sludge) sites, GE1-4 (graphite electrode sludge), and OC1-4 (residue in o-chloranil) sites. The sites labelled PC (residue in pentachlorophenol) and NPCP (residue in Na-pentachlorophenol) had similar pollution sources and were also loosely grouped together. The urban air sites (UA) from Germany (UA1-8) and those from the USA (UA9-15) arranged in the same area, together with the OL (chimney soot from oil central heating) and H (soils from scrap wire and car incineration) sample types.

Because Wenning *et al.* (1993) reported and discussed only three factors, the same approach was followed when the South African data was incorporated into the Wenning data (Table 4.6). 70.1% of the variance was explained by the three factors.

Table 4.6: The varimax-rotated factor loadings of the PCDD/F congeners contributing to the three factors explaining 70.1% of the variance in the South African data together with the Wenning data. The congeners are arranged with factor loadings in decreasing order.

Factor 1		Factor 2		Factor 3	
Congener	Factor loading	Congener	Factor loading	Congener	Factor loading
PeF1	0.937	TeD	0.911	HxF3	0.555
PeF2	0.908	PeD	0.699	HxF1	0.542
TeF	0.861	HxD1	0.651	HpF2	0.534
HxF2	0.755	HxD3		OF	
HxF4	0.546	HxD2		HpF1	
HxF1	0.516	TeF		TeD	
HxF3	0.054	PeF2		HxF2	
PeD		PeF1		PeF2	
HpF2		HpD		PeF1	
TeD		HxF3		HxF4	
HxD1		OD		TeF	
HxD3		HxF4		PeD	
HpF1		HxF2		HxD1	
HxD2		HxF1		OD	
OF	-0.630	OF	-0.502	HpD	-0.586
HpD	-0.759	HpF2	-0.594	HxD2	-0.642
OD	-0.820	HpF1	-0.604	HxD3	-0.692
%Total variance	33.7		18.8		17.5
Cumulative %	33.7		52.5		70.1

Only factor loadings $\geq |0.5|$ are reported.

The same congeners contributed to the same factors when compared with the Wenning only data (Table 4.5). The only exception was OF that also contributed to factor 2 of the combined dataset (cf. Tables 4.5 & 4.6).

Investigation of the biplots (Figures 4.7, 4.8 & 4.9) together with the factor scores table (Table 4.7) revealed which Wenning sample types associated with the South African sites, and therefore shared similar congener profiles and presumably had the same type of

Table 4.7: The factor scores for the Wenning and South African data arranged in decreasing order for each of the factors.

Sites	Factor 1	Sites	Factor 2	Sites	Factor 3
C1	2.192	D1	3.946	A3	4.304
B1	2.184	A4	2.375	A1	3.324
PCBk2	1.883	A2	2.316	PCBa8	2.665
GE3	1.717	A1	2.155	D1	2.410
GE2	1.715	A3	1.800	PCBk4	2.274
PCBa7	1.710	PCBa5	1.531	OC4	2.227
GE1	1.679	OL16	1.305	WT4	2.109
PCBk3	1.541	D3	1.289	A4	2.086
PCBk1	1.536	UA10	1.185	A2	1.773
B4	1.519	D2	1.184	PCBk3	1.721
B3	1.513	OL12	1.133	GE2	1.665
PCBa6	1.481	OL13	1.079	GE1	1.605
P2	1.436	UA11	1.010	GE3	1.579
CL3	1.294	CL4	0.985	PCBk1	1.504
OL2	1.289	CL5	0.957	OC1	1.494
CL1	1.255	SS7	0.784	B1	1.384
PV4	1.192	OL7	0.773	PCBa7	1.303
P1	1.131	OL14	0.762	OC2	1.303
CL4	1.069	OL18	0.750	PCBk2	1.271
OL8	1.049	H7	0.743	D2	1.186
OL1	1.027	OL6	0.726	PV6	1.075
H10	0.985	TB	0.722	PV2	1.064
PV2	0.982	OL15	0.699	OL12	0.708
H17	0.978	UA9	0.697	PV1	0.667
PCBa8	0.958	PCBa6	0.696	NCPC3	0.651
CL2	0.947	OL9	0.690	TT7	0.644
OL6	0.925	LskpD	0.667	B4	0.608
PV5	0.912	OL19	0.654	OL13	0.599
PV3	0.835	UA8	0.647	OrangeM	0.592
PCBk4	0.823	CL3	0.622	D3	0.585
PCBa5	0.773	SS9	0.585	PV3	0.581
P3	0.759	TT1	0.578	TT4	0.545
H9	0.721	UA4	0.567	PCBa6	0.491
H5	0.712	OL8	0.566	OC3	0.488
TB	0.709	OL3	0.540	PCBa5	0.366
H6	0.692	PV1	0.537	UA10	0.362
P4	0.678	SS10	0.529	TT8	0.357
OL10	0.608	H4	0.520	LskpD	0.352
OL12	0.584	SldnhBy	0.506	Bfalo	0.312
TT2	0.574	H10	0.499	SS3	0.302
WT4	0.571	OL4	0.497	H2	0.287
CL5	0.563	OL17	0.489	TT6	0.286
OL14	0.528	H2	0.478	UA4	0.276
H14	0.507	OL10	0.465	H4	0.250
OL4	0.498	VaalDam	0.457	SldnhBy	0.236
OL3	0.474	OL11	0.452	PV5	0.230
OL5	0.466	VaalRiv	0.448	CL2	0.217
OL20	0.466	P2	0.438	P3	0.179
H12	0.451	RchrdBy	0.429	UA12	0.176
ASH8	0.434	UA2	0.417	VaalDam	0.143
H16	0.434	H9	0.380	H12	0.140
H7	0.377	SS11	0.359	UA9	0.139
H11	0.367	Olifant	0.351	UA2	0.131
H13	0.345	CL2	0.339	PC2	0.129
OL15	0.339	Grt	0.339	SS7	0.126

H15	0.314	SS4	0.331	P4	0.108
OL9	0.288	CL1	0.330	OL7	0.102
E2	0.263	SS8	0.330	ASH10	0.086
UA3	0.262	UA1	0.321	WT3	0.077
H1	0.244	SS5	0.316	Gr ^t	0.068
TT6	0.239	OL1	0.295	OL9	0.066
OL18	0.234	OrangeM	0.291	ASH9	0.055
OL11	0.211	Bfalo	0.286	RchrdBy	0.045
E3	0.211	SS2	0.285	PV4	0.037
OL17	0.187	SS12	0.278	TT3	0.004
OL7	0.183	Thlzhlk	0.263	H7	-0.001
UA6	0.169	UA14	0.258	UA13	-0.022
PV1	0.147	SS3	0.241	OL11	-0.024
PV6	0.137	UA6	0.232	AIR23	-0.031
SS14	0.129	UA12	0.213	OL19	-0.042
OL13	0.127	SS6	0.201	H8	-0.076
SldnhBy	0.125	Mooi	0.194	H16	-0.087
D2	0.113	SS14	0.185	CL1	-0.097
E1	0.110	E1	0.164	OL4	-0.104
OL16	0.103	TH2ODam	0.161	H11	-0.126
H3	0.097	UA13	0.153	WT1	-0.143
WT3	0.095	UA5	0.141	OL20	-0.144
OL19	0.080	OL5	0.141	Berg	-0.150
UA7	0.055	P3	0.130	B3	-0.153
Thlzhlk	0.046	SS15	0.118	OL15	-0.157
TT1	0.022	UA3	0.111	OL3	-0.164
TT3	0.013	H6	0.107	SS9	-0.170
TT4	0.010	P4	0.102	OL16	-0.173
UA5	-0.013	H17	0.044	H10	-0.179
UA13	-0.018	WT3	0.044	Olifant	-0.184
TT8	-0.019	H5	0.042	UA14	-0.194
RtsprCh	-0.019	UA7	0.027	OL14	-0.196
UA15	-0.040	SS13	0.026	H6	-0.207
TT5	-0.045	RietSpr	0.012	H13	-0.217
RchrdBy	-0.071	SS16	0.011	OL17	-0.220
AIR23	-0.083	H8	-0.028	PC1	-0.224
Olifant	-0.084	SS1	-0.029	TT2	-0.233
SS16	-0.095	TT7	-0.044	VaalRiv	-0.234
VaalDam	-0.105	TT3	-0.046	TT5	-0.236
H4	-0.141	TT5	-0.056	Mooi	-0.236
LochV	-0.170	UA15	-0.068	H5	-0.238
UA8	-0.176	PV5	-0.088	P2	-0.255
UA12	-0.214	TT2	-0.091	CL5	-0.274
Modder	-0.218	H11	-0.149	UA15	-0.285
D3	-0.229	Berg	-0.154	TT1	-0.302
UA11	-0.235	OL20	-0.169	UA8	-0.369
HrtbDam	-0.262	SwkpEst	-0.186	H14	-0.372
UA9	-0.267	WT4	-0.190	UA5	-0.385
SS13	-0.284	TT6	-0.195	UA11	-0.391
VaalRiv	-0.285	RtsprCh	-0.206	H15	-0.392
TT7	-0.293	H14	-0.207	TH2ODam	-0.399
RietSpr	-0.301	E2	-0.207	Thlzhlk	-0.414
UA1	-0.312	E3	-0.252	RietSpr	-0.421
Mooi	-0.320	P1	-0.263	C1	-0.426
UA4	-0.341	Croc	-0.266	OL10	-0.429
Bfalo	-0.388	HrtbDam	-0.275	P1	-0.437
SwkpEst	-0.393	TT8	-0.283	CL3	-0.477
SS15	-0.401	OL2	-0.285	OL5	-0.491

H2	-0.404	ASH8	-0.299	H9	-0.495
OrangeM	-0.407	H13	-0.304	E1	-0.497
Umlazi	-0.446	Umgeni	-0.328	UA1	-0.516
WT2	-0.451	PV3	-0.339	OL2	-0.527
UA14	-0.452	PCBa7	-0.344	NPCP2	-0.528
Berg	-0.458	H1	-0.370	SS1	-0.531
Grt	-0.462	Lochv	-0.387	UA6	-0.546
Umgeni	-0.478	AIR23	-0.393	Umgeni	-0.554
Lskpd	-0.487	Umlazi	-0.413	H3	-0.567
H8	-0.501	PCBk1	-0.420	SS6	-0.571
TH2ODam	-0.528	WT2	-0.474	OL1	-0.605
SS2	-0.529	Modder	-0.482	Modder	-0.646
UA10	-0.549	PCBk2	-0.517	RtsprCh	-0.661
UA2	-0.696	H15	-0.528	Lochv	-0.668
ASH9	-0.764	WT1	-0.541	Croc	-0.677
SS10	-0.802	OC3	-0.554	SS10	-0.696
Croc	-0.853	H12	-0.557	CL4	-0.706
D1	-0.923	TT4	-0.563	OL18	-0.729
SS6	-0.923	H16	-0.598	HrtbDam	-0.743
SS12	-0.977	PCBk3	-0.623	SS8	-0.768
SS1	-1.052	PV4	-0.657	E3	-0.792
SS11	-1.066	PCBk4	-0.663	OL8	-0.833
SS7	-1.082	PV2	-0.671	E2	-0.843
SS9	-1.090	NPCP2	-0.789	Umlazi	-0.857
ASH10	-1.116	H3	-0.819	UA7	-0.900
SS4	-1.122	PCBa8	-0.956	OL6	-0.912
SS5	-1.184	OC1	-1.048	SS11	-0.966
WT1	-1.251	B4	-1.071	SS4	-0.995
SS8	-1.301	ASH9	-1.351	ASH8	-1.034
NPCP1	-1.361	C1	-1.384	UA3	-1.035
SS3	-1.398	NPCPC3	-1.397	H17	-1.101
A2	-1.491	PV6	-1.465	WT2	-1.143
A3	-1.501	B1	-1.832	SwkpeEst	-1.218
A1	-1.509	NPCP1	-1.954	NPCP1	-1.227
A4	-1.834	ASH10	-2.035	H1	-1.252
OC4	-1.863	GE1	-2.421	SS5	-1.296
NPCP2	-1.920	OC4	-2.597	TB	-1.369
OC2	-2.286	OC2	-2.607	SS12	-1.483
OC3	-2.313	GE2	-2.682	SS16	-2.073
OC1	-2.403	GE3	-2.724	SS2	-2.162
PC1	-3.072	PC2	-2.926	SS13	-2.263
NPCPC3	-3.078	B3	-3.046	SS14	-2.398
PC2	-3.401	PC1	-3.168	SS15	-2.569

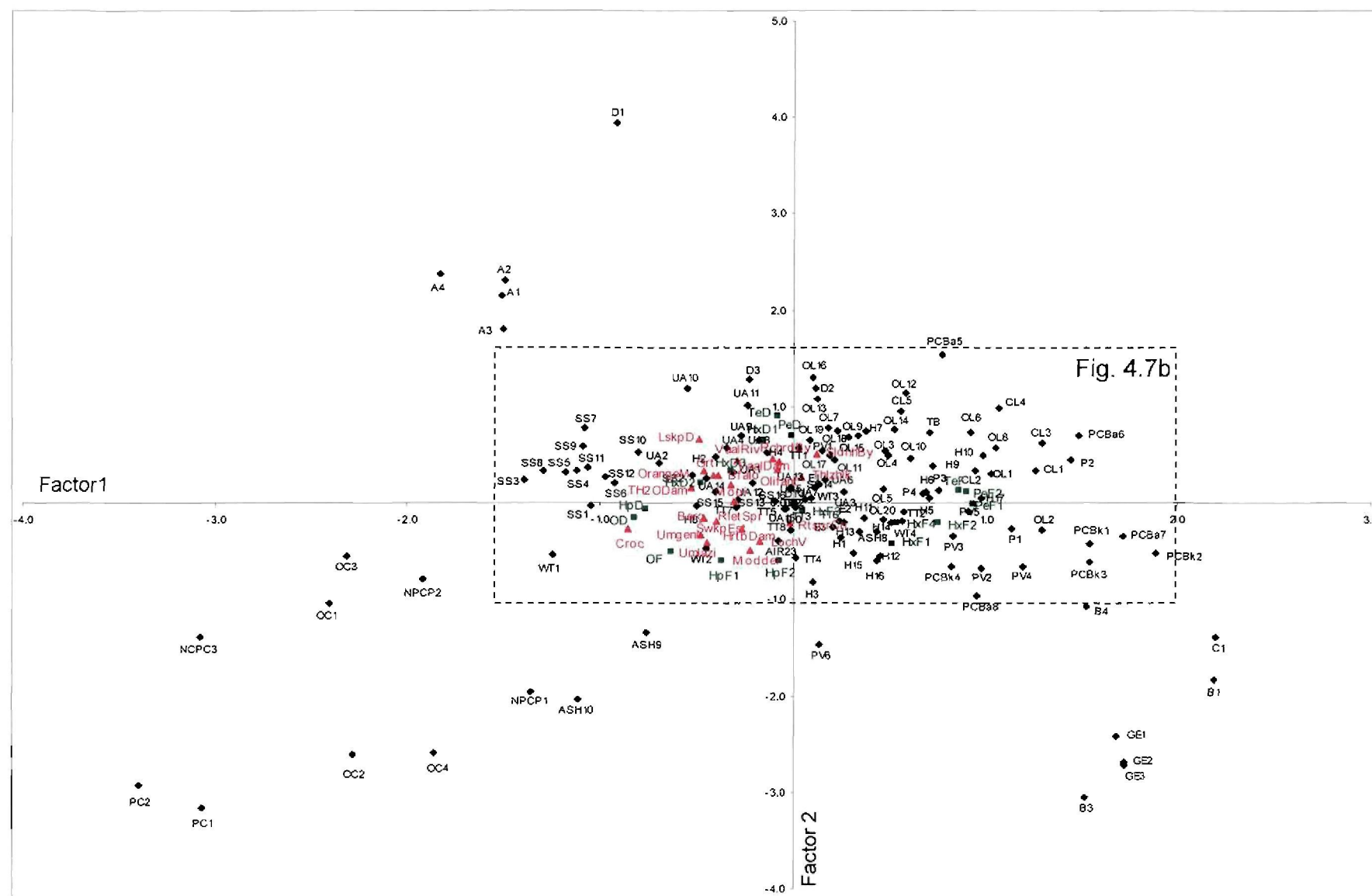


Figure 4.7a: A PCA biplot of factors 1 and 2 of the Wenning and SA data. ◆ = Wenning sites/type of samples; ▲ = SA sites; ■ = PCDD/F congeners. All sites/type of samples included on the biplot. (Broken line rectangle magnified in Figure 4.7b).

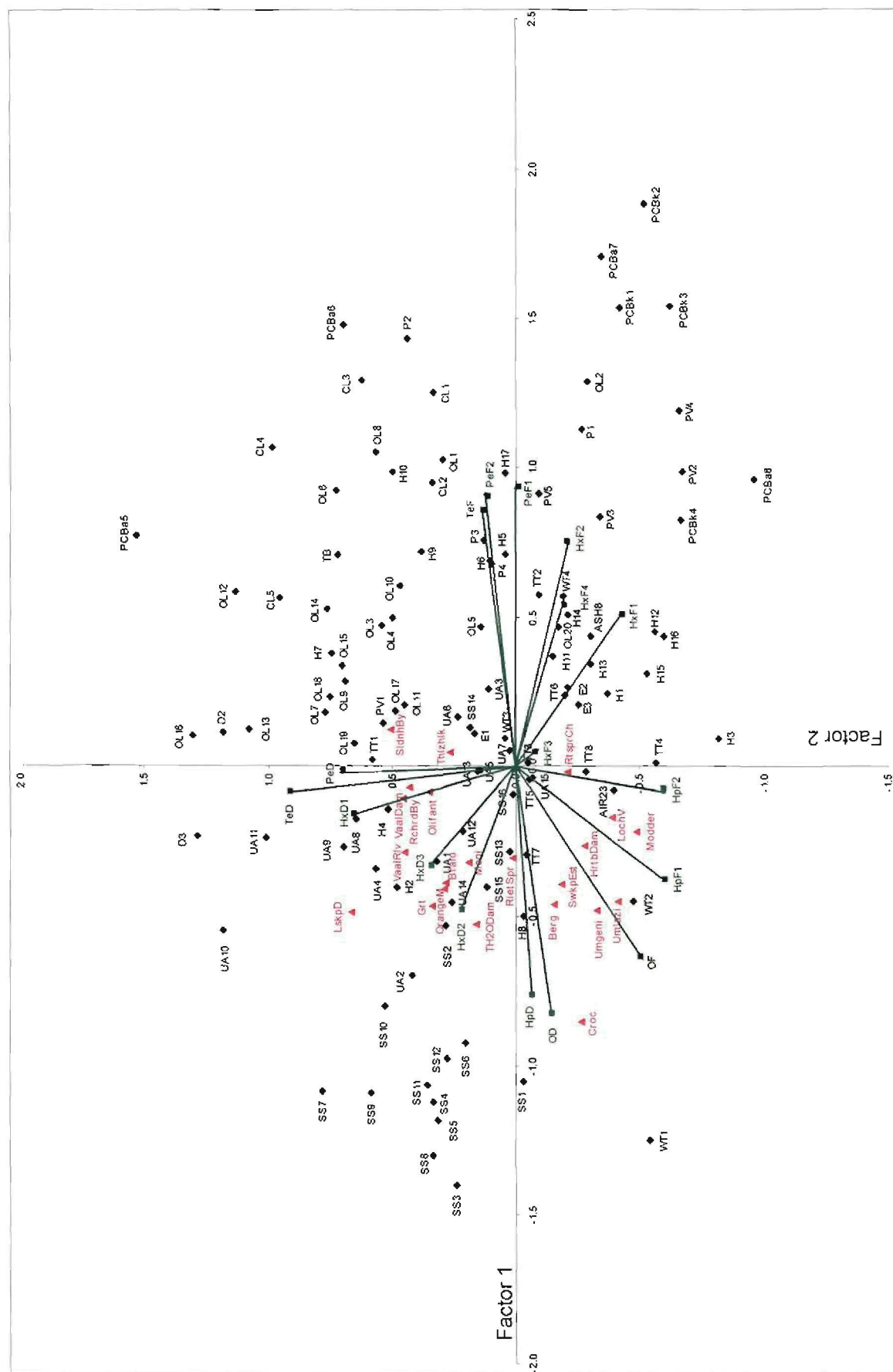


Figure 4.7b: A PCA biplot of factors 1 and 2 of the Wenning and SA data. ♦ = Wenning sites/type of samples; ▲ = SA sites; ▴ = PCDD/F congeners. Only sites/type of samples surrounding the origin.

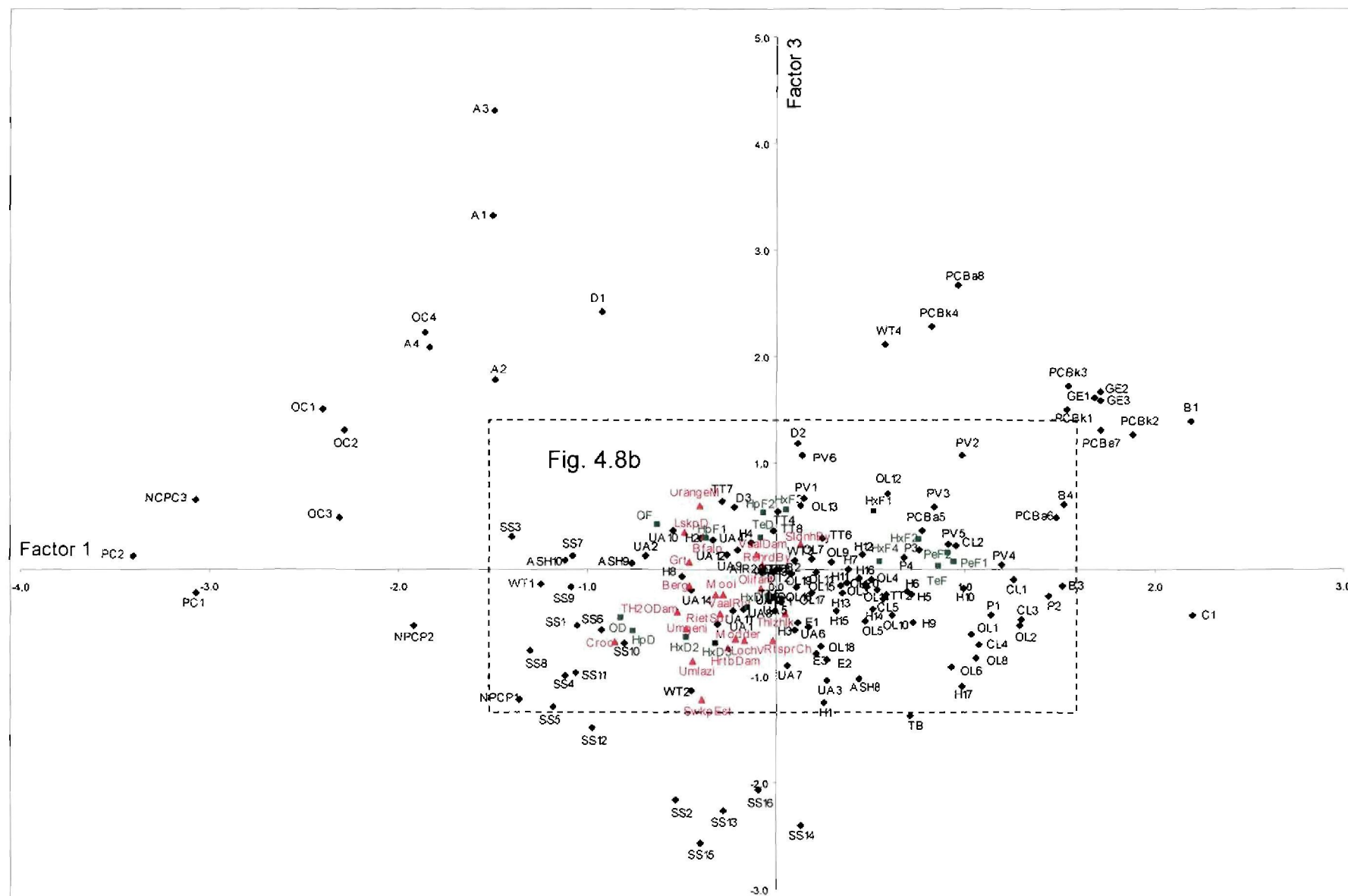


Figure 4.8a: A PCA biplot of factors 1 and 3 of the Wenning and SA data. ◆ = Wenning sites/type of samples; ▲ = SA sites; ■ = PCDD/F congeners. All sites/type of samples included on the graph. (Dotted line rectangle magnified in Figure 4.8b).

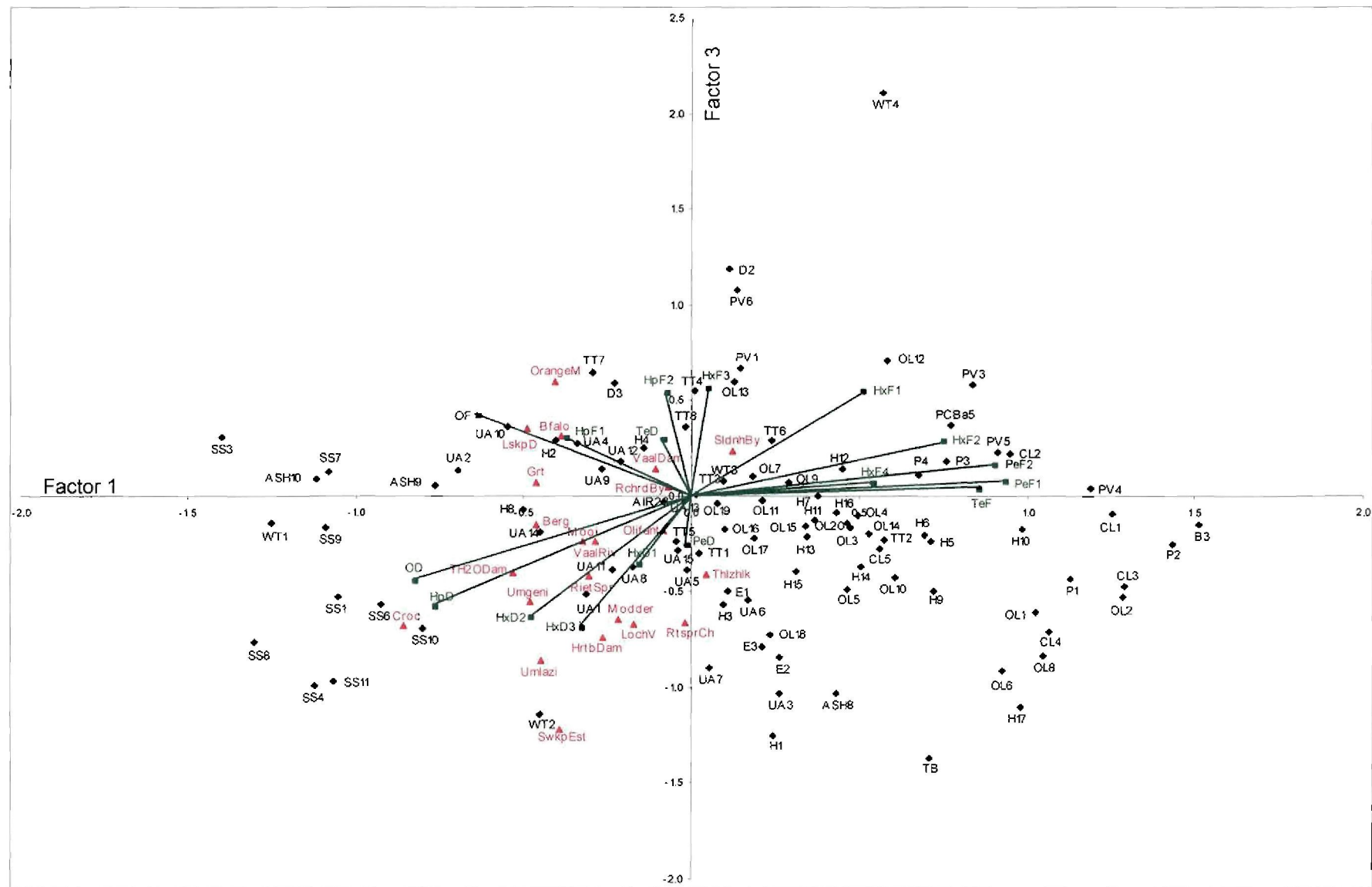


Figure 4.8b: A PCA biplot of factors 1 and 3 of the Wenning and SA data. ♦ = Wenning sites/type of samples; ▲ = SA sites; ■ = PCDD/F congeners. Only sites/type of samples surrounding the origin.

