CHARACTERIZATION OF POLYCHLORINATED BIPHENYL RESIDUES IN THE NORTH END LAKE AND PORT ELIZABETH HARBOUR, SOUTH AFRICA

By

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Submitted in fulfilment of the requirements for the degree of Philosophiae Doctor in the Faculty of Science at the Nelson Mandela Metropolitan University

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DECLARATION

I, *Edwige Kampire* (212438883), hereby declare that the *thesis* for *Philosophiae Doctor* is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

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DEDICATION

“To my husband Eugène Nzambitare and our children Nice Ukeye Nzambitare and Nick Nzambitare for their unfailing love, encouragement, advice and sacrifice during the completion of this study”

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ABSTRACT

Persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs) are widespread in aquatic systems, they can bioaccumulate in the tissues of aquatic organisms, especially fish as they occupy a position near the top of the aquatic food web. PCBs are among the most toxic substances and have been shown to cause many adverse effects to humans and wildlife. High health risks are associated with populations that follow a diet high in fat content such as fish and shellfish in which PCBs bioaccumulate. Given the importance of industries and the potential health concerns of exposure to PCBs, the lack of information on environmental levels of PCBs in South Africa (SA) is significant and concerning. Less attention has been directed to analysis of PCBs in the South African environments due to high cost and lack of appropriate equipment. This study was the first conducted in order to assess the levels of PCBs in the environment of Nelson Mandela Bay Municipality (Port Elizabeth). The research was directed at determining the PCB levels in water, sediments, fish and mussels collected in the Nelson Mandela Bay Municipality. The sampling sites, North End Lake (NEL) and Port Elizabeth Harbour (PEH) were selected based on their location and the importance of activities taking place in these areas. In total 456 samples of water (dissolved and particulate phases), sediments, mussels and tissues of fish were analysed. Suitable analytical methods were based on the equipment and materials available at the Nelson Mandela Metropolitan University. Gas chromatography-mass spectrometry (GC/MS) was used to quantify PCBs in the samples using the internal standard method. Six indicator congeners (PCB nos. 28, 52, 101, 138, 153, 180) were chosen because they are mainly present in most PCB mixtures in environmental samples and they are recommended for regular monitoring. These congeners represent about 50% of the total non-dioxin like (NDL) PCBs in food. All these congeners were detected in the samples analysed. The total PCB concentrations in the sediments from the NEL and PEH ranged from 1.60 to 3.06 and from 0.56 to 2.35 ng g^{-1} dry weight, respectively. The concentrations of total PCBs in *Mytilus galloprovincialis* ranged from 14.48 to 21.37 ng g^{-1} wet weight and from 20.84 to 31.34 ng g^{-1} wet weight in *Perna perna*. Total PCBs in water ranged from 0.18 to 0.355 ng L^{-1}. The concentrations of total PCBs in the liver, gonads, gills and muscle were 95.69, 57.49, 44.63, 34.14 ng g^{-1} lipid weight in *Cyprinus carpio* and 119.73, 59.21, 49.78, 34.63 ng g^{-1} in *Oreochromis*

mossambicus, respectively. Fish liver was the most contaminated organ due to its high lipid content compared to other parts of fish analysed. The NEL was found to be more contaminated than the PEH. The main potential sources of PCB pollutants in both areas of this study are industrial and municipal discharges. The NEL is surrounded by many industries and serves as a potential pollutant sink due to wastewater and inflow entering into this lake. Four 0.227 kg meals of the edible part of fish (muscle) per month were recommended based on the non-cancer health endpoint and one 0.227 kg meal per month was recommended based on the cancer health endpoint. This research contributed to notify the public and relevant governmental departments on the PCB pollution status of sediments, water and aquatic life in the PEH and NEL.

Keywords: PCBs, tissues, mussels, sediments, dissolved phase, bioaccumulation, GC/MS, biodegradation, adverse human health effects.

ACRONYMS AND ABBREVIATIONS

AAP : American Academy of Pediatrics

ADI : acceptable daily intake

Ah : aryl hydrocarbon

AhR : aryl hydrocarbon receptor

ANOVA : Analysis of variance

ASE : Accelerated solvent extraction

ATSDR : Agency for Toxic Substances and Disease Registry

BW : body weight

CAS : Chemical Abstract Service

CB : chlorobiphenyl

CBA : Chlorobenzoic acid

CCREM : Canadian Council of Resource and Environment

CEPA : Canadian Environmental Protection Act

CF : condition factor

CI : chemical ionization

CR : consumption rate

CSF : cancer slope factor

CSIR : Council of Scientific and Industrial Research

dc : direct current

DCM : dichloromethane

DDT : dichlorodiphenyltrichloroethane

DEAT : Department of Environmental Affairs and Tourism

DL-PCBs : dioxin-like PCBs

dw : dry weight

EC : European Commission

ECD : electron capture detector

ECNI : electron negative capture ion

EFSA : European Food Safety Authority

EI : electron ionization

EIP : Entrepreneurship and Innovation Programme

ELISA : Enzyme-linked immunosorbent assay

EPA : Environmental Protection Agency

eV : electron volt

FDA : Food and Drug Administration

FID : flame ionisation detector

FIFA : International Federation of Association Football

GC : gas chromatography

GC/MS : gas chromatography/mass spectrometry

GPC : Gel permeation chromatography

GPS : global positioning system

HBCD : hexabromocyclododecane

HCB : hexachlorobenzene

HPLC : high-performance liquid chromatography

HRGC/HRMS: high-resolution gas chromatography/high-resolution mass

IUPAC : International Union of Pure and Applied Chemistry

K_{OW} : Octanol water partition coefficient

LLE : liquid-liquid extraction

LOD : Limit of detection

LOI : loss on ignition

LOQ : Limit of quantification

LRMS : low resolution mass spectrometry

lw : lipid weight

m/z : mass-to-charge ratio

MAE : microwave-assisted extraction

MDLs : maximum detection limits

$MeSO_2$: methylsulfonyl

MS : mass spectrometry

MSD : mass spectrometer detector

MSPD : matrix solid-phase dispersion

NCI : negative chemical ionisation

NDL-PCBs : non-dioxin-like PCBs

NEL : North End Lake

NEMA : National Environmental Management Act

NIOSH : National Institute of Occupational Safety and Health

NIP : National Implementation Plan

NMMU : Nelson Mandela Metropolitan University

NOAA : National Oceanic and Atmospheric Administration

OCPs : organochlorine pesticides

OEHHA : Office of Environmental Health Hazard Assessment

OH-PCBs : hydroxylated polychlorinated biphenyls

Pa : pascal

PAHs : polycyclic aromatic hydrocarbons

PBBs : polybrominated biphenyls

PCBs : polychlorinated biphenyls

PCDDs : polychlorinated dibenzo-p-dioxins

PCDFs : polychlorinated dibenzofurans

PCI : positive chemical ionization

PCNs : polychlorinated naphthalenes

PCTs : polychlorinated terphenyls

PEH : Port Elizabeth Harbour

PEN : PCB Elimination Network

PFOS : perfluorooctane sulfonyl fluoride

POPs : persistent organic pollutants

QA/QC : quality assurance/quality control

RF : response factor

RfD : reference dose

RSD : relative standard deviation

RT : retention time

SD : standard deviation

SFE : supercritical fluid extraction

SIM : selected ion monitoring

SOPs : Standard operating procedures

SPE : solid phase extraction

SPMDs : semi-permeable membrane devices

TCDD : tetrachlorodibenzodioxin

TC-*m*-X : tetrachloro-*meta*-xylene

TEQ : toxic ecological quotient

TEF : toxic equivalency factor

TSCA : Toxic Substances Control Act

UNEP : United Nations Environment Program

USA : United States of America

USDA : United States Department of Agriculture

USE : ultrasonic extraction

USEPA : United States Environmental Protection Agency

UV : ultra-violet

WHO : World Health Organization

ww : wet weight

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LIST OF PAPERS AND PRESENTATIONS

This thesis is based on the papers and presentations listed below.

1. **Kampire, E**., Rubidge, G., & Adams, J.B. (2015). Distribution of polychlorinated biphenyl residues in sediments and blue mussels (*Mytilus galloprovincialis*) from Port Elizabeth Harbour, South Africa. *Marine Pollution Bulletin* 91(1): 173–179. (Chapter 7).

2. **Kampire, E**., Rubidge, G., & Adams J.B. (2015). Distribution of polychlorinated biphenyl residues in several tissues of fish from the North End Lake, Port Elizabeth, South Africa. Water SA 41(4): 559–570. (Chapter 4).

3. **Kampire, E**., Rubidge, G., Adams J.B. & Human, L. Inflow and outflow water concentrations and congener profiles of PCBs and the effect on marine mussels at an outfall site (Manuscript No. 3266 submitted to Water SA, 2015). (Chapter 6).

4. **Kampire, E**., Rubidge, G., Adams, J.B. Characterization of polychlorinated biphenyls (PCBs) in surface sediments of the North End Lake, Port Elizabeth in South Africa (Manuscript). (Chapter 5).

Conference proceedings

Polychlorinated biphenyl residues in fish and sediments of the North End Lake and Port Elizabeth Harbour. South African Chemical Institute (SACI), 41st National Convention, East London, South Africa. December 2013. Poster.

CHAPTER 1

GENERAL INTRODUCTION

“No matter how old we are, where we live, or what we do, none of us can escape exposure to man-made chemicals in our everyday lives that threaten our health. We are exposed through food and water, through cosmetics that we rub into our skin and through the fumes from cleaning products and polluted city air. The unborn child and children are most at risk because they have fewer defences and longer periods of life ahead of them in which cancer and other health problems may emerge” (Veillerette and Jensen, 2012).

1.1 PREAMBLE

Chemicals are important determinants used worldwide especially in industries to sustain development. The supply of food has become more plentiful and the occurrence of insects, fungi, rodents or other animals living in or on the crops has been dramatically reduced by the use of herbicides and pesticides (Barr and Needham, 2002). Industrialization has contributed to quality of life; such as increase of personal incomes, health, greater social wealth, and services, particularly transport and communications. However, industrial activities carry the risk of adverse health consequences for the workforce and the general population, either directly, through exposure to harmful agents or practices, or indirectly, through environmental degradation. Industrial emissions and products also threaten the global environment.

Some chemicals known as persistent organic pollutants (POPs), including industrial chemicals such as polychlorinated biphenyls (PCBs), may become environmental contaminants through their use and application. POPs have been the subject of internationally agreed protocols to ensure that their impact on humans and the environment are minimized. More specifically, PCBs were first introduced in 1929 (Jensen, 1972), and they became the most widespread environmental contaminants found in air, water, sediments, and soils around the globe (Marku et al., 2011). Monsanto Corporation was the principal U.S. manufacturer of PCBs from 1929 until Congress enacted the Toxic Substances Control Act (TSCA) in 1976, which prohibited the manufacture, processing and distribution of PCBs (Erickson, 1997).

No natural sources of PCBs are known, and they are therefore exclusively anthropogenic. Current atmospheric levels of PCBs in the environment are primarily due to production and disposal, or accidental releases of products or materials containing PCBs, volatilisation from environmental reservoirs or *via* the combustion processes of some congeners (Breivik et al., 2002). The primary sources of PCB exposure are from the environment and the workplace. Breathing indoor air and consuming food contaminated with PCBs have been identified as major sources of exposure (U.S.EPA, 1997). PCBs exhibit several qualities including non-flammability, chemical stability, high boiling point, and electrical insulation which made them ideal for use in a multitude of products over the years. PCBs were used worldwide in industrial and commercial applications, including electrical equipment (transformers and capacitors), heat transfer, and hydraulic equipment; as plasticizers in paints, plastics, and rubber products; in pigments, dyes, and carbonless copy paper; and many other industrial applications (ATSDR, 2000). However, PCBs in electrical equipment are considered as a priority source of environmental pollution due to leaks from operating installations, storage or disposal in several emission inventories.

In South Africa, chemicals have been used extensively by various sectors for many years; however PCBs are not produced in South Africa. PCB oils and equipment containing PCB oils were imported mainly for electricity generation use (NIP, 2011). Owing to the toxicity, bioaccumulation and other dangers, the intentional production of PCBs is now banned globally under the Stockholm Convention of 2001 (UNEP, 2006). However, because of human activities and the chemical characteristics of these substances, they can still be found in the environment and continue to pose potential health risk to humans, marine ecosystems, and wildlife all over the world. PCBs enter the environment from point sources, such as runoff from landfills, discharge of industrial wastes, leaks and spills from PCB devices, and through global transport. Once in the environment, they do not freely break down and, therefore, may remain for long periods of time cycling between air, water, and soil. PCBs have been shown to exhibit negative effects on wildlife; some PCBs have been shown to bioaccumulate through food chains resulting in high concentrations in top predators (Ross and Birnbaum, 2003).

PCB contamination was first recognized more than 45 years ago by Sören Jensen (1966), who detected high levels of PCBs in a sea eagle found dead in the Stockholm archipelago in Sweden. Since that period, they have been identified in almost every compartment of the global ecosystem. Most human exposure to PCBs comes from the diet, absorption through the skin, and inhalation. Intake of PCBs through food is estimated to account for more than 90% of the total human exposure (Ribeiro et al., 2008). PCBs are slowly metabolized in the body, and with time the level of these contaminants can rise in fatty tissue. Exposure to PCBs has been reported to cause adverse effects on immune, reproductive, and nervous systems, while long-time exposure to some congeners also affects liver function and may lead to developmental effects resulting in cancer (WHO, 2003). Negative associations between prenatal PCB exposure and cognitive functioning and motor development in childhood have been reported (Schantz et al., 2003). In addition, in humans, chronic exposure to certain POPs *via* food chains has led to the accumulation of both parent compounds and its metabolites in lipid rich tissues, such as adipose tissues (Minh et al., 2004). Such compounds and metabolites have also been detected in human breast milk (Minh et al., 2004). Humans and wildlife can be exposed to PCBs either directly from contact with contaminated air, sediments, and water or indirectly through the diet.

1.2 MOTIVATION AND AIM OF THE STUDY

The presence of anthropogenic pollutants throughout all parts of the aquatic environment has been of national and international concern for a number of decades. PCBs, one of the most toxic and widespread compounds in the aquatic environment, can have adverse effects on humans and wildlife. Most PCBs may persist for years in sediments and can be bioaccumulated by aquatic and terrestrial organisms, and can enter the food web. Consumption of PCB-contaminated foods is the most significant route of exposure to PCBs for the general human population (Fitzgerald et al., 1996). Pollution of the environment poses a threat to the health and wealth of every country. Consequently, it is essential to monitor the levels of organic pollutants in the environment.

In South Africa, less information is available on the concentrations and types of organic pollutants in the South African environment (Wepener and Degger, 2012) due to the high cost and lack of sufficient modern equipment (DEAT, 2008). Research conducted by Grobler et al. (1996) on PCBs in the Isipingo Estuary, and De Kock and Randall (1984) in the Wilderness Lakes, confirmed the presence of PCBs in South African aquatic environments. No studies conducted between 1960 until the present have focused on analysis of different tissues of fish from the North End Lake. In other areas, the muscle tissue was mostly the only target tissue analysed and PCBs were reported mostly on Aroclor which is the common name representing mixtures of PCBs (Breivik et al., 2002). Aroclor may contain up to 157 congeners (Frame et al., 1996).

This project is the first to characterize and evaluate the distribution of six indicator PCB congeners in several tissues of fish (liver, muscle, gills and gonad), sediments, water and mussels from the North End Lake and Port Elizabeth Harbour within the Nelson Mandela Bay Municipality. Fish can be, and often are entirely consumed, particularly those with small size. This poses a problem since some organs are highly contaminated by PCBs (OEHHA, 2003). It is in this regard that the quantitative and qualitative analysis of PCBs in tissues of fish is of interest. The six indicator PCB congeners represent about 50% of the total non-dioxin-like (NDL) PCBs in food, and they are recommended for regular monitoring (EFSA, 2010). PCBs include 197 non-dioxin-like and 12 dioxin-like (DL) PCBs. The NDL-PCBs are referred to as the “non-coplanar” or “ortho-substituted” congeners while the dioxin-like PCBs are non- or mono-*ortho* substituted congeners (Henry and De Vito, 2003). Some NDL-PCBs have been shown to elicit different types of health responses than the dioxin-like PCBs. According to Cimenci et al. (2013), some indicator congeners exhibit specific health effects such as neurological (PCBs 180 and 138), neuroendocrinological (PCBs 101, 138, and 153), immunological (PCBs 101, 153, 180) and carcinogenic problems (PCBs 180, 153).