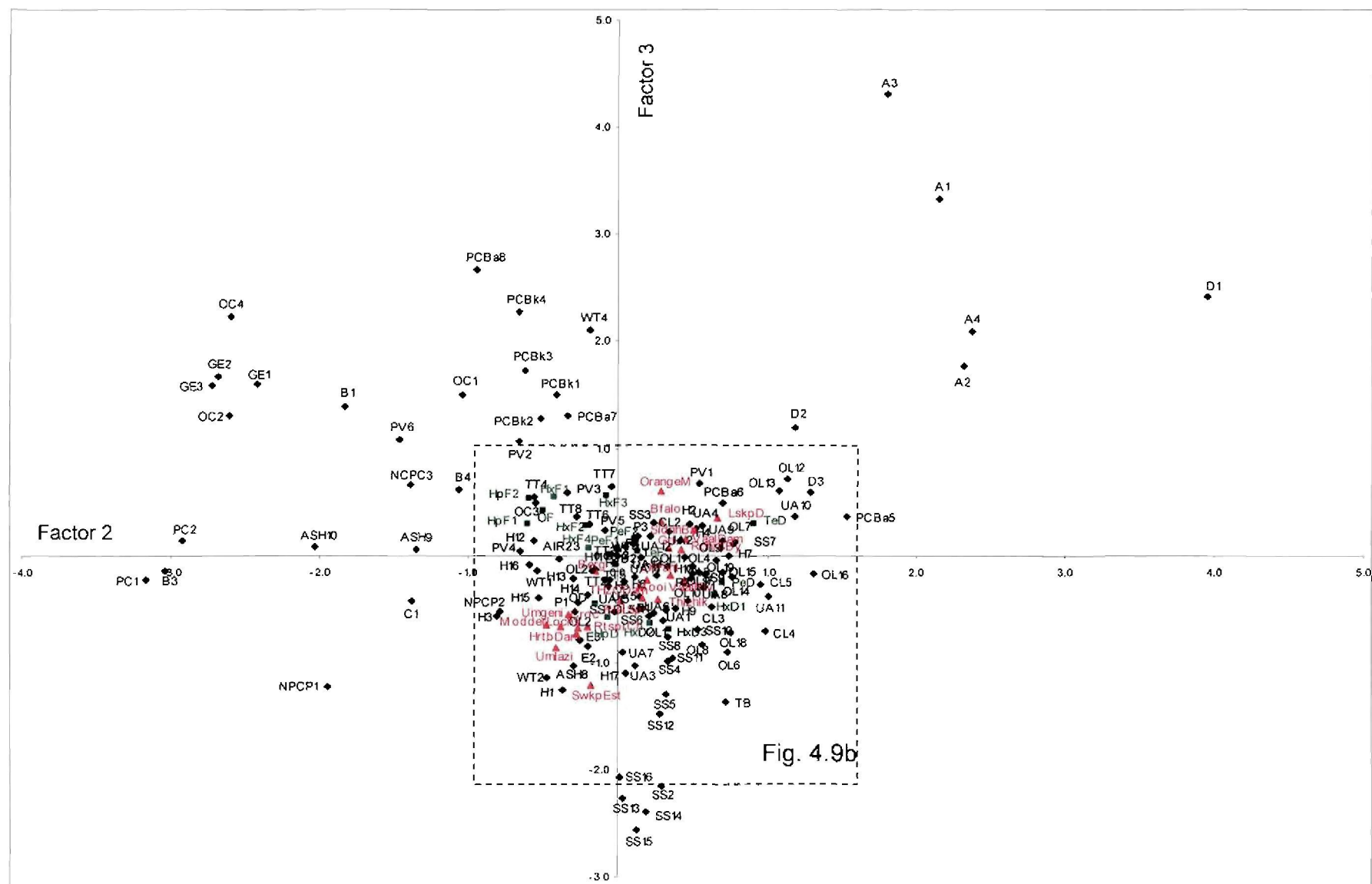


Figure 4.9a: A PCA biplot of factors 2 and 3 of the Wenning and SA data. ◆ = Wenning sites/type of samples; ▲ = SA sites; ■ = PCDD/F congeners. All sites/type of samples included on the graph. (Dotted line square magnified in Figure 4.9b).

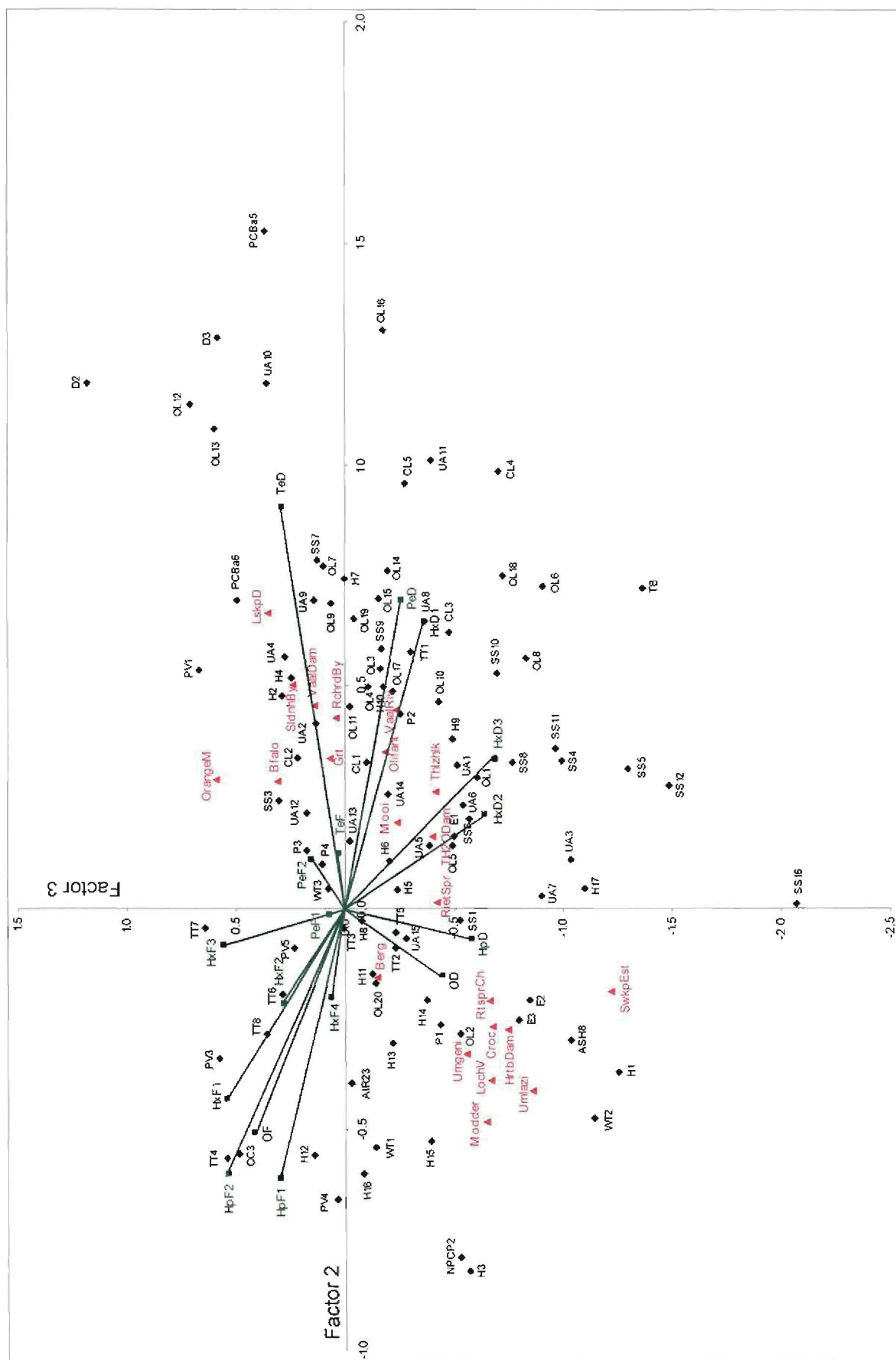
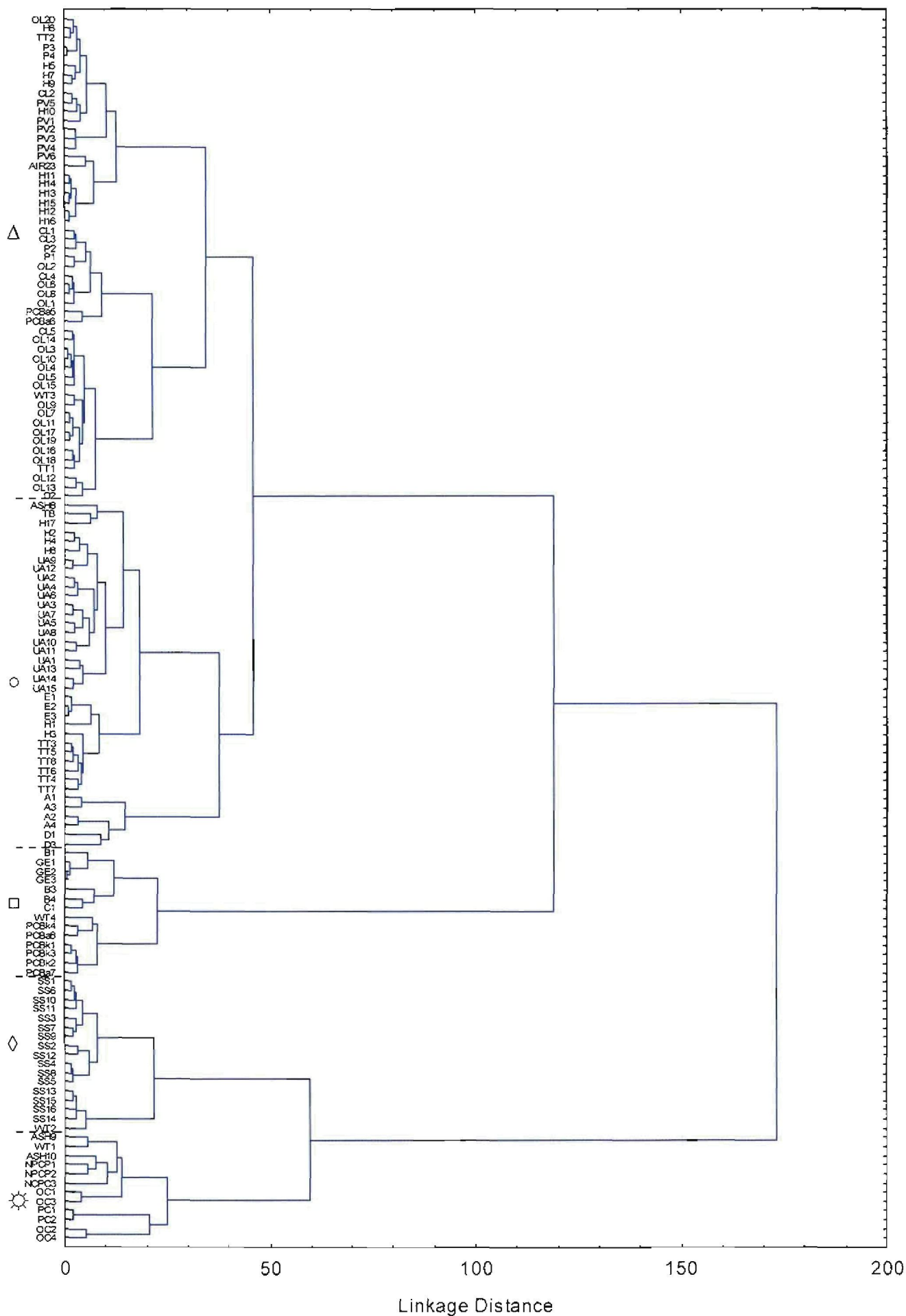


Figure 4.9b: A PCA biplot of factors 2 and 3 of the Wenning and SA data. ♦ = Wenning sites/type of samples; ▲ = SA sites; ▪ = PCDD/F congeners. Only sites/type of samples surrounding the origin.



pollution source. With the South African sites included in the PCA (Figures 4.7 to 4.9), the Wenning sample types that originally clustered together, did so again (A1, A2, A3 and A4; NCPC and PC; PCBk and PCBa; GE1-3; SS; OC, and PV). This means that the pattern of sample type distribution in the biplots of the Wenning data conformed to the site distribution in the combined data set: the inclusion of the South African dataset therefore did not disturb the pattern. However, all 22 of the South African sites were clustered closely together (Figures 4.7 to 4.9).

Because too many sites/types of samples crowded around the origins of the biplots (**a**-versions of the biplots) and to allow better inspection of the plots, many of the outliers were removed to obtain a better spread of the South African sites (**b**-versions of the biplots).

For all three factors (Table 4.7; Figures 4.7 to 4.9), the South African sites appeared between the OL (chimney soot from oil central heating), UA (urban air), H (soils from scrap wire and car incineration sites), WT (municipal water treatment plant sludge and chlorinated tap water), AIR (ambient air near municipal sewage sludge), TT (air from automobile traffic tunnel) and SS (municipal sewage sludge) sites/sample types. Although PCBk and PV also featured, the previously mentioned sample types were the most common.

Performing HCA with the Wenning data separately, and then combined with the South African data, confirmed the association between those sites with similar pollution profiles (Figures 4.10 and 4.11). Linkage distances of 46 and 50 were used as the criterion to define clusters in the Wenning HCA (Figure 4.10) and the HCA of the combined dataset (Figure 4.11), respectively. For both datasets, the following smaller clusters were identified: (Each of these clusters were labelled with a symbol. This was done to prevent any confusion with other numerical or alphabetic labels. The South African sites are indicated in brackets.)

□ = B; GE; PCBa+k

Δ = PCBa5,6; P; CL; OL; DT; PV; H; (Thlzhlk)

○ = TT; E; H; (remaining 21 South African sites); UA; D; A

◇ = SS

☼ = ASH; NCPC; OC; PC

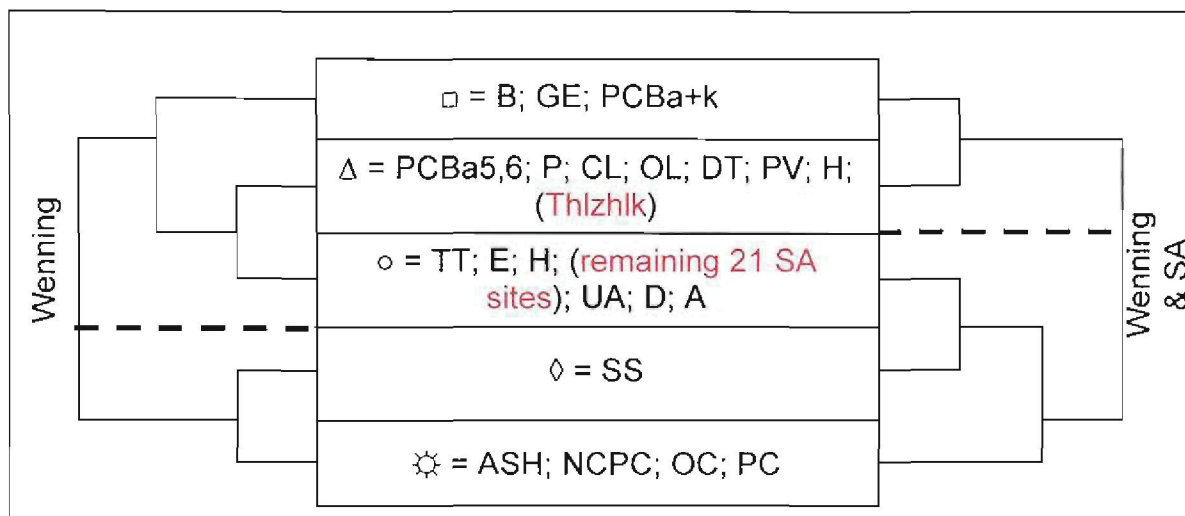


Figure 4.12: A simplified summary of how the small clusters in the two datasets (Wenning and Wenning and SA) combined in the HCA.

Most of the South African sites clustered in the midst of UA (urban air) and H (soils from scrap wire and car incineration) sample types. The single WT (municipal water treatment plant sludge) sample type between the South African sites is but one of four similarly labelled sample types which were distributed among four different clusters and might not be of significance. Wenning sites/types of samples: TT, H and UA, that clustered with the 21 South African sites in the dendrogram (Figure 4.12), corroborated the pattern observed in the biplots (Figures 4.7 to 4.9).

There was a slight difference between the two HCA's in how these smaller clusters were constituted. This difference was attributed to the effect the addition of the South African data had on the Wenning data (Figure 4.12). When the South African sites were added, all but one - the Thlzhlk - grouped with the TT, E, H, UA, D, A group. Not just did Thlzhlk cluster with a different small cluster, but that particular site clustered in a different major cluster away from the rest of the South African sites (Figures 4.11 & 4.12). The dendrogram appeared to be separating Thlzhlk from the rest of the South African sites, but this separation was not all that obvious on the biplots (Figures 4.7 to 4.9). As will be observed in the next sections where only the South African data will be taken into consideration, the Thlzhlk site seemed to be "changing sides"; grouping with different South African sites in consecutive PCAs. Thlzhlk was the site with high %OC but low pollution levels (Figure 4.2).

4.2.2 PCDD/F profiles of South African sites only

When a PCA of only the South African sites was performed, the congeners contributing to the different factors (Table 4.8) differed from those observed for the previous two that had larger data sets (Tables 4.5 & 4.6). Factor 1 of Table 4.8 consisted mainly of PCDF

congeners, contrasting with higher chlorinated PCDDs (OD and Hp) and PCDFs (OF and HpF1). Also, contributing with the PCDFs was TeD, the lowest chlorinated PCDD analysed for. The congeners that contributed to factor 1 in both Table 4.6 (Wenning & SA) and Table 4.8 (South Africa) were PeF1, HxF2, HxF1, HxF3, OF, HpD and OD. Those congeners that were unique to factor 1 of Table 4.8 (South African data only), were HpF2, TeD and HpF1. Factor 1 of Table 4.8 explained 39.9% of the variance in the South African data. Those congeners constituent of factor 1 for the South African data only, generally seemed to be the same as those for factor 1 of the larger data sets. Thus, even for a small sample size, the PCDD/F type of pollution of South Africa was similar to what had been found for the northern hemisphere.

Table 4.8: The varimax-rotated factor loadings of the PCDD/F congeners contributing to the three factors explaining 70.1% of the variance in the South African data. The congeners were arranged with factor loadings in decreasing order.

Factor 1		Factor 2		Factor 3	
Congener	Factor loading	Congener	Factor loading	Congener	Factor loading
TeD	0.885	HxD1	0.709	HxF4	0.641
HpF2	0.871	HxD2	0.702	PeF2	0.579
HxF2	0.749	HxF1		HxF1	0.497
HxF1	0.574	HpF1		PeF1	0.486
PeF1	0.574	HxF2		HxF2	
HxF3	0.551	HxF4		TeF	
PeF2		PeD		HpF1	
PeD		HxD3		OF	
HxD2		HpF2		HxD1	
HxD3		OF		TeD	
HxF4		TeD		HpF2	
HxD1		HxF3		PeD	
TeF		HpD		HpD	
HpF1	-0.749	OD		HxD2	
OF	-0.882	PeF2		OD	
OD	-0.906	PeF1		HxD3	-0.681
HpD	-0.932	TeF	-0.890	HxF3	-0.721
%Total variance	39.9		16.2		14
Cumulative %	39.9		56.1		70.1

Only factor loadings $\geq |0.5|$ were reported.

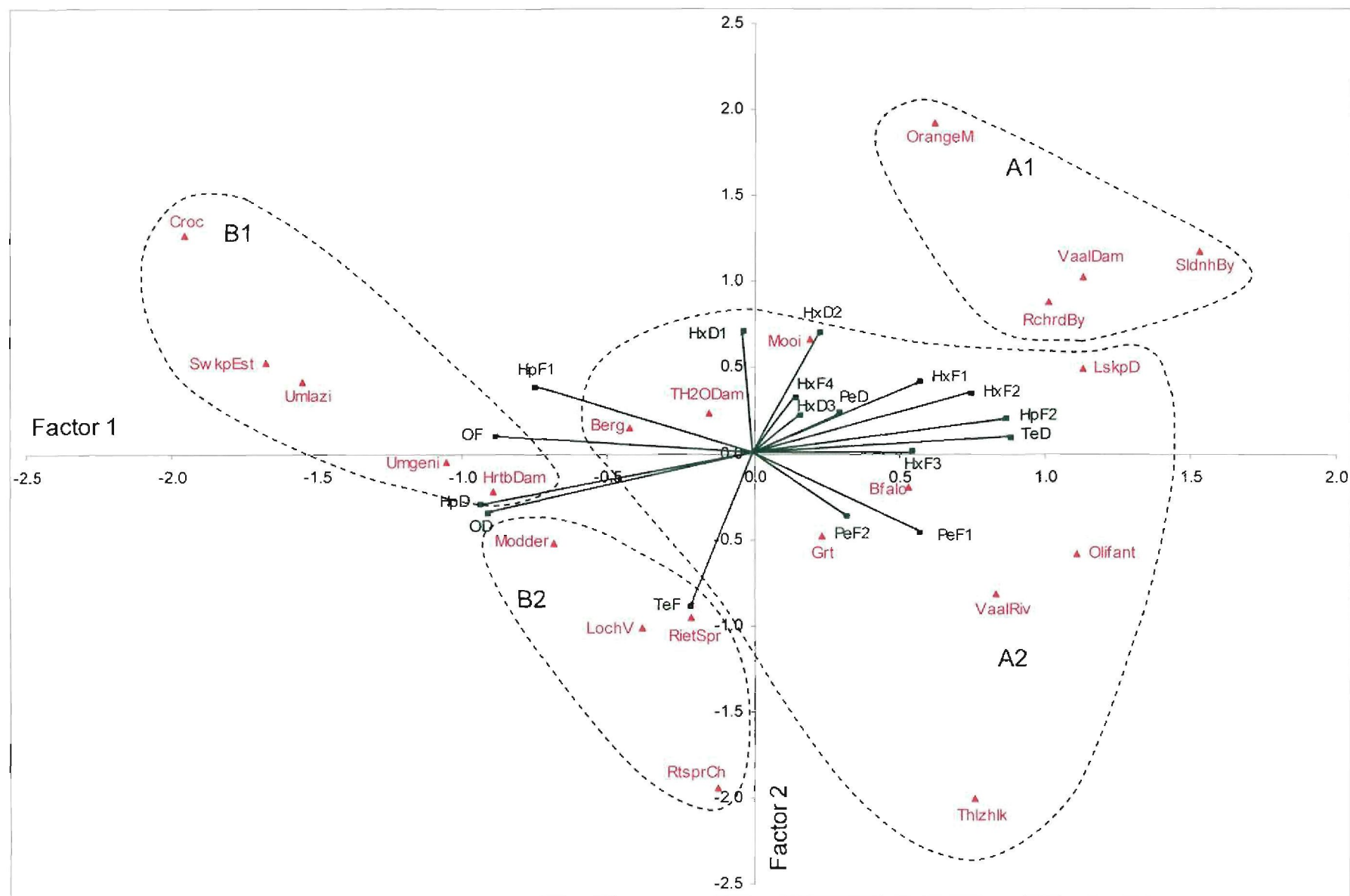


Figure 4.13: A PCA biplot of factors 1 and 2 of the PCDD/Fs of the SA data. ▲ = SA sites; ■ = PCDD/F congeners. Dotted lines and A1, A2, B1 and B2 refer to clusters identified on dendrogram (Figure 4.16).

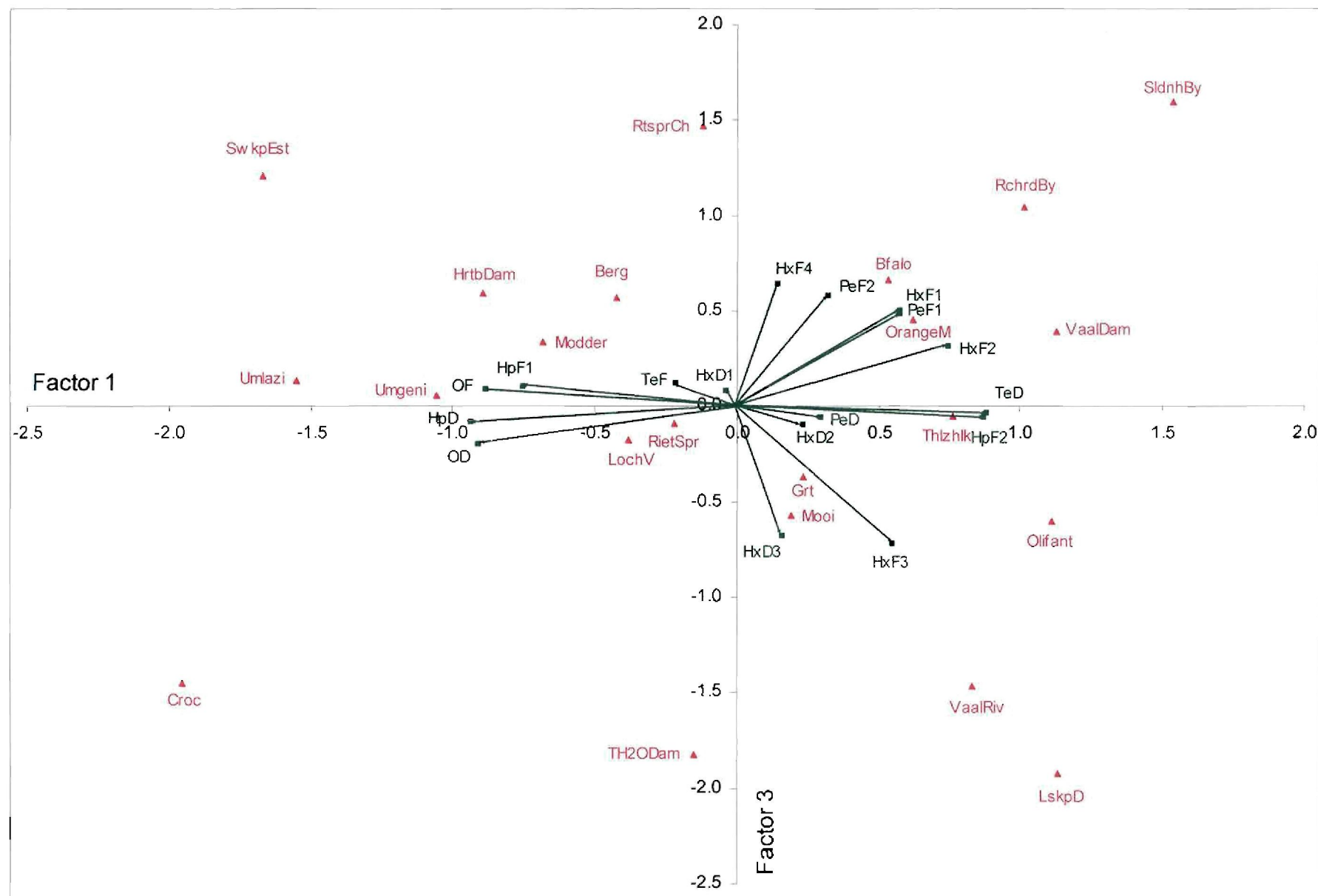


Figure 4.14: A PCA biplot of factors 1 and 3 of the PCDD/Fs of the SA data. ▲ = SA sites; ■ = PCDD/F congeners.

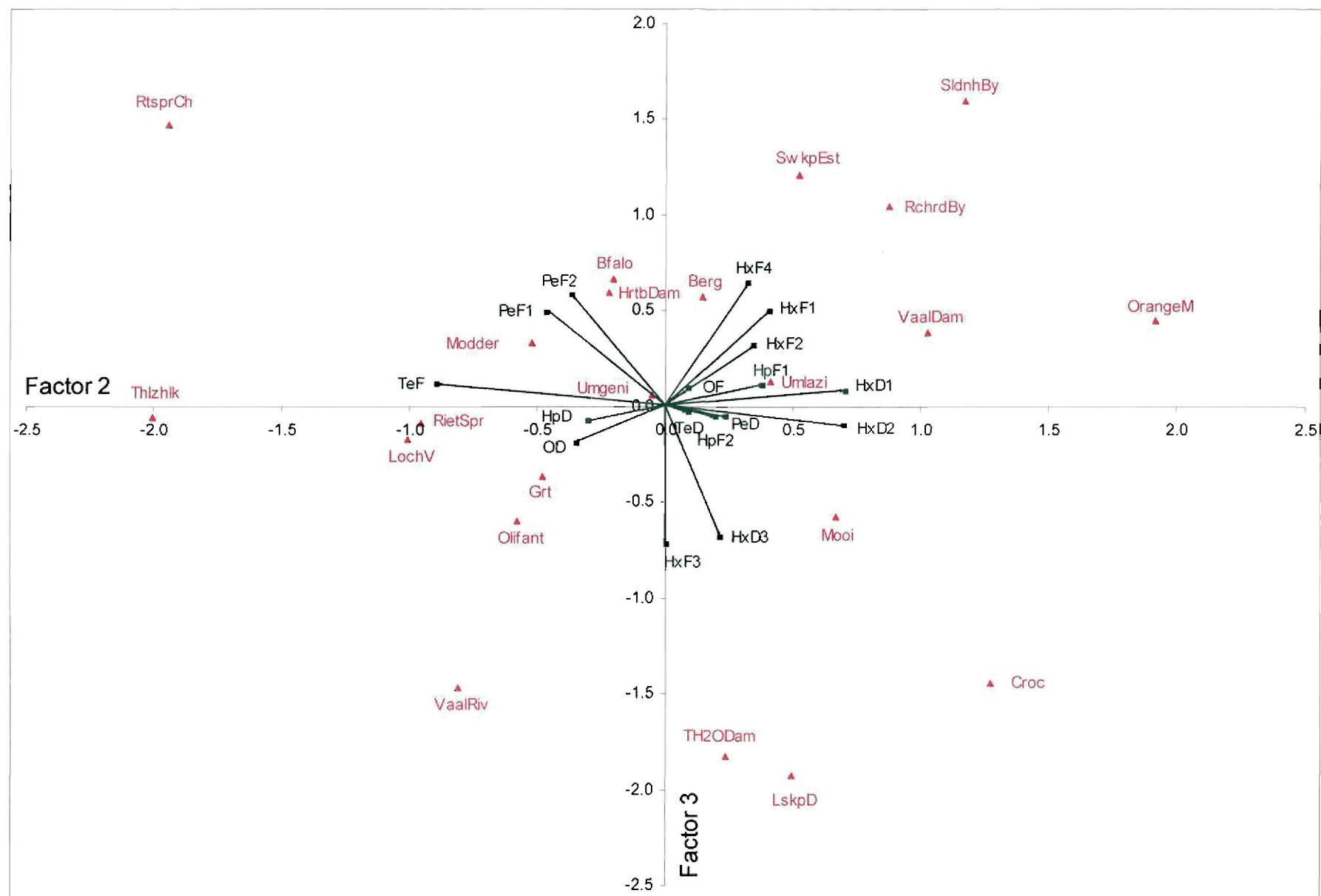


Figure 4.15: A PCA biplot of factors 2 and 3 of the PCDD/Fs of the SA data. ▲ = SA sites; ■ = PCDD/F congeners.

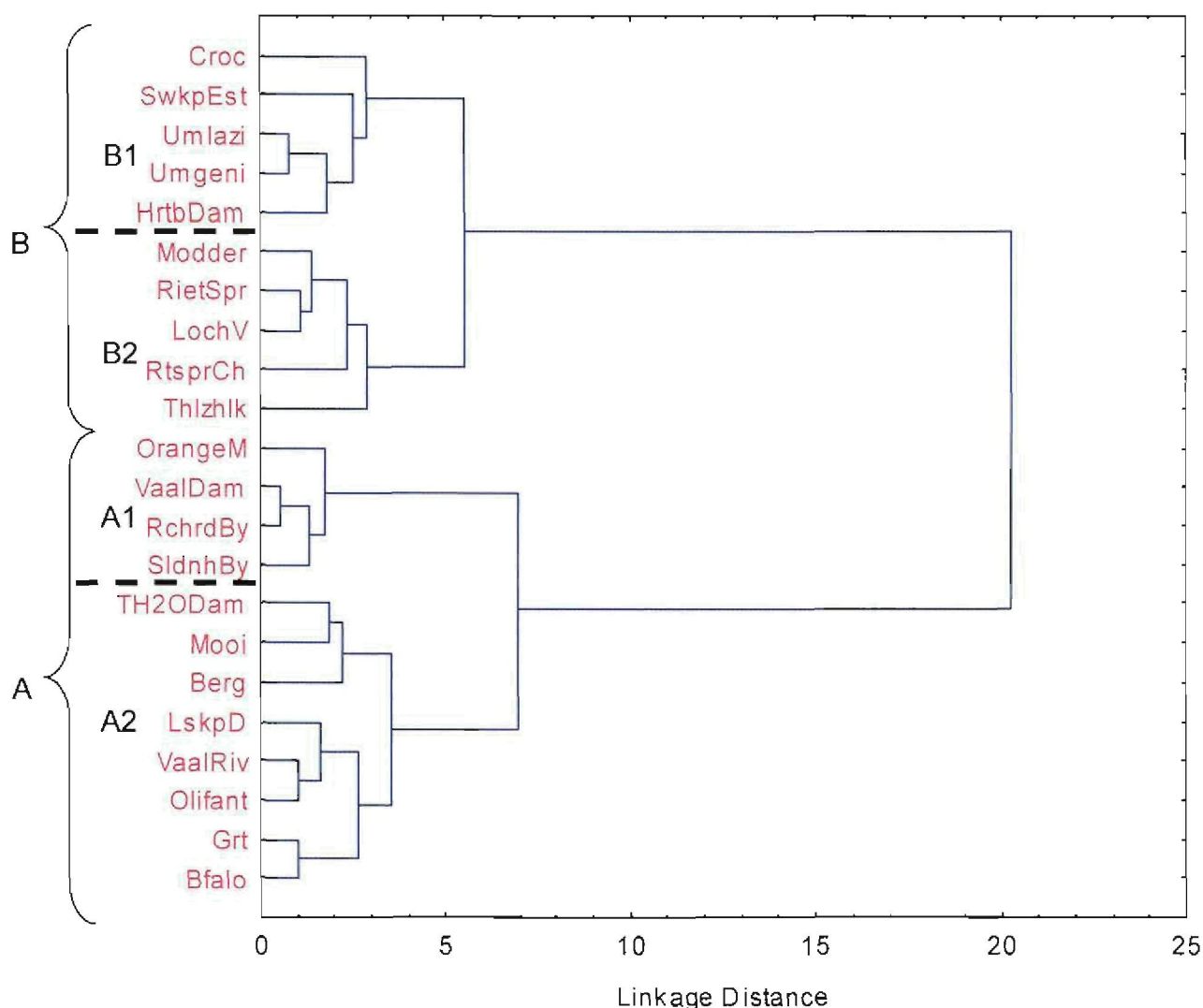


Figure 4.16: HCA of the PCDD/Fs of the SA data. Linkage rule: Ward's method. Distance measure: Euclidean. Dotted lines between sites separate the sub-clusters.

The second factor in Table 4.8 explained 16.2% of the observed variance and showed the contrast between two hexa chlorinated PCDDs (HxD1 and HxD2) and the tetra chlorinated dibenzofuran, TeF. Compared to the congeners of factor 2 in Table 4.6, HxD1 was the only congener in factor 2 of Table 4.6 (Wenning and South Africa) that also contributed to factor 2 in Table 4.8 (South Africa). The third factor in Table 4.8 explained 14% of the data. This factor also showed a distinct contrast: a group of moderately chlorinated congeners (HxF4, PeF2, HxF1 and PeF1) contrasted with HxD3 and HxF3, two other moderately chlorinated congeners. Even though both HxF1 and PeF1 had factor loadings slightly lower than 0.5, they were still taken into consideration. Three of these congeners (HxF3, HxF1 and HxD3) also contributed to factor 3 in Table 4.6 (Wenning and South Africa).

Investigating the biplots (Figures 4.13 to 4.15) showed which South African sites had similar PCDD/F congener profiles. In all three of the biplots, the SldnBy, RchrdBy and the

VaalDam sites were grouped closely together, with the OrangeM not too far away. Of the sites occurring downstream of each other (RtsprCh, RietSpr, LochV, the VaalRiv and the OrangeM), it was only the LochV and RietSpr sites that appeared close to each other in all of the biplots.

The HCA of the same data confirmed that the SldnhBy, RchrdBy, VaalDam and OrangeM sites had similar PCDD/F profiles because they all grouped together in the same cluster (Figure 4.16). Only clusters at linkage distance 5 were considered for interpretation (Figure 4.16). This resulted into four sub-clusters (as indicated by the broken lines in Figure 4.16). Sites downstream of each other (RtsprCh, RietSpr and LochV) appeared in the same cluster. With only 22 samples available in the study, not much can be learned from the further branching of the four mentioned sub-clusters.

The two main HCA clusters of the South African sites were grouped in the same manner as in the biplot of both factors one and two (Figure 4.13). All four of the sub-clusters distinguished on the dendrogram (Figure 4.16) were identified on the biplot of factors 1 and 2. Sites belonging to the different sub-clusters could be outlined without overlapping one another. Cumulatively, factors 1 and 2 explained more than half of the variance (56.1%; Table 4.8). It was therefore not surprising to have found strong corroboration between the biplot (Figure 4.13) and the dendrogram (Figure 4.16).

4.2.3 PCB profiles of South African sites

Northern hemisphere data was not included here because the Wenning *et al.* (1993) publication did not include PCB congeners. Similar papers, with a list of dioxin-like PCB congener concentrations could not be found. Although data from a combination of papers could be compiled, it was deemed beyond the scope of this thesis and will be addressed in later publications.

In order to determine the effect the co-planar PCB profile had on the grouping of the South African sites, PCA and HCA were performed using only the PCBs. During this PCA, only two factors were deemed significant according to their eigenvalues, and together they explained 69.2% of the observed variance in the data (Table 4.9).

For both factors, the same three lower chlorinated congeners (PCB77, 114 and 81; Table 4.9) contrasted with different higher chlorinated PCB congeners. In factor 1, the higher chlorinated congeners included the two penta chlorinated PCBs (PCB126 and 123) as well as the hexachlorinated PCBs (PCB156 and 167). For factor 2, the lower chlorinated congeners of factor 1 were joined with PCB105, also a penta chlorinated congener. They

contrasted with PCB169 (a penta chlorinated PCB) and two hexa chlorinated PCBs (PCB157 and 189).

Table 4.9: The varimax-rotated factor loadings of the co-planar PCB congeners contributing to the two factors explaining 69.2% of the variance in the South African data. The congeners were arranged with factor loadings in decreasing order.

Factor 1		Factor 2	
Congeners	Factor loading	Congeners	Factor loading
PCB167	0.901	PCB105	0.821
PCB156	0.898	PCB81	0.586
PCB126	0.874	PCB77	0.535
PCB123	0.641	PCB114	0.492
PCB189		PCB118	
PCB157		PCB126	
PCB118		PCB156	
PCB105		PCB167	
PCB169		PCB123	
PCB114	-0.683	PCB157	-0.544
PCB81	-0.731	PCB189	-0.738
PCB77	-0.738	PCB169	-0.874
% Total variance	40.5		28.7
Cumulative %	40.5		69.2

Only factor loadings $\geq |0.5|$ are reported.

In the biplot (Figure 4.17), the South African sites distributed into all four quadrants of the graph, but sites that grouped together for the PCDD/F data (Figure 4.13), grouped together in this graph as well (RietSpr and LochV; Modder and HrtbDam; and OrangeM, RchrdBy, VaalDam and SldnhBy). However, added to the latter group were Grt, Bfalo and LskpD, clustered closely together. As it did for the PCDD/F data (Figures 4.13 to 4.15), for PCBs the Croc site also appeared isolated from the other South African sites.

In the dendrogram (Figure 4.18) of the HCA, again, only clusters at linkage distance 5 were considered for interpretation. This allowed for two main clusters, A and B, and four sub-clusters. All of the sites that grouped towards the negative side of factor 1 (Figure 4.17) occurred in cluster A, and all of those sites on the positive side of factor 1 clustered into group B of the HCA (Figure 4.18).

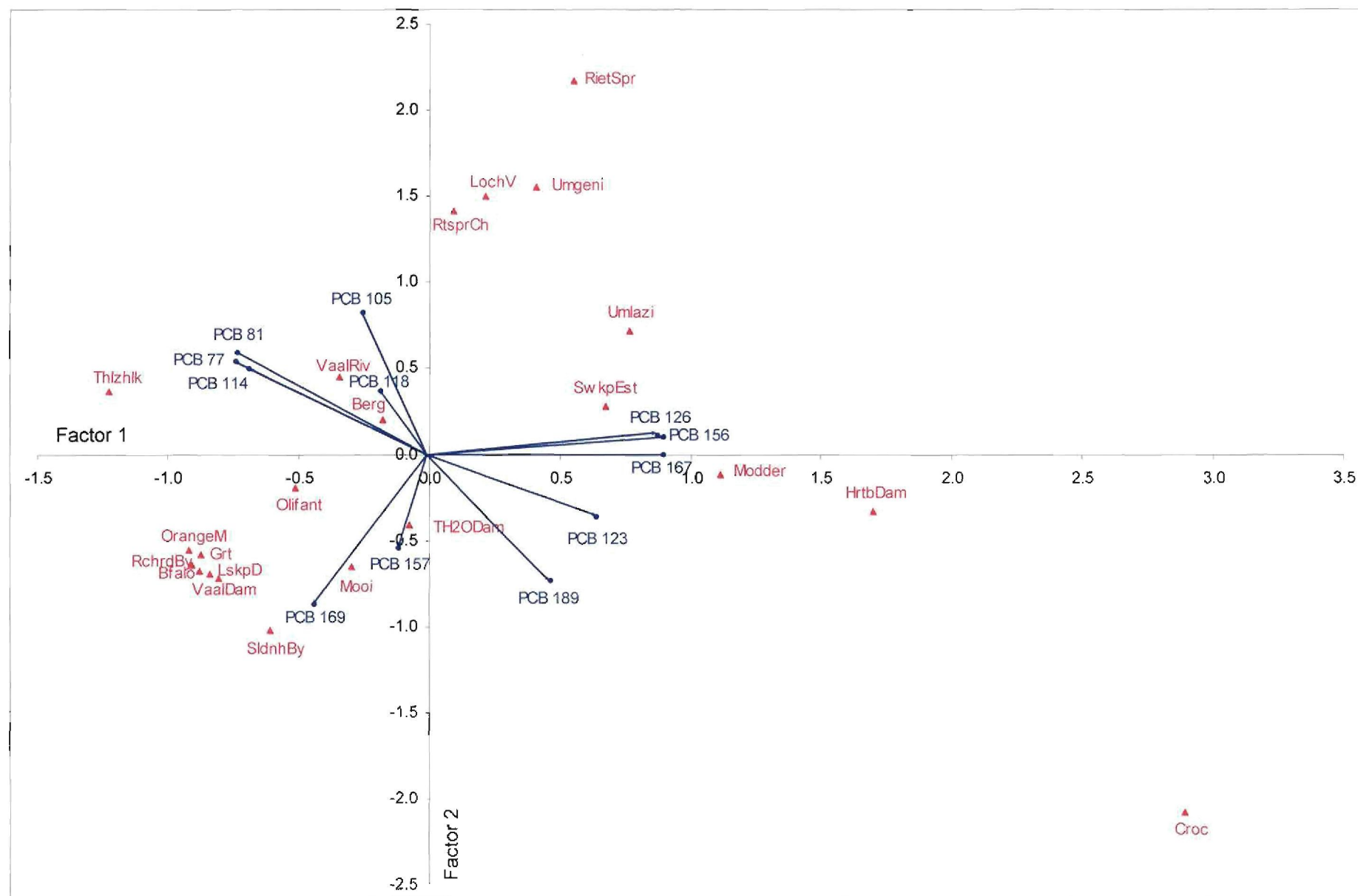


Figure 4.17: A PCA biplot of factors 1 and 2 of the PCBs of the SA data. ▲ = SA sites; • = PCB congeners

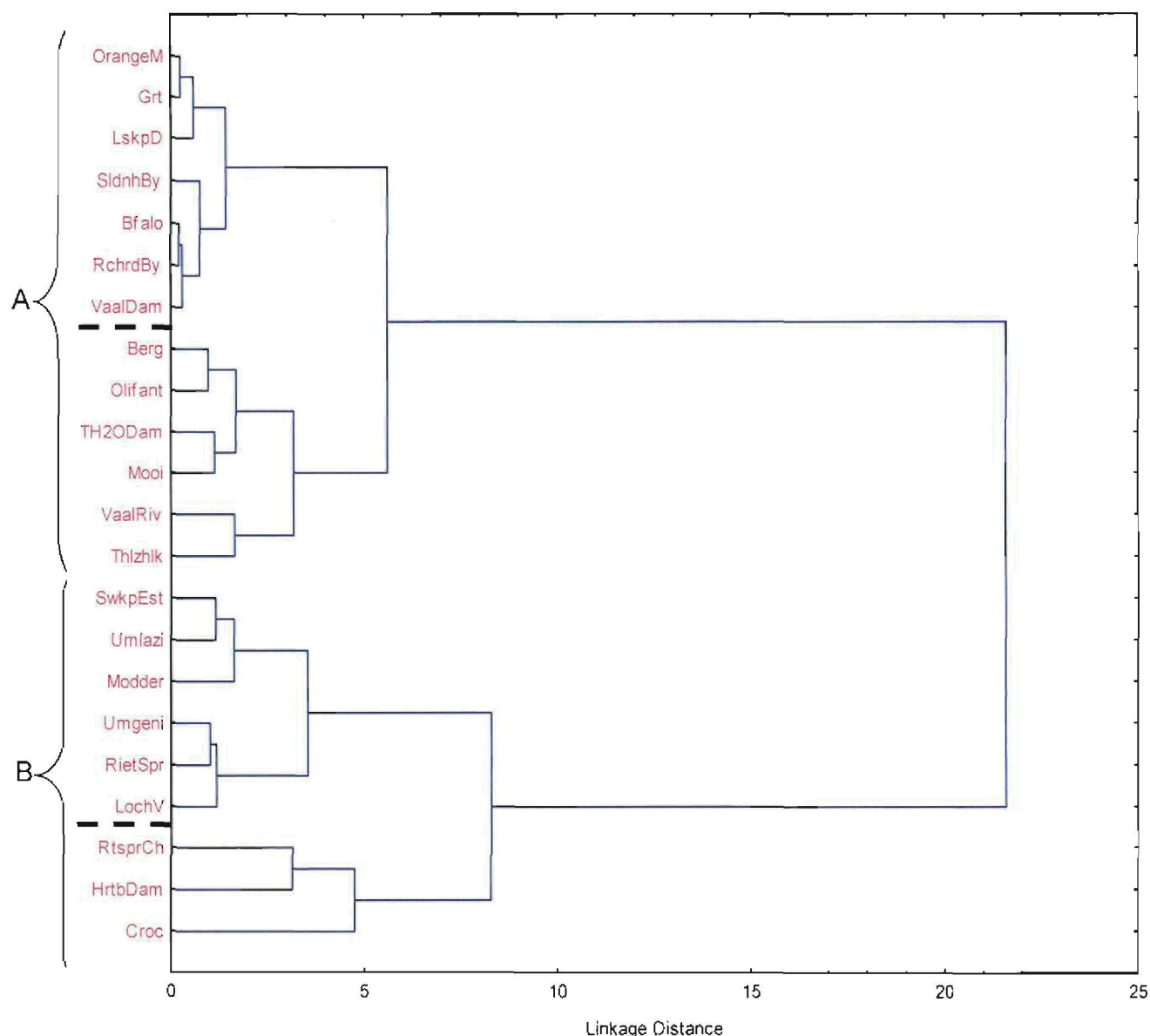


Figure 4.18: HCA of the PCBs of the SA data. Linkage rule: Ward's method. Distance measure: Euclidean. Dotted lines between the sites separate the sub-clusters.

Those sites that grouped together in the two main clusters A and B of Figure 4.16 (PCDD/F data) also grouped together into the two main clusters of Figure 4.18 (PCB data) except for Thlzhlk. The latter site grouped with cluster B (Figure 4.16) when the PCDD/F data was considered, and with group A (Figure 4.18) when the PCB data was considered. When the PCDD/F and PCB congeners were added together in a dendrogram, the Thlzhlk site remained in cluster A - see next section.

4.2.4 PCDD/Fs and PCBs of South African sites

Combining the South African PCB and PCDD/F data would allow for investigating the relationships between the groups of congeners, and to establish whether there were differences in congeners contributing to the different PCA factors.

Again, with PCA, three factors were extracted, explaining 45, 18.2 and 12.6% respectively of the variance of the data (Table 4.10). Factor 1 showed the contrast between most PCDD/F congeners and PCB congeners (column 1 of Table 4.10; Figure 4.19). PCB77

Table 4.10: The varimax-rotated factor loadings of the PCDD/F and co-planar PCB congeners contributing to the three factors explaining 76 % of the variance in the South African data. The congeners were arranged with loading factors in decreasing order.

Factor 1		Factor 2		Factor 3	
Congeners	Factor loading	Congeners	Factor loading	Congeners	Factor loading
HpF2	0.852	HxD1	0.702	HxF1	
HxF2	0.850	PCB169*	0.690	TeD	
TeD	0.832	HpF1	0.539	PCB169	
HxF1	0.758	HxD2	0.486	PeF1	
PeD	0.745	HxD3		HpF2	
PeF1	0.741	PCB189		HxF2	
HxD2	0.710	PeD		PeF2	
HxF3	0.692	HxF3		HxF4	
HxD3	0.683	HxF2		PCB114	
PeF2	0.651	HxF1		PCB77	
HxF4	0.577	HpF2		PCB189	
PCB77*	0.572	OF		PCB81	
HxD1		TeD		HxD1	
TeF		HxF4		PCB157	
HpF1		PCB157		HxD2	
PCB81		PCB123		PCB123	
OF		PCB167		PeD	
PCB169		PCB126		HxF3	
HpD		PCB156		PCB118	
PCB114		OD		PCB105	
OD		PeF2		TeF	
PCB105		HpD		PCB126	
PCB157	-0.833	PeF1		HxD3	
PCB118	-0.833	PCB118		PCB156	
PCB189	-0.879	PCB77		PCB167	
PCB156	-0.914	TeF	-0.729	HpF1	
PCB167	-0.915	PCB105	-0.731	OF	-0.660
PCB126*	-0.923	PCB114	-0.758	OD	-0.843
PCB123	-0.971	PCB81	-0.912	HpD	-0.886
% Total variance	45.0		18.2		12.6
Cumulative %	45.0		63.3		75.9

Only factor loadings $\geq |0.5|$ were reported.

was the only PCB congener contributing together with the PCDD/Fs in factor 1. The higher chlorinated PCDD/Fs (OD, OF, HpD and HpF1) were the PCDD/Fs that did not contribute to factor 1. The majority of both the PCDD/Fs and PCBs congener groups contributed to factor 1. Only three of the possible 12 PCB congeners and six of the possible 17 PCDD/F

congeners did not contribute significantly, i.e. with factor loadings $\geq |0.5|$. Some of these congeners contributed to the second factor, and again this factor showed the contrast between mainly PCB congeners (with negative loadings) and PCDD/F congeners (with positive loadings). The congeners contributing to the opposing sides of the factor were, however, not exclusively of the one or other type: included in the negatively loaded PCB group (PCB81, 114 and 105), was the tetra chlorinated furan (TeF) (Figure 4.20). Those congeners contributing with positive loadings were HxD1, PCB169, HxD2 and HpF1. HxD2 was included because its loading was approximately 0.5. PCB81, 114, 105 and TeF are lower chlorinated congeners, while HxD1, PCB169 and HpF1 are higher chlorinated congeners. The PCDD/F congeners with the highest chlorination - OF, OD and the HpD contributed to factor 3 (column 3 of Table 4.10; Figure 4.20). This factor was the only one which did not show a contrast between groups of congeners.

Comparing the PCDD/F congeners that contributed to the various factors when the PCBs were included in the PCA, to those PCDD/Fs that contributed to the same factors when only the PCDD/Fs were in the PCA (Table 4.8) revealed similar contribution patterns: of the ten PCDD/Fs that contributed to factor 1 of Table 4.8 (PCDD/Fs only), six contributed to factor 1 of Table 4.10 (PCDD/Fs and PCBs). They were HpF2, HxF2, TeD, HxF1, PeF1 and HxF3. All three PCDD/Fs that contributed to factor 2 of Table 4.8 (PCDD/Fs), also contributed to factor 2 of Table 4.10. The three PCDD/Fs that contributed to factor 3 of Table 4.10, also contributed to the contrast in factor 1 of Table 4.8. Thus, even though they contributed to a different factor, they were still associated with each other - as they were for factor 1 in Table 4.8, and they still had negative loadings.

Considering the PCB congeners, the five that contributed to factor 1 of Table 4.10 also contributed to factor 1 of Table 4.9. They included PCB77, 156, 167, 126 and 123. Three of the PCBs contributing to factor 2 of Table 4.10 (PCB77 (again), 157 and 189) also contributed to factor 1 of Table 4.10. All four of the PCBs contributing to factor 2 of Table 4.10 (PCB81, 114, 105 and 169), contributed to the corresponding factor of Table 4.9. It seems that when both congener groups - PCDD/Fs and PCBs - were included in the PCA, they contributed similarly to the factors as when they were considered separately.