So far, no work has been done on possible PCB contamination in the North End Lake. Possible sources of PCBs in this lake include waste discharges from industries and stormwater runoff from residential areas. Therefore, inflow water discharged into the lake through runoff and effluents were sampled and analysed. Lake water as well

as outflow of this lake into the Indian Ocean were analysed. The water from the North End Lake is treated and utilised for irrigating green areas in the Nelson Mandela Bay Soccer stadium. Repeated watering of the grass with PCB contaminated water could result in bioaccumulation of these compounds in the sports field inside the stadium. PCBs are attached strongly to organic particles in the water column (Beyer and Biziuk, 2009), they thus ultimately settle out in bottom sediments. As the bottom-dwelling organisms feed, they could ingest and accumulate PCBs from the contaminated sediments, and hence entering them into the food chain.

The analysis of PCBs in sediments was proposed since they can be a source of contamination of aquatic organisms. Fish consumption is a potential route of exposure for environmental contaminants, and their dietary uptake has contributed significantly to increasing human health risks (Turyk et al., 2006). The public of Port Elizabeth are unaware of the potential impact of PCBs in seafoods. The aim of this project was to analyse PCB congeners both quantitatively and qualitatively in the organs of fish, water, and sediments of the North End Lake, mussels at the point source of the lake outfall into the Indian Ocean as well as the sediments and mussels within the Port Elizabeth Harbour. Sediments and mussels were analysed to verify if the PCB levels would pose potential risks to human health. Thereafter, it would be important to notify the public and relevant governmental departments as to the quality of water and aquatic life, particularly fish as nutritional food, as the North End Lake is targeted for recreational uses.

1.3 HYPOTHESES AND OBJECTIVES OF THE STUDY

The overall hypothesis of this study is the following:

The North End Lake (NEL) and Port Elizabeth Harbour (PEH) are contaminated by PCBs.

To address the overall hypothesis, this project was divided into four objectives with their respective hypotheses. Each objective has been carefully chosen in order to answer the overall hypothesis.

- Objective 1. To investigate the occurrence and distribution of PCBs in several tissues of fish.

    - Hypothesis 1.1. The fish from the North End Lake are contaminated by PCB compounds.
    - Hypothesis 1.2. The fatty tissue of fish is the most contaminated organ with PCBs.
    - Hypothesis 1.3. PCB concentrations were expected in tissues of fish due to transfer of PCBs from water and sediments in fish.

- Objective 2. To characterize and evaluate the PCB levels in sediments from the NEL.

    - Hypothesis 2.1. The North End Lake surficial sediment is contaminated with PCBs.

- Objective 3. To investigate the levels of PCBs in water of the NEL and PCB levels of brown mussels from the discharge point of the North End Lake's overflow into the Indian Ocean.

    - Hypothesis 3.1. The North End Lake water is contaminated by PCBs from runoff and other waste discharges from the industries and residential areas.
    - Hypothesis 3.2. PCB levels in mussels from the outfall of the NEL into the Indian Ocean are higher than harbour mussels due to the transfer of PCBs from the NEL to these mussels.

- Objective 4. To assess the PCB contamination levels of biota (blue mussels) and sediments from PEH.

    - Hypothesis 4.1. Mussels from PEH are contaminated by PCBs
    - Hypothesis 4.2. The PEH sediments are contaminated by PCBs.

1.4 OUTLINE OF THE STUDY

The thesis was divided into 8 chapters: **Chapter 1** provides an introduction to the proposed project and includes the need of conducting such an investigation. **Chapter 2** provides an overview of PCBs in the environment as a background to understanding their history of use, sources of input into the environment, distribution in the environment, and their toxic effects to humans and other organisms. **Chapter 3** deals with an overview of the methodology for PCB determination in environmental matrices. Different techniques used while performing PCB analysis in the present study for the determination of indicator PCB congeners in tissues of fish (liver, muscle, gills, and gonads), sediments, water and mussels are also given in this chapter. Chapters **4, 5, 6 and 7** present the main findings of the analysed samples. **Chapter 4** assesses the distribution of PCBs in muscle, gonad, liver and gills of fish from the North End Lake; it addresses objective 1 and hypotheses 1.1 and 1.2 and 1.3. **Chapter 5** investigates PCB levels in surface sediments from the North End Lake and addresses the objective 2 and hypothesis 2.1. **Chapter 6** focuses on the investigation of the levels of PCBs in water from the North End Lake and PCB levels of brown mussels *(Perna perna*) from the discharge point of the North End Lake's overflow into the Indian Ocean. In this chapter, the objective 3 and hypotheses 3.1 and 3.2 are addressed. **Chapter 7** reports PCB residues in sediments and blue mussels (*Mytilus galloprovincialis*) from Port Elizabeth Harbour where objective 4, hypotheses 4.1 and 4.2 are discussed. **Chapter 8** gives conclusions and recommendations for future research. Supplementary information is given in the appendices.

CHAPTER 2

OVERVIEW OF POLYCHLORINATED BIPHENYLS (PCBs): CHEMISTRY, PROPERTIES, TOXICITY AND HEALTH EFFECTS

2.1 BRIEF HISTORY OF POLYCHLORINATED BIPHENYLS (PCBs)

Over the past several decades PCBs have been classified as chemicals belonging to the most persistent, bioaccumulative, and toxic pollutants that have been identified worldwide in diverse environmental matrices (Hu et al., 2010a; Cimenci et al., 2013; Gdaniec-Pietryka et al., 2013). First discovered in the late nineteenth century, PCBs began to be widely produced and used commercially since 1929 in industry as heat transfer fluids, dielectric fluids for transformers and capacitors, adhesives, plasticizers in paints, plastics and sealant due to the high stability and excellent electrical insulation (Erickson and Kaley II, 2011). Globally 1.3 million tonnes of PCB compounds have been produced in the U.S. between 1929 and 1977 (Breivik et al., 2007; Cave et al., 2010).

In the mid-1960s, the first environmental occurrence of PCBs was reported by Jensen who discovered PCB compounds while analysing the insecticide dichloro-diphenyl-trichloethane (DDT) in environmental samples (Jensen, 1966). Two years later, in Japan (Yusho), an incident occurred and was attributed to the consumption of rice oil contaminated with PCBs from leakage of food-processing equipment. A similar incident happened in 1979 in Taiwan (Yucheng). The findings showed that adverse health effects accredited to PCBs were observed in those people who had consumed the rice oil as well as the children later born to women who had consumed the contaminated rice oil during their pregnancies. Subsequent studies of Kuratsune et al. (1987); Safe (1994) and others indicated that exposure to PCBs are linked to many adverse effects and can damage immune, reproductive, nervous and endocrine systems, and cause hepatotoxicity leading to cancer and other possible threats to humans and environment. Therefore, in 1979, the United States Congress banned its production in the U.S. and the intentional production and use of PCBs were banned globally under the Stockholm Convention of 2001. However due to the high stability, bioaccumulation and the potential ability of PCB compounds to be transported through trophic chains, they can still be found in the environment (El-

Shahawi, 2010; Nikonova and Gorshkov, 2011). Recent studies proved that PCBs can be detected in almost every component of the global ecosystem including water, air, soil, aquatic and marine sediments, fish, wildlife, domestic animals, human blood, adipose tissue and milk (van den Brink et al., 2000; Banudevi et al., 2006). The findings of the above-mentioned authors showed that PCBs are broken down slowly into the environment and can still be produced from energy production, combustion from industries, production processes, and waste (landfill, incineration, waste treatment, and disposal) (Urbaniak, 2007; Webster et al., 2013; Kumar et al., 2013). Bioaccumulation and biomagnification of PCB compounds through the food chain lead to their occurrence in aquatic ecosystems. Consequently, animals at the top of the food chain, such as whales, dolphins and humans, can store PCBs at highly concentrated levels.

2.2 CHEMISTRY AND PROPERTIES OF PCBs

2.2.1 The chemistry of PCBs

PCBs are synthetic chlorinated compounds formed by two covalently linked phenyl rings with single carbon-carbon bond. The empirical formula of PCBs is $C_{12}H_{10-n}Cl_n$ (n = 1- 10). Chlorines can be substituted for hydrogen at the 10 possible sites on the biphenyl rings and 209 possible congeners can be produced by varying the degree of substitution of chlorine atoms around the biphenyl (Figure 2.1) (Wiegel and Wu, 2000). The positions 2, 2'; 6, and 6'; 3, 3', 5, and 5'; 4 and 4' are called *ortho, meta*, and *para*, respectively (Figure 2.1). The PCB congeners without chlorine atoms at the *ortho* positions can assume a coplanar conformation and have dioxin-like activity. The dioxin-like PCBs (DL-PCBs) have toxicological properties similar to dioxins and are therefore called dioxin-like (Viluksela et al., 2012) (see section 2.3.2). PCBs with chlorine at *ortho* positions are referred to non-dioxin-like PCBs (NDL-PCBs). There are 12 co-planar (DL-PCBs) congeners and 197 non-planar (NDL- PCBs).

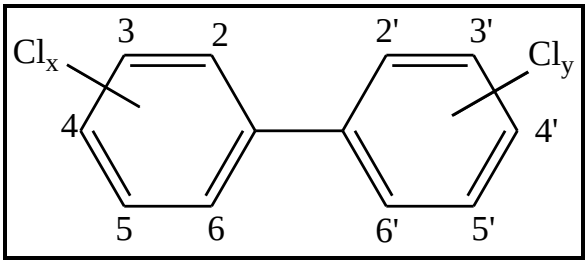


Figure 2.1: Chemical structure of PCB molecule.

The 209 congeners can be subdivided by their degree of chlorination, resulting into ten groups named “homologs”. PCBs of a given homolog having the same number of chlorine atoms are called “isomers” and PCBs with different chlorine substitution positions are called congeners (Pellequer et al., 2005). Figure 2.2 showed two isomers 2, 3, 5-trichlorobiphenyl and 3, 3’, 4-trichlorobiphenyl of trichlorobiphenyl homolog.

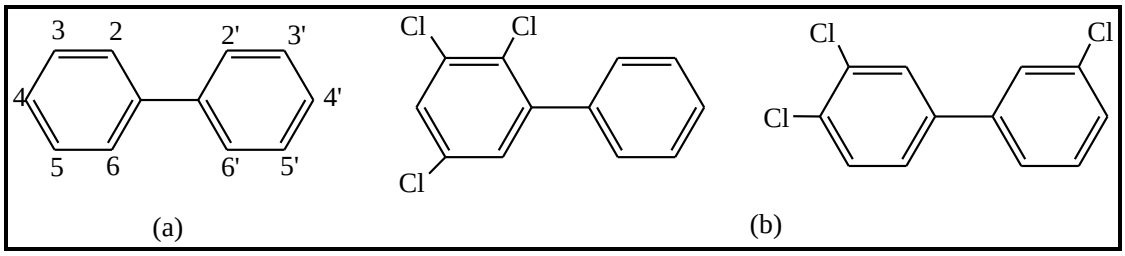


Figure 2.2: Distribution of chlorine atoms in the two rings of biphenyls: (a) possible chlorine substitution positions; (b) examples of two isomers (2, 3, 5-trichlorobiphenyl and 3, 3', 4-trichlorobiphenyl).

PCBs are numbered according to the International Union of Pure and Applied Chemistry (IUPAC) which specifies the sites where chlorines are attached to the two benzene rings. The order for assigning priorities to the chlorine substituent in the PCB ring is (i) unprimed number is assigned lower order than the corresponding primed number, as 2 vs. 2’; (ii) lower number is assigned to a point of attachment in equivalent position, as 2 vs. 6, for a substituent in one of the ortho positions; (iii) when the number of substituents in the two ring systems is the same, unprimed numbers are assigned to the ring system with smaller numbered substituents.

Ballschmiter and Zell (1980) established a simple nomenclature of PCBs based on the congener’s chlorination (Table 2.1). This nomenclature was adopted by IUPAC and distinct numbers from 1 to 209 were assigned to each PCB congener. A list of

all 209 PCB congeners, including IUPAC name, IUPAC No., CAS No. and octanol-water coefficient (log K_{ow}) are provided in Appendix 2.

Table 2.1: IUPAC nomenclature of PCBs

IUPAC name	No. of isomers	Molecular weight (g mol^{-1})	Cl (% w/w)
Monochlorobiphenyl	3 (1 - 3)	188.7	19
Dichlorobiphenyl	12 (4 – 15)	223.1	32
Trichlorobiphenyl	24 (16 – 39)	257.6	41
Tetrachlorobiphenyl	42 (40 – 81)	292.0	49
Pentachlorobiphenyl	46 (82 – 127)	326.4	54
Hexachlorobiphenyl	42 (128 – 170)	360.9	59
Heptachlorobiphenyl	24 (171 – 193)	395.3	63
Octachlorobiphenyl	12 (194 – 205)	429.8	66
Nonachlorobiphenyl	3 (206 – 208)	464.2	69
Decachlorobiphenyl	1 (209)	498.7	71
All PCBs	209	-	-

w: weight

2.2.2 Dioxin-like and non-dioxin-like PCBs

PCBs are divided into two groups based on their structural characteristics and toxicological effects: the “DL-PCBs” (coplanar with non- or mono-*ortho* substitution PCBs) and “NDL-PCBs” (non-coplanar with di-*ortho* chlorine substitution). The DL-PCBs have a minimum of four chlorines in the lateral positions (i.e., 3, 3', 4, 4', 5, 5') and none (non-) or only one (mono-) of the *ortho* positions (i.e., 2, 2', 6, or 6') of the biphenyl (Table 2.2). The DL-PCBs elicit a dioxin-specific toxicity and the NDL-PCBs elicit broad health effects (Henry and DeVito, 2003). Figure 2.3 shows the planar spatial orientation of DL-PCB 126 (a), and the non-planar spatial orientation of NDL-PCB180 (b) (Antunes Fernandes, 2011).

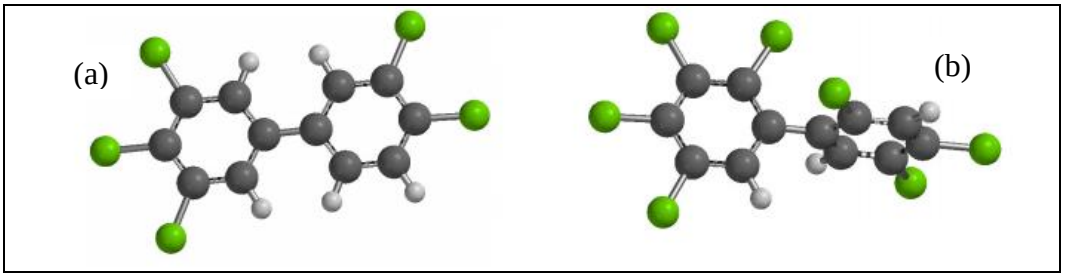


Figure 2.3: Spatial orientation of DL-PCB 126 (a) and NDL-PCB 180 (b): carbon atoms (dark grey spheres), hydrogens (light grey spheres) and chlorine (green spheres).

Recently, (Kim et al., 2010; Eichbaum et al., 2014) indicated that the DL-PCBs exhibit their toxic effects through binding and activation of aryl hydrocarbon receptor (AhR), a ligand transcription factor that controls the expression of a diverse set of genes. In addition, DL-PCBs have been assigned an international toxic equivalent factor (TEF) relating their toxicity to the most toxic compound: 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Van den Berg et al., 2006). AhR controls the enzyme production and hormone regulation especially the metabolism of the cytochrome P450 enzyme. The eradication of foreign compounds (xenobiotics) such as POPs, drugs and other toxins from the body is an essential process designed to protect the potential toxicity from the foods. Therefore, the cytochrome P450 is responsible for the biotransformation of several toxins including PCBs. If DL-PCBs bind to AhR, the metabolism of the cytochrome P450 enzyme can be induced. Even if there are no given TEF values to NDL-PCBs, they can induce adverse health effects. The Ah-receptor are found in many animal species, including humans. DL-PCBs are among the most toxic PCB congeners, they exhibit toxic effects at relatively lower concentrations than those of NDL-PCBs (Giesy and Kannan, 1998). However, NDL-PCBs are commonly found in blood and tissues of humans, wildlife and fish at much higher levels than the DL-PCBs (Storelli and Perrone, 2010). Therefore, it is difficult to distinguish the effects of NDL-PCBs from DL-PCBs because all are often present in technical mixtures.

Table 2.2: DL-PCBs and their recommended toxic equivalency factors (TEFs)

IUPAC No.	Homolog group	Substitution group	IUPAC name	WHO_{1998}-TEF	WHO_{2005}-TEF
non-*ortho* substituted PCBs					
77	Tetra-CB	non-ortho	3, 3', 4, 4'-tetra-CB	0.0001	0.0001
81	Tetra-CB	non-ortho	3, 4, 4', 5-tetra-CB	0.0001	0.0003
126	Penta-CB	non-ortho	3, 3', 4, 4', 5-penta-CB	0.1	0.1
169	Hexa-CB	non-ortho	3, 3', 4, 4', 5, 5'-hexa-CB	0.0001	0.03
mono-*ortho* substituted PCBs					
105	Penta-CB	mono-ortho	2, 3, 3', 4, 4'-penta-CB	0.0001	0.00003
114	Penta-CB	mono-ortho	2, 3, 4, 4', 5-penta-CB	0.0005	0.00003
118	Penta-CB	mono-ortho	2, 3', 4, 4', 5-penta-CB	0.0001	0.00003
123	Hexa-CB	mono-ortho	2, 3', 4, 4', 5'-penta-CB	0.0001	0.00003
156	Hexa-CB	mono-ortho	2, 3, 3', 4, 4', 5-hexa-CB	0.0005	0.00003
157	Hexa-CB	mono-ortho	2, 3, 3', 4, 4', 5'-hexa-CB	0.0005	0.00003
167	Hexa-CB	mono-ortho	2, 3', 4, 4', 5, 5'-hexa-CB	0.00001	0.00003
189	Hepta-CB	mono-ortho	2, 3, 3', 4, 4', 5, 5'-hepta-CB	0.0001	0.00003

Sources: (Giesy and Kannan, 1998; van den Berg et al., 2006).

CB: chlorobiphenyl.

2.2.3 Physical and chemical properties of PCBs

PCBs are complex mixtures and their properties depend on the individual congeners that constitute the mixtures. The toxicity of PCBs increases as the number of chlorine atoms increases. This results in a change of physical properties throughout the ten PCB homolog groups as shown in Table 2.3 (Erickson, 1997). At room temperature, PCBs vary in physical state from colourless or slightly yellowish, oily liquids for the lower chlorinated compounds, to more viscous and increasingly darker liquids, to yellow and then black resins for the most highly chlorinated types. The water solubility and vapour pressures decrease with increasing degree of chlorination. An investigation of Loganathan and Kannan (1994) revealed that the water solubilities of monochlorobiphenyl congeners are in the range of 1 to 5 g L^{-1}, while that of decachlorobiphenyl is 0.015 mg L^{-1}. Further studies of Zhang et al. (2013); Salem et al. (2014) showed that most PCBs are soluble in organic solvents, oils, and fats and the bioaccumulation property is observed for POP compounds with Log K_{OW} values > 3. The high thermal and chemical resistance; non-flammability and chemical inertness of PCBs means that they do not easily break down when

exposed to heat or chemical treatment (Watnick, 2010). Therefore, these properties lead to increase the probability of PCB compounds in soils, sediments and biota (Covaci et al., 2005) which in turn are bioconcentrated and transferred upwards in the food chains. PCBs are recalcitrant compounds in aquatic systems which mean that even if PCBs are banned globally by the Stockholm Convention, the major source of exposure to PCBs today is the redistribution of PCBs already present in soil/sediment and water.

Table 2.3: Physico-chemical properties of PCB homolog groups

Homolog	Melting point (°C)[a]	Boiling point (°C)[a,b]	Vapour pressure (Pa)[b]	Aqueous solubility (g/m3)	Log K_{OW}[b]	Log K_{OC}
Biphenyl	71	256	4.9	9.3	4.3	3.4
Mono-CB	25 – 77.9	285	1.1	14.0	4.7	3.47
Di-CB	24 – 149	312	0.24	1.6	5.1	3.62
Tri-CB	28 – 87	337	0.054	0.65	5.5	3.9
Tetra-CB	47 – 180	360	0.012	0.26	5.9	4.81
Penta-CB	76.5 – 124	381	2.6×10^{-3}	0.099	6.3	4.98
Hexa-CB	77 – 150	400	5.8×10^{-4}	0.038	6.7	6.08
Hepta-CB	122.4 – 149	417	1.3×10^{-4}	0.014	7.1	6.22
Octa-CB	159 – 162	432	2.8×10^{-5}	5.5×10^{-3}	7.5	6.56
Nona-CB	182.8 – 206	445	6.3×10^{-6}	2.0×10^{-3}	7.9	7.1
Deca-CB	305.9	456	1.4×10^{-6}	7.6×10^{-4}	8.3	-

Sources: Erickson, 1997; Mills, 2001**;** [a]Average properties of all isomers in group; [b]Mean value for liquid; K_{OC} = (K_d * 100)/% organic carbon; K_d is the adsorption coefficient, it measures the amount of chemical adsorbed onto soil per amount of water.

2.3 PRODUCTION AND USE OF PCBs

Prior to the public concern about environmental occurrence of PCBs, they were produced since 1929 and were marketed worldwide under various trade names: Aroclor (Monsanto, United States), Clophen (Bayer, Germany), Phenoclor (Caffaro, Italy), Pyralene (Prodelec, France), Kanechlor (Kanegafushi, Japan), and Sovol (Russia) (Metcalfe and Haffner, 1995; ATSDR, 2000). PCBs were produced until 1977 in the United States, and until the 1990's in some developing countries (Mills, 2001). Industrial synthesis of PCBs was carried out by direct chlorination of biphenyl with chlorine gas (Figure 2.4). In North America, the Monsanto Industrial Chemical

Corporation (St. Louis, Missouri, USA) was the primary producer of PCBs (Aroclor). Table 2.4 provides information on the composition of the six indicator congeners in various commercial PCB mixtures.

Table 2.4: Composition of six indicator PCBs in various Aroclors (% ww)

PCBs	Aroclor 1016	Aroclor 1242	Aroclor 1248	Aroclor 1254	Aroclor 1260
PCB 28	8.50	6.86	3.59	0.06	0.19
PCB 52	4.63	3.53	6.93	0.83	5.38
PCB 101	0.04	0.69	2.22	5.49	8.02
PCB 138	-	0.10	0.38	5.95	5.80
PCB 153	-	0.06	0.23	3.29	3.77
PCB 180	-	-	0.02	0.42	0.67

The two main sources of PCBs are commercial production, and by-products in combustion processes as thermodynamically stable compounds. Until production ceased, a large amount of PCBs were produced world-wide between 1929 and 1977 but most of the PCBs were produced between 1950 and 1983 (WHO, 2003). The global official use of PCBs ranged from 1.2 to 1.5 million tonnes. However, Urbaniak (2007) indicated that this value is underestimated relative to the true value due to unknown and illegal production. Fiedler (2001) reported that about 2.0 million tonnes have been produced and used worldwide since 1930 while Holoubek (2000) assumed that this amount was about 1.2 million tonnes globally. In addition, EFSA (2011) indicated that an estimated total world production of 1.2 - 1.5 million tonnes of PCBs was massively produced from 1929 until they were banned. In general, most of PCBs produced were not pure compounds but were mixtures containing diverse isomers. Some reports showed that during the PCB production, Aroclor mixtures were contaminated by small amounts of polychlorinated dibenzofurans (PCDFs). Naert (2007) indicated that PCDFs and polychlorinated dibenzodioxins (PCDDs) may also be formed during combustion of PCBs. In addition, Addison (1986) and Eisler (1986) reported that an amount of 0.8 to 33 mg kg^{-1} of PCDFs may occur as post-production toxic impurities in PCBs. Due to the desirable properties of PCBs as reported by numerous authors (Hwang et al., 2008; Kim et al., 2010a; Erickson and Kaley, 2011), they were commonly used in various industrial and commercial applications. UNEP Chemicals (1999) indicated that about 60% of all PCBs produced were mainly used as dielectric fluids, about 28% were used as ingredients

in manufactured products while about 12 % were used as hydraulic and heat transfer fluids (Table 2.5). According to the World Health Organization (WHO), applications of PCBs were categorized into three systems: (i) completely closed systems (i.e. electrical equipment such as transformers and capacitors) to avoid any leak, (ii) uncontrollable closed system where release of PCBs might result from leakage and replacement of PCBs (i.e., hydraulic or heat transfer systems with PCBs as hydraulic fluid or heat transfer media), and (iii)open applications (i.e., plasticizers, paints, inks and additives) (Erickson, 1997).

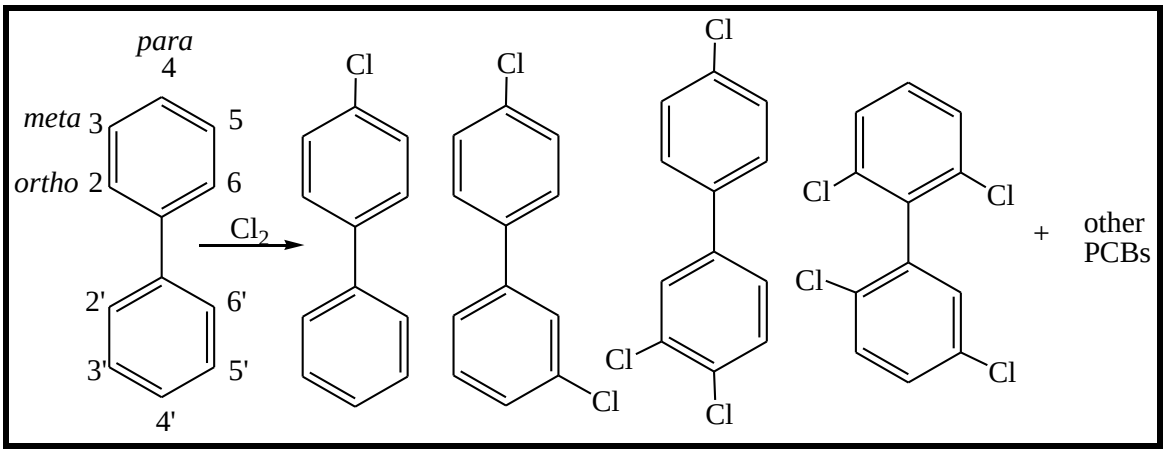


Figure 2.4: Production of PCB congeners by chlorination of the biphenyl compound

Table 2.5: Primary uses of PCBs

Uses of PCBs	% of total production	Quantity (10^6 pounds)
Closed system: electrical and heat transfer fluids (transformers, capacitors, fluorescent, light ballasts)	60	850
Plasticizers (additives in plastics that maintain softness and pliability)	25	350
Hydraulic fluids and lubricants	10	140
Miscellaneous use (flame retardants, paints, inks, sealants, carbonless copy paper)	5	71
Total	**100**	**1400**

Source: (EIP Associates, 1997)

2.4 PCB ENVIRONMENTAL FATE, DISTRIBUTION AND TRANSFORMATION

2.4.1 Environmental fate and distribution of PCBs

It is well known that the fate of PCBs in the environment is a function of chemical, physical and biological processes and is dependent on the degree of chlorination of the molecule. PCBs have been distributed around the globe by various transport mechanisms. Two important routes of entry into the environment include losses during the process of manufacture and leakage from electrical equipment and other products containing PCBs. Because of their stability and hydrophobic nature, atmospheric transport is the primary distribution pathway moving PCBs from atmospheric emission sources to terrestrial and aquatic ecosystems (Wang et al., 2007). Mahugija (2013) showed that the semi-volatility of PCBs is the property that confers them a degree of mobility through the atmosphere and they can be transported over long distances. Other researchers indicated that aquatic organisms and sediments become contaminated through discharges of contaminants by atmospheric deposition, terrestrial runoff, industrial waste leakage and sewage discharges (Nogales et al., 2011). Therefore, once released into the environment, PCBs tend to partition to the organic components and are adsorbed onto organic matter in soils and sediments and can enter the food web. Figure 2.5 shows the distribution of pollutants in the aquatic environment (Redfern, 2006).

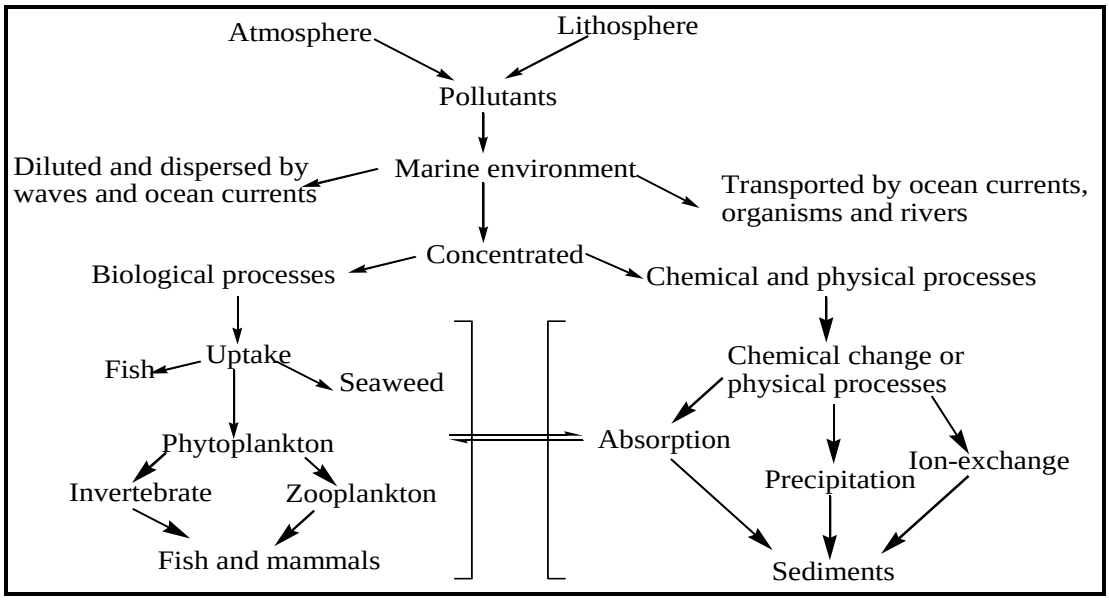


Figure 2.5: Movement of pollutants in marine environment.

The transport and distribution of PCBs in the environment is extremely complex, and involves air, water, soil, fish, birds, and other routes (Moodley and Carsky, 2013). Factors such as air temperature, wind speed, storm frequency, rainfall rates and the volatility of individual PCB isomers influence the pattern and rates of PCB movement in the atmosphere. They are deposited from air by rain, snow, dry fallout, and vapour-phase deposition. In the atmosphere, PCBs are present in both the vapour phase or adsorbed on the atmospheric particles. The vapour phase appears to be more mobile than particle-bound PCBs**.** Therefore, higher chlorinated congeners are more expected to sorb, while lower chlorinated congeners can probably volatilize (Wania and Mackay, 1996). The results lead to higher levels of highly chlorinated PCBs in soils/sediments while air is dominated by lower-chlorinated fractions (Hornbuckle et al., 2004). In water, PCBs are present due to the transfer from sediments and other suspended particles by diffusion and currents. Their removal from the water column is attributed to sorption by suspended solids and sediments as well as by volatilization from the water surface (Pearson et al., 1996). The principal transport route of PCBs through aquatic systems is from waste streams into receiving waters, with further downstream movement occurring by solution and readsorption onto particles as well as by the movement of sediments. This leaves the marine environment as one of the final sinks for PCBs.

According to Wania and Mackay (1996), volatile monochlorobiphenyls remain primarily in the atmosphere; congeners with 1 to 4 chlorines and *ortho*-rich (i.e., number of *ortho* chlorines > 1) tend to migrate toward polar latitudes by series of volatilization/deposition cycles between the air and the water and/or soil. PCBs with 4 to 8 chlorines remain in mid-latitudes, and those with 8 to 9 chlorines remain close to the source of contamination (ATSDR, 2000). The low water solubility and the low vapour pressure of PCBs, in the presence of air, water, or sediment favour the transport processes from contaminated sites to remote areas.

2.4.2 Bioaccumulation and biomagnifications of PCBs

Persistent hydrophobic chemicals may accumulate in aquatic organisms through different mechanisms: *via* the direct uptake from water by gills or skin (bioconcentration), *via* uptake of suspended particles (ingestion) and *via* the

consumption of contaminated food (biomagnification). Louis et al. (2014) studied the bioaccumulation of PCBs in humans and animals. The bioaccumulation was related to the lipophilic nature of these compounds. Aken et al. (2009) revealed that the concentration of PCBs in fatty tissue can accumulate to higher levels than the surrounding environmental concentrations even at low exposure levels. This explained that fatty tissues (for example liver) of fish will experience higher levels of PCBs rather than muscles and other organs. The degree of PCB bioaccumulation over time depends on how quickly they are taken up and eliminated by the organism, and on the ability of the organism to decompose the pollutants. For aquatic organisms, bioaccumulation of PCBs depends on the species, its habitat, and on the type of PCB congener. The concentration of PCBs in sediments is typically several times higher than in water, therefore PCB levels detected in bottom-feeding species are often high (WHO, 2003; Hedman, 2008).