Some of the sites that grouped together when the two congener groups were considered in separate PCAs (such as RietSpr, LochV and OrangeM, RchrdBy, VaalDam and SldnhBy) again appeared in close proximity on the biplots when all congener groups were used in the PCA (Figures 4.19 and 4.20). It was unnecessary to include the biplot of factor 1

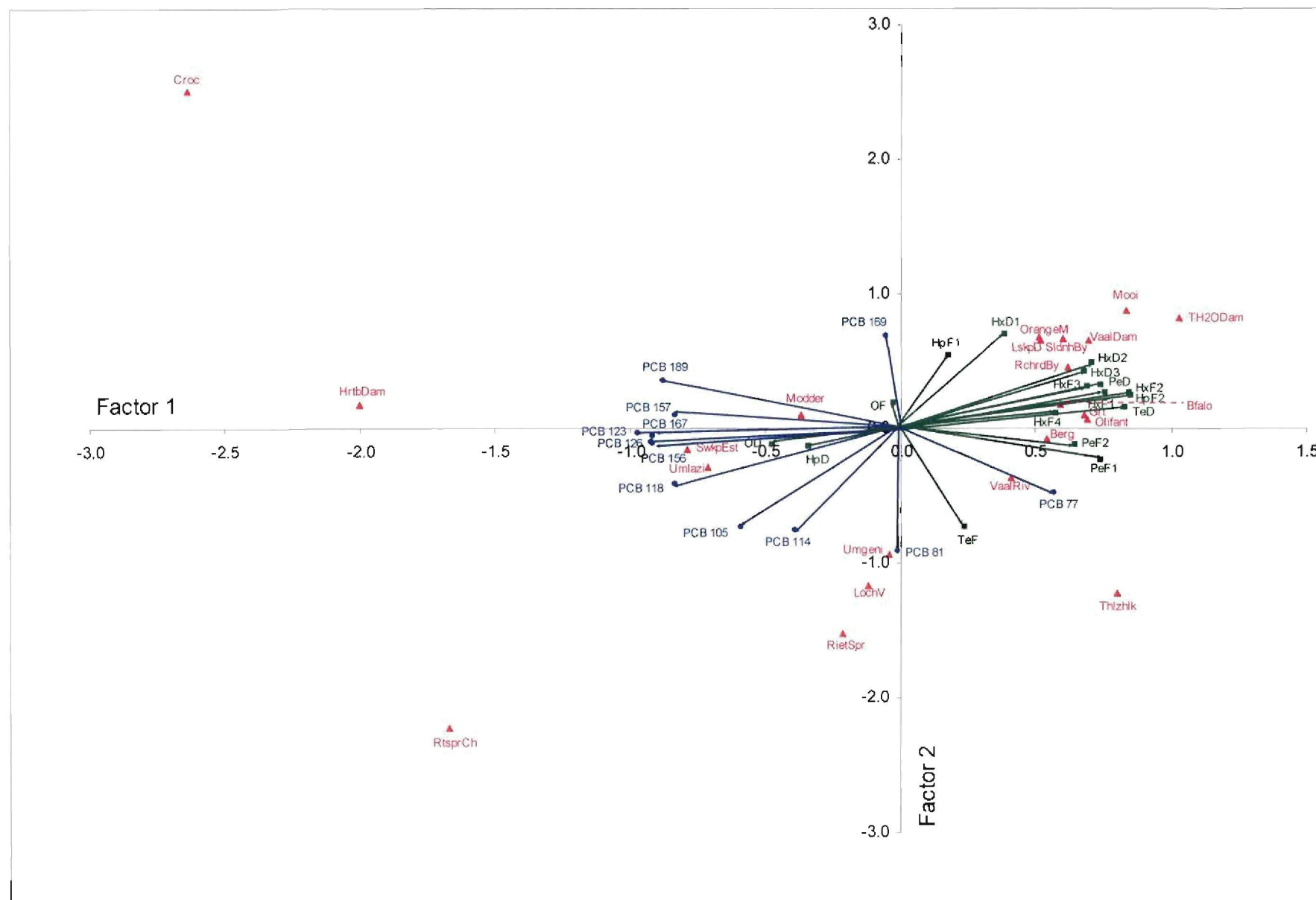


Figure 4.19: A PCA biplot of factors 1 and 2 of all congeners of the SA data. ▲ = SA sites; • = PCB congeners; ■ = PCDD/F congeners.

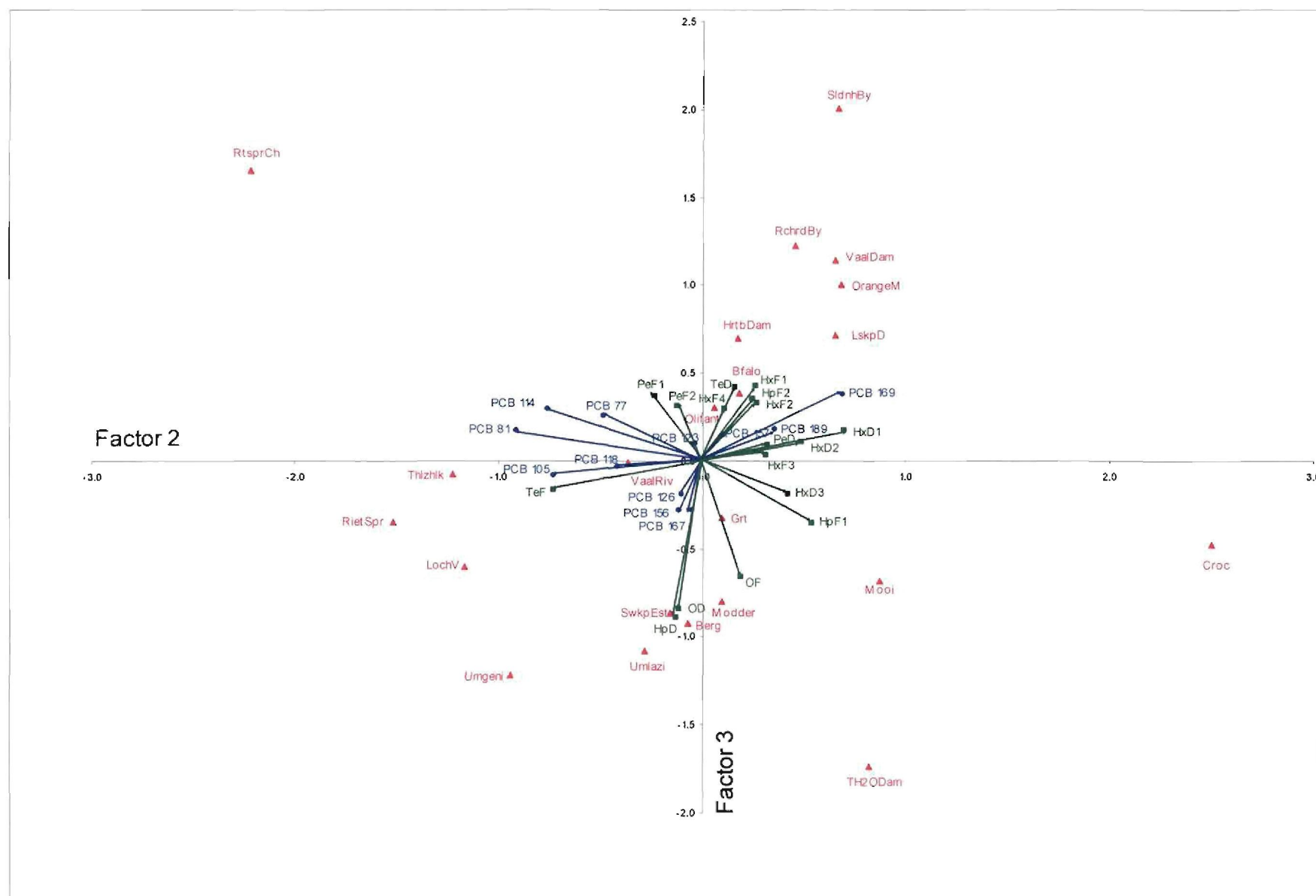


Figure 4.20: A PCA biplot of factors 2 and 3 of all congeners of the SA data. ▲ = SA sites; • = PCB congeners; ▪ = PCDD/F congeners.

and 3, because all observed phenomena were expressed on Figures 4.19 and 4.20. One group of sites (Modder and HrtbDam) that seemed to associate with each other in the previous PCAs did not group together in the last PCA. There was at least one biplot in each PCA where the Croc site arranged far removed from any of the other sites including Figure 4.20. This site also clustered separately in the HCA (Figure 4.21).

The South African sites grouped into two main groups on the biplot (Figure 4.19) when factors 1 and 2 were plotted. Those sites characterised by PCBs (to the left of the y-axis), and those sites characterised by a majority of PCDD/Fs (to the right of the y-axis). Both factors 1 and 2 showed the contrast between PCBs and PCDD/Fs (Table 4.10), and the two of them explained 63.3% of the variance in the data. The "PCB"-loading sites included Croc, HrtbDam, RtsprCh, SwkpEst, Umlazi, Modder, Umgeni, LochV and RietSpr. Other sites were rather characterised by PCDD/F congeners. These were TH20Dam, Thlzhlk, Olifant, Mooi, Grt, VaalDam, Berg, SldnhBy, RchrdBy, Bfalo, LskpD, OrangeM, VaalRiv. In the HCA, the sites also grouped into these two main groups (A and B; Figure 4.21). Sites on the positive side of factor 1 (Figure 4.19) clustered in group A (Figure 4.21). The Berg site is the only exception: it appeared on the positive side of factor 1 (Figure 4.19) too, but clustered in group B (Figure 4.21). The Berg site also changed from group A in Figure 4.18 (only PCBs) to group B in Figure 4.21 (PCBs and PCDD/Fs). Sites that had high levels of OF, OD and HpD (thus characterised by factor 3) came from both the "PCDD/F" and the "PCB" groups and included sites such as TH20Dam, Umgeni, Umlazi, Berg, SwkpEst, Modder, Mooi and LochV (Figure 4.20). Thus, as soon as the third factor was included in a biplot, the division of the South African sites into two groups was not evident anymore (Figure 4.20).

Factor 1 explained 45% of the variance in the data (Table 4.10) and seemed to have the power to distinguish between two groups of sites (Figure 4.19). The same two groups were recognised in the dendrogram (Figure 4.21), except for the Berg site. The difference between the two groups was investigated for statistical significance. The Berg site was omitted from both groups. The names of the sites as well as the Σ PCDD/F and Σ PCB concentrations are summarised in Table 4.11. The raw data was not distributed normally, for this reason, the data was log transformed before two-tailed Student's t-tests (Statistica 7.0) were performed on (i) Σ PCDD/F and Σ PCB congener concentrations; (ii) Σ PCDD/F concentrations; and (iii) Σ PCB concentrations. In each case, the difference between the means was statistically and practically significant ($p < 0.001$; effect size > 0.8) (Ellis & Steyn, 2003) (Table 4.12). Therefore, the pollution was, in practice, larger for group B than for group A, irrespective of the congeners under consideration. By investigating the raw

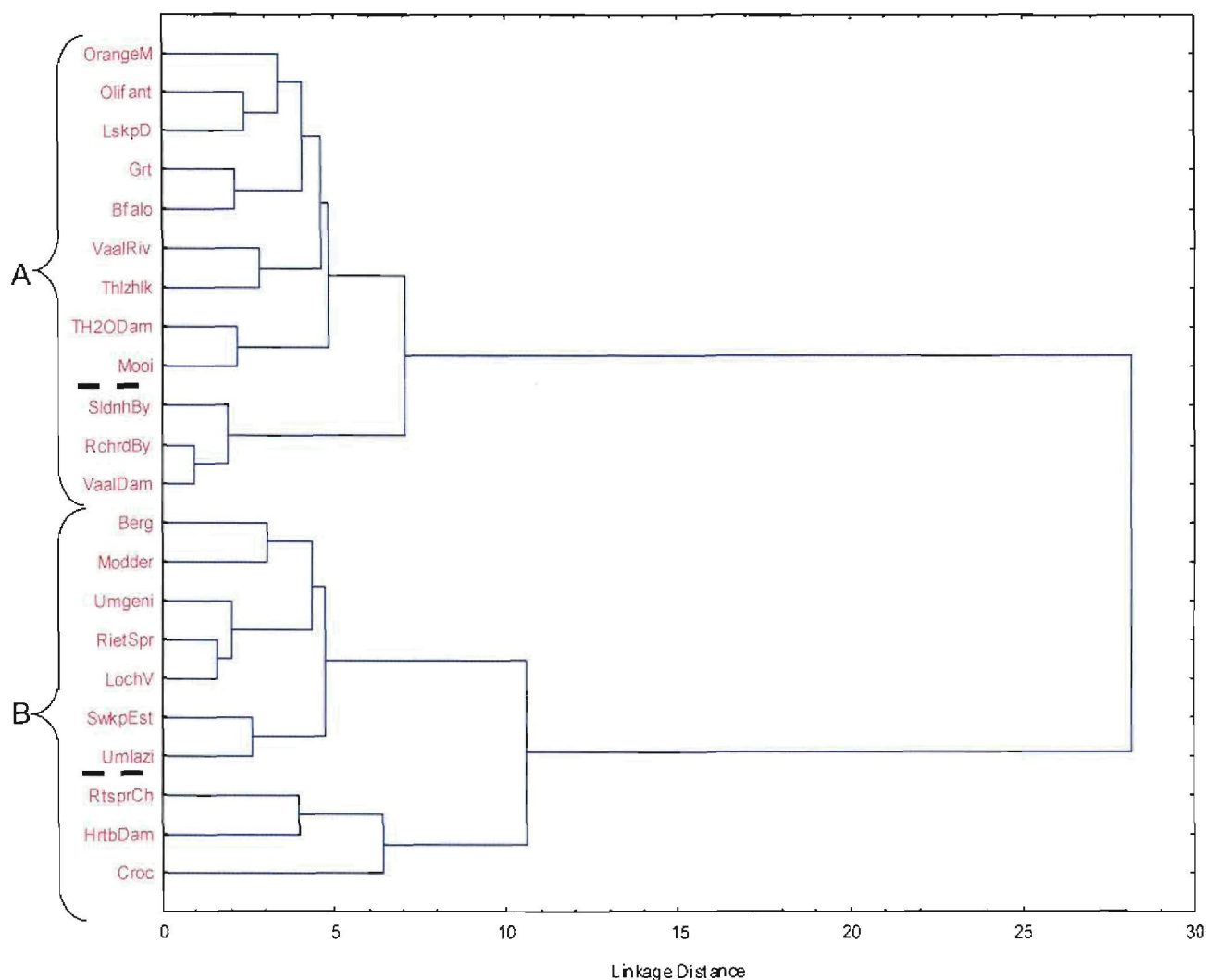


Figure 4.21: HCA of the PCBs and PCDD/Fs of the SA data. Linkage rule: Ward's method. Distance measure: Euclidean. Dotted lines separate the sub-clusters.

Table 4.11: The two groups of SA sites that were identified from the PCA and HCA. Berg was omitted because it grouped differently between the PCA and the HCA.

Group A*			Group B*		
Sites	$\Sigma\text{PCDD/F (ng kg}^{-1}\text{ d.w.)}$	$\Sigma\text{PCB (ng kg}^{-1}\text{ d.w.)}$	Sites	$\Sigma\text{PCDD/F (ng kg}^{-1}\text{ d.w.)}$	$\Sigma\text{PCB (ng kg}^{-1}\text{ d.w.)}$
OrangeM	1.3	3.8	SwkpEst	10.8	237.2
SldnhBy	1.5	3.1	Umlazi	6.5	155.9
TH2ODam	1.6	22.0	Umgeni	9.1	188.9
Grt	1.4	7.8	RietSpr	9.0	79.6
VaalRiv	1.5	7.7	RtsprCh	219.0	1136.9
Bfalo	1.4	6.3	LochV	23.4	247.5
Mooi	1.8	13.2	Croc	7.9	250.8
RchrdBy	1.4	3.8	HrtbDam	4.6	61.2
Thlzhik	3.8	20.5	Modder	30.0	535.7
VaalDam	1.3	3.7			
Olifant	1.4	5.7			
LskpD	1.2	4.2			

*Clusters as identified on dendrogram (Figure 4.21)

data tables (Table 4.2a & b), this result was corroborated: those nine sites with the highest total concentration were all in group B.

Table 4.12: A summary of Student's t-test results between groups A and B (Figure 4.21).

	Σ PCDD/F & PCB	Σ PCB	Σ PCDD/F
Mean (A)	1.55	1.46	0.80
Mean (B)	3.17	3.09	2.35
t-value	-10.12	-9.96	-10.09
df	19	19	19
p	0.0000	0.0000	0.0000
Valid N (A)	12	12	12
Valid N (B)	9	9	9
Stdev (A)	0.22	0.21	0.37
Stdev (B)	0.50	0.52	0.39
F-ratio variances	4.96	5.83	1.50
Effect size	3.27	3.17	4.00

stdev = standard deviation; df = degrees of freedom

4.2.5 Effect of sediment characteristics on PCA

As was discussed in the literature review chapter (section 2.4.3), sediment characteristics - such as organic carbon and clay content - improve the sediment's capacity to retain PCDD/Fs and dioxin-like PCBs. Therefore, sediment characteristics might have influenced the distribution of the sites in the biplots. To determine if this was the case for the South African sites, all of the PCAs were repeated, but this time with the sediment characteristics

Table 4.13: The sediment characteristics of the South African sites.

Sites	% > 2 mm	(% < 2 mm)			%OC
		%Sand	%Silt	%Clay	
OrangeM	0.2	49.5	42.3	8.2	0.42
SldnhBy	0.1	90.9	2.5	6.6	0.35 [#]
Berg	0.0	94.6	0.6	4.8	0.35 [#]
TH2ODam	11.9	63.3	21.6	15.1	1.35
Grt	1.2	89.9	0.7	9.4	2.39
SwkpEst	3.7	76.3	13.8	9.8	1.75
VaalRiv	3.9	48.8	23.1	28.0	1.84
Bfalo	2.3	75.3	12.2	12.5	0.55
Mooi	4.3	66.6	16.5	16.9	2.71
Umlazi	0.2	85.3	5.0	9.7	1.53
Umgenei	1.9	78.5	8.6	12.9	1.19
RchrdBy	1.6	86.5	4.6	8.9	0.56
Thlzhlk	0.5	25.0	66.3	8.7	6.29
VaalDam	7.7	89.7	4.9	5.3	0.35 [#]
RietSpr	0.3	61.9	13.5	24.5	2.59
RtsprCh	0.3	22.0	68.5	9.5	9.26
LochV	0.0	5.4	31.7	63.0	3.01
Croc	3.1	60.1	16.8	23.2	1.84
Olifant	0.2	43.6	21.7	34.7	1.44
LskpD	1.5	83.2	8.2	8.6	0.57
HrtbDam	3.7	68.9	17.5	13.6	0.90
Modder	4.6	64.5	17.6	17.9	3.40

[#] Walkley-Black results: 0% oxidisable organic carbon

(Table 4.13) included as variables. Before the centred log-ratios of the characteristics were calculated to be incorporated into the PCAs, their values were expressed as fractions of one, rather than percentages.

Because the approach was to establish whether sediment characteristics had any influence on the association of the sites, the following PCAs were not discussed in the same detail as before, especially since the variables generally contributed in the same manner as before.

The factor loadings tables (Table 4.14) revealed the association of congeners with each other and with the sediment characteristics. To establish the influence of the sediment characteristics on the association of sites to each other, biplots were created.

When the sediment characteristics were added to the PCDD/F data, five factors with

Table 4.14: The varimax-rotated factor loadings of the PCDD/F congeners and the sediment characteristics explaining 72.4% of the variance in the South African data. The congeners were arranged with loading factors in decreasing order.

Factor 1		Factor 2		Factor 3		Factor 4		Factor 5	
Variables	Factor loading	Variables	Factor loading	Variables	Factor loading	Variables	Factor loading	Variables	Factor loading
OD	0.959	HxF1	0.859	PeF1	0.738	HxD1	0.529	%silt	0.514
HpD	0.944	HxF2	0.831	TeF	0.679	OF		HxD2	
OF	0.808	HxF4	0.728	PeF2	0.578	HpF1		%> 2mm	
HpF1	0.713	HpF1	0.499	HxF4		HxF4		OF	
TeF	0.499	PeF1	0.441	HxF2		PeF2		HpF1	
HxD3		OF	0.401	OD		%> 2mm		HxD1	
HxD1		HxD2	0.397	HxF1		HxD2		PeD	
HxD2		PeD	0.387	HpD		HxF1		HxD3	
PeD		PeF2	0.385	HxD2		%sand		HpD	
PeF2		TeF		PeD		PeD		OD	
HxF4		HpF2		%OC		HpD		HxF1	
HxF3		TeD		HxD3		OD		TeF	
PeF1		HpD		TeD		HxF2		HxF2	
HxF1		OD		OF		PeF1		HxF3	
HxF2		HxD1		HxD1		%OC		HpF2	
%> 2mm		HxF3		HxF3		HpF2		HxF4	
%OC		%OC		HpF2		TeD		%clay	
%clay	-0.384	HxD3		HpF1		TeF		TeD	
%silt	-0.456	%silt		%silt		HxD3		PeF1	
%sand	-0.539	%clay		%> 2mm		%clay	-0.522	PeF2	
HpF2	-0.690	%sand		%clay	-0.633	%silt	-0.599	%sand	
TeD	-0.759	%> 2mm	-0.841	%sand	-0.709	HxF3	-0.664	%OC	-0.905
% Total variance									
24.8		19.5		12.5		9.5		6.1	
Cumulative %									
24.8		44.2		56.7		66.2		72.4	

Only factor loadings $\geq | -0.4 |$ were reported

eigenvalues greater than one were extracted. Between them, they explained 72.4% of the variance in the data (Table 4.14). The expectation was that %sand and %>2mm would

associate with one other, while %clay, %silt and %OC would associate with each other (Table 4.3b; R^2 for %OC and mud was 0.45); i.e. those characteristics that have inherently higher carbon contents would associate with each other, as would those with expected less carbon. This was, however, not the case. The factor where only sediment characteristics contributed the most, was factor 5 (%silt and %OC) (Table 4.14). This factor explained only 6.1% of the variance. Three characteristics (%sand, %silt and %clay) contributed with negative loadings to factor 1. The same was observed for factor 3 (%clay and %sand). Some of the congeners, such as HpF2 and TeD in factor 1, also associated with sand, one of the characteristics least expected to associate with PCDD/F congeners. %silt and %OC contributed to opposite sides of factor 5.

When the PCA was repeated with the sediment characteristics added to the PCB data, only three factors were extracted that explained 80% of the variance in the data (Table

Table 4.15: The varimax-rotated factor loadings of the dioxin-like PCBs and sediment characteristics contributing to three factors explaining 80% of the variance in the South African data. The congeners were arranged with factor loadings in decreasing order.

Factor 1		Factor 2		Factor 3	
Variables	Factor loadings	Variables	Factor loadings	Variables	Factor loadings
PCB126	0.883	PCB77	0.892	%silt	0.360
PCB123	0.880	PCB114	0.787	PCB157	
PCB167	0.871	PCB81	0.783	PCB126	
PCB156	0.870	PCB105	0.736	PCB189	
PCB189	0.838	PCB118	0.600	PCB167	
PCB157	0.791	PCB123		PCB105	
PCB118	0.713	PCB156		PCB156	
PCB105	0.523	PCB126		PCB118	
PCB114		PCB167		%> 2mm	
PCB81		PCB157		PCB123	
PCB77		PCB189		PCB77	
PCB169		%silt		PCB114	
%OC	-0.416	%OC		PCB81	
%> 2mm	-0.458	%clay		%clay	
%sand	-0.683	PCB169	-0.373	PCB169	
%silt	-0.851	%sand	-0.573	%sand	
%clay	-0.859	%> 2mm	-0.846	%OC	-0.882
% Total variance	60.2		8.5		11.3
Cumulative %	60.2		68.7		80

Only factor loadings $\geq | \sim 0.4 |$ were reported

4.15). The same trends evident from Table 4.14, were also observed for Table 4.15: sediment characteristics with different amounts of carbon contributed together, not only to the same factor but to the same side of the factor (see factor 1 of Table 4.15). Factor 1 explained 60.2% of the variance and most of the PCB congeners had positive loadings, while all five of the sediment characteristics had negative loadings for the same factor. Factor 2 had %sand and %>2mm both contributing to the negative side of the factor. This association is within the range of what would be expected for these two sediment

characteristics, because both were expected to have less carbon. What was unexpected however, was that PCB169 would associate with them. Factor 3 showed the two sediment characteristics, %silt and %OC, contributing to opposite sides of that factor. This corroborated what was observed for the last factor of the previous PCA (Table 4.14).

In the PCA, where both PCBs and PCDD/Fs were included (Table 4.16), six factors explained 80.7% of the variance in the data. The factors that were influenced by sediment characteristics only were factors 5 and 6, explaining 3.8% and 3.2% of the variance respectively. Factor 1 corroborated what was observed for factor 1 of Table 4.15: most of the PCB congeners contributing to the factor with positive loadings and two sediment characteristics (%silt and %clay) contrasted with them with negative loadings. Associated with these two sediment characteristics were HxD3 and HxF3. In factor 3 both HxD1 and PCB169 associated with the two least likely sediment characteristics (%>2mm and %sand).

Table 4.16: The varimax-rotated factor loadings of the PCB and PCDD/F congeners and the sediment characteristics explaining 80.7% of the variance in the South African data. The congeners were arranged with factor loadings in decreasing order.

Factor 1		Factor 2		Factor 3		Factor 4		Factor 5		Factor 6	
Variables	Factor loading	Variables	Factor loading	Variables	Factor loading	Variables	Factor loading	Variables	Factor loading	Variables	Factor loading
PCB 157	0.825	HxF1	0.902	PCB 77	0.885	OF	0.877	%silt	0.411	PCB 189	
PCB 123	0.803	HxF2	0.834	TeF	0.865	HpF1	0.776	PCB 157		PCB 123	
PCB 189	0.773	HpF2	0.728	PCB 81	0.857	HpD	0.670	PCB 189		HpF1	
PCB 118	0.669	TeD	0.703	PCB 114	0.717	OD	0.588	PCB 114		PCB 167	
PCB 126	0.659	HxF4	0.686	PCB 105	0.704	PCB 126		%> 2mm		PCB 156	
PCB 156	0.580	PeF1	0.576	PCB 118	0.479	PCB 118		PCB 105		PCB 169	
PCB 114	0.577	PeF2	0.568	PeF1	0.420	PCB 156		OF		%clay	
PCB 167	0.565	PeD	0.516	HpD		PCB 105		PCB 118		HxF3	
PCB 105	0.521	HxD2	0.468	OD		PCB 157		PCB 77		%silt	
PCB 81		%sand	0.389	PCB 156		PCB 167		PCB 126		PCB 118	
PCB 169		%OC		PCB 167		HxF4		%clay		PCB 126	
OF		%clay		PCB 126		PCB 123		PCB 123		HxF1	
PCB 77		HxD1		PCB 123		PeD		HxD2		HxF2	
HpF1		HxF3		PeF2		PCB 77		HpF1		HxF4	
Table abbreviated to fit the page											
PeF2		PCB 77		HxF1		HxD3		HxF3		OF	
PeF1		OF		PeD		PeF2		HpD		HxD2	
HxF2		%> 2mm		TeD		HxF2		OD		OD	
%OC		PCB 169		%silt		%sand		HxD3		HxD1	
HxD2		PCB 105		HpF1		PCB 81		HxF1		HpF2	
PeD		PCB 157		HxD3		%clay		HxD1		TeD	
%> 2mm	-0.396	PCB 118	-0.373	HpF2		%OC		TeD		HpD	
%sand	-0.436	PCB 123	-0.436	%OC		HpF2		%sand		%sand	
TeD	-0.461	PCB 189	-0.462	HxD2		HxF3		HxF2		PeD	
HpF2	-0.464	PCB 126	-0.590	%clay	-0.440	%> 2mm		TeF		TeF	
%silt	-0.610	HpD	-0.598	PCB 169	-0.466	PeF1		HxF4		PeF1	
HxD3	-0.713	PCB 156	-0.671	HxD1	-0.695	TeD	-0.380	PeF2		PCB 81	
HxF3	-0.732	OD	-0.695	%> 2mm	-0.702	PCB 169	-0.411	PeF1		PeF2	-0.386
%clay	-0.759	PCB 167	-0.707	%sand	-0.740	%silt	-0.572	%OC	-0.836	%> 2mm	-0.469
% Total variance	22.5		21.9		19.1		10.2		3.8		3.2
Cumulative %	22.5		44.4		63.5		73.8		77.5		80.7

Only factor loadings $\geq | \sim 0.4 |$ are reported.