Fish that are close to the top of the aquatic food web have a relatively long life expectancy and concentrate high amounts of PCBs (Bervoets and Blust, 2003). In the aquatic environment for example, levels of PCBs are found to be greater in shellfish than in the plankton on which they feed, and even greater in animals near the top of the food chain such as large predatory fish or mammals (seals, dolphins, and whales). On land, the biomagnification occurs through the accumulation of PCBs from soil or plant leaves to worms and finally to birds and mammals. When aquatic organisms with high lipid content serve as food sources for terrestrial organisms, food chain effects can become predominant and allow further bioaccumulation. The very high partition coefficients between water and lipids favour extremely high uptakes by aquatic organisms. The uptake of PCBs in aquatic biota is usually higher than the concentrations of these pollutants in terrestrial animals (Erickson, 1997).

2.4.3 Environmental degradation of PCBs

Some properties such as thermal and chemical stability of PCBs showed that they are ubiquitous in the environment. When organic chemicals including PCBs are discharged into the environment, they are subjected to various reactions which consequently may lead to further toxic by-products. A study of Abraham et al. (2002) showed that microbes are able to metabolize and utilize PCBs as carbon and/or

energy sources under aerobic or anaerobic conditions. However, Abou-Arab et al. (2010) and Magan et al. (2010) reported that the degradation is a specific process and the growth of some microorganisms can even be inhibited by a xenobiotic. The work conducted by Hussain et al. (2009) showed that pesticides can inhibit the growth of certain microorganisms by interfering with enzymatic activity while nitrogenase activity involved in nitrogen fixation by plants can be inhibited by some types of organophosphate pesticides and the productivity is reduced. However, various animal species, such as insects, crabs and vertebrates, including some birds, fish, and mammals are able to break down some PCB isomers (WHO, 2003). In addition, Nie et al., (2006) mentioned that the rate and physiological mechanism of PCB metabolism depend on both animal species and specific PCB congener. For example, congeners with chlorine atoms in positions 2, 4, and 5 in one (PCB 138) or both rings (PCB 153 and PCB 180) have a greater resistance to break down and be eliminated from the organism than the lower congeners such as 28, 52, and 101. Although, photolysis and biodegradation appear to be significant degradation processes of PCBs.

2.4.3.1 Photolysis of PCBs

The photolysis is a possible route of environmental degradation for PCBs. There are two types of photolysis: a direct and an indirect photolysis. Direct photolysis involves the absorption of energy above 290 nm for the PCB molecules to get excited increasing the probability of a chemical reaction to occur (usually oxidation) (Ackerman et al., 1983). However, indirect photolysis of substances occurs by involving a reaction with three most important photo-oxidizing agents in the atmosphere such as OH-radicals, ozone or NO_2. Further, PCB reaction with OH radical seems to be the only important tropospheric loss process for the gaseous phase (Tehrani and Van Aken, 2014). Several steps are observed through the mechanism of radical formation. The initial step of the photolytic reaction usually involves fission of the parent molecule to form free radicals as intermediate species. These intermediates are unstable and react further with the solvent to produce other organic molecules, inorganic species and other free radicals. The resulting product is a complex mixture in which isomerisation, substitution, oxidation or reduction process can occur (Das, 1997). Different Research conducted on the transformation

of PCBs indicated that many factors influence the photodegradation of PCBs. Wong and Wong (2006) studied the decomposition of the six indicator PCB congeners by UV-catalysed photolysis individually and in combination with the organic solvents, the results indicated that biphenyl was the major product with minor amounts of hydroxylated PCBs. On the other hand (Huang et al., 1996; Lores et al., 2002; Xue et al., 2008) mentioned that photolysis of PCBs happens using organic solvents. Various solvents such as methanol, ethanol, and 2-propanol can be used to decompose PCBs. Dasary et al. (2010) showed that the decomposition of highly chlorinated PCBs on octadecylsilylated silica particles by light irradiation showed a tendency to lose chlorine from *meta*- or *para* and the lower congeners are formed. These studies revealed that most PCBs degrade slowly with long half-lives under UV light. Photolysis of PCBs occurs by photolytic cleavage of a carbon-chlorine bond followed by a stepwise replacement of chlorine with hydrogen which degrades PCBs (Barr et al., 1997). In water, photolysis appears to be the main chemical degradation process as hydrolysis and oxidation do not significantly degrade PCBs (Urbaniak, 2007).

2.4.3.2 Biodegradation of PCBs

Researchers have identified two different biological processes able of biodegrading PCBs in the environment: aerobic oxidative and anaerobic reductive processes. According to Bedard and Quensen (1995), the transformation of PCBs by microorganisms is considered as an important environmental endpoint. The biodegradation of a PCB molecule depends upon the degree of chlorination and the position of the chlorine atoms on the biphenyl ring (*ortho, meta* and *para*). PCBs containing all chlorines on one ring are biodegraded faster than those which contain chlorines throughout both rings. PCBs with chlorine atoms in the *para*- positions are more readily biodegraded (WHO, 1993). Abramowicz et al. (1993) investigated the biodegradation of PCBs and reported that the kinetics of PCB degradation depends mainly on PCB concentration, microbe type population, temperature and nutritients. Furthermore, Pham et al. (2015) showed that the duration of PCB degradation depends on various conditions such as optimal or non-conducive conditions. The biological degradation of PCBs consists of the removal of chlorine from the biphenyl

ring followed by cleavage and oxidation of the formed compound resulting into the formation of carbon dioxide (CO_2), chloride and water (Boyle et al., 1992).

2.4.3.2.1 Anaerobic degradation of PCBs

Studies conducted over the years discovered that highly chlorinated congeners were not transformed under aerobic conditions but they are dechlorinated under anaerobic conditions leading to the formation of less-chlorinated congeners (Abramowicz, 1990, Mohn and Tiedje, 1992). During this process, the organohalogen compound serves as electron acceptor (Figure 2.6) (Borja et al., 2005). A study by Ye et al. (1992) found that by-products of anaerobic dechlorination are less toxic and can usually be degraded by the aerobic microbes.

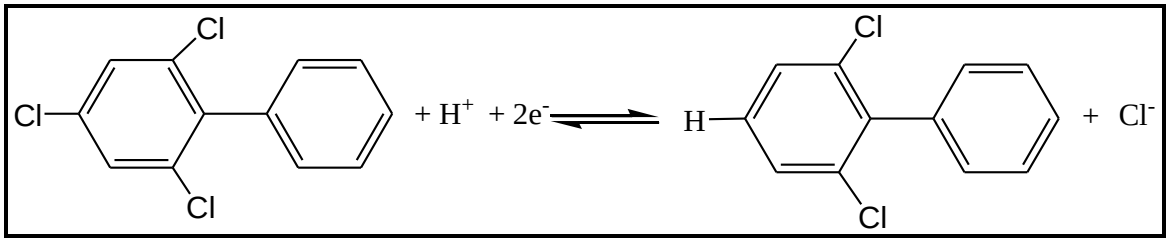


Figure 2.6: Reductive dehalogenation of PCBs by anaerobic microorganisms.

The biodegradability of PCBs is strongly influenced by the substitution of chlorine atoms on the biphenyl ring. This is supported by Fish and Principe (1994) who found that the *para*- and *meta*-substituted congeners are more commonly degraded than *ortho*-substituted congeners. Figure 2.7 shows the degradation process of a higher chlorinated congener into lower ones. The dechlorination of one PCB isomer can produce several types of PCBs. For example, 2,3,5,6-tetrachlorobiphenyl can be transformed into 2,3,6-trichlorobiphenyl or 2,3,5-trichlorobiphenyl. In all the cases, the ring with the higher degree of chlorination is the primary site where dechlorination occurs (Barr et al., 1997).

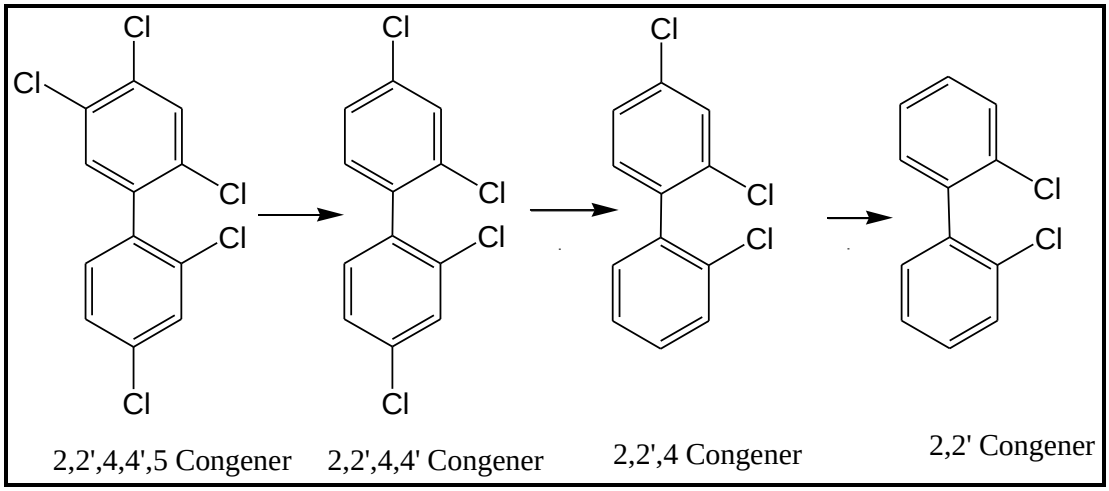


Figure 2.7: Anaerobic dechlorination of a highly chlorinated congener to a lower chlorinated congener.

2.4.3.2.2 Aerobic biodegradation of PCBs

The aerobic biodegradation of PCBs is generally limited to congeners with five or fewer chlorines and two adjacent unsubstituted carbon atoms (Boyle et al., 1992). Aerobic oxidative destruction involves two steps. The first step is responsible for the transformation of PCB congeners to chlorobenzoic acid as an intermediate compound. The second cluster is responsible for the degradation of the chlorobenzoic acid as mineralization degradation (Figure 2.8). The oxidation of the ring by a 2, 3-dioxygenase, which substitutes two hydrogen with two hydroxyl groups at adjacent *ortho* and *meta* positions on the molecule is observed. The process yields the chlorinated 2, 3-dihydro-2, 3-dihydroxybiphenyl which is further degraded to a chlorinated 2, 3-dihydroxybiphenyl, followed by enzymatic cleavage of the hydroxylated ring to form the corresponding chlorobenzoic acid (CBA) and a five-carbon fragment (Flanagan and May, 1993). Further, the CBA formed can be readily degraded by indigenous bacteria, producing carbon dioxide, water, chloride and biomass (Robinson and Lenn, 1994).

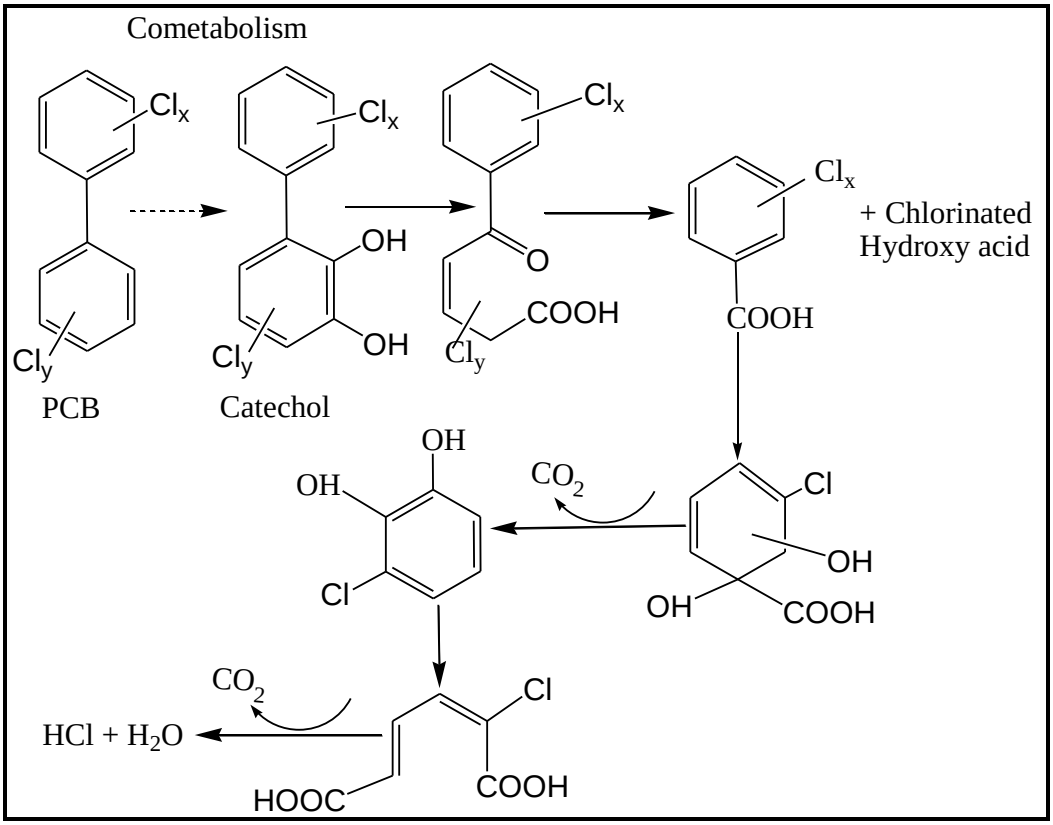


Figure 2.8: Pathways for aerobic degradation of PCBs.

A common growth substrate for PCB-degrading bacteria is biphenyl or monochlorobiphenyl by aerobic microorganisms, especially by bacteria of the genus *Pseudomonas.* This process involves the addition of oxygen to the biphenyl ring (Boyle et al., 1992) and *ortho*- ring cleavage occurs (Figure 2.9). According to Bevinakatti and Ninnekar (1992), a similar degradation of biphenyls by the *Micrococcus* is possible and the metabolic pathway resembles that of the *Pseudomonas*. Harkess et al. (1993) studied the aerobic biodegradation of PCBs in Hudson River sediments. They found that both aerobic and anaerobic degradation occurred but the field biodegradation rates were two to three times slower than those determined in the laboratory. In addition, no more than 60% of the PCBs in the sediments were degraded in both field and laboratory studies. Both pH and redox potential can influence the dechlorination of PCBs in sediments. Pardue et al. (1988) reported higher biodegradation under moderately aerobic conditions (maximum influence at + 250 mV) than either aerobic conditions (+ 500 mV) or anaerobic conditions (- 200 mV). The degradation rates were 30 to 40 times higher in moderately aerobic sediments than in anaerobic sediments. This indicates that

oxygen availability to the microbial population is the limiting factor for PCB biodegradation.

Research of Anyasi and Atagana (2013) on the microbial degradation of commercial PCB mixtures revealed that certain forms of chlorine substitution delay PCB degradation. Borja et al. (2005) showed that sequential enzymatic steps involving degradation for the lower chlorinated PCB congeners are observed (Figure 2.10).

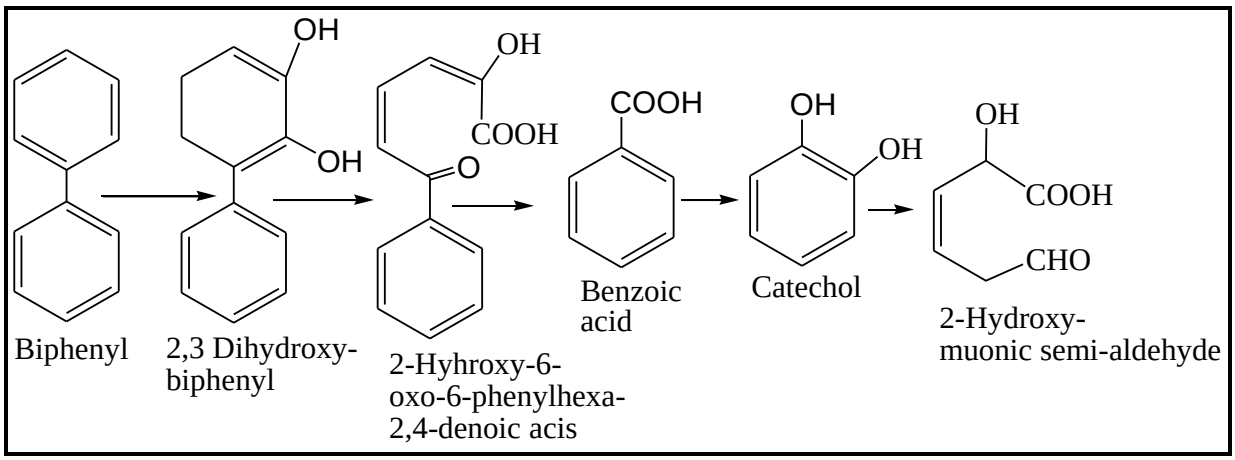


Figure 2.9: Aerobic oxidative dehalogenation of PCBs.

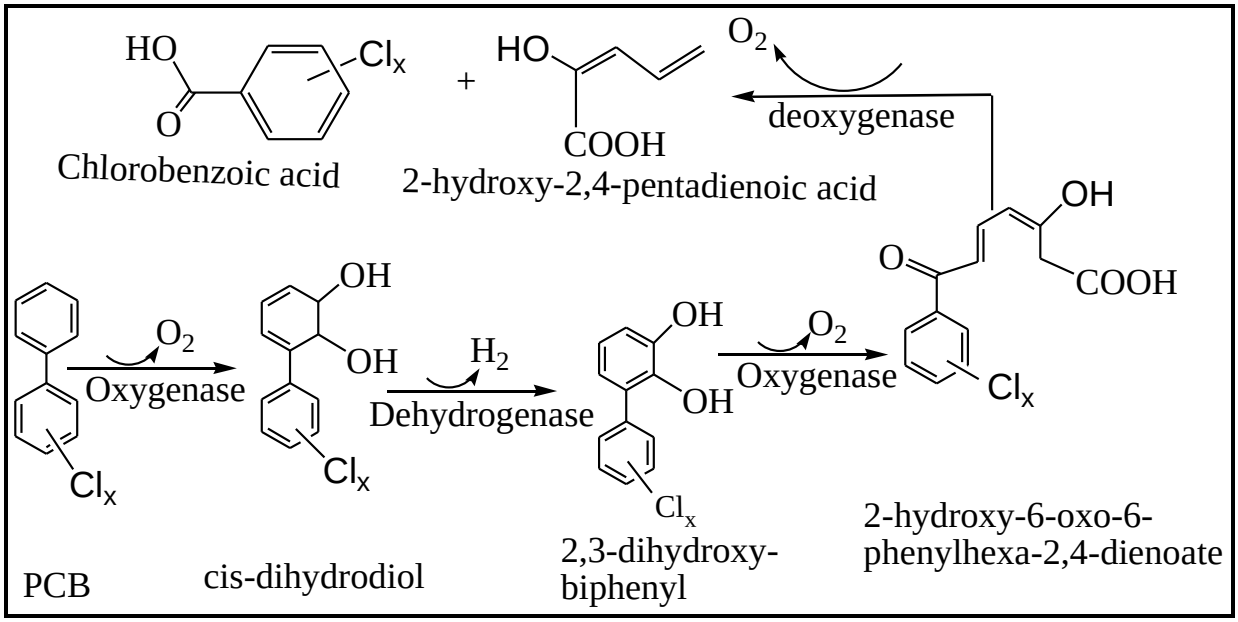


Figure 2.10: Major steps in the conversion of PCBs into chlorobenzoates.

Studies (Seeger et al., 1999; Sinkkonen and Paasivirta 2000) proposed a general pathway of PCB transformation by the microorganisms (Figure 2.11). In general the biodegradation process of PCBs displayed that there are two biologically mediated

PCBs degradation routes: anaerobic and aerobic. The anaerobic process removes chlorine atoms of highly chlorinated PCBs, which are then mineralized under aerobic condition. The degradation way is dependent on the complexity of the PCB congener (number of chlorine atoms) combined with the type of microorganism present and the interaction among the microorganisms.

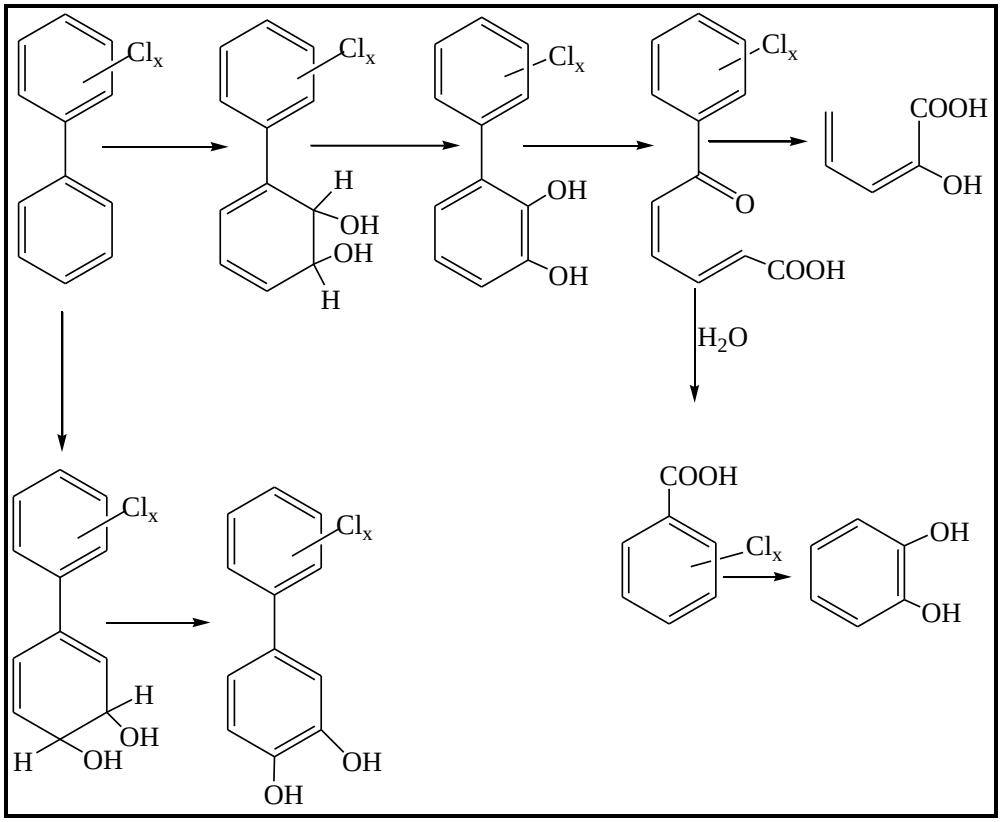


Figure 2.11: Pathways for the aerobic biodegradation of PCBs.

2.5 TOXICOKINETICS AND METABOLISM OF PCBs

Toxicokinetics refers to the combined physiological and behavioural factors which control contaminant uptake, distribution, metabolic alteration, elimination and delivery to the site of toxic action (Drouillard et al., 2001). Bu et al. (2015) indicated that the absorption of PCBs by humans is through diverse routes (e.g. inhalation, ingestion, dermal), but ingestion is considered the main route of exposure pathway since PCBs and other POPs can bioaccumulate along the food chain. It is approved that individual PCB congeners and their mixtures are readily absorbed from the gastrointestinal tract. Tanabe et al. (1981) reported that gastrointestinal absorption of

individual congeners in rats varies between 66% and 96%. Further, Hu et al. (2010) indicated that the degree of absorption depends on the degree of chlorination. In addition, the physico-chemical properties of PCBs including other factors such as diet, age, gender and type of species lead to their different toxicokinetics in biota (Delistraty, 2013). Researches on PCB congeners or mixtures have demonstrated effective dermal absorption.

(Wester et al., 1983) mentioned that in guinea pigs, absorption of PCB mixtures was found to be at least 33 – 56% during 16 days of exposure, whilst monkeys absorbed 20% during 28 days of exposure. Jackson et al. (1993) revealed that up to 60% of PCB 77 was absorbed by rats after three days of exposure. In 1978, the estimated dietary intake of PCBs by adults in the USA was 0.027 µg kg^{-1} body weight per day, but it declined to 0.0005 µg kg^{-1} body weight per day in 1982–1984 and < 0.001 µg kg^{-1} body weight per day for the period 1986 – 1991 (WHO, 2003). Once absorbed, PCBs are readily distributed to all body compartments, where the storage rate is proportional to the fat content of the organ.

The distribution of PCBs in the body depends on the blood flow to the tissues. There is usually an initial uptake of PCBs in the liver due to the high blood perfusion in the liver. Once in the blood, PCBs are distributed among cellular and serum components by passive partitioning, with most of the PCBs becoming associated with plasma lipoproteins. Some studies on the metabolism and excretion of PCBs have been carried out. (Jandacek et al., 2013; Faniband et al., 2014) showed that the main route of PCB excretion is *via* the faeces (biliary excretion), urine and breast-milk. The metabolism of PCBs involves different steps. Hovander (2006) indicated that activation by enzymes is the first step for the biotransformation process of PCBs. The metabolism of PCBs occurs *via* the hepatic cytochrome P-450-dependent monooxygenase system, and varies according to the chlorine content of the congener. Hydroxylated products are major metabolites, with hydroxylation occurring primarily at the *para* or *meta* positions if these sites are unsubstituted. Coristine et al. (1996) found that PCBs with more than five chlorines and *para*-chlorine atoms are less susceptible to hydroxylation and show the longest half-lives while PCBs with chlorine-unsubstituted *meta-para*-carbons are more susceptible to hydroxylation. Secondly, PCB metabolism involves the deactivation of the arene oxide either

spontaneously to a dihydrodiol that subsequently releases water to yield a polychlorinated biphenylol (Tehrani and Van Aken, 2014). The last step involves conjugation to increase the water solubility of metabolites and leads to the formation of a large number of metabolites, e.g. hydroxylated polychlorinated biphenyls (OH-PCBs), methylsulfonyl- ($MeSO_2$) PCBs and glucuronide or sulphate conjugates. However, some OH-PCBs and $MeSO_2$-PCBs are persistent within the body and are retained in the blood of animals as well as human tissues (Naert, 2007). It has been observed from the different studies that the number of chlorine atoms in the PCB molecule influence the toxicokinetics of PCBs. The metabolism of PCBs in some cases lead to the formation of other toxic substances.

2.6 TOXICITY OF PCBs

Recently, the studies proved that the toxicity of a chemical compound to humans can be evaluated based on its acute (short term) exposure and/or chronic (long-term) exposure (White and Martin, 2010). The work done by Gonzalez et al. (2011) showed that the toxicity of PCBs depends on numerous factors such as the type of PCB (i.e. mixture or congener), its structure, chlorine content, dose, and route of exposure. The chronic toxicity of a chemical is usually of primary concern for environmental toxicants (USEPA, 2000). In addition, the exposure to mixtures of PCBs may lead to different effects such as hormonal, immunological, neurological and reproductive system damage. They are known to be among the major cause of tumour activity and cancer of adipose tissues (Pavuk et al., 2004). Specific PCB congeners can act through diverse mechanisms and have different toxic potentials (Safe, 1994). The mechanisms of the latter compounds involve both arylhydrocarbon (Ah) receptor dependent and Ah receptor independent. DL-PCBs are able to disrupt the modulation of normal gene expression *via* their binding to the cytosolic arylhydrocarbon-receptor (AhR) (see section 2.3.2). Additional studies reported the presence of tumors in rats (Ross, 2004) and acute toxicity of PCBs to *Photobacterium phosphoreum* (Chu et al., 1997). Though NDL-PCBs are less toxic than DL-PCBs, they have shown to induce neurotoxicity, carcinogenicity and changes in hormones (Cooke et al., 2001; Hardell et al., 2006). On the other hand, PCB 138 and 153 have been found to initiate histological and neurological damages in the liver (Duffy and Zelikoff, 2006) whereby, PCB 153 is a dominant PCB

congener found in wildlife and human tissues. It accounts approximately 25% of all PCBs present in the environment (Sipka et al., 2008); and its half-life may exceed 100 years in marine sediments (Jonsson et al., 2003). This property attracted many researchers to investigate its effects on invertebrate fecundity and development in *Chironomus riparius* (Hwang et al. (2001), on hepatic injury, global gene expression in livers of female rats (Vezina et al., 2004), on livers, lungs and brains damage of mice (Sipka et al., 2008). Dillon et al. (1990) reported the toxicity observed in invertebrates *Daphnia magna* after exposure of NDL-PCB congeners 52, 101, 138, 153, 180 and (DL-PCB 118). The information here above indicated that PCB compounds are toxic to humans and other organisms. Minimising the introduction of PCBs in the environment can play an important role in decreasing its levels of toxicity.

2.7 HUMAN EXPOSURE TO PCBS

Nowadays, it is assumed that the main route of human exposure to PCBs is *via* contaminated food items. Since PCBs are lipophilic and bioaccumulate through the food chain, mostly fatty foods of animal origin such as meat, fish, seafood and dairy products are considered to be an important source of PCB exposure to the general population. In addition, recent studies (Frazzoli et al., 2010; Di Stefano, 2014) confirm that only some exception of specific cases of accidental or occupational exposure can lead to human exposure to PCBs. A study of Carpenter (2008) showed that as PCBs evaporate into the air, inhalation becomes the most important route of exposure. The exposure to PCB congeners depends on different food products. Though, vegetables account for a major part of the intake of lower chlorinated congeners, whereas fatty foods such as fish, dairy products and meat play a greater role for exposure to higher chlorinated PCBs. On the other hand, Djien Liem et al. (2000) indicated that ingestion of PCBs through food is estimated to account for more than 90% of the human total exposure. Therefore, breastfed infants are considered to be at the very top of the food chain due to their source of nourishment because breast tissue is an area rich in lipids and fatty tissue. PCB concentrations in human milk are higher than in cow's milk or other infant foods. Consequently, Mead (2008), Ferreira and Alves (2015) suggested that infants feeding with human milk as the dominant food source will consequently have higher PCB intake than adults

because when a mother nurses her infant, PCBs and other lipophilic compounds leave the fatty tissue and enter the breast milk. It has been shown that PCB levels in breast milk of women living in industrialized countries are higher than of those living in developing countries (Minh et al., 2004). However, due to the ban of PCB use and precautions implemented, PCB levels in breast milk have decreased significantly in the past decades in some countries. Becker et al. (2002) found that PCB levels in breast milk from Germany showed a 60 % decrease in the time frame 1986 – 1997. Similarly, Norén and Meironyté (2000) also disclosed that in comparison to the levels of PCBs detected in 1972, a decrease of 30 % was observed in 1997 in Swedish human milk. Even if it is well known that human exposure to PCBs is mainly through contaminated food, with extensive spread use of PCBs, people that handled or worked with PCB containing products such as fluorescent light transformers or electric transformers, may have higher PCB body burdens.

2.8 HEALTH EFFECTS OF PCBs

Due to their persistence, PCBs pose severe threat to biota, laboratory animals, humans and wildlife. A number of acute and chronic health effects have been linked to PCB exposure both directly and indirectly. Cogliano (1998) indicated that the chronic toxicity is more dangerous because its symptoms appear in the body after bioaccumulation over extended periods of time in the environment. Recently, the work done by (Hooper et al., 2013; Murínová and Dercová, 2014) revealed that PCBs are distributed across cell membranes inside the body and can alter biochemical, molecular and physiological processes at the cellular level. The human health effects depend on the concentration of PCBs, the type and extent of exposure. The reported health effects are based on studies of community exposures from ingestion of contaminated foodstuffs, occupational exposures during the manufacture of PCB mixtures (e.g. Aroclors) or PCB-containing components, and various animal studies (WHO, 2003).

Various studies showed that the health effects associated with PCBs in humans and laboratory mammal studies include cancer, neurological damage, and reproductive disorders, reduced cognitive function accompanied by adverse behavioural effects and disruption, and other effects (Jacobson and Jacobson (1996); Carpenter (2006);

Bhupander et al., (2011)). In addition, Schantz et al., (2003) found that PCBs are associated with intellectual problems and a reduction in the ability to learn and remember. Further, various studies indicated that children are more exposed to the effects of PCBs. Santiago et al. (2013) pointed out that reproductive effects, neurobehavioral and developmental birth defects in new-borns and older children are linked to a mother's intake of unhealthy substances in food, beverages and medications throughout pregnancy. In addition, prenatal exposure to environmental toxins profoundly affects the developmental biology of the foetus. On the other hand, in Michigan, Jacobson (2002) investigated the adverse effects on children at 11 years who were in the group most highly exposed to PCBs prior to birth. The findings showed that the intelligence quotient (IQ) of those children was six points lower on average than those in the lowest exposed group to PCBs. Similarly in the Netherlands, a study of Patandin (1999) proved that children who were exposed pre-birth to PCBs also exhibited reduced function of the immune system while Ward et al. (2009) investigated effects of PCB exposure in children and concluded that the risk of developing acute lymphocytic leukaemia is increased by two-fold when PCBs were detected in the dust of a room in which the child spent a significant amount of time. Many other health effects were found to be associated to exposure of PCBs including cardiovascular disease, hypertension, diabetes; acne and rashes, asthma and other infectious respiratory diseases (Everett et al., 2011; Norman et al., 2013). The evaluation of health effects of PCBs is mainly carried out using laboratory animals such as rats, mice and monkeys. A primary criticism of the animal studies is the typically large doses administered to initiate a response. Matthiessen (2003) found that PCB levels above 120 ng g^{-1} ww in ovaries of the Baltic flounder (*Platichthys flesus*) were correlated with impaired egg development. Sipka et al. (2008) indicated that liver, lungs and brain of mice were damaged after administration of 150 µmol kg^{-1} of PCBs 77, 104 and 153.