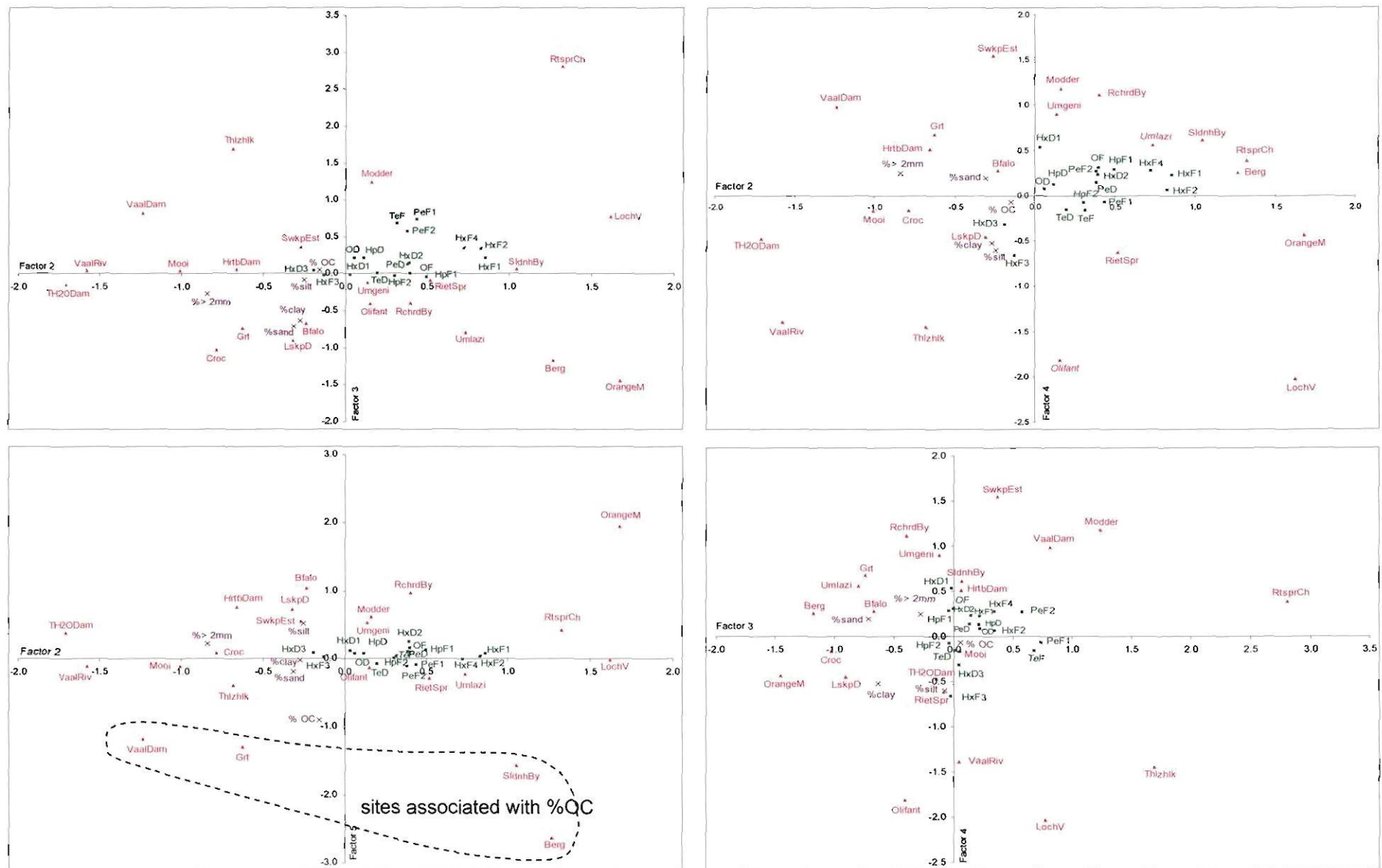


Figure 4.23: Biplots of factors 2x3 (top left); 2x4 (top right); 2x5 (bottom left) and 3x4 (bottom right) of the PCA on the PCDD/Fs and sediment characteristics.

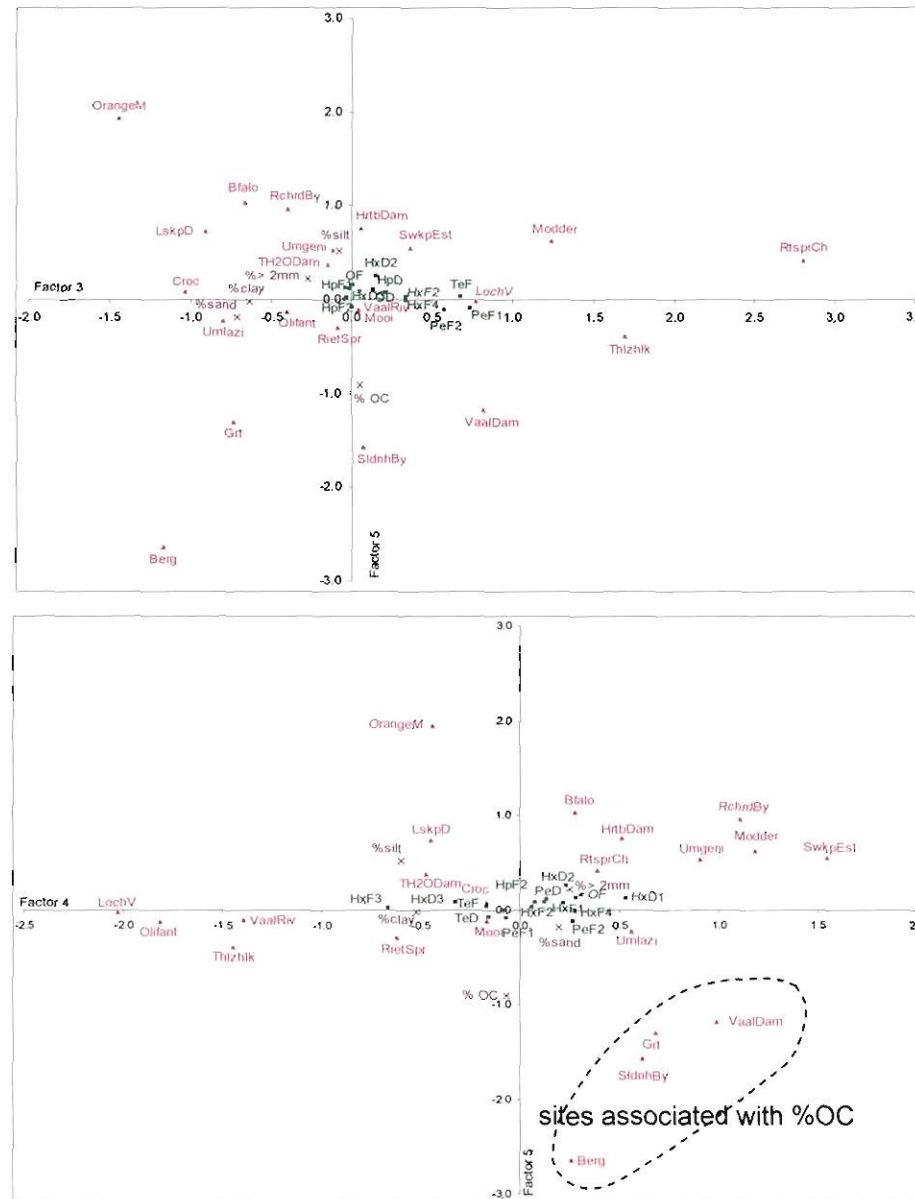


Figure 4.24: Biplots of factors 3x5 (top) and 4x5 (bottom) of the PCA on the PCDD/Fs and sediment characteristics.

As was the case with the two previous PCAs where the sediment characteristics were included, %OC again contributed to one of the smaller factors (factor 5; Table 4.16). It contrasted with %silt as it did for factor 5 in Table 4.14 and factor 3 in Table 4.15.

Investigating all of the biplots (15 biplots for the six factors that were identified for the PCDD/Fs, PCBs and sediment characteristics, 10 for the five factors of the PCDD/Fs and sediment characteristics and three for the three factors of the PCBs and sediment characteristics) revealed no particular patterns of site distribution, except for one. In all three of the combinations of congeners and sediment characteristics, four sites always associated with that factor to which %OC contributed. Figures 4.22 to 4.24 contain the 10 biplots of the PCA of the PCDD/Fs together with the sediment characteristics. On all of the biplots that included factor 5, sites Grt, VaalDam, SldnhBy and Berg were associated with the %OC. %OC had a factor loading with a negative sign and was found on the negative side of factor 5 on the biplots. The four named sites occurred on the same side of factor 5 and three of these sites had 0% oxidisable organic carbon (Table 4.13).

On the biplots of the PCBs together with the sediment characteristics, the same four sites: Berg, Grt, SldnhBy and VaalDam formed a group on the negative side of factor 3 (Figure 4.25). The negative side of factor 3 is associated with %OC (Table 4.15).

Not all 15 of the biplots possible for the PCDD/F, PCB and sediment characteristics PCA were included in this chapter (Figure 4.25 to 4.28), but enough were shown to illustrate the association of only Berg, Grt, SldnhBy and VaalDam. Biplots with the majority of the more important factors as well as one biplot containing factor 6 (Figure 4.28) were included.

When a dendrogram of an HCA of the sediment characteristics with the congener data was investigated (Figure 4.29), sites that associated with %OC, Berg, Grt, VaalDam and SldnhBy, clustered together with those sites that had a TEQ ≤ 0.308 ng kg⁻¹ d.w. (section 4.1.1). These sites clustered in group A of all previous dendrograms of the South African data (cf. Figures 4.16, 4.18 and 4.21). All nine of the sites in cluster B were those sites with the higher pollution load. In this HCA, Berg clustered with cluster A, instead of cluster B as it did in the dendrogram of Figure 4.21.

When the major clusters of all the HCA done for the South African data-set to this stage were compared (Table 4.17), two sites changed "sides" regularly: Berg and Thlzhlk. Thlzhlk initially clustered in group B when only PCDD/Fs were included (Table 4.17), but moved to group A, and remained with this group throughout all other combinations of variables. The Berg site clustered in group A most of the time and only clustered in group B when, PCDD/Fs and PCBs were considered. Berg was one of those sites that had

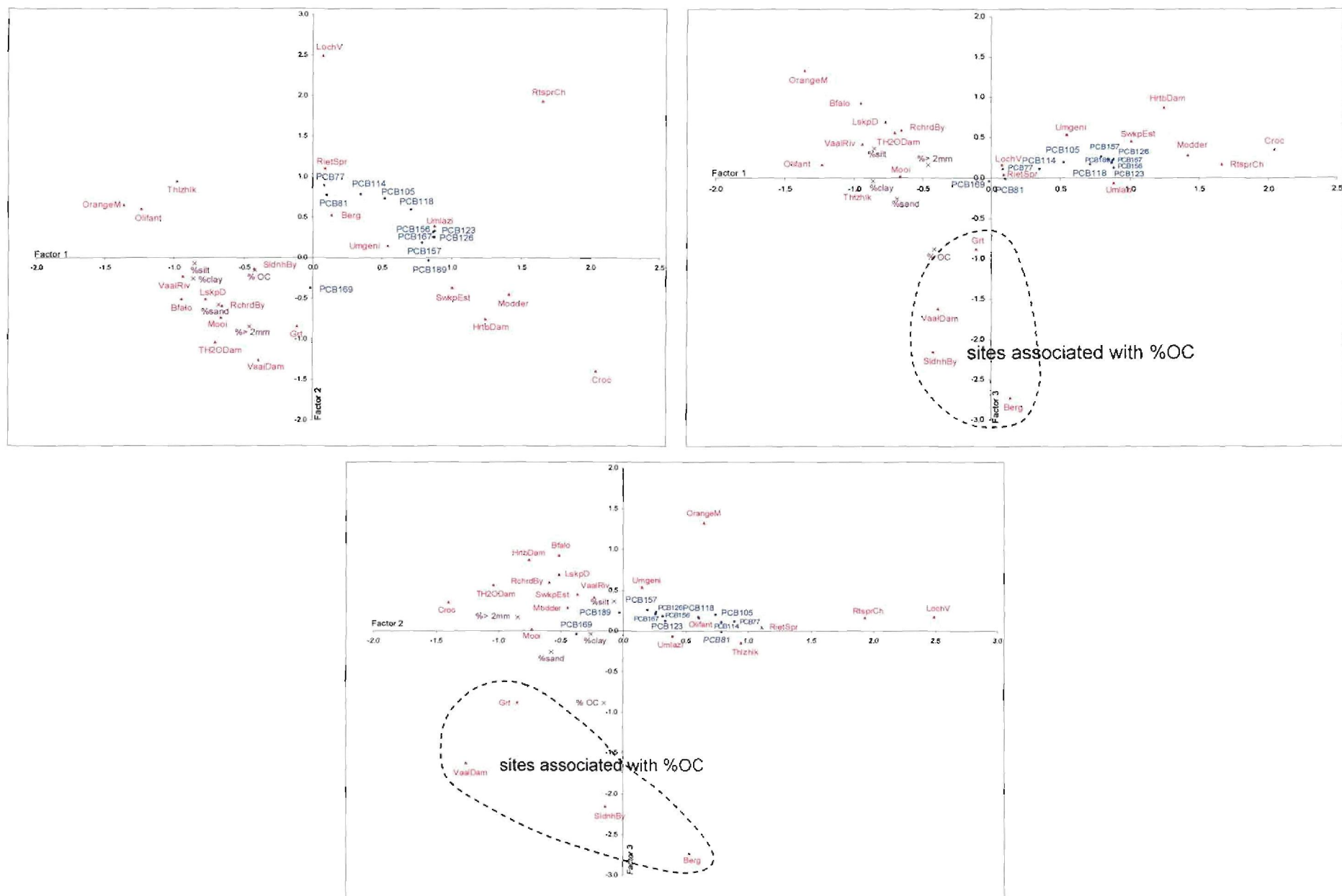


Figure 4.25: Biplots of factors 1x2 (top left) ; 1x3 (top right) and 2x3 (bottom) of the PCA on the PCBs and the sediment characteristics.

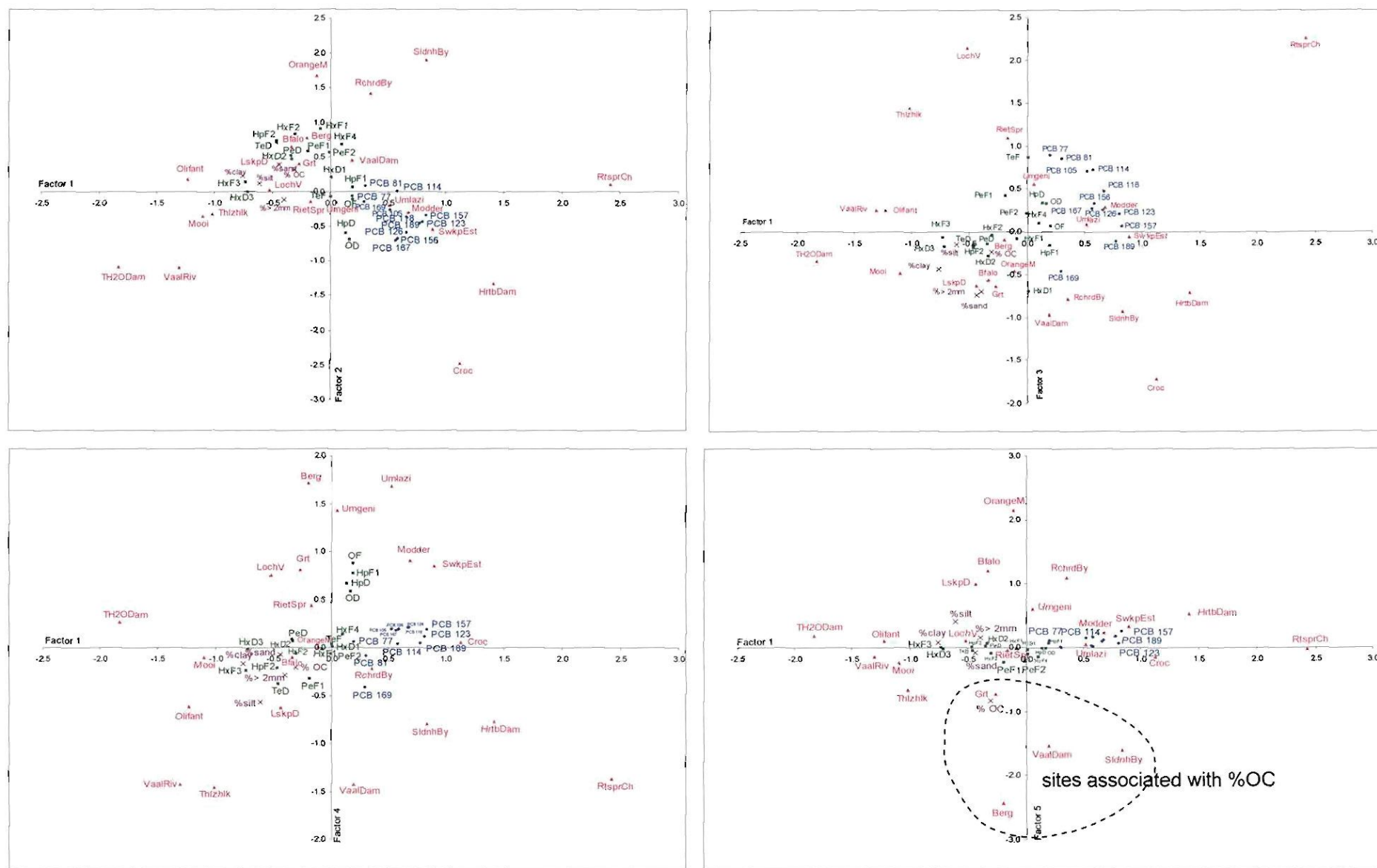


Figure 4.26: Biplots of factors 1x2 (top left); 1x3 (top right); 1x4 (bottom left) and 1x5 (bottom right) of the PCA on the PCDD/Fs, PCBs and sediment characteristics.

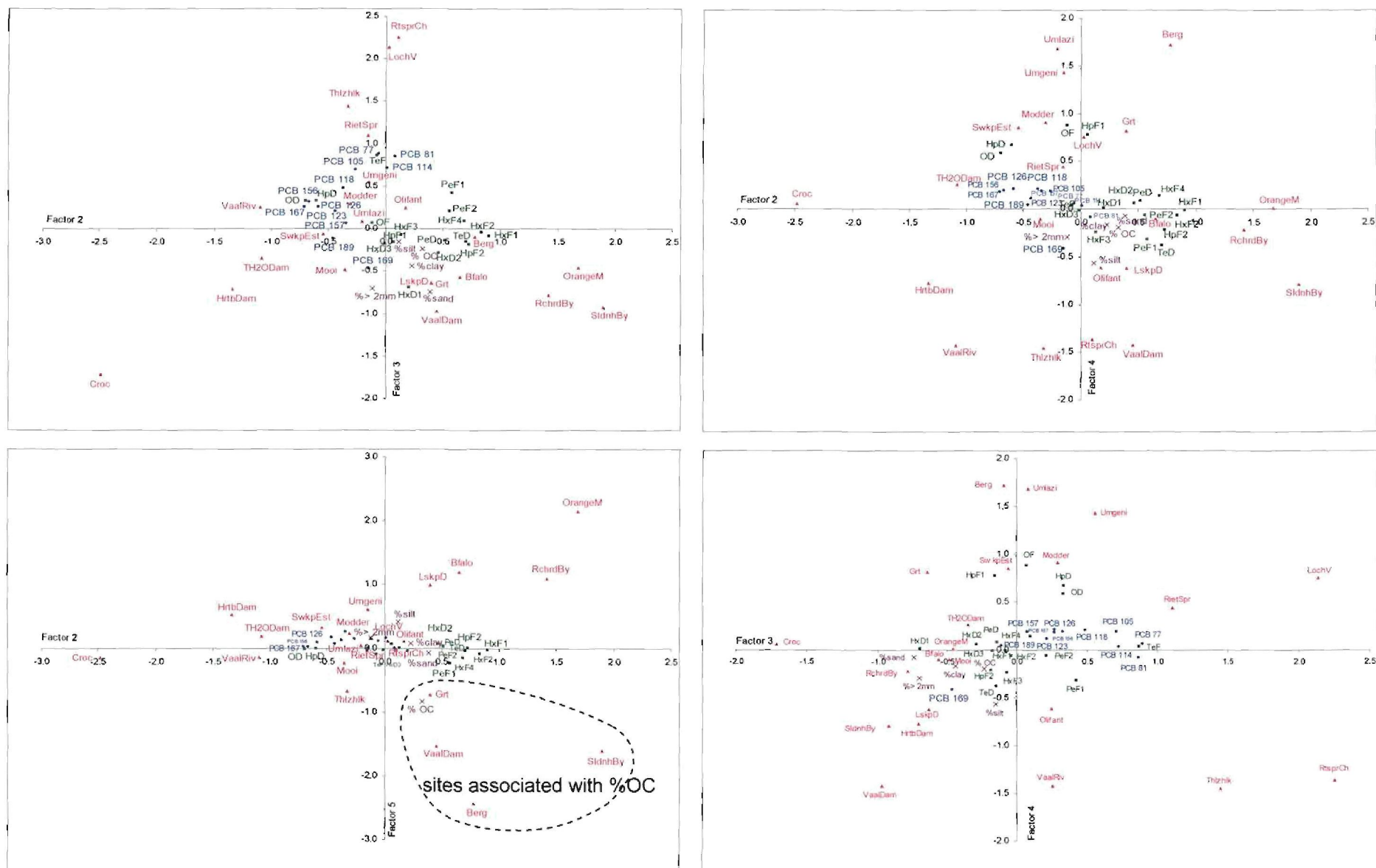


Figure 4.27: Biplots of factors 2x3 (top left); 2x4 (top right); 2x5 (bottom left) and 3x4 (bottom right) of the PCA on the PCDD/Fs, PCBs and sediment characteristics.

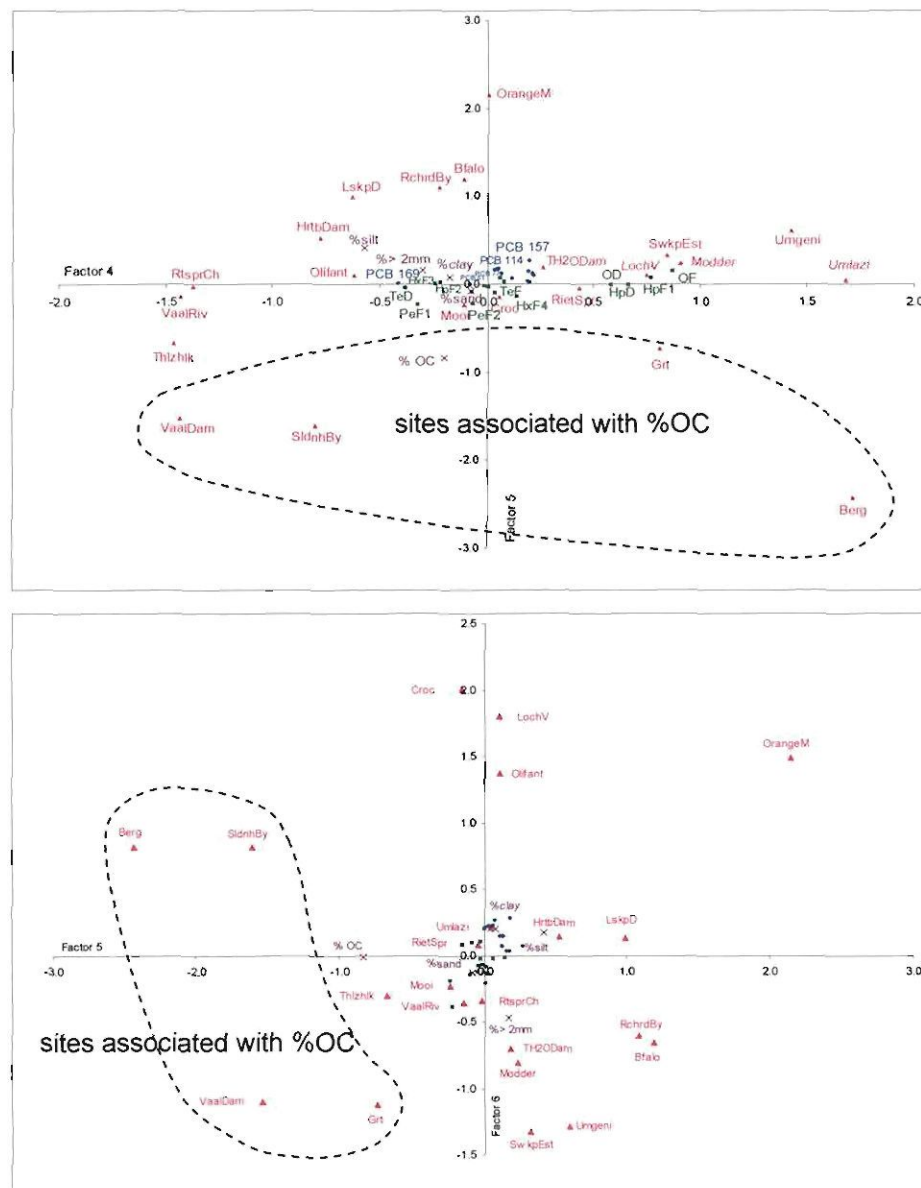


Figure 4.28: Biplots of factors 4x5 (top) and 5x6 (bottom) of the PCA on the PCDD/Fs, PCBs and sediment characteristics

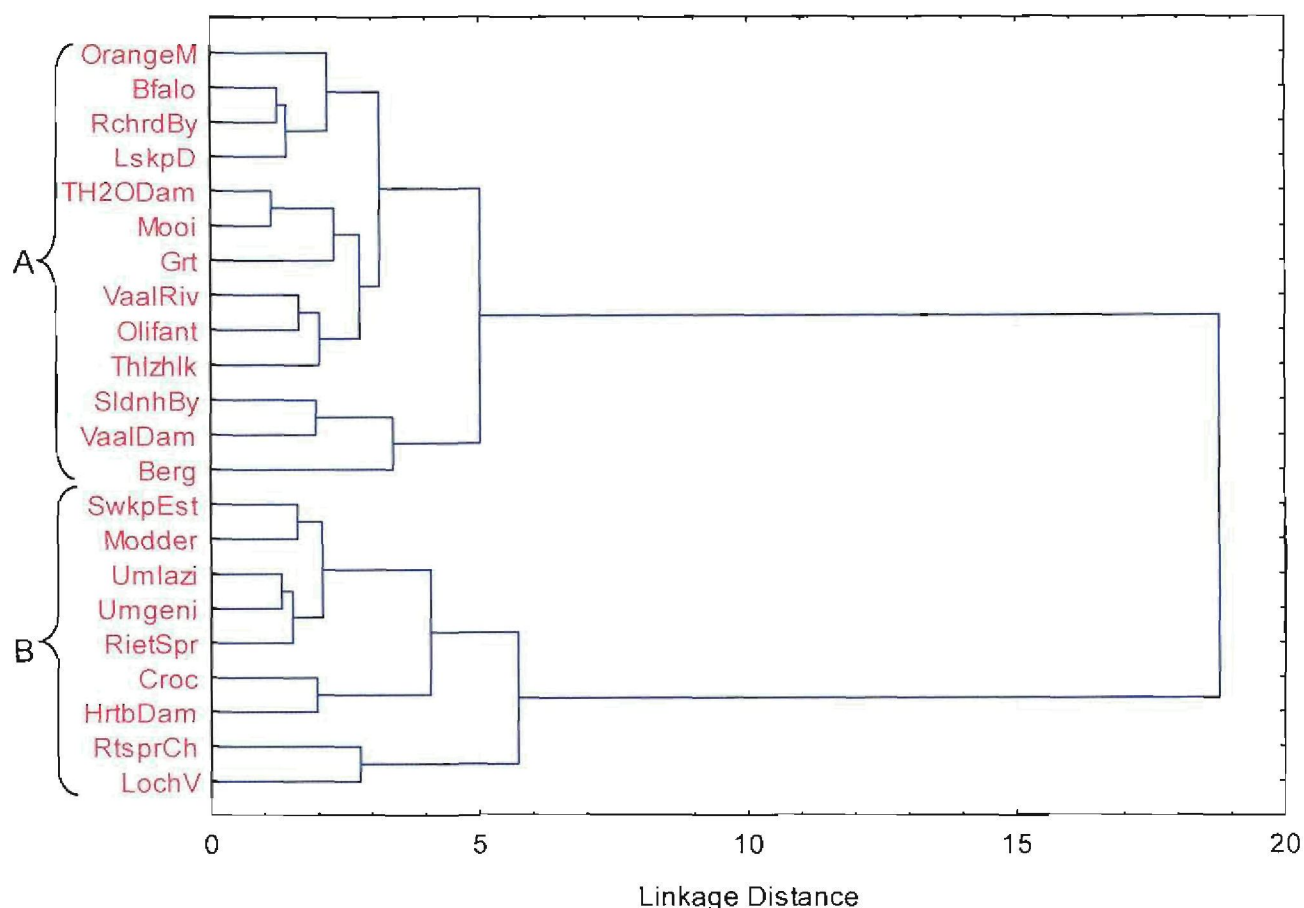


Figure 4.29: HCA of the PCBs, PCDD/Fs and sediment characteristics of the SA data. Linkage rule: Ward's method. Distance measure: Euclidean.