As indicated by Carpenter et al. (2014), PCBs are still of global concern since they are stable and are able to remain in the environment for prolonged periods of time due to their persistence, bioaccumulative potential, and adverse effects on humans and wildlife. Although, it would be prejudiced to suggest that people will not be concerned about the potential harmful effects of PCBs which are still present in the global environment according to different studies.

2.9 SOME STUDIES ON PCBs IN THE SOUTH AFRICAN ENVIRONMENT

Despite the bans of PCB globally, residues of PCB are still found in the South African environment. In Africa, research on environmental PCBs has not been as intensive as in other continents (UNEP, 2002). PCBs were quantified in water, sediment, eggs and biota in the 1980s and 1990s in some African countries. In the South African environment, a number of specific studies were undertaken to monitor the levels of POPs including PCBs in environmental media. Major studies were undertaken between 1960 and 1990 for the assessment of the levels of contaminants in sediments and biota along the South African coastline (Wepener and Degger, 2012). Some studies have investigated environmental organochlorine pesticide levels in South Africa (Vosloo and Bouwman, 2005), but few studies have measured PCB levels in the South African environment due to the high costs of analysis, limited capacity to measure POPs in terms of both laboratory capacity as well as a lack of trained and skilled staff (NIP, 2011). Tables 2.6, 2.7, 2.8 and 2.9 summarized some studies on PCB levels in South Africa.

Shoreline areas provide important benefits to humans in terms of food resources and ecosystem services. However, human activities which take place mainly around the coastline may have significant negative impacts on the health of ecosystems. The results from Table 2.6 indicated that the aquatic and marine environment of South Africa is contaminated by PCBs. Aquatic/marine environments are easily accessible as dumping sites from the surrounding population and industries. PCBs from industrial chemicals and waste from industries are suspected to be the main source of these compounds in the environment. As indicated in the Table 2.6, a variation of PCB concentrations in different species of fish analysed is found. Sea mammals are more contaminated than fish from rivers, estuary or dams. This is explained by the bioaccumulation and biomagnification of PCB compounds through the food chain.

For mussel samples, Degger et al. (2011) analysed DL-PCBs from different harbours and Bays and the results showed that South African Harbours are contaminated by PCBs.

Some investigations on contamination levels of PCBs in soils, sediments and air from the environment of South Africa showed the presence of these pollutants (Table

2.7). Soils and sediments investigated by Nieuwoudt et al. (2009) were highly contaminated by PCBs. These levels can be attributed to the areas of the study. The sampling sites were classified in three categories; non-industrial, industrial and rural areas. The main areas of concern identified in this survey were the Klerksdorp residential area, the industrial area of Vanderbijlpark, and Riet Spruit. PCBs are transported by wind, water fish, migratory mammals and other means, thus areas not impacted by waste discharges can also show some contamination. Durban South Industrial Basin (DSIB) has one of the highest concentrations of industrial activity in Africa comprising two large petroleum refineries, a paper mill, an international airport, large chemical tank farm, landfill sites, incinerators, processing and manufacturing industries, major trucking, harbour and rail facilities, and other industries. In addition to soil and air samples collected in the industrialized urban areas of KwaZulu Natal, levels of PCBs in air and soil detected by Batterman et al. (2007, 2009) are accredited to waste discharges from those industries and other human activities. Thereby humans who are working in the environment of Durban/KwaZulu Natal can be at high risk of exposure to PCBs by inhalation or dermal route of exposure to contamination.

Vosloo and Bouwman (2005) analysed DL-PCBs in sediments from 22 sites across the country. The highest TEQ-value (toxic ecological quotient) of 22 ng kg^{-1} was determined for the Riet Spruit that is close to an iron and steel refinery in Vanderbijl Park. The Modderfontein Spruit had a TEQ of 6 ng kg^{-1}. This site is also located in close proximity to a highly industrialised site. The lowest TEQ was recorded at the Loskop dam (0.22 ng kg^{-1}) and not the Mooi River (0.34 ng kg^{-1}) which was selected because of its expected low impact status due to its location. The PCB concentrations did not exceed the level of 50 ng kg^{-1} sediment, which was the set limits determined for the USA.

Table 2.6: Mean and range of PCB levels (ng g^{-1} ww) in some freshwater/marine biota samples

Location	Samples (Fish/mussels)	Mean	Range	References
East coast of South Africa	Sea mammals:		450 – 30600	Gardner et al. (1983)
	Tursiops aduncus	12900		
	Arctocephalus pusillus	1700		
Coast of KwaZulu-Natal	Blubber of bottlenose dolphins:			Cockcroft et al. (1989)
	South coast animals	8400	-	
	Central coast animals	13000	-	
	North coast animals	20000	-	
East coast of South Africa	Four adult cape fur seals	1.80	0.39 – 3.49	Cockcroft and Ross (1991)
	Blubber of common dolphins	4.04		Cockcroft et al. (1990)
Eastern Cape coast (1993-1994)	Blubber of 12 adult male Cape fur seals: *Arcfocephalus pusillus pusillus*	nd	-	Stewardson et al. (1999)
west and east coasts of southern Africa (1977-1987)	Blubber of bottlenose dolphins	-	100 – 15510	De Kock et al. (1994)
Olifants River (1990)	*Clarias gariepinus, Eutropius depressirotris* and *Oreochromis mossambicus*	41	-	Grobler (1994)
Isipingo Estuary, KwaZulu-Natal (1991)	8 fish species	96.5	5.7 – 869	Grobler et al. (1996)
Cape Cross/Namibia	South African fur seals (*Arctocephalus pusillus pusillus*)	104		Vetter et al. (1999)
Urban Nature Reserve (Pretoria) (2004-2005)	Catfish: *Clarias gariepinus*		5 – 25	Borman et al. (2007)
South African Bays and harbours	Brown mussels (*Perna perna*)		34000 – 131000[a]	Degger et al. (2011)

[a]Results are expressed on lipid weight

Table 2.7: Mean and range of PCB levels in South African soil/sediments (ng g^{-1} dw) and air samples (ng TEQ L^{-1})

Location	Types of samples	Mean	Range	References
Gauteng, North-West and the Free State	Soils and sediments	-	120 – 4700	Nieuwoudt et al. (2009)
Durban South Industrial Basin	Air	0.087	0.021 – 0.289	Batterman et al. (2007)
KwaZulu-Natal	Air (vapour plus particulate phase)	128 ± 47		Batterman et al. (2009)
	Soil: surface soils (0 - 0.5 cm depth)	109.64 ± 116.07		
	Shallow soil (1 - 2 cm depth)	19.22 ± 33.23		
Limpopo Province, North West Province and Gauteng	Soil and ash	< 1000		Umlauf et al. (2011)
Olifants River in the Mpumalanga province	Sediments	nd	nd	Grobler (1994)
Aquatic environments throughout South Africa: sediments	Sediment from Riet Spruit	22000		Vosloo and Bouwman (2005)
	Sediment from Modderfontein Spruit	6000		
	Sediments from Loskop dam	220		
	Sediments from Mooi River	340		

TEQ: Toxic ecological quotient

The reported findings in the Table 2.8 from different authors showed that birds as well as eggs of birds or crocodiles are contaminated by PCBs. Higher levels of PCBs were detected in eggs of birds collected from Vaal River by Van Dyket al. (1982). The Vaal River forms a boundary between the Free State and the provinces of Gauteng, North West and Northern Cape. This indicated that this river may receive waste from these bordering provinces. However, the levels of PCBs detected by Bouwman et al. (2008) in eggs of birds from the same river (Vaal River) showed a decline from 11000 to 300 ng g^{-1} ww. A study of Bouwman et al. (2014) showed that almost all chlorinated compounds analysed were detected in crocodile eggs from the Kruger National Park. The lower concentrations of total PCBs suggest that the Nhlanganini tributary of the Olifants River had much less influence from industrial pollution. These findings indicated that repeated annual episodes of Nile crocodile deaths in two isolated areas of the Kruger National Park were related to the contamination of PCBs and other possible organohalogen pollutant. Crocodile eggs collected were close to one of the mortality sites (Gorge) as well as from a crocodile farm as reference. All the eggs and birds analysed indicated areas impacted by anthropogenic compounds. Higher levels were detected in eggs of birds compared to eggs of crocodiles. The birds and crocodiles accumulate PCBs from their diet including small fish which are already contaminated through the food chain.

Table 2.8: Mean and range of PCB levels (ng g^{-1} ww) in eggs of birds and crocodiles

Location, year	Samples	Mean	Range	References
Vaal River	Eggs of birds	11000		Van Dyk et al. (1982)
Along the Natal coast	Seabirds eggs		nd – 4600	Gardner et al. (1983)
Kruger National Park	Nile crocodile eggs			Bouwman et al. (2014)
	Eggs from crocodile farm (n = 10)	2.4	0.097 – 8.8	
	Eggs from Letaba (n = 6)	7.0	6.5 – 7.7	
	Eggs from Olifant River 1 (n = 3)	7.3	6.0 – 7.9	
	Eggs from Olifant River 2 (n = 3)	6.1	4.6 – 7.5	
	Eggs from Olifant River 2 (n = 3)	4.9	4.4 – 5.5	
	Nhlanganimi (n = 3)	3.4	3.3 – 3.6	
Eastern Cape Province	Seabird eggs		210 – 890	De Kock and Randall (1984)
Sedgefield (1985)	African marsh harrier eggs		200 – 2240	De Kock (1988)
Western Cape Province	One egg of *G. coprotheres*	1800[a]		Pieters and Focant (2014)
South African sites	Eggs of birds		5.7 – 300	Bouwman et al. (2008)
Barbespan and Vaal River	Cattle egret (n = 20)	5.7		
Western Cape Province	Kelp gull (n = 1)	103		
Vaal River	African darter (n =14)	300		
	Reed cormorant (n = 3)	110		
	African sacred ibis (n = 2)	59		
	Crowned plover (n = 1)	9.7		
	Little grebe (n = 1)	9.7		
	white plover (n = 1)	33		

[a]Result is given as total PCB levels

The results in the Table 2.9 indicated that except water from the Olifants River, all other samples were contaminated by PCBs. The levels of PCBs in mother's milk were not high; the authors suggested that the low levels of PCBs in the breast milk from Limpopo Province probably reflect the rural and non-industrial character of this region. Another study analysed the blood samples taken from 96 pregnant women admitted for delivering in South Africa. The study took place in seven geographical regions of South Africa (rural, urban, industrial, fishing (Atlantic Ocean coast), mining, coastal endemic malaria (Indian Ocean) and inland endemic malaria). The levels of PCB congeners detected by Röllin et al. (2009) in blood were found to be low in all samples and across all sites. Both PCB 138 and PCB 153 congeners were detected in all the study sites. In addition, Batterman et al. (2009) analysed cow's milk samples delivered from a local dairy of KwaZulu Natal, out of 36 target congeners, 28 were found at quantifiable levels, including dioxin-like PCB 77 (80 pg L^{-1}) and PCB 126 (30 pg L^{-1}). The levels detected in cow's milk should be attributed to the localization area of the study which was an industrialized and urban area of KwaZulu-Natal. For water samples analysed by Amdany et al. (2014), the levels of PCBs detected were attributed to atmospheric deposition because the samples collected during the winter season exhibited higher contaminant concentrations for most compounds studied than those recorded in all of the other seasons. In the aquatic environment, water is subjected to serve as dumping site from the surrounding areas where runoff, wastewater and other discharges are introduced into lakes/rivers by accidental or illegal dumping. DL-PCBs are known to be the most toxic congeners than NDL-PCBs. However, in the study of Pieters and Focant (2014), levels of NDL-PCBs detected in serum of people exposed to burning solid biofuels and those relying on electricity, gas and paraffin were higher than that of DL-PCBs. Ten groups corresponding to 421 human subjects were exposed to burning solid biofuels and eleven groups comprising 272 human subjects were people relying on electricity, gas and paraffin. Serum levels of PCBs increased with age. The higher levels of NDL-PCBs detected can be explained by routine application of toxic equivalency factors, which assume that dioxin like mechanisms and aryl hydrocarbon receptor involvement, may not adequately reflect the effects of NDL-PCBs in the mixture (Serdar et al., 2014). The levels of DL-PCBs in the blood of this

Tswana population were similar to those of non-exposed adult general populations in other countries.

Table 2.9: Mean and range of PCB levels in water (ng L^{-1}), blood, breast and cow's milk samples (ng g^{-1} lw)

Location, year	Types of samples	Mean	Range	References
Limpopo Province	Breast milk (n = 28)	10	3.1 – 42	Darnerud et al. (2011)
Durban South Industrial Basin	Blood	0.91	0.31 – 2.7	Röllin et al. (2009)
KwaZulu-Natal	Cow's milk	22[b]		Batterman et al. (2009)
Hartbeespoort dam (2011)	Water		0.038 – 0.150[a]	Amdany et al. (2014)
Twana population	Serum: for those relying on burning solid biofuels (DL)	10.73 ± 16.14		Pieters and Focant (2014)
	Those relying on electricity, gas and paraffin	4.34 ± 1.6		
	Those relying on burning solid biofuels (NDL)	74.76 ± 83.17		
	Those relying on electricity, gas and paraffin	62.86 ± 52.98		
Olifants River in the Mpumalanga province	Water	nd	nd	Grobler (1994)

[a]Results are expressed in ng L^{-1}, [b]Results are expressed as total PCB concentrations in ng L^{-1}

The data from all the studies above cited illustrate that the environment of South Africa investigated is contaminated by PCB contaminants. The results from these and other studies predict the consequences of human activities on marine and estuarine ecosystems. Freshwater environments basically include rivers, dams, wetlands and lakes while marine water includes harbours, bays, and seas/oceans. Contaminants loads in fresh/marine water is mainly attributed to aerial deposition, sewage, industrial and municipal waste, and other accidental/illegal wastes discharged into the environment. The occurrence of these industrial chemicals in different compartments of the aquatic environment, even at trace levels is of ecological and environmental health concern. None of the above-mentioned authors studied the environmental effects associated with PCBs. It would be of great value if intensive studies be conducted in the South African environment on the probable adverse effects of PCBs on humans and wildlife.

2.10 SOME STUDIES ON PCB CONTAMINATION LEVELS IN DEVELOPING AND DEVELOPED COUNTRIES

Over the last decade, public concern about the adverse human health effects of persistent organic pollutants including PCBs led to strict regulations on their use in developed nations. However, amount of PCBs are still found in the environment due to their persistence and lipophilic nature. Therefore, developing countries need to build and strengthen the chemicals management systems that are already in place in developed parts of the world. Up to now, researchers continue to detect PCBs in various environmental matrices such as water, biota, plants, vegetables, sediments, breast milk and adipose tissue but research on PCBs is not intensive in developing countries as in developed countries.

Some studies indicated that PCBs are of concern in developing countries due to the amount detected in environmental samples such as fish, mussels, water and sediments. Table 2.10 and 2.11 summarized some work done on PCBs in developing and developed countries, respectively.

The findings from these studies confirmed the presence of PCB contaminants in all the matrices analysed at some levels. Due to the use of PCBs mainly in transformers and capacitors for electricity generation, Bentum et al. (2012) investigated soil samples collected around twenty six transformers in the central region of Ghana. The findings revealed the presence of PCBs with mean levels of 8.17 ± 2.96 ng g^{-1} dw. Similar mean levels of PCBs (7.69 ± 0.90 ng g^{-1} dw) were detected in sediments from Yamuna in Delhi, India by Bhupander et al. (2011). The contamination of Yamuna River is probably due to its location near the Delhi city where various activities generating waste including urban centers and industries are situated. On the other hand, Eqani et al. (2012) investigated the contamination levels of PCBs (sum of 31 congeners) in sediments collected from the River Chenab during two years of monitoring. The levels detected were higher than the levels detected by Bhupander et al. (2011). The PCB levels detected in winter and summer were similar and ranged from 9.33 to 129.45 ng g^{-1} and from 12.55 to 144.23 ng g^{-1} dw, respectively. The sediments from the mainstream sites were dominated by tetra-CBs and penta-CBs, while hepta-CBs and octa-CBs were predominant in industrial and urban sites near tributaries. In addition, Barakat et al. (2013) revealed that PCB levels noticed in sediment samples collected from Lake Qarun in Egypt were attributed to its location particularly near urban areas reflecting the local usage and input of these pollutants due to the presence of many industries in the urban areas. In China, Zhang et al. (2007) assessed the levels of PCBs in 198 agricultural soil samples collected from Zhangjiagang and Changshu in Southern Jiangsu in China. The average concentration in all the soil samples was 4.13 ng g^{-1} dw, indicating low-level of contamination compared to Bhupander et al. (2011) and Bentum et al. (2012). Tetra, penta-, and hexa-chlorinated biphenyls were dominant in the soil samples, accounting for more than 75% of all PCBs analysed. Recently, Zeng et al. (2014) measured PCB concentrations in five types of vegetables, soil, and settled air particle samples from two sites (at a domestic waste incinerator and at 20 km away from the incinerator) in Guangzhou, South China. The total PCB concentrations in aerial parts of vegetables were greater than those in rhizosphere soils and roots with median values of 108 and 47.08 ng g^{-1} dw,

respectively. Among the five types of vegetables studied, the highest concentration of PCBs was found in bitter lettuce. A study of Nie et al. (2005) investigated the concentrations and distribution of PCBs in water, surface sediments and fishes from the estuaries of Pearl River in China. The results found (Table 2.10) showed that the feeding habits of fish were linked to the accumulation of PCBs and pattern of congeners. Carnivorous and benthic fishes such as eel (*Anguilla japonica*) and Chinese sea catfish (*Arius sinensis*) were detected at higher concentrations and with higher chlorinated congeners, while herbivores such as shad (*Clupanodon punctatus*) and mullet (*Mugil cephalus*) exhibited an opposite trend. Another study of Masmoudi et al. (2011) indicated that fish species *Liza aurata* collected at the outlet of Khélij channel in Tunisia was contaminated by PCBs with predominance of hexachlorobiphenyl congeners accounting for 25 – 52% of the total profiles. The higher chlorinated congeners such as hexachlorobiphenyls are the most dominant in biota samples due to their resistance to degradation. El-Kady et al. (2007) examined the contamination levels of PCBs and other pollutants in fish samples collected from different locations in the River Nile in Egypt. For eighteen fish samples analysed, the mean concentration of PCBs detected was low and ranged from 695 to 853 pg g^{-1} fresh weight. Mihale et al. (2013) analysed dioxin-like PCBs in sediments from the Kizinga River basin in Tanzania. No significant difference was observed between PCB levels detected in the wet and dry season. The DL-PCB levels in sediments were probably attributed to open burning of plastic scraps, household burning of wood or charcoal and traffic related emissions, all of which occur in the Dar es Salaam region as well as the denser population and the more intense industrial activities in the Kizinga River basin. These studies showed that the urban areas where most of industries are situated are severely impacted by PCB contaminants.

Table 2.10: PCB levels in soils/sediments (ng g^{-1} dw), water (ng L^{-1}) from some developing countries

Location, year	Types of samples	Range	References
Kizinga River basin, Tanzania	Sediments (wet season)	0.0002 – 0.00053	Mihale et al. (2013)
	Sediments (dry season)	0.0002 – 0.00059	
Central region of Ghana	Soils	1.32 – 12.94	Bentum et al. (2012)
Khélij channel, Tunisia	Fish (*Liza aurata)*	77 – 180	Masmoudi et al. (2011)
Yamuna, Delhi, India.	Sediments	0.16 – 30.05	Bhupander et al. (2011)
River Chenab, Pakistan	Sediments collected in Winter	9.33 – 129.45	Eqani et al. (2012)
	Sediments collected in Summer	12.55 – 144.23	
Lake Qarun, Egypt	Sediments	1.48 – 137.2	Barakat et al. (2013)
River Nile, Egypt	Fish	0.695 – 0.853	El-Kady et al. (2007)
Pearl River, China	Water	2.47 – 6.75	Nie et al. (2005)
	Sediments	11.13 – 23.23	
	Fish[a]	68.64 – 316.85	
Guangzhou, South China	Soil around a domestic waste incinerator	17.2 – 77.7	Zeng et al. (2014)
	Soils from 20 km far away the incinerator	5.48 – 25.57	

[a]Results are expressed on lipid weight

In developed countries, van der Oost et al. (1988) determined the concentrations of six indicator PCB congeners in sediments and four classes of biotic species of the aquatic food chain in a freshwater lake near Amsterdam. Despite the low concentrations of the contaminants in the plankton, significant amounts of total PCBs were found in sediments, macro-invertebrates and fish (Table 2.11). The higher chlorinated congeners (PCB 138, 153 and 180) were the most dominant. A study of Korrick and Altshul (1998) detected higher PCB levels in breast milk among four women living adjacent to a PCB-contaminated waste site in southeastern Massachusetts. Among 122 mother-infant pairs, four milk samples were identified with total PCB levels that were significantly higher than the rest, with total PCBs ranging from 1100 to 2400 ng g^{-1} milk fat compared with an overall mean of 320 ng g^{-1} milk fat for the 122 women. The higher levels of PCBs detected were attributed to contamination by effluents from local electronics manufacturing facilities. On the other hand, She et al. (2007) analysed PCBs in breast milk

from the Pacific Northwest in US. The total PCBs (82 congeners), the mean PCB levels detected was 147 ppb with median of 126 ppb.

Rodziewicz et al. (2004) inspected the contamination levels of seven PCBs in sediment samples collected at different sites along the Odra River and its tributaries. The levels detected were not high compared to the findings of Kanzari et al. (2012) in France. The lowest concentration was measured in the Odra for Widuchowa while the highest was in the Odra Braid. Kanzari et al. (2012) conducted a research on PCBs and other organic compounds in surface sediments from the Arc River and the Berre lagoon, France. The sum of seven PCB concentrations revealed higher levels of PCBs and ranged from 0.3 to 466.8 μg kg^{-1} dw. In the same country (France), Syakti et al. (2012) investigated the distribution of PCBs in marine sediments directly exposed to wastewater from Cortiou, Marseille. PCB concentrations was expressed as equivalent to Arochlor 1260 and varied from 9.1 to 226.9 ng g^{-1} dw. The hexachlorobiphenyls (PCBs 153, 138) and Pentachlorobiphenyl (PCB 101) were the dominant congeners detected at higher levels. In addition, Moret et al. (2005) evaluated the degree of PCB contamination of Venice lagoon water in France. The samples were collected at six sites and were selected to represent all the pollution situations present in the lagoon, and at a site in the Adriatic Sea. The dissolved fraction concentrations (0.250 – 0.792 ng L^{-1}) were greater at six sites than that of the particulate fraction (0.105 – 0.1273 ng L^{-1}). Chemometric analyses suggested that the PCB congener pattern in the Venice Lagoon was different from that in the Adriatic Sea. On the other hand, Balasubramani et al. (2014) analysed PCBs in wastewater effluent samples sampled in the summer of 2009 from 16 different locations including municipal and industrial wastewater treatment plants and petrochemical industrial outfalls in the Houston area. Higher levels of PCBs were found in water compared to the results of the above mentioned-author. Lighter PCB congeners exhibited highest concentrations in the dissolved phase whereas, in the suspended phase, heavier PCBs exhibited the highest concentrations. This is due to the water solubility of the lower PCB congeners than the heavy ones. Levels of PCBs were also detected in fish samples. Nicola et al. (2014) investigated and analysed PCBs and DDT in populations of endemic Iberian

barbel (*Barbus bocagei*) in the Jarama River in Spain *via* a pollution gradient from well-preserved areas upstream to contaminated downstream areas. Upstream to downstream PCB concentrations ranged from 3.4 to 101.4 ng g^{-1} ww. The higher chlorinated congeners such as PCBs 153, 138 and 180 congeners were dominant and the less chlorinated had a relatively high contribution upstream. Jaffal et al. (2011) assessed concentrations of PCBs in the muscle of 48 brook trout and 38 brown trout caught during summer and spring 2006 in the rivers, lakes and ponds of Kerguelen Island. The sum of 29 PCBs detected was high (Table 2.11). For dioxin-like PCB analysed, was 19 and 69 ng g^{-1} lipid, in brook and brown trout, respectively. The values showed a high variability and some fish accumulated PCBs at levels similar to those of fish from impacted areas.

Table 2.11: Range of PCBs in sediments (ng g^{-1} dw), breast milk (ng g^{-1} lw or fat basis), fish (ng g^{-1} ww), water (ng L^{-1}) from some developed countries

Location, year	Types of samples	Mean	Range	References
Jarama River in Spain	Fish (*Barbus bocagei*)		3.4 – 101.4	Nicola et al. (2014)
New Bedford Harbor and estuary in southeastern Massachusetts	Breast milk[b]		1100 – 2400	Korrick and Altshul (1998)
Pacific Northwest in US	Breast milk[a]	147	49 – 415	She et al. (2007)
Odra River and its tributaries,1998	Sediments collected in May 1998		1.3 – 13.6	Rodziewicz et al. (2004)
	Sediment collected in November 1998		1.3 – 28	
Arc River and the Berre lagoon, France	Surface sediments		0.3 – 466.8	Kanzari et al. (2012)
Kerguelen Island	Brook trout[a]	404		Jaffal et al. (2011)
	Brown trout[a]	358		
Cortiou, Marseille	Marine sediments		9.1 – 226.9	Syakti et al. (2012)

Location, year	Types of samples	Mean	Range	References
Houston area, USA	Wastewater (dissolved phase)		1.01 – 8.12	Balasubramani et al. (2014)
	Wastewater (particulate phase)		2.03 – 31.2	
Venice lagoon water in France	Water (Dissolved plus particulate phases)		355 – 1868	Moret et al. (2005)
Freshwater lake near Amsterdam	Sediments		0.22 –13.74	van der Oost et al. (1988)
	Plankton		0.22 – 0.69	
	Molluscs		0.52 – 5.77	
	Crustaceans		0.80 – 3.98	
	Fish		3.98 – 97.35	

[a]Results expressed on lipid weight, [b]Results expressed on fat basis,

The findings from the above studies with others showed that PCB levels detected in developed countries in general are higher than that detected in developing countries. This indicated that humans and other organisms are highly exposed to PCBs in those countries. However, due to the transport of PCBs over long distances from contaminated sites to remote areas, no one could say that the environment of the developing countries is safer than that of the developed countries.

2.11 PCB REGULATORY FRAMEWORK

Environmental contamination by POP chemicals, including PCBs, has been recognised by the international community as a significant problem. As a result, a number of international procedures and protocols for elimination, restriction, and prohibition of the manufacturing, distribution, usage, and emissions of PCBs have been developed. The result is that PCBs are one of the most highly regulated organic compounds, worldwide. Many countries have set their regulations based on the requirements ratified by the Stockholm Convention.

2.11.1 The Stockholm Convention

Phase-out and control of persistent organic pollutants (POPs) is one of the predominant features in implementing the Stockholm Convention (Sharma, 2013). The Stockholm Convention is a global agreement to protect human health and the environment from persistent organic pollutants (POPs). The Stockholm Convention originally listed twelve POPs, referred to as the "dirty dozen" that can be grouped into three categories (Bouwman, 2004). The POPs are listed under various Annexes of the Convention, namely: Annex A (elimination), Annex B (restriction) and Annex C (unintentional production) (Sharma, 2014).

- Pesticides including aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene are listed under Annex A except DDT which is listed under Annex B;
- Industrial chemicals: hexachlorobenzene and polychlorinated biphenyls (PCBs) are listed under Annex A and Annex C;
- By-products: hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), are listed under Annex C.

Due to the adverse effects of PCBs on human and the environment, the Stockholm Convention established a global ban of these harmful and toxic compounds, and requires its parties to take measures to eliminate or reduce the release of POPs into the environment. The Stockholm Convention was adopted, and put into practice, by the United Nations Environment Programme (UNEP) on May 22 - 23, 2001 (UNEP, 2006). The Convention strictly enforces the PCBs protocol of 23 October 2003 and sets out plans for their elimination. The Convention was ratified by the European Council, under EC Decision 2006/507/CE, on the 14th of October 2004. The United States of America has signed the Stockholm Convention but has not yet ratified it, as of 2014, however most countries in the world have ratified the convention. By 2025, all equipment containing PCBs must be listed, labeled and removed. Furthermore, they must be correctly stored before being eliminated in an environmentally sound manner by 2028. In general, all wastes containing at

least 50 mg kg^{-1} (50 ppm) of PCBs are considered as PCB waste. The ppm system is based on regulations in place for the United States while the percent by weight system is based on regulations in place for Sweden (0.1 percent by weight = 1000 ppm) (UNEP, 1999).

In 2009, nine additional POPs were added to the Stockholm Convention (Muir and Wit, 2010). These include: chlordecone, lindane, hexabromobiphenyl, pentachlorobenzene, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride (PFOS), tetrabromodiphenyl ether and pentabromodiphenylether (commercial pentabromodiphenyl ether), hexabromodiphenyl ether and heptabromodiphenyl ether (commercial octabromdiphenyl ether). The new nine POPs are listed under Annex A except pentachlorobenzene listed in Annex A and Annex C and perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride (PFOS) listed in Annex B. The Convention was amended in 2011 to include the pesticide endosulfan, making a total of 22 substances regulated under the Convention. This amendment entered into force on 27 October 2012. In May 2013, hexabromocyclododecane (HBCD) was included as 23rd POPs. The amendment for the listing of HBCD to Annex A of the Stockholm Convention entered into force on November 26, 2014 for most parties (Sharma, 2014).

2.11.2 Basel Convention

The Basel Convention is an international treaty signed on the 22nd of March 1989, designed to protect the environment and mankind from the harmful effects of transboundary hazardous waste. Its goal was to encourage the environmentally sound management of hazardous waste by controlling the wastes' movements in the environment and to prevent and minimize the generation of waste containing POPs, notably PCBs. Wastes containing a concentration of greater than 50 ppm of PCBs, polychlorinated terphenyls (PCTs), polybrominated biphenyls (PBBs) or polychlorinated naphthalenes (PCNs) were considered as hazardous waste, and have to be destroyed by safe means (Krueger, 2001).

2.11.3 PCB Elimination Network (PEN)

After signing the Stockholm Convention, developing countries may be faced with problems in fulfilling their obligations to destroy PCBs, due to lack of treatment capacity, logistical difficulties, limited resources or difficulty to access information. To overcome these difficulties, PEN was created in 2009 to establish linkages within regions, to improve and exchange information to ensure if environmentally sound management of PCBs is achieved worldwide (Harris, 2013).

2.11.4 Canadian Environmental Protection Act (CEPA)

In Canada, PCBs are regulated under the Canadian Environmental Protection Act, 1999. CEPA has established a prohibition on the release, manufacture, processing, use, import, export, and sale of PCBs and products that contain certain concentrations of PCBs, and also provide exceptions to these prohibitions and the duration of these exceptions (Garett et al., 2010). Other requirements comprise environmentally sound management, equivalent levels of safety and waste reduction plans for exports for final disposal. CEPA's new PCB Regulations of 2008 (SOR/2008-273) retracts and replaces the existing PCB regulations and the storage of PCB material regulations. They set specific deadlines for ending the use of remaining products containing PCBs in concentrations at or above 50 milligrams per kilogram (mg kg^{-1}). The use of equipment containing PCBs at a concentration ≥ 500 mg kg^{-1} was phased out by 31 December 2009. Similar equipment containing PCBs in a concentration of at least 50 mg kg^{-1} but less than 500 mg kg^{-1} and located at any other place in Canada will be eliminated from use by 31 December 2025 (CEC, 2010). The exportation of waste containing PCB with a concentration of 50 mg kg^{-1} (ppm) or more to any country other than the United States was prohibited.

2.11.5 Regulations in France

A decree has been created governing the labeling and packaging of PCB substances classified as "toxic substances" on October 10, 1983**.** In France, production and uses of PCB were restricted since 1975 in open systems (e.g.,

hydraulic fluids, coatings) and 1987 in closed systems (e.g., transformers, capacitors) to ensure the prohibition of the hazardous waste (Arnich et al., 2009; Mourier et al., 2014). In 2001, the deadline of December 31, 2010 was set out for the elimination of equipment containing concentrations of PCBs above 500 mg/kg. Other measures prohibit any material containing more than 50 ppm of PCBs (http://www.tredi-pcb-treatment.com/en/page/4/regulations.html, accessedon 1st June 2015).

2.11.6 Regulations in South Africa

South Africa, as a party to the Stockholm Convention on persistent organic pollutants, has published on the 10th July 2014 in Government Gazette No. 10232, Vol. 589 the regulations on phasing out the use of PCB materials and PCB contaminated materials. These regulations come into force immediately. The National Environment Management Act (NEMA), 1998 (Act No. 107 of 1998) proposed regulations to phase out the use of PCB materials and PCB contaminated materials which stipulate:

- The prohibition to use, process or produce PCB materials or PCB contaminated materials.
- The import of PCB materials or PCB contaminated materials or PCB waste into the country, or export PCB materials or PCB contaminated materials from South Africa.
- The trade of PCB materials or PCB contaminated materials.