Table 4.17: A summary of how the SA sites grouped into the two major clusters during HCA with different variables under consideration

Group	PCDD/Fs (Figure 4.16)	PCBs (Figure 4.18)	PCDD/Fs & PCBs (Figure 4.21)	PCDD/Fs, PCBs & sediment characteristics (Figure 4.29)
A	OrangeM	OrangeM	OrangeM	OrangeM
	Bfalo	Bfalo	Bfalo	Bfalo
	RchrdBy	RchrdBy	RchrdBy	RchrdBy
	LskpD	LskpD	LskpD	LskpD
	TH20Dam	TH20Dam	TH20Dam	TH20Dam
	Mooi	Mooi	Mooi	Mooi
	Grt	Grt	Grt	Grt
	VaalRiv	VaalRiv	VaalRiv	VaalRiv
	Olifant	Olifant	Olifant	Olifant
	VaalDam	VaalDam	VaalDam	VaalDam
	SldnhBy	SldnhBy	SldnhBy	SldnhBy
	Berg	Thlzhk	Thlzhk	Thlzhk
	Thlzhk	Berg	Berg	Berg
	SwkpEst	SwkpEst	SwkpEst	SwkpEst
B	Modder	Modder	Modder	Modder
	Umlazi	Umlazi	Umlazi	Umlazi
	Umgeni	Umgeni	Umgeni	Umgeni
	RietSpr	RietSpr	RietSpr	RietSpr
	Croc	Croc	Croc	Croc
	HrtbDam	HrtbDam	HrtbDam	HrtbDam
	RtsprCh	RtsprCh	RtsprCh	RtsprCh
	LochV	LochV	LochV	LochV

measurable pollution, but no measurable organic carbon (Table 4.3a). Thlzhlk, on the other hand, had a low pollutant level for its relatively high %OC (Figure 4.2).

The important conclusion made from all of the included biplots (Figures 4.22 to 4.28) was that the sites did not group into any particularly obvious pattern due to sediment characteristics added to the PCA, except for the indicated Berg, VaalDam, SldnhBy and Grt. These sites were enclosed by the dotted line on the biplots. When two-tailed Student's t-tests were performed on the raw sediment data to compare whether the sediment characteristics of the two groups (A and B) differed significantly, neither a statistically nor a practically significant difference was found (Table 4.18). There was an indication that %clay and %OC might have been different for the two groups (cf. the effect sizes in Table 4.18), but the difference was not practically significant (cf. section 3.4).

The sediment characteristics had a small influence on the distribution and association of the South African sites. The consistency of where the majority of the South African sites clustered in all of the dendograms (Table 4.17) also supported the finding that sediment characteristics were not influential in the pollutant clustering of the South African sites.

Table 4.18: Summary of Student's t-tests between groups A and B as identified for Figure 4.21.

	Mean (A)	Mean (B)	t- value	df	p	Valid N (A)	Valid N (B)	Stdev (A)	Stdev (B)	F-ratio variances	Effect size
%>2mm	2.95	1.98	0.74	19	0.47	12	9	3.60	1.84	3.83	0.27
%sand	67.71	58.09	0.91	19	0.37	12	9	21.91	26.81	1.50	0.36
%silt	18.73	21.44	-0.32	19	0.75	12	9	19.04	19.12	1.01	0.14
%clay	13.56	20.47	-1.21	19	0.24	12	9	9.06	16.92	3.49	0.41
%OC	0.99	2.02	-1.36	19	0.19	12	9	1.38	2.07	2.25	0.49

When the biplot of factor 1 and factor 2 of the PCA of all congeners without the sediment characteristics was compared to the corresponding biplot of all congeners with the sediment characteristics (Figures 4.30a & b) the same sites still clustered around the same type of congener. However, when the sediment characteristics were included (Figure 4.30b), the entire orientation of variables and sites changed from that in Figure 4.30a.

It is important to reiterate that PCA is an explorative statistical technique during which a great many variables are taken into consideration in this analysis, and also that 22 sites constitute a relatively small sample size. One must also keep in mind that most of the concentrations for 13 of these sites (section 4.1.1.) contained less than the limit of quantification values when the PCAs were performed, which meant that the contamination levels had been fairly low for many of the sites.

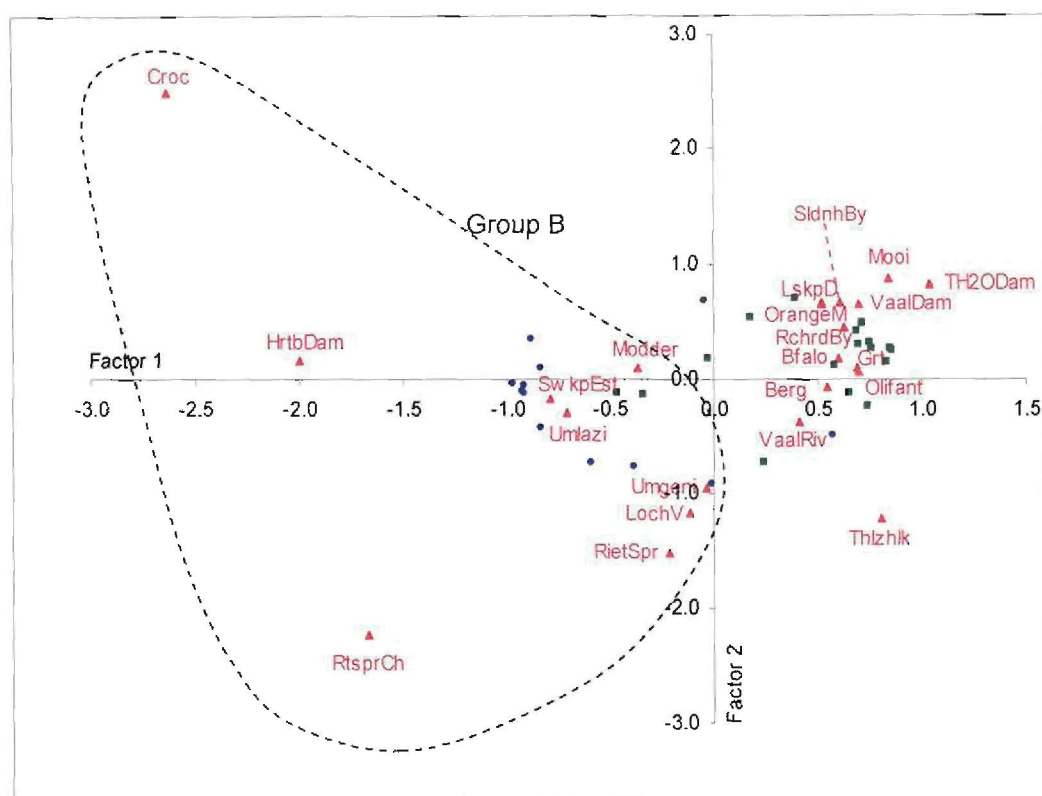


Figure 4.30a: The biplot of the first two factors (explaining 63.3% of the variance; Table 4.10) of the PCA with congener variables only. ▲ = SA sites; • = PCB congeners; ■ = PCDD/F congeners. See text of explanation of "Group B".

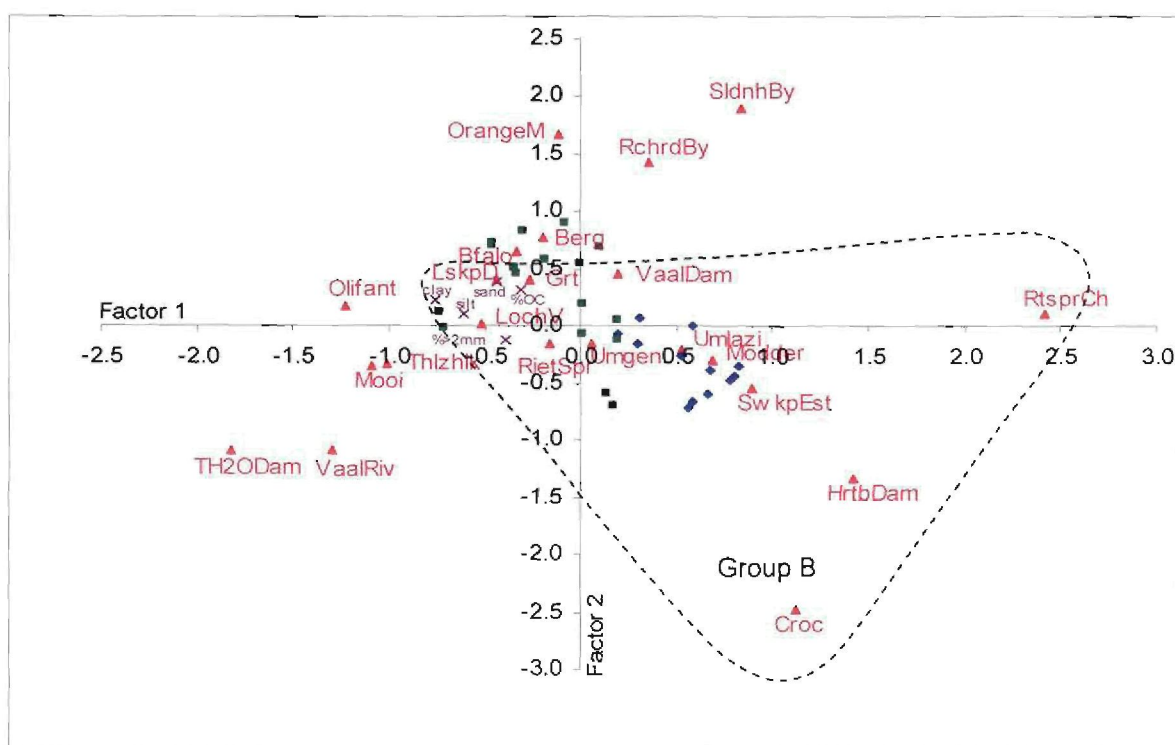


Figure 4.30b: The biplot of the first two factors (explaining 44.4%; Table 4.16) of the PCA with congeners and sediment characteristics included. ▲ = SA sites; • = PCB congeners; ■ = PCDD/F congeners; X = sediment characteristics. See text for explanation of "Group B".

5 DISCUSSION

During this study, the levels of PCDD/Fs and dioxin-like PCBs were determined for 22 aquatic sites in South Africa. In this chapter, these levels will be compared to sediment quality guidelines found in literature, to assess their possible risk. The usefulness and applicability of the H4IIE-*luc* bio-assay in South African research laboratories will be also referred to.

The nature of the PCDD/F pollution profiles, as gleaned from the PCA and HCA, will be discussed. The nature of the dioxin-like PCBs profiles in the sediment is compared to profiles from literature using histograms to indicate concentration levels.

The possible effects of sediment characteristics on the clustering of the sites in HCAs will also be discussed.

5.1 Sediment characteristics and sediment quality guidelines

Knowledge of the magnitude of the dioxin-like pollution in sediments facilitates assessment regarding its risk to wildlife and human health, i.e. providing an answer to the "so what?"-question: What is the meaning of the determined dioxin-like levels in sediment? To understand the risk of the dioxin levels in the sediment of the 22 South African sites, the concentrations had to be expressed in terms of %OC in the sediment before comparisons to sediment quality guidelines could be drawn. Biological responses of benthic organisms to non-ionic organic chemicals in sediments are different across sediments when the sediment concentrations are expressed on a dry weight basis, but similar when expressed on an organic carbon normalised basis (US EPA, 2000).

The oxidisable organic carbon content of the sediments collected at the South African sites was determined by the Walkley-Black method and converted to total organic carbon (%OC) as described in section 3.3.4 of the Materials and Methods chapter. The correlation of the PCDD/Fs and dioxin-like PCBs to the %OC was positive ($R^2 = 0.63$; Figure 4.2). This was expected because POPs preferably sorb to the organic fraction in sediment and soil. Koh *et al.* (2006a) reported a significant relationship between total organic carbon content and total PCBs ($r = 0.55$, $p < 0.01$) for harbour sediment from Korea. A significant correlation between %OC and concentrations of PCDD/Fs ($R^2 = 0.90$, $p = 0.004$) was also reported for a Korean river (Koh *et al.*, 2004), but the same authors could not observe a significant correlation between the concentrations of PCBs and the sediment %OC ($R^2 = 0.50$, $p = 0.117$). Also, Hilscherova *et al.* (2003) reported that concentrations of total PCDD/Fs were not significantly correlated with organic carbon content in sediment or soils ($p > 0.05$) from the Tittabawassee River and floodplain in Michigan (USA). Jeong *et al.* (2001) again

reported a positive correlation between %OC and total PCBs for another Korean river ($R^2 = 0.85$). For the 22 South African sites involved in this study, there was a positive association between the dioxin pollution in sediment and %OC of the sediment.

For the present study, a practical correlation was also found between the %OC and the fine particle contents (mud) of the sediments (Table 4.3b). The fine particle content includes the silt and clay fraction of each sediment sample, which was also higher in organic carbon than the sand fraction. It was therefore not surprising that the %OC would correlate significantly ($p < 0.05$) with the mud fraction. The mud fraction also correlated significantly ($p < 0.05$) with the Σ PCDD/F concentrations, but did not correlate significantly with the Σ PCB concentrations ($p > 0.05$) (Table 4.3b). These correlations between sediment characteristics such as %OC and the silt and clay, warranted the inclusion of the sediment characteristics into the PCA to investigate their possible influence on the congener profiles of the sites and, consequently, how the sites would associate on the biplots. The effect of sediment characteristics on the grouping of the South African sites is discussed below (section 5.5).

For the development of sediment quality guidelines (SQG), the effect-level concentrations from several guidelines of similar narrative intent were averaged to yield consensus-based lower and upper effect values for contaminants of concern (Macdonald, Ingersoll & Berger, 2000). These SQGs are an attempt to predict the toxicity of the pollutant(s) present (Wisconsin Department of Natural Resources, 2003) and include different levels of concern, such as threshold effect concentration (TEC), minimal effect concentration (MEC) and probable effect concentration (PEC).

For Wisconsin and the Canadian freshwater SQG (Canadian Sediment Quality Guidelines, 2002) the TEC, MEC and PEC levels for 2,3,7,8 TCDD are $0.85 \text{ ngTEQ kg}^{-1}$, $11.2 \text{ ngTEQ kg}^{-1}$ and $21.5 \text{ ngTEQ kg}^{-1}$, respectively. (South Africa does not have similar guidelines yet.) Since the guidelines are calculated to represent concentration per 1% OC, the concentrations of the present study had to be divided by the %OC of the sites, for example 0.226 ng kg^{-1} (WHO₂₀₀₅-TEQ for OrangeM; Table 4.3a) divided by 0.42 = 0.53 ng kg^{-1} for 1% OC (Wisconsin Department of Natural Resources, 2003) to facilitate comparison to the SQG. This is the same as dividing the last column of Table 4.3a by 100.

All of the South African sites were below the MEC of $11.2 \text{ ngTEQ kg}^{-1}$ (Table 5.1). There were seven sites with levels between the TEC and MEC, namely Swartkops Estuary, Umgeni River mouth, Riet Spruit channel, Loch Vaal, Crocodile River, Hartbeespoort Dam and Modderfontein Spruit. Compared to these guidelines, only some of the South African

Table 5.1: Recalculated TEQ values for comparison to SQG for freshwater of Canada and Wisconsin (USA).

Sites	%OC	WHO ₂₀₀₅ -TEQ (LOQ included) (ng kg ⁻¹ d.w.)			Normalised TEQ (ng kg ⁻¹ d.w.)	TEQ (ng kg ⁻¹ %OC*)
		PCB	PCDD/F	Total		
OrangeM	0.42	0.014	0.212	0.226	53.27	0.53
SldnhBy	0.35 [#]	0.014	0.241	0.255	72.88	0.73
Berg	0.35 [#]	0.015	0.246	0.261	74.68	0.75
TH2ODam	1.35	0.014	0.286	0.300	22.26	0.22
Grt	2.39	0.014	0.199	0.214	8.93	0.09
SwkpEst	1.75	0.532	1.515	2.047	116.84	1.17
VaalRiv	1.84	0.025	0.203	0.228	12.38	0.12
Bfalo	0.55	0.014	0.204	0.218	39.90	0.40
Mooi	2.71	0.014	0.294	0.308	11.35	0.11
Umlazi	1.53	0.227	0.848	1.075	70.23	0.70
Umgeni	1.19	0.264	1.089	1.353	114.04	1.14
RchrdBy	0.56	0.014	0.221	0.235	42.03	0.42
Thlzhik	6.29	0.030	0.438	0.469	7.45	0.07
VaalDam	0.35 [#]	0.014	0.216	0.230	65.62	0.66
RietSpr	2.59	0.255	0.760	1.014	39.18	0.39
RtsprCh	9.26	7.372	10.437	17.809	192.42	1.92
LochV	3.01	0.525	2.038	2.563	85.25	0.85
Croc	1.84	1.574	0.795	2.369	128.89	1.29
Olifant	1.44	0.015	0.214	0.228	15.80	0.16
LskpD	0.57	0.014	0.186	0.200	34.94	0.35
HrtbDam	0.90	0.378	0.483	0.861	95.31	0.95
Modder	3.40	1.412	4.056	5.469	160.82	1.61

Walkley-Black results: 0% oxidisable organic carbon

*Values to be compared to SQG

sites were above the threshold effect concentration, which means that the chemicals are present and may cause moderate impairment to benthic communities since these chemicals can be toxic to sensitive species. These sites warrant further investigation because only a composite sample from a single area from that aquatic body was analysed for this study. An in-depth study would better reveal how much one should be concerned about the quantity of dioxin-like pollution measured at these sites. When the South African levels were compared to the sediment guidelines of 3.6 ng kg⁻¹ %OC, which is the SQG for oceanic sediments (endorsed by the National Oceanic and Atmospheric Administration (NOAA) of the USA (Buchman, 1999)), none of the South African sites exceeded that level. This 3.6 ng kg⁻¹ level represents the apparent effects threshold (AET). The NOAA does not have guideline concentrations for dioxins in the lower risk levels as they do for other types of pollutants.

The top layer of the sediment that was sampled during the present study is one of the exchangeable layers, meaning that the contaminants could have a wide range of impacts on the plants and animals that live within and upon the sediments. Sediment-associated contaminants also have the potential to accumulate in the tissue of aquatic organisms, and high tissue concentrations in benthic or other aquatic organisms often result in the bio-accumulation of chemicals in higher levels of the aquatic food web (Jeong *et al.*, 2001).

The bio-accumulation of toxic compounds such as dioxins and PCBs in aquatic organisms presents a potential hazard to sensitive wildlife species, such as birds and humans that rely on these organisms for food, as reported by many authors (Eljarrat, Caixach & Rivera, 2001; Fattore *et al.*, 2002).

5.2 Comparing South African sediment PCB and PCDD/F levels to others

("PCBs" and "PCDD/Fs" in this section refer to the 29 dioxin-like congeners analysed for in the present study, unless stated otherwise.)

The Σ PCB-concentration at each site was higher than the sum concentration measured for the PCDD/F congeners at each site (Table 4.3a). This is consistent with trends seen elsewhere in the world (Chi, Chang & Kao, 2007; UNEP, 2003). However, when comparing the TEQ-values for the two groups of congeners, the contribution by the Σ PCDD/F-component was higher at all the sites, except for the Crocodile River site (Figure 4.1). The higher TEQ-values for Σ PCDD/Fs are not surprising because their TEF values are higher than those of the PCBs (Table 2.5). In general PCDD/Fs congeners are more toxic than dioxin-like PCBs.

The sediments from industrial areas in the interior of the country, i.e., Riet Spruit channel, Loch Vaal, Hartbeespoort Dam and Modderfontein Spruit, showed higher concentrations than sediments from less industrialised inland areas such as Berg River, Theewaterskloof Dam, the Vaal River (at the confluence with the Orange River), the Buffalo River and the Mooi River (Table 5.2).

Table 5.2: A categorisation of the 22 South African sites and their corresponding WHO₂₀₀₅-TEQ (ng kg⁻¹ d.w.)

Coastal				Interior			
Harbours		Estuaries/River mouths		Rivers		Dams	
RchrdBy	0.235	SwkpEst	2.045	RtsprCh	17.809	LochV	2.563
SldnhBy	0.255	Umgeni	1.353	Modder	5.469	HrtbDam	0.861
		Umlazi	1.075	Croc	2.369	Thlzhlk	0.469
		OrangeM	0.226	RietSpr	1.014	TH20Dam	0.300
		Grt	0.214	Mooi	0.308	VaalDam	0.230
				Berg	0.261	LskpD	0.200
				VaalRiv	0.228		
				Olifant	0.228		
				Bfalo	0.218		

Generally, the sites in estuaries or in harbours showed lower concentrations and this might have been due to the diluting effect of the ocean. At these sites, there were also less organic carbon and a higher sand content, lowering the sorption capacity of the sites. But even for these sites, the industrialised areas (Swartkops Estuary, the Umlazi River mouth and the Umgeni River mouth) had higher concentrations than the less industrialised area

(the Orange River mouth). The two harbours that were sampled, namely Saldanha Bay and Richard's Bay, seemed to have little of these particular contaminants, but this could be explained by their high sand percentage and very low organic carbon content (Table 4.3a).

5.2.1 Coastal water bodies

The two sites flanking Durban (Umgeni and Umlazi River mouths) had measured concentrations higher than many of the other South African sites, as could be expected for an industry-rich city. There was, however, no major difference between the sites in the two rivers. The site in the Umlazi River was selected for its close proximity to a paper mill and is also downstream of the Umlazi Township and industry. One would therefore expect a higher TEQ for this site when compared to the Umgeni River site.

The coastal site with the highest dioxin-like PCB and PCDD/F concentrations was the Swartkops Estuary near Port Elizabeth (another highly industrialised city). Its TEQ-value was also the highest of all the coastal sites (Table 5.2). I-TEQ values reported for PCDD/F congeners in the Masan Bay of Korea varied between 1 and 76 ng kg⁻¹ (Im *et al.*, 2002). The Swartkops Estuary was the only coastal site that had a WHO-TEQ level for PCDD/Fs (1.52 ng kg⁻¹) that was within the range reported for Masan Bay. For Tokyo Bay, Japan, Yao *et al.* (2002) reported a WHO-TEQ of 41 ng kg⁻¹ for PCDD/Fs and 31 ng kg⁻¹ for dioxin-like PCBs. Wenning, Dodge, Peck, Shearer, Luksemburg, Della Sala and Scazzola (2000) reported even higher levels of 334.24 ngWHO-TEQ kg⁻¹ (my calculation) for PCDD/F congeners of the Venice Lagoon next to the Adriatic Sea. Koh *et al.* (2006a) reported levels for non-, *mono*- and di-*ortho* PCBs in Yeongil Bay, Korea, that ranged between 220 and 3740 ng kg⁻¹ d.w. (my conversion). Three of the South African coastal sites (Swartkops Estuary 794.2 ng kg⁻¹ d.w.; Umlazi River mouth 637.4 ng kg⁻¹ d.w.; Umgeni River mouth 549.9 ng kg⁻¹ d.w.) had levels of dioxin-like PCBs that would compare to the lower range of Yeongil Bay.

5.2.2 Interior water bodies

The concentrations of the sites in the Vaal River (Vaal Dam, Loch Vaal and the Vaal River confluence with the Orange River, and the Orange River mouth) reflected the expected effect of industry on the aquatic sediment. The downstream order of the sites is Vaal Dam, Loch Vaal, the Vaal River and the Orange River mouth (Figure 5.1). The Vaal Dam is upstream of the major industries and has lower concentrations (Table 5.2) than the Loch Vaal site. This site is situated downstream and next to industries expected to contribute towards dioxin-like PCB and PCDD/F pollution and therefore the highest concentrations in this river system were measured at this site. The Vaal River site, at the confluence of the Orange River with the Vaal River, is downstream from industry and reflected lower

concentrations again, probably because of dilution, but also indicates transport. The concentrations are similar to those measured at the Vaal Dam site. The Orange River mouth's concentration was similar to that at the confluence, indicating long-range riverine transport and the persistence of the compounds of interest. However, the Orange River mouth site's normalised TEQ (Figure 5.1) was more than four times greater than the Vaal River site's. This suggested a possible addition to the PCDD/Fs and dioxin-like PCBs along the flow of the Orange River. This section of the Orange River flows through a part of the country with a low population density and little industrialisation compared to the Vaal Triangle and cities such as Durban and Port Elizabeth. Agricultural herbicides containing pentachlorophenol and chloronitrofen might have contributed (Masunaga, Takasuga & Nakanishi, 2001) to the PCDD/F levels in the Orange River as well as the diamond mining industry along the banks of the river close to its mouth in the Atlantic Ocean. However, the

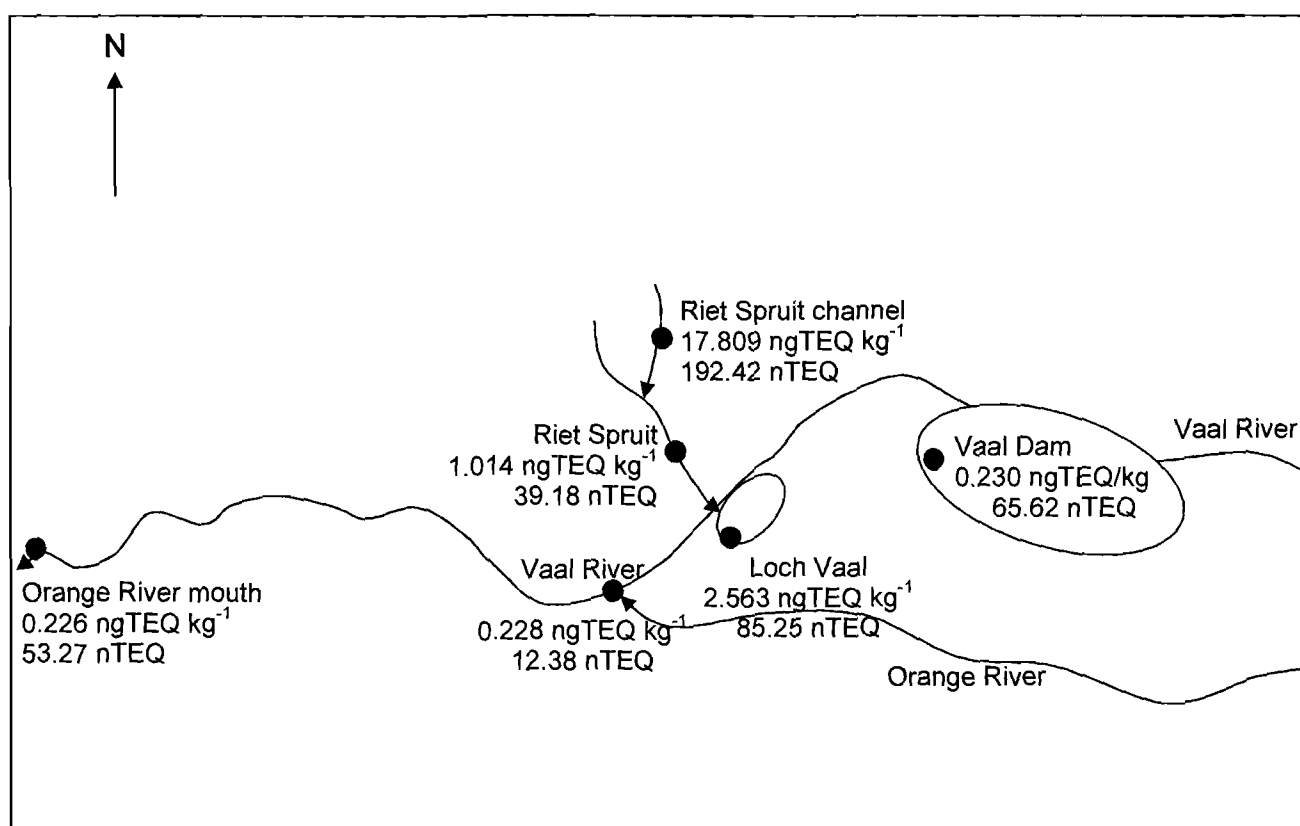


Figure 5.1: A diagrammatic representation (not on scale) of the six interior Vaal River catchment sites and the measured WHO₂₀₀₅-TEQ at each site. (nTEQ = normalised TEQ ng kg⁻¹ (Table 5.1))

process of solvent depletion might also explain this increased normalised TEQ. In this process, solvent (in this case organic carbon) is removed by some mechanism (such as natural degradation) and the contaminants become more and more concentrated in the decreasing solvent pool (Macdonald, Mackay, Li & Hickie, 2003). The 0.42% OC at the Orange River mouth is 4.4 times less than the 1.84%

measured for the Vaal River at the confluence with the Orange River (Table 4.3a). The Riet Spruit channel is upstream of the Riet Spruit site itself and had the highest of all the measured concentrations (Table 5.2). The site in the Riet Spruit had slightly lower concentrations, probably due to the dilution or chromatographic effect when moving downstream. The Riet Spruit empties into Loch Vaal (Figure 5.1). The concentrations in Loch Vaal were higher than those at the Riet Spruit site, which could indicate other sources of dioxin-like PCB and PCDD/Fs, or might be an indication of the higher sedimentation rates of lakes. The Riet Spruit channel site and the Loch Vaal site were the only two sites of the system represented in Figure 5.1 that had levels higher than the TEC of the Canadian sediment quality guidelines (Table 5.1).

Another site associated with industry is the Modderfontein Spruit that drains an area well known for its production and storage of explosives and manufacture of fertilizer, but also insecticides, paints and varnishes. Of the 22 sites of this study, this was the second most polluted site. It had the second highest concentration for both dioxin-like PCBs, PCDD/Fs and normalised TEQ (Table 4.3a).

The Crocodile River site, close to Nelspruit in Mpumalanga, had high concentrations that might be explained by the papermill upstream, at Ngodwana, or possibly by other unknown sources. The Groot River was selected due to it being inside a forestry area with wood treating facilities, a known source of dioxins. This river was sampled at its mouth and it is possible that its high sand content (90%; Table 4.13) limited the sorption of the PCB and PCDD/F congeners. Since no other sites from this river were sampled, this low level might also have been due to low pollution levels to start with, or it might be another example of solvent depletion.

Rivers that were selected to be sampled because of their association with coal mining and electricity producing areas, such as the Buffalo River and the Olifants River - including the Loskop Dam – had relatively low PCB and PCDD/F concentrations (Table 4.2a & b).

Another site above the Canadian TEC level is the Hartbeespoort Dam site with 0.862 ngTEQ kg⁻¹ d.w., and a PCB concentration level of 814.4 ng kg⁻¹ d.w. Greichus *et al.* (1977) reported 0.32 mg kg⁻¹ d.w. PCB (3.2 x 10⁵ ng kg⁻¹ d.w.) for bottom sediment from this dam. This included all detected PCB congeners. The same authors reported 70 000 ng kg⁻¹ d.w. PCB (my conversion) in the bottom sediments of the Voëlvelei Dam which is connected to the Berg River via a canal. In the present study, the Berg River only had a PCB concentration of 50.6 ng kg⁻¹ d.w.

Mean PCDD/F TEQ-values for sediment from Lake Ontario (USA) were 91 ngTEQ kg⁻¹ TEQ (Marvin *et al.*, 2002) with four sites in excess of 200 ngTEQ kg⁻¹ TEQ. Not one of the sites in the present study had any PCDD/F TEQ-levels even close to this (cf. Table 4.3a). The highest was the 10.4 ngTEQ kg⁻¹ of the Riet Spruit channel site, which was nine times lower than measured at Lake Ontario. High concentrations of PCDD/Fs were also reported for the Tittabawassee River, Michigan (USA) that varied from 102 to 53 6000 ng kg⁻¹ d.w. (Hilscherova *et al.*, 2003). Local interior levels within that range included Loch Vaal (247.5 ng kg⁻¹ d.w.), the Crocodile River (250.8 ng kg⁻¹ d.w.), Modderfontein Spruit (535.7 ng kg⁻¹ d.w.) and the Riet Spruit channel site (1 136.9 ng kg⁻¹).

The PCDD/F TEQ-values of the present study were generally within the same range as some of the European sites: PCDD/F TEQ-values reported for surface sediments from Arctic lakes in Norway and Sweden showed concentrations ranging from 1.4 to 4.2 ngTEQ kg⁻¹ d.w. (UNEP, 2003). Loch Vaal and the Modderfontein Spruit had values of 2.04 and 4.06 ngTEQ kg⁻¹ d.w. (Table 4.3a), respectively, all within the range reported for the Arctic Lakes. PCDD/F concentrations for the Po River (Italy) varied between 121 and 814 ng kg⁻¹ d.w., which is 1.3 to 13 ngWHO-TEQ kg⁻¹ d.w. (Fattore *et al.*, 2002). The Riet Spruit channel site had a PCDD/F concentration of 1 136.9 ng kg⁻¹ d.w., which was higher than anything measured for the Po River, but the Loch Vaal (247.5 ng kg⁻¹ d.w.), the Crocodile River (250.8 ng kg⁻¹ d.w.) and the Modderfontein Spruit (535.7 ng kg⁻¹ d.w.) sites were all within the range reported for the Po River. Comparing local interior sites' TEQ values (for both PCBs and PCDD/Fs) to what was reported for a Czech river basin (1 to 5 ng kg⁻¹ d.w.), Modderfontein Spruit (5.5 ng kg⁻¹ d.w.), the Crocodile River (2.4 ng kg⁻¹ d.w.), Loch Vaal (2.6 ng kg⁻¹ d.w.) and Riet Spruit (1.0 ng kg⁻¹ d.w.) were within the same range, while Riet Spruit channel (17.8 ng kg⁻¹ d.w.) had a TEQ level three times higher (Hilscherova *et al.*, 2001).

Koh *et al.* (2004) reported an average of 475.18 ng kg⁻¹ d.w. PCDD/F and 2 319 ng kg⁻¹ PCB (my calculations) for the Hyeongsan River in Korea. All but two of the South African sites had comparable PCDD/F levels: Modderfontein Spruit (535.7 & 1 505.0 ng kg⁻¹ d.w.) and the Riet Spruit channel (1 136.9 & 24 936.2 ng kg⁻¹ d.w.). The WHO-TEQ for PCDD/F in a Taiwanese reservoir was 0.663 ng kg⁻¹ d.w. and the WHO-TEQ for the PCBs was 0.041 ng kg⁻¹ d.w. (Chi *et al.*, 2007). At least five interior South African sites had higher PCDD/F TEQ: Modderfontein Spruit, the Crocodile River, Loch Vaal, Riet Spruit and the Riet Spruit channel (Table 4.3a), and four of the same sites (Riet Spruit excluded) also had higher PCB TEQ levels. Masunaga, Yao, Ogura, Nakai, Kanai, Yamamuro and Nakanishi (2001) reported PCDD/F WHO-TEQ of 16.4 ng kg⁻¹ d.w. for a lake in rural Japan. This

level was higher than for any of the South African sites.

5.3 Evaluation of the H4IIE bio-assay results

Comparing the measured and extrapolated TCDD-EQ values of the H4IIE-*luc* bio-assay to the corresponding WHO-TEQ as was chemically determined (Table 4.4) shows a correlation of $R^2 = 0.654$, with one outlier removed. Concentrations of WHO-TEQ, generally, are not directly comparable to bio-assay derived TCDD-EQ concentrations because the TEFs promulgated by WHO were designed for use in risk assessment and are meant to be protective rather than predictive (Coady, Jones & Giesy, 2001). The expert panel assembled to evaluate the TEFs consistently made conservative assumptions about relative toxicity and determined that values were always rounded up to the next higher order of magnitude (Van den Berg *et al.*, 1998). As a result, the TEFs derived thus only estimate the relative biological potency of the congeners.

Derivation of a mass balance relationship between concentrations of TEQs and TCDD-EQs can be accomplished by applying relative effects potency (REP) to detected congener concentrations. The REPs are derived from the same endpoint in the same bio-assay system (H4IIE-*luc*). Equivalent concentrations (REP-TEQ) derived using REPs and individual PCDD/F and PCB concentrations can be compared directly to concentrations of TCDD-EQ measured in the same bio-assay (Coady *et al.*, 2001). The REP values used in this study were taken from a previous study in which REP values were calculated with the H4IIE-*luc* cell line (Giesy *et al.*, 1997). Concentrations of REP-TEQ can be compared to concentrations of the instrumentally measured PCDD/Fs and PCBs:

$$\text{REP-TEQ} = \sum C_i \times \text{REP}_i$$

where C_i indicates the concentration of the congener and REP_i is the REP for that congener.

The REP-TEQs and the WHO-TEQ corresponded better than the WHO-TEQ and the TCDD-EQ and are in the same order of magnitude (Table 5.3). The poor correspondence between the WHO-TEQ and TCDD-EQ can be explained by the fact that the bio-assay measures total activity of complex mixtures, and accounts for interactions among congeners, while instrumental analyses measured only target analytes (Sanderson, Aarts, Brouwer, Froese, Dennison & Giesy, 1996). The H4IIE-*luc* bio-assay can also detect additional dioxin-like activity from compounds not specifically measured in analytical chemistry procedures. In all of the samples in Table 5.3, concentrations of REP-TEQ were greater than concentrations of TCDD-EQ. This can be explained by the fact that congeners, when tested as a mixture in the bio-assay, can antagonise one another,

causing interference and reduced measures of dioxin-like activity that are not predictable in the additive REP-TEQ model (Sanderson *et al.*, 1996). Thus, in the absence of unquantified AhR-active

Table 5.3: Comparison between the TCDD-EQ of the assay, the WHO-TEQ of the chemical analysis and the REP-TEQ

Site name	TCDD-EQ20 (ng kg ⁻¹ d.w.)	WHO-TEQ (ng kg ⁻¹ d.w.)	REP-TEQ (ng kg ⁻¹ d.w.)
RchrdBy	2.33 × 10 ^{-6*}	2.35 × 10 ⁻¹	3.7 × 10 ⁻¹
Thlzhlk	8.31 × 10 ^{-6*}	4.69 × 10 ⁻¹	8.37 × 10 ⁻¹
RietSprS	1.02 × 10 ^{-2*}	1.01	1.86
RtsprCh	5.17 × 10 ^{-3*}	1.78 × 10	2.66 × 10
LochV	1.72 × 10 ⁻¹	2.56	5.54
Croc	2.59 × 10 ^{-3*}	2.37	3.38
LskpD	2.32 × 10 ⁻²	2.0 × 10 ⁻¹	2.3 × 10 ⁻¹
HrtbDam	7.39 × 10 ⁻²	8.61 × 10 ⁻¹	1.3
Modder	2.01 × 10 ⁻¹	5.47	1.19 × 10

compounds, the concentration of TCDD-EQ would be expected to be less than that of REP-TEQ (Coady *et al.*, 2001).

The WHO-TEQ was also greater than the TCDD-EQ (Table 5.3). The difference in magnitude of the concentrations between TCDD-EQ and WHO-TEQ was in some cases more than twofold, which is the minimum proposed to conclude that the concentrations of bio-assay and instrumental TEQs are not equivalent (Hilscherova *et al.*, 2003). Studies have reported that especially mono- or di-*ortho*-PCBs can act as antagonists or partial agonists for the AhR, thus reducing the total potency of the mixture. The mono-*ortho*-PCBs such as PCB118 may act as AhR antagonist (Sanderson & Van den Berg, 1999). This might not be sufficient to explain the big difference observed in this study. The real explanation might be found in the different extraction techniques: when extracts were prepared for GC/MS analysis in the German laboratory, an ASE was used, and when extracts were prepared for bio-analysis in the USA laboratory, Soxhlet apparatus was used. In both laboratories, toluene was the organic solvent, but had to be replaced from the extract with hexane for the bio-assay because toluene is cytotoxic at the dosage concentrations. It is possible that sample was lost during this step, explaining the low TCDD-EQ concentrations of this study. Another contributing factor to poor assay results might have been the lack of treatment of the extracted samples with activated copper. Sediment extracts specifically need to be treated with activated copper to remove the sulphur that might be present (Koh, *et al.*, 2005). This step was not introduced during the training period in the US laboratory. Sulphur is cytotoxic and might have been responsible for poor, non-usable dose-response curves. Cytotoxicity prevents the cells in the assay from responding to AhR ligands in the extracts.

The effectiveness of the H4IIE-*luc* bio-assay to quantify TCDD-EQs within the same order of magnitude as predicted by the WHO-TEQ cannot be supported by the limited data of this study. That there are too many differences in interpretation at best only promotes the assessment that the two methods provide complementary, not necessarily comparative data (Coady *et al.*, 2001). There are, however, enough peer-reviewed papers that report good correlations between the TCDD-EQ of the H4IIE-bio-assay and WHO-TEQ. In a study where the dioxin-like activity of sediments from a Czech river basin was characterised, the two EQs correlated significantly ($R^2 = 0.8$; $p < 0.01$) (Hilscherova *et al.*, 2001). Koh *et al.* (2004) also reported a significant correlation between TCDD-EQs and WHO-TEQs for sediment from the Hyeongsan River in Korea ($R^2 = 0.994$, $p < 0.05$). Sediments from the Tittabawassee River also produced a significant correlation between concentrations of TCDD-EQs derived from the H4IIE bio-assay and TEQs estimated from instrumental analysis ($R^2 = 0.94$; $p < 0.05$) (Hilscherova *et al.*, 2003). Nording *et al.* (2007) reported that results from bio-analytical techniques, which included the H4IIE-bio-assay, were, in principle, not significantly different from the GC/MS data ($p = 0.05$). Therefore this type of assay was found to be sufficiently sensitive, selective and accurate to be used in connection with soil remediation activities when aiming at the remediation goal recommended by the US EPA (i.e., $< 1 \text{ ngTEQ kg}^{-1}$).

5.4 Nature of pollution

The patterns of relative concentrations of PCDD/Fs alone were insufficient to determine the exact source of PCDD/Fs as PCDD/Fs undergo congener-specific degradation in soil/sediments and volatilisation in the environment (Hilscherova *et al.*, 2003). Sites with levels lower than the detection limit need therefore be re-sampled several times to confirm its low levels. The results of the principal components analysis of relative proportions of congeners (section 4.2.1), combined with the absolute concentrations, indicate that the source of PCDD/Fs in the 22 samples were due to everyday human activity (trash burning, traffic emissions and sewage sludge from waste water) rather than specific industrial processes (graphite refining, sintering plants and pesticide production).

An assumption that underpins the interpretation of the PCA results and the subsequent source identification is that variance in the congener space of the sediment profile is caused only by source variation. Other possible causes of variance such as environmental breakdown were not considered because PCDD/Fs were reported to be resistant to microbial attack in the environment (Hutzinger, Blumich, Berg & Olie, 1985). Even though microbial breakdown has been reported, (see end of section 2.4.3) their photodegradation in sediment is not considered to be significant (Yao *et al.*, 2002). Other authors that

followed the same approach were Froese *et al.* (1998) and Jones *et al.* (2005). There are a myriad of other environmental conditions that may impact on the pollution profile, such as a combination of pollution sources, different environmental partitioning conditions (soluble organic content such as fulvic acids or other pollutant concentrations (e.g. surfactants) that may differ from water body to water body), or geomorphological factors (such as the mixing energy and flow pattern), and the turbidity of the water column. By using PCA and HCA in this study, it was assumed that the variation in pollution profile observed was due to the source input, rather than all the factors that might have contributed to a changing pollution profile.

The multivariate analytical techniques, PCA and HCA, used to investigate the nature of the pollution were of limited use due to the small number of South African samples, and dioxin-like concentrations were below the quantification limit for more than half of the sites (Table 4.2a & b). In biplots of only South African data, no obvious clustering of any of the South African sites occurred. More information could however, be garnered from the biplots where the South African sites were merged with data from Wenning *et al.* (1993).

5.4.1 PCDD/F pollution

Comparing the South African PCDD/F data to that of Wenning *et al.* (1993), all the South African sites were clustered together on the biplots (cf. Figure 4.7 to 4.9) with the Wenning types of samples/sites, and arranged around the origin. All 22 of the South African sites therefore shared the same PCDD/F profile. This was corroborated by the factor loadings (Table 5.4). When the South African data was added to the Wenning data: (i) those congeners that contributed to a particular factor, did so again; and (ii) congeners associating with each other in the Wenning data set, did so again when the South African data was added. Even when only the South African data set was investigated, a similar pattern of congener contribution was observed.

The South African sites grouped between the OL (chimney soot from oil central heating), UA (urban air), H (soils from scrap wire and car incineration sites), WT (municipal water treatment plant sludge and chlorinated tap water), AIR (ambient air near municipal sewage sludge), TT (air from automobile traffic tunnel) and SS (municipal sewage sludge) of the Wenning data (Figure 4.7 to 4.9). In the HCA, the same sample types were identified (Figure 4.11 & 4.12); highlighting TT, H and UA. These sources can be described as resulting from everyday human activity (non-industrial) (e.g. traffic, urban air, sewage and waste water treatment plants), and are not specific to any particular industrial processes or wastes such as P (pyrolysis of PCBs and polyvinylchloride), GE (graphite electrode sludge)

Table 5.4: Comparison between the factor loadings of three data sets containing PCDD/Fs.

	F1			F2			F3		
	W	W & SA	SA	W	W & SA	SA	W	W & SA	SA
+	PeF1	PeF1	PeF1	TeD	TeD		HxF3	HxF3	
	PeF2	PeF2		PeD	PeD		HxF1	HxF1	HxF1
	TeF	TeF		HxD1	HxD1	HxD1	HpF2	HpF2	
	HxF2	HxF2	HxF2			HxD2			PeF1
	HxF4	HxF4							PeF2
	HxF1	HxF1	HxF1						HxF4
		HxF3	HxF3						
-			TeD						
			HpF2						
	OF	OF	OF	HpF1	HpF1		HpD	HpD	
	HpD	HpD	HpD	HpF2	HpF2		HxD2	HxD2	
	OD	OD	OD		OF		HxD3	HxD3	HxD3
			HpF1			TeF			HxF3
% Total variance	33.7	33.7	40	18.7	18.8	16.2	18.6	17.5	14
Cumulative %	33.7	33.7	40	52.4	52.5	56.1	71	70.1	70.1

W: Wenning data only;

W & SA: Wenning and SA data;

SA: SA data only;

+: positive loadings;

-: negative loadings

and D (soil from beneath waste electrical equipment incineration). However, sources at individual South African sites might vary from this generally observed trend. The South African sites might also have grouped with other types of pollution, had they been included in addition to the Wenning data (for example, effluents from sintering plants and paper mills and releases from the petroleum industry).

Conducting this type of investigation, using only 17 PCDD/F congeners, also limits the linking of patterns with sources. Research from Masunaga *et al.* (2003) on PCDD/F profiles in the sediment of Tokyo Bay basin showed that source identification was clearer with all of the congeners, rather than with only 17 congeners. In general, most of the South African sites had higher PCDD concentrations than PCDF (Table 5.5), and individual congener contributions decreased from OD>HpD>OF>HpF (Figure 5.2a).

The very high OD concentration found in the South African sediments coincided with results from Czuczwa and Hites (1986) for the Great Lakes, Chi *et al.* (2007) for a Taiwanese reservoir, and Masunaga *et al.* (2003) for Tokyo Bay. The PCDD/F congener pattern that Czuczwa and Hites (1986) detected for particulate matter in urban air, was reflected in the sediment samples from the Great Lakes. They ascribed the high OD levels to combustion sources. However, direct sampling from the combustion sources themselves showed a great variety of congener profiles. It was in the environmental samples (sediments) that OD dominated. The less chlorinated PCDD/Fs have higher

Table 5.5: Comparison between the total PCDD and PCDF contribution at each of the South African sites.

Sites	Σ PCDD (ng kg ⁻¹ d.w.)	Σ PCDF (ng kg ⁻¹ d.w.)
OrangeM	1.51	2.29
SldnhBy	1.30	1.75
Berg	16.96	3.31
TH2ODam	19.66	2.34
Grt	6.09	1.71
SwkpEst	218.58	18.62
VaalRiv	6.28	1.39
Bfalo	4.57	1.71
Mooi	10.84	2.38
Umlazi	140.51	15.36
Umgeni	168.55	20.37
RchrdBy	2.17	1.60
Thlzhlk	16.76	3.71
VaalDam	2.02	1.65
RietSpr	70.59	8.96
RtsprCh	1 021.80	115.10
LochV	220.59	26.9
Croc	235.41	15.38
Olifant	4.15	1.56
LskpD	2.88	1.28
HrtbDam	55.09	6.06
Modder	467.92	67.78
Total	2 694.20	321.21

vapour pressures and higher water solubilities than OD (Table 2.4). The relative content of OD seemed to increase from air, through atmospheric deposition, to soil (Masunaga *et al.*, 2003). Thus, less of the lower chlorinated congeners would bind to particles in the air or in

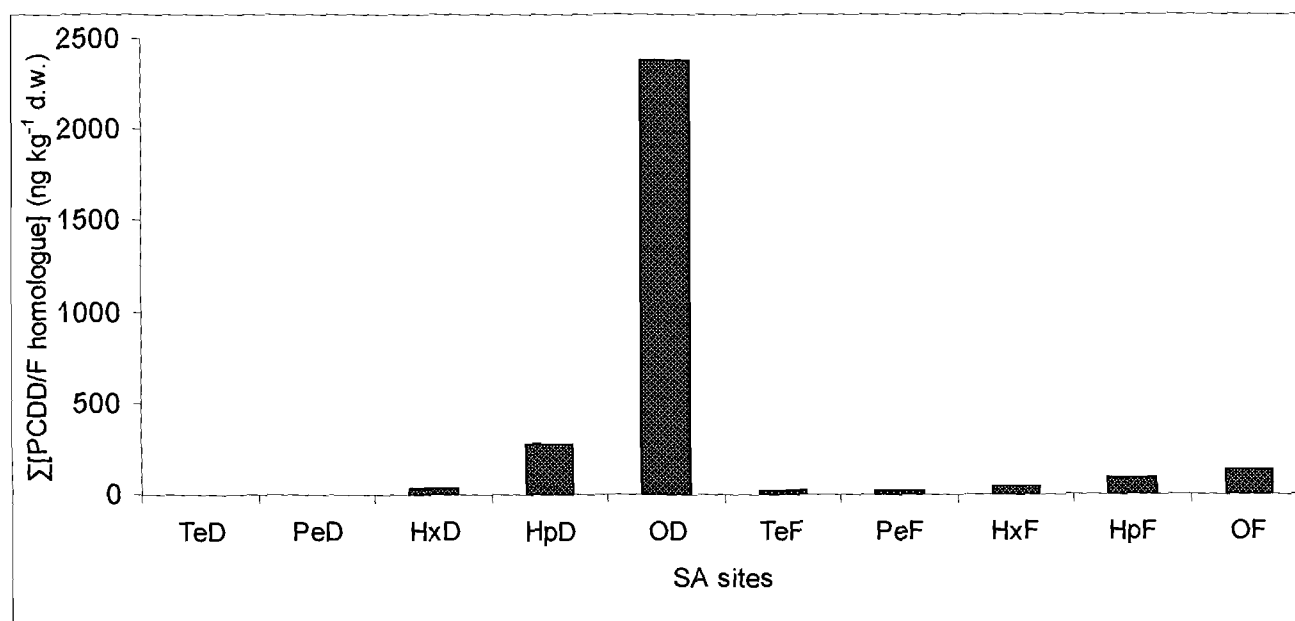


Figure 5.2a: Contribution of the different congener homologues (17 congeners) for the 22 South African sites.

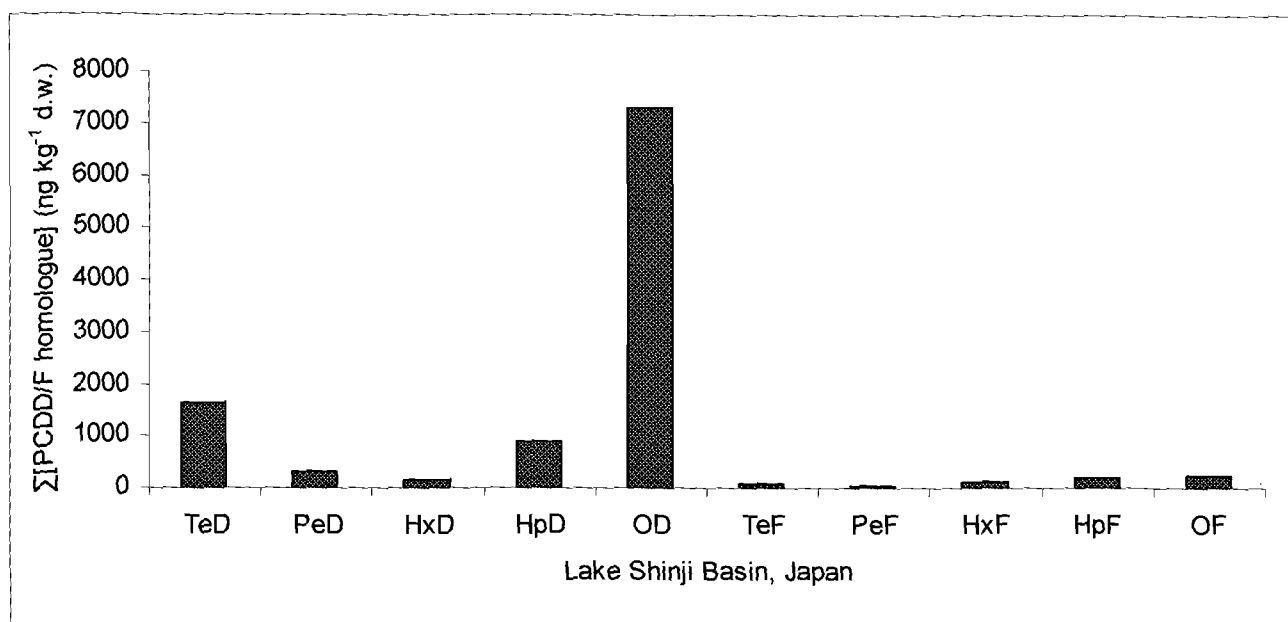


Figure 5.2b: Contribution of the different congener homologues (78 congeners) for Lake Shinji Basin, Japan (Masunaga *et al.*, 2001)

the water and more of the higher chlorinated compounds such as OD, explaining its high level relative to the other congeners. The less chlorinated PCDD/Fs also have more C-H bonds than OD and are likely to be more easily degraded, either biologically or chemically.

Results from a study by Masunaga *et al.* (2001) showed a very similar homologue pattern (Figure 5.2b) with that found in the present study, except for the relatively high TeD level absent from the South African pattern. These authors also performed PCA on all PCDD/F congeners found at sites in Lake Shinji basin, Japan. Characteristic congeners in their factor 1 that had high factor loadings were OD, all the HpD isomers, OF and most of the HpF isomers. According to them, these corresponded well with the impurities in PCP, a wood preservative, insecticide and herbicide. (Baker and Hites (2000) noted that the photochemical synthesis of OD from PCP in atmospheric condensed water might be the most significant portion of OD in the environment.) In addition to OD, HpD, OF and HpF, Yao *et al.* (2002) also attributed HxDs and some HxFs in sediment to be a result of PCP. In the present study, whether considering Wenning, Wenning and South African or only South African data, the first factor for all three data sets was also characterised by OD, HpD and OF. HpF and HxF also contributed to factor 1 of the South African data. These congeners contrasted with other congeners (Table 5.4) in the present study, while Masunaga *et al.* (2001) did not report any contrasts.

Comparing relative concentrations at sites (as in a PCA), factor 1 of the present study seems to share similarities, something which is indicative of PCP pollution in sediment (Masunaga *et al.*, 2001). However, in the biplots (Figure 4.7 to 4.9) and HCA (Figure 4.11 & 4.12) of the present study, the South African sites did not group or cluster with the PCP

associated types of pollution (PC and NPCP - Figure 4.7 to 4.9), but the type of samples reported in the Wenning *et al.* (1993) paper, came from the preparations themselves. Once released into the environment, a change in homologue pattern occurs, favouring the higher chlorinated homologues (Czuczwa & Hites, 1986). Im *et al.* (2002) concluded that high OD levels in the sediment of Masan Bay, Korea, might be due to both incineration and PCP sources.

Therefore, based on the homologue pattern of a typical combustion process reported by Hutzinger and Fiedler (1993), in which PeFs and HxFs were the main components, it was assumed that factor 3 of the South African data (Table 4.8), rather than factor 1, corresponded with combustion processes in which incineration was the main factor.

Bar graph profiles of 17 PCDD/F concentrations in soil surrounding a United States municipal waste incinerator, expressed as fractions of the total pollution concentration, had a similar pattern as Figure 5.2a (Lorber, Pinsky, Gehring, Braverman, Winters & Sovocool, 1998). The air samples from the Lorber *et al.* (1998) study also presented the same profile. According to a study in the United Kingdom on atmospheric emissions by waste incinerators, cement kilns, sinter plants and sewage sludge incinerators, OD and HpD dominated, followed by HpF and OF (Alcock, Gemmill & Jones, 1999). This also supports the conclusion that PCDD/F pollution for the 22 South African sites were due to combustion and chemical releases such as PCPs.

Another conclusion Im *et al.* (2002) arrived at, was that PCDF levels in sediment were higher than PCDD levels when the possible source had been Kanechlor (Japanese PCB formulations). Hagenmaier, Lindig and She (1994) already reported that PCB preparations were sources of PCDFs in the environment. If it is assumed that all PCB formulations have the same tendency, irrespective of its country of origin, PCB formulations were probably not the main source of dioxin pollution at the South African sites, because PCDD pollution was approximately eight times higher than the PCDF pollution (Table 5.5). On an individual level, the Orange River mouth and Saldanha Bay harbour had PCDF levels slightly higher than the PCDD levels, but the margin and levels were so small that they might be of no consequence.

The PCDD/F profile observed for the South African sites was probably due to combustion processes in general, and non-industrial human activity specifically, and these PCDD/Fs were transported to the sites through air deposition. The PCA results, however, also seemed to hint at the contribution of chemical manufacturing processes, but this would only become clearer if sites are investigated individually and effluents and emissions of possible sources in their direct proximity are also analysed.

5.4.2 Dioxin-like PCBs

Dioxin-like PCBs might have the same sources as PCDD/Fs. Amongst others, municipal waste incinerators (Sakai, Hiraoka, Takeda & Shiozaki, 1994), "combustion systems" (Lemieux, *et al.*, 2001) and metal reclamations and sintering plants (Boers *et al.*, 1994) are sources of co-planar PCBs. Since there was no indication in the previous section that PCB preparations were mainly responsible for PCDD/F pollution in general in the South African sites, the question as to the source of the determined dioxin-like PCBs presented itself. Since only dioxin-like PCB concentrations were determined, and there was no indication as to the levels of the other PCBs, a definitive answer would be impossible. However, comparing HCA dendograms might provide an indication. Again, the biplots were of limited help only.

In an investigation by Alcock, Behnisch, Jones and Hagenmaier (1998), Aroclor formulations (1221, 1232 and 1242) were dominated by PCB105, PCB118 and PCB77, with PCB126 and PCB156 present in smaller quantities. Emissions from combustion sources contributed towards additional PCB126, a non-*ortho* substituted PCB. According to Chi *et al.* (2007), non-*ortho* PCBs, in particular, are formed during coal combustion and industrial waste incineration and do not originate solely from commercial PCB mixtures. The Japanese commercial PCB preparations Kanechlor 300, 400 and 500 were responsible for PCB118, PCB105 and PCB77 (Kannan, Tanabe, Wakimoto & Tatsukawa, 1987). Municipal solid waste incinerators and medical waste incinerators typically release PCB118 and PCB123 into the atmosphere (Dyke, Foan & Fiedler, 2003). Ogura, Masunaga & Nakanishi (2004) ascribed the presence of PCB189, PCB126 and PCB169 in air samples to thermal processes, and PCB118, PCB77, PCB167 and PCB123 to volatilisation from commercial PCB products. The dioxin-like PCBs in the sediment of the 22 South African sites that had the highest levels were PCB118 > PCB105 > PCB156 > PCB77 (Figure 5.3). From this bar graph profile it seems likely that the main source of dioxin-like PCBs in the South African sites were from commercial PCB preparations. Although combustion processes also contributed, it was to a smaller extent.

Irrespective of the type of congener group used in a HCA (i.e. PDD/Fs, PCBs or both groups) the groupings of the South African sites were very similar (Table 4.17). On the biplot of the first two factors of the PCA with all congeners included (Figure 4.19) it seemed as if the South African sites were separated due to either PCB, or PCDD/F content. However, results from the t-tests performed on the two groups revealed that the distinguishing factor was not type of pollution (whether a site had higher either PCB or PCDD/F levels), but rather magnitude of pollution (Table 4.12). Those sites associated

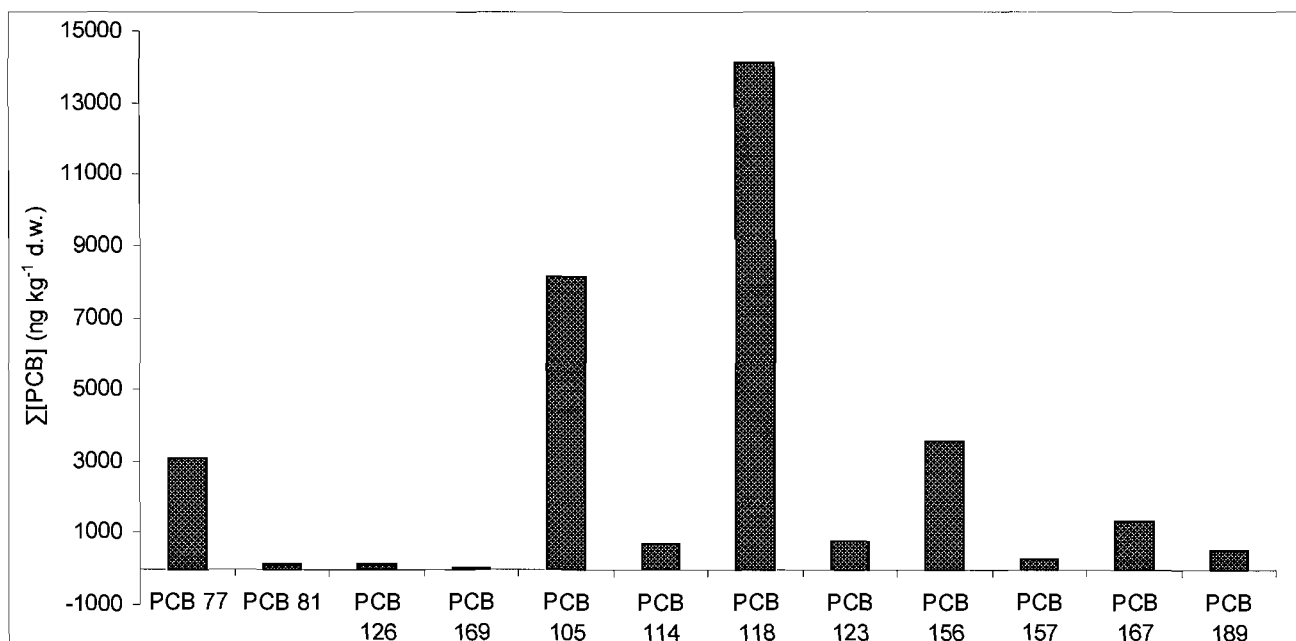


Figure 5.3: Dioxin-like PCB concentrations at the 22 South African sites.

with the PCBs on the biplots (Figure 4.19) had the highest levels of both PCBs and PCDD/Fs. This conclusion was corroborated by inspection of the congener concentrations in Tables 4.2a and 4.2b.

5.5 Effect of sediment characteristics

The sediment characteristics of the sites from which samples were collected for the present study did not seem to play an import role in the nature of the pollution. When the sediment characteristics were added to the PCA, no obvious grouping of sites appeared on the biplots except for the Vaal Dam, Saldanha Bay, the Berg River and the Groot River mouth. Of the four, only the Groot River mouth had a measurable level of oxidisable organic carbon that was detected by the Walkley-Black method (Table 4.3a). The other three did not show any measurable organic carbon. It is therefore clear why the Vaal Dam, Saldanha Bay and the Berg River would group together in the biplots, but it is not clear why the Groot River mouth would be associated with them. Its %OC was small, but not the smallest, so that one could conclude that the bottom four sites regarding %OC clustered together. In fact, there were 11 other sites with lower %OC. It seems, therefore, that the distribution of the sites was due to congener profiles rather than sediment characteristics. One distinction these four sites share, but which is not unique to them, is that all four of these sites were included in the group of sites with low pollution levels, i.e. group A in Table 4.17.

Comparing all five the sediment characteristics of the two groups: A (with low pollution levels) and B (with higher pollution levels) (Table 4.17), neither statistical nor a practically significant difference was found (Table 4.18). There was a slight indication that %clay and

%OC might have been different between the two groups because their effect sizes were 0.41 and 0.49 respectively, but the difference was not practically significant (c.f. section 3.4).

When the sediment characteristics were added to the PCA of the South African data the tables listing the factor loadings for the different combinations of congener groups with the sediment characteristics (Tables 4.14 to 4.16) revealed an unexpected phenomenon. As was briefly explained in section 4.2.5, the expectation was that %sand and %>2mm would associate with one other, while %clay, %silt and %OC would associate with each other, i.e. those characteristics that have inherently higher carbon contents would associate with each other, as would those with expected less carbon. This was, however, not the case. It was not expected that %clay and %silt would associate with %sand (factor 3, Table 4.14; factor 1, Table 4.15; factor 1, Table 4.16). Another unexpected association was that some of the congeners, such as HpF2 and TeD in factor 1 (Table 4.14) associated with sand and PCB169 with %sand and %>2mm (factor 2, Table 4.15).

This observation might have been a real result from the physical environment such as the fact that some congeners prefer to associate with sand rather than with sediment compartments with a higher organic carbon content. However, this conclusion could not be confirmed by the literature. The generally low %OC in all the sediments collected for this study might be the reason that there was no clear disassociation between %>2mm and %sand on the one hand, and %silt, %clay and %OC on the other. One also has to keep in mind that a PCA calculates factor loadings (which are correlations) with whatever data was available and, if more congeners were to be included, or additional sediment characteristics, the observed associations between %sand and %silt or %clay might have improved the resolution.

5.6 In summary

- PCDD/Fs and dioxin-like PCBS are present in detectable quantities in the aquatic environment of South Africa.
- The total PCB concentration was higher than the total PCDD/F concentrations at all sites, similar to global patterns.
- Dioxin-like levels in the sediments of the 22 South African sites were within the lower concentration range reported in literature for other (mainly North American, European and Eastern countries).
- Seven sites (Swartkops Estuary, the Umgeni River mouth, the Riet Spruit channel, Loch Vaal, the Crocodile River, Hartbeespoort Dam and Modderfontein Spruit) had

levels higher than the threshold level of the Canadian sediment quality guidelines, yet lower than the minimal effect level.

- The high level of the octa-chlorinated PCDD (OD) confirms global observations.
- Across the 22 sites, the PCDD/F sources were probably combustion processes associated with human activities, rather than specific industrial processes, with a small contribution from chemical processes. This observation might be different when individual sites are investigated more intensively.
- The dioxin-like PCBs present at the South African sites were likely from commercial PCB preparations, but a small contribution was also made by combustion processes.
- Sediment characteristics did not seem to play a major role in the clustering of the sites.
- The process of solvent depletion might be responsible for the increase in normalised TEQ observed for the Orange River mouth.
- The use of the H4IIE-*luc* bio-assay as a screening tool seems promising.

Abbreviation list for sites and congeners referred to in the Wenning and SA data.

Abbreviations for samples	Site or type of sample from Wenning et al. (1993)	Abbreviations for SA sites	SA site names
A	Soil from former 2,4,5-T plant	Berg	Berg River
AIR	Ambient air near municipal sewage waste incinerator	Bfalo	Buffalo River
ASH	Municipal sewage waste incinerator fly ash	Croc	Crocodile River
B	Soil contaminated with graphite sludge	Grt	Groot River
C	Soil from chlorine production plant	HrtbDam	Hartbeespoort Dam
CL	Chimney soot from coal ovens	LochV	Loch Vaal
D	Soil beneath waste electrical equipment incineration	LskpD	Loskop Dam
DT	Residue in commercial detergents	Modder	Modderfontein Spruit
E	Semi-rural British soils	Mooi	Mooi River
GE	Graphite electrode sludge	Olifant	Olifants River
H	Soils from scrap wire and car incineration sites	OrangeM	Orange River mouth
NPCP	Residue in Na-pentachlorophenol	RchrdBy	Richard's Bay
OC	Residue in o-chloranil	RietSpr	Riet Spruit
OL	Chimney soot from oil central heating	RtsprCh	Riet Spruit channel
P	Pyrolysis of polychlorinated biphenyls and polyvinylchloride	SldnhBy	Saldanha Bay
PC	Residue in pentachlorophenol	SwkpEst	Swartkops Estuary
PCB	Residues in Aroclor® & Kanechlor® products	Thlzhk	Thulazihleka Pan
PV	Pyrolysis of polyvinylchloride	TH20Dam	Theewaterskloof Dam
SS	Municipal sewage sludge	Umgeni	Umgeni River
TB	Residue in trichlorobenzene	Umlazi	Umlazi River
TT	Air from automobile traffic tunnel	VaalDam	Vaal Dam
UA1-8	Urban air from Hamburg, Germany	VaalRiv	Vaal River
UA9-15	Urban air from Bridgeport, USA		
WT1-3	Municipal water treatment plant sludge		
WT4	Chlorinated tap water		
Name of congener		Abbreviation	
2,3,7,8-TCDD		TeD	
1,2,3,7,8-PeCDD		PeD	
1,2,3,4,7,8-HxCDD		HxD1	
1,2,3,6,7,8-HxCDD		HxD2	
1,2,3,7,8,9-HxCDD		HxD3	
1,2,3,4,6,7,8-HpCDD		HpD	
OCDD		OD	
2,3,7,8-TCDF		TeF	
1,2,3,7,8-PeCDF		PeF1	
2,3,4,7,8-PeCDF		PeF2	
1,2,3,4,7,8-HxCDF		HxF1	
1,2,3,6,7,8-HxCDF		HxF2	
1,2,3,7,8,9-HxCDF		HxF3	
2,3,4,6,7,8-HxCDF		HxF4	
1,2,3,4,6,7,8-HpCDF		HpF1	
1,2,3,4,7,8,9-HpCDF		HpF2	
OCDF		OF	
3,4,4',5'-TCB		*PCB81	
3,3',4,4'-TCB		*PCB77	
3,3',4,4',5'-PeCB		*PCB126	
3,3',4,4',5,5'-PeCB		*PCB169	
2,3,3',4,4'-PeCB		PCB105	
2,3,4,4',5'-PeCB		PCB114	
2,3',4,4',5'-PeCB		PCB118	
2',3,4,4',5'-PeCB		PCB123	
2,3,3',4,4',5'-HxCB		PCB156	
2,3,3',4,4',5'-HxCB		PCB157	
2,3',4,4',5,5'-HxCB		PCB167	
2,3,3',4,4',5,5'-HxCB		PCB189	

* non-ortho substituted PCBs

6 CONCLUSION

The four aims that were stated at the end of the introductory chapter have been met:

- The levels of the most toxic PCDD/F congeners and the dioxin-like PCBs in the sediments of selected aquatic areas in South Africa have been determined successfully.
- The possible risk associated with these levels was determined by comparison to the Canadian sediment quality guidelines (due to a lack of local guidelines), and seven sites were identified as having low level risk. The levels at the other 15 sites were too low and did not reach the lowest risk levels.
- Possible sources responsible for the PCDD/F pollution were identified as combustion processes related to non-industrial human activities, with a smaller contribution from chemical and industrial sources. Sources possibly responsible for dioxin-like PCB levels were established to be mainly due to commercial PCB preparations, but also combustion sources.
- Even if the applicability of the H4IIE-*luc* reporter gene bio-assay has not been proven beyond any doubt from data specifically presented in this thesis, it has been established as a useful research tool in laboratories across the world and now also at the North-West University.*

6.1 Levels, risk and sources

The Vaal Triangle had three sites, namely the Riet Spruit channel, Riet Spruit and Loch Vaal. The Vaal Triangle is one of the most industrialised areas of the country and it was not surprising that it was home to the site with the highest levels (Riet Spruit channel). Two of the aforementioned sites, Loch Vaal and the Riet Spruit channel, also had levels comparable to the TEC of the Canadian SQG. This means that there is a risk posed to the benthic organisms. Wildlife (birds of prey) and humans (subsistence fishermen) at the top of the food chain that is based on the benthic organisms found at these sites may be at risk too, since these pollutants have been proven to bio-accumulate in organisms and to bio-magnify in the food chain.

Other sites that had levels above the Canadian SQG included sites from highly industrial areas:

- the Swartkops Estuary downstream of the industrial area of Port Elizabeth;

* Since the onset of the research reported here, three Master's Degree students have successfully completed their studies, also employing the assay, with much more success.

- the Modderfontein Spruit in an industrial area northeast of Johannesburg (Gauteng), known for its explosives and fertiliser plants;
- the Umgeni River mouth in Durban, also an industrial and highly populated city in KwaZulu-Natal; and
- the Hartbeespoort Dam that is fed by the Hennops, Magalies and Crocodile (West) Rivers. (This Crocodile River is the river draining Roodepoort/Krugersdorp urbanised areas in Gauteng, not the same as the Crocodile River (East) of the study.)

The Crocodile River (East) site downstream of a paper mill in Mpumalanga is in a less populated and industrial part of the country, compared with the previously mentioned sites, but apart from possible pollution by the paper mill, the Crocodile River drains a part of Mpumalanga Province known for its forestry and sub-tropical fruits such as mango and avocado, and macadamia and pecan nuts (Joyce, 2004). Wood preservatives used at saw mills in the forestry industry and herbicides used by the farmers might have contributed to the dioxin levels in the river. The Crocodile and Olifants Rivers were included in this study, not just because of their possible pollution levels, but also because they leave South Africa through the Kruger National Park and enter Moçambique, transporting pollutants into the neighbouring country.

The sites in the same riverine system (Figure 5.1) illustrated the effect of the industrial area's input. The Vaal Dam (upstream of the industrial area) had a lower concentration than Loch Vaal, which is situated directly next to the industries. Downstream of Loch Vaal, at the confluence of the Vaal and Orange Rivers, the levels dropped again. This might be due to a dilution effect because of the confluency, or dechlorination of the compounds by microbial activity or even the chromatographic effect, which is the separation of the initial mixture into fractions along a stretch of river. The concentration seems to have remained the same through out the rest of the lower Orange River, until it flows into the ocean - indicating riverine transport. The normalised TEQ, however, tells a different story. The normalised TEQ at the Orange River mouth indicated additional input of PCDD/Fs and dioxin-like PCBs along the lower Orange River. The Orange River is extensively mined for diamonds in the Northern Cape, and crops such as fruits, maize, peanuts and cotton are irrigated on its banks (Joyce, 2004). These activities might be contributing to the observed dioxin pollution. Alternatively, or in combination, the removal of organic carbon from the sediment, a phenomenon known as solvent depletion, might also have been responsible for the available pollutants to have accumulated onto to the only available organic carbon.

6.2 Maintaining perspective

For this study, 22 sites were selected to represent the entire South Africa. This number of sites was determined by budget constraints - the GC/MS analysis of one sample in the German laboratory cost €980 in 2002. For most of the aquatic water bodies, one sample per water body was collected, which is not really representative of a river or a dam the size of the Vaal Dam (cf. section 3.1 in the Materials and methods chapter). Sites were selected because of their proximity to known dioxin sources, thus representing an intentionally biased approach. From this alone one would expect high levels at all of the sites (except for the reference site), but sampling a single area in a water body cannot provide accurate data regarding the levels of a particular water body. Run-off, rainfall and unknown point source pollution might influence the pollution at a single site. However, it does provide a first set of data on which to base an assessment.

Although the reference site, namely the Mooi River, was selected because of its distance from suspected pollution sources, it had levels comparable to other sites with expected pollution levels (the Vaal and Olifants Rivers). The Mooi River site might have been contaminated by unlawful dumping of burned trash or some other kind of point source pollution. The Mooi River originates in the Drakensberg Mountain range, and airborne pollutants carried from the industrial Gauteng Province might have been deposited on the mountains through the annual winter snowfall, finding their way into the many streams flowing from the mountain range.

The two harbours that were included in the study, namely Saldanha Bay and Richard's Bay, also showed very little PCDD/F and PCB contamination. Again, a single sample from a harbour can never be entirely representative. Samples were collected from the shore, while samples collected by dredging from the bottom of the harbours might present a clearer picture.

The geography of the water bodies was not taken into consideration, except for determining the sediment characteristics of every sediment sample. Factors such as sedimentation rate (in the dams), and flow rate of the various rivers at the point of sampling, nor the specific surface area of the sediment found at each site, as was explained in section 3.3.3 have been considered. Other environmental conditions such as wind direction, rainfall and temperature, that might influence pollution levels at sites, were also not noted, but as this was a cross-sectional survey, it was not deemed necessary.

Furthermore, pollution patterns are not just a function of the composition of the pollutants initially released into the environment: the original pollution profile might be modified by a number of factors as was mentioned in section 5.4.

In spite of the obvious gaps in this study - which is most often the situation with environmental research - it was the first of its kind for South Africa (and Africa) and the study did establish that this country has dioxin-like pollution.

6.3 The way forward for South Africa

Since the completion of this research project for the WRC, a report has been published: "Survey of certain persistent organic pollutants in major South African waters" (WRC Report no 1213/1/05). Due to the results of this study, current research aimed at better understanding the distribution and extent of dioxin-like pollution in the Vaal Triangle (which was one of the most polluted areas in the previous study) is being conducted. A number of rivers draining into the Vaal River were included, as well as fish to determine bio-accumulation. This approach was expanded to also include the aquatic environment of cities such as Cape Town, Bloemfontein, Richard's Bay and Durban. Rivers that cross South Africa's borders into neighbouring countries were also included, because according to South Africa's National Water Resource Strategy (DWAF, 2004), 11 of the 19 water management areas share international rivers, and South Africa shares the responsibility regarding the quality and quantity of these resources. Compounds such as PAHs, short and medium chained chlorinated paraffins and some chlorinated pesticides are now included. The findings of this research have already lead to further, more informed, investigations.

Research such as this, and the capacity to determine TCDD-EQs with the H4IIE bio-assay, contribute towards South Africa's obligations with regards to the Stockholm Convention. The required National Implementation Plan can be formulated, based on scientific knowledge such as the WRC reports and others. A beginning on the NIP has been made during the second half of 2007. UNEP Chemicals has also begun a Global Monitoring Programme (GMP) that intends to provide guidance and support to developing countries and regions with limited POPs monitoring programmes. Participation by South Africa to such an initiative will contribute towards establishing regional monitoring structures (Bouwman, 2004). The Global Environment Facility (GEF) has approved the African Stockpiles Programme (ASP) to rid the continent of more than 50 000 tonnes of obsolete pesticides and contaminated soils associated with these stockpiles within 15 years. (Pesticide stockpiles are known sources of POPs.) South Africa is one of the initial target countries, with the DEAT playing a leading role (Bouwman, 2004).

Although the national priorities of South Africa are HIV/AIDS, poverty, housing and unemployment, its international responsibilities, such as the Stockholm Convention, also have to be obliged.

6.4 The way forward for this research in South Africa

As new information becomes available, the Stockholm Convention can take action and add new chemicals to their list. The POPs Review Committee has already started its activities and candidate chemicals include hexachlorohexane (HCH), perfluorooctane sulphonates (PFOS) and polybrominated diphenyl ethers (PBDEs) (Bouwman, 2004). Knowing this, any future research on POPs in South Africa would need to include these chemicals.

No information is currently available on precisely what happens to dioxin-like chemicals in the South African situation. The fate, distribution and deposition patterns of these chemicals have been well established for the northern hemisphere, but not for sub-Saharan Africa, much less for Southern Africa. South Africa has high temperatures and little precipitation for the greater part of the year; both conditions that would promote volatilisation and long range transport of lesser chlorinated compounds. This would also further OH⁻ breakdown of the compounds in the air.

A component that needs to be addressed would be other possible sources and exposure venues of POPs that are unique to South Africa. An example would be to determine the exposure levels of people who burn trash as fuel for indoor cooking and heating. Another would be to determine the level of dioxin-like pollution produced by the many mining activities in South Africa. Metals such as copper, iron, zinc, aluminium, chromium and manganese are known catalysts for the production of PCDD/Fs (UNEP, 2004) and South Africa is mining all of these, but nothing is known about miners' exposure to dioxins. South Africa is also the world's largest producer of platinum-group metals and dioxins have been measured in a pilot study (Jordaan *et al.*, 2007) on a platinum mine, but more information is needed as to exactly which component(s) of the refining process is/are responsible for the dioxin levels.

From preliminary results of the second WRC project on POPs in the aquatic environment, dioxins seem to be trapped in wetlands, rather than arriving in the sediments of the rivers. In a paper delivered at a local conference by Quinn, Pieters, Kylin and Bouwman (2007) it was indicated that there are high PCDD/F and dioxin-like PCB levels in soils, rather than sediments from rivers and dams. The level of exposure of subsistence farmers through their produce from areas with known dioxin deposition is also unknown. Except for a number of papers on some POPs in bird tissues and birds' eggs (Bouwman, Polder, Venter & Skaare, *in press*; Lötter & Bouwman, 2001; Van Wyk *et al.*, 2001) nothing is known about the levels of POPs in the tissues of other wildlife, terrestrial or aquatic.

These are a few examples of avenues for further research. The sustainability of continued research into POPs, specifically the dioxin-like POPs, in South Africa (and the African

continent) depends on the availability of a well equipped and accredited GC/MS laboratory with well trained staff for the region. This is sadly lacking at this stage. At least the H4IIE-*luc* reporter gene bio-assay is fully functional and operational at the NWU. A step that is included in the standard operating procedure is the treatment of all sediment derived extracts with activated copper to remove sulphur. A viability test, in addition to the microscopic inspection of the cells, has been introduced and is performed simultaneous to the assay, as well. The next step would be participation in international inter-laboratory calibration tests, to standardise the assay and validate its results.

Therefore, this study has shown the presence of measurable quantities of chlorinated dioxins, furans and dioxin-like PCBs in sediments from 22 aquatic sites in South Africa, and that some of these levels are cause for concern. The sources seem to be mainly due to human activities rather than industries, which would guide future research into a better characterisation of the sources, so as to reduce the formation of these compounds, but also to better understand the exposure and risk scenarios, if humans are to be in close contact with these sources. A better characterisation of the sources would also improve the global understanding of POPs contribution from the southern hemisphere.

To achieve a better living environment for South Africans, as well as a cleaner environment, more research, based in part on my findings, and supported by an improved analytical infrastructure, would be required.

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