PCB contaminated materials refer to oil or articles with PCB concentrations greater than 51 ppm, but less than 500 ppm; while PCB materials refer to oil or articles with PCB concentration greater than 500 ppm (http://www.polity.org.za/article/national-environmental-management-act-regulations-to-phase-out-the-use-of-polychlorinated-biphenyls-pcsbs-materials-and-polychlorinated-biphenyl-pcbs-contaminate-materials-g-37818-rg-10232-gon-549-2014-07-10, accessed on 20th October, 2014).

2.11.7 EPA Regulations

The Environmental Protection Agency (EPA) regulates PCBs through rules issued to the Toxic Substances Control Act (TSCA) of 1976. These regulations generally control the use, manufacture, commercial distribution, and disposal of chemicals including PCBs. (http://www.epa.gov/oppt/newche-ms/pubs/chem-pmn/appendix.pdf, accessed on 18th May 2015). There are millions of pieces of equipment in operation in the U.S. which were manufactured prior to these regulations and which contained PCBs. Items such as transformers and hydraulic fluids were identified as high-risk sources and were targeted for accelerated phase-out. The regulation addresses two types of equipment containing PCB concentrations greater than 500 ppm, and PCB-contaminated equipment containing 50 – 500 ppm PCBs. The concentrations below 50 ppm were not regulated and were considered as non-PCB liquids (Fisher et al., 1984). For example, a PCB transformer is a transformer that contains PCBs at concentrations greater than 500 ppm. EPA stipulates that any liquids containing concentrations of PCBs greater than 500 ppm be disposed of in special landfills. The storage and disposal facilities of PCBs were required to maintain extensive records (Erickson, 1997).

This chapter highlighted the general information on PCB compounds and its regulation in some countries. PCBs are characterized by high persistence and bioaccumulative properties. They have adverse health effects on humans, animals and other ecosystems. Being semi-volatile they can travel great distance due to wind, water currents and exposed species (birds, fish, migratory mammals, etc.). The main route of exposure of PCBs to humans is through ingestion of contaminated food.

CHAPTER 3

ANALYTICAL METHODS FOR PCB RESIDUES DETERMINATION IN ENVIRONMENTAL MATRICES AND METHODS USED IN THIS STUDY

3.1 INTRODUCTION

The analytical methods and techniques that are commonly used for the assessment of PCBs in environmental samples are presented in this chapter. The methods are approved by federal agencies and organizations such as Standard Operating Procedures (SOPs), Environmental Protection Agency (EPA) and the National Institute for Occupational Safety and Health (NIOSH) (ATSDR, 2000). Traditionally, the analyses of PCBs in environmental matrices were based on Aroclor mixtures, but with the improvement of laboratory techniques, PCB congener specific methods have been introduced (Fikslin and Santoro, 2003).

3.2 ANALYTICAL PROCEDURES FOR THE DETERMINATION OF PCBs IN ENVIRONMENTAL MATRICES

The determination of organic pollutants including PCBs in environmental samples (water, sediments, biota and others) generally involves four major steps including sampling and sample preparation, extraction, clean-up, identification and quantification of target analytes (Tadeo et al., 2012; Guhl et al., 2014).

3.2.1 Extraction techniques

The extraction is the first step applied for the determination of PCBs in environmental matrices using an organic solvent (Msagati and Mamba, 2011) with the purpose of isolation of the target compounds from the sample matrix. According to Muir and Sverko (2006), different types of extraction techniques may be used depending on the sample matrix. Khan et al. (2005) showed that the accuracy of results is influenced by many factors including the use of a

convenient type of extraction. Other factors such as the solubility of the analytes in the extraction mixture, the accessibility of the matrix to the extraction medium, the extraction time and temperature may also influence the efficiency extraction procedures (Naert, 2007). Common extraction techniques for solid matrices include Soxhlet extraction, sonication extraction, supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and pressurized liquid extraction (or accelerated solvent extraction). For aqueous matrices such as water, PCBs are separated from the sample matrix using separatory funnel extraction, continuous liquid/liquid extraction or solid phase extraction (SPE).

3.2.1.1 Soxhlet extraction

Soxhlet extraction is frequently used to isolate the extract due to its easy manipulation and low costs (Iozza, 2010). Soxhlet extraction has been used for more than 100 years (Khan et al., 2005) and the technique has been adopted by the U.S. Environmental Protection Agency (EPA) as method 3540C (U.S. EPA, 1996) for extracting semi-volatile and non-volatile compounds from solid matrices (Banjoo and Nelson, 2005). The Soxhlet extraction involves heating of the organic solvent to continuous reflux through the sample contained in a porous thimble (Khan et al., 2005) until all analyte in the sample is completely extracted into the lower flask (Figure 3.1). Once the solvent is boiled, the vapour passes through a bypass arm into the condenser, where it condenses and drips back onto the solvent and sample in the thimble (Lau et al., 2010). Soxhlet extraction is performed at temperatures below the boiling point of the solvents and the extraction times with a regular Soxhlet apparatus may vary between 6 h and 48 h. The disadvantages of Soxhlet extraction include lengthy time and the consumption of large amount of solvent (200 - 500 mL). The extract volume is relatively large and the evaporation step is usually needed to concentrate the analyte prior to clean-up and analysis.

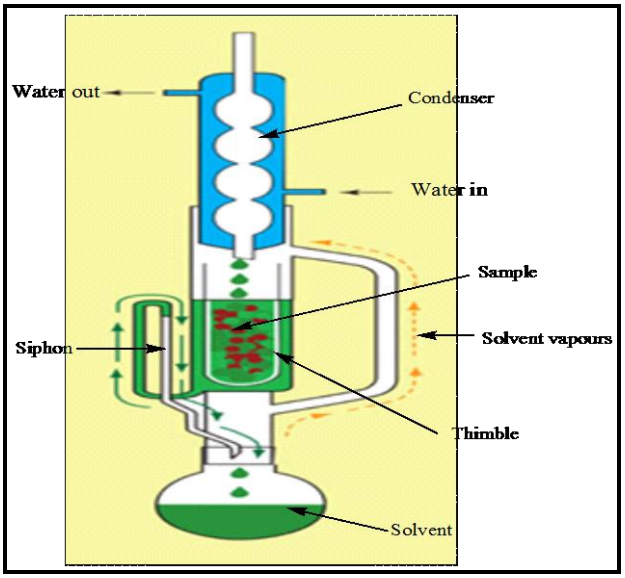


Figure 3.1: Soxhlet apparatus: solvent vapour passes external to the sample containing thimble, this results in cooled organic solvent passing through the sample.

3.2.1.2 Ultrasonic extraction (USE)

USE is performed using an appropriate organic solvent. The sample is immersed in a vessel and placed in an ultrasonic bath (Bayen et al., 2004). The effectiveness of ultrasonication depends on the polarity of the solvent, the homogeneity of the matrix and the time to complete the extraction. In contradiction to Soxhlet extraction, the ultrasonic extraction takes about 30 to 60 minutes to perform and several extractions can be done simultaneously. However, the extract requires filtration and the method is not automated (Camel, 2000; Eskilsson and Björklund, 2000).

3.2.1.3 Supercritical fluid extraction (SFE)

SFE was first introduced by Stahl in 1976 (Sporring et al., 2005) but it was only used to extract persistent organic pollutants (POPs) in environmental samples in 1986 (Hawthorne and Miller, 1987). SFE has been adopted by the U.S.EPA as method 3562 for the extraction of PCBs in solid environmental samples. Carbon dioxide (CO_2) is used as solvent and a co-solvent (usually ethanol) may be added to increase the polarity. The carbon dioxide is brought above its critical pressure (73 atm) and critical temperature (31.1 °C) to diffuse through the solid samples like a gas and to dissolve the matrix

components like a liquid (Figure 3.2) (Lopez-Avila, 1999). The important properties offered by a supercritical fluid for extraction are: good solvating power; low viscosity and minimal surface tension. Turner et al. (2002) mentioned that the collection device (Figure 3.2) can be an empty vessel, a vessel containing a small volume of organic solvent, a solid-phase trap, or a cryogenically cooled capillary leading to many possibilities for achieving collection in SFE. The high cost of SFE is a disadvantage.

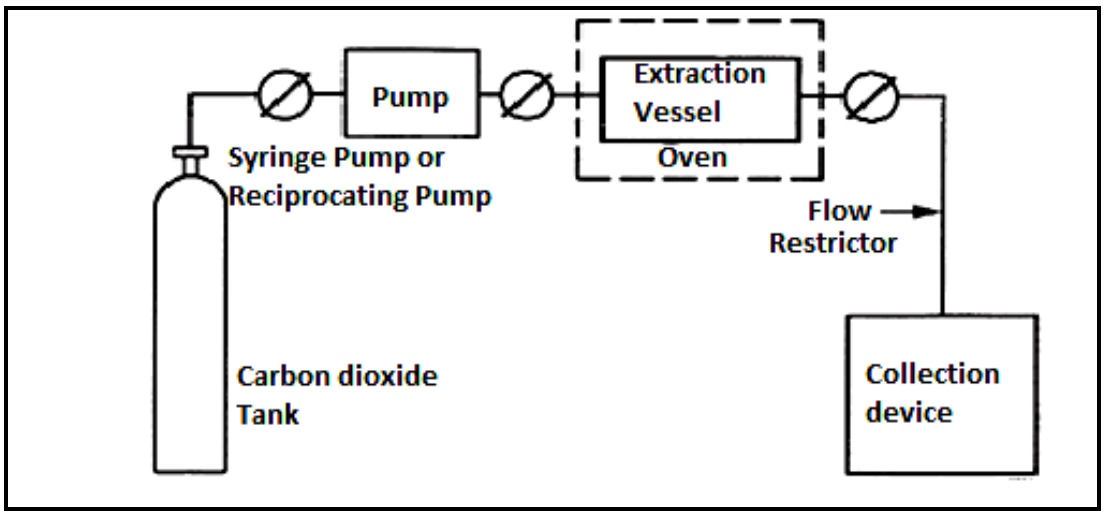


Figure 3.2: Basic components of supercritical fluid system.

3.2.1.4 Accelerated solvent extraction (ASE)

Researches indicated that ASE is one of the most recent extraction technique introduced for solid samples (Ahmed, 2001; Björklund et al., 2002). The system consists of a stainless-steel extraction cell, where a programmed temperature and pressure are controlled by electric heaters and pumps. In opposition to SFE, ASE uses organic solvents in place of carbon dioxide under pressures ranging from 102.06 to 204.13 atmospheres (atm) and temperatures (50 to 200 °C) which reduce the time of the extraction. In ASE, simultaneous extraction and clean-up can be carried out (Covaci et al., 2003). It can be accomplished in the static mode, the dynamic mode, or a combination of both (Carabias-Martínez et al., 2005). In the static mode, the sample and the solvent are maintained for a specified time at constant pressure and temperature, whereas in the dynamic mode the solvent flows through the sample in a continuous manner. This technique presents some

drawbacks, the equipment is very expensive, and a stable power source is required. It is also difficult to control selectivity in the ASE process (Giergielewicz-Możajska et al., 2001). For fatty matrices, the presence of large co-extracted lipids is present, and an extra post-clean-up step is required for the lipid removal in order to enhance the subsequent chromatographic performance for PCB analysis (Beyer and Biziuk, 2008).

3.2.1.5 Microwave-assisted extraction (MAE)

MAE utilizes the microwave energy as a source of heat to enhance the rotation of the sample molecules with a permanent dipole and disorganised movement, leading to rapid heating of the solvent and samples (Eskilsson and Björklund, 2000). The extraction is advantageous in that requires a short time because the entire sample is simultaneously heated without heating the vessel and the solution quickly reaches its boiling point (Camel, 2000). Further advantages of microwave extraction include the low vessel temperature, high extraction rate, amenability to automation and the possibility of extracting different samples at the same time without interferences (Camel, 2000; Zanella et al., 2012). Nowadays, the novel extraction techniques such as microwave-assisted extraction, supercritical fluid extraction, and accelerated solvent extraction are very attractive because they are faster and use smaller amounts of solvents.

3.2.1.6 Matrix solid-phase dispersion (MSPD)

Since 1989, MSPD has been used as a sample preparation method for the analysis of organic pollutants (Barker, 2007). MSPD extraction is carried out when the analysis comprises a variety of solid and semi-solid/or highly viscous biological matrices (Barker, 2007; Zhang et al., 2014). The technique involves homogenization of solid or viscous samples by direct mechanical grinding of a small amount of the sample with a sorbent material (mostly florisil, silica and octadecylsilyl -derivatized silica (C18)) using a mortar and pestle until a dry free-flowing powder is produced (Figure 3.3) (Ferrer et al., 2005; Bogialli et al., 2007). The homogenized sample is then packed into a solid phase extraction column followed by washing with a small amount of

solvent and elution to extract a range of compounds of interest (Łozowicka et al., 2012). The advantage of this technique is that extraction and clean-up can be performed in one step leading to a quicker time and lesser consumption of solvents (Dassanayake et al., 2009). However, the efficiency of the MSPD depends on multiple factors, particularly the sorbent type and the appropriate eluting solvent. The method requires a smaller sample size (0.5 g) has a shorter analysis time (40 min) and uses less and reduced toxic organic solvent (10 mL) (Ahmed 2001; Parvathamma et al., 2012).

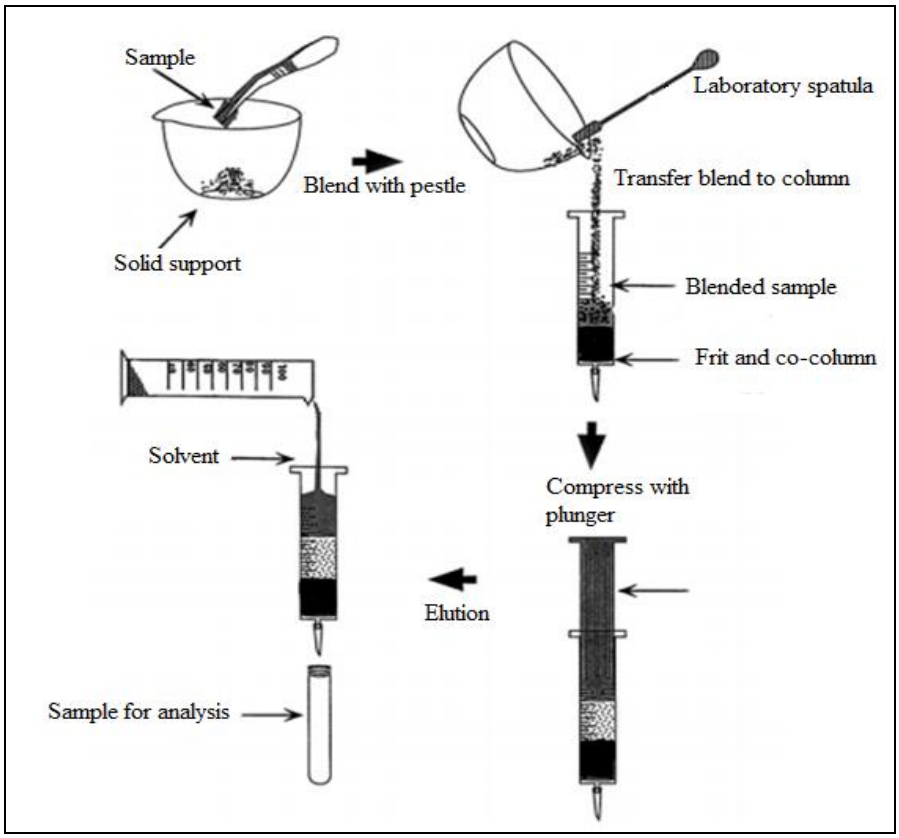


Figure 3.3: Schematic representation of the MSPD extraction procedure.

3.2.1.7 Extraction of aqueous samples

The commonly used extraction techniques for the determination of PCBs in aqueous samples include liquid-liquid extraction (SW-846 Method 3510), continuous liquid-liquid extraction (Method 3520) and solid phase extraction (SPE) (Method 3535) (USEPA, 1997). Liquid-liquid extraction technique

involves adding a solvent to the sample that is immiscible followed by the partitioning of analytes versus contaminants between the two phases. The mixture of the two phases is agitated by shaking using a separatory funnel. A Study of Hasset (2001) indicated that this technique is less likely to cause emulsion problems due to dirty matrices such as blood, food and wastewater. For solid phase extraction, water sample is passed through a sorbent column, filter, or disk where the target compounds are extracted onto the sorbent. The adsorbed organics are then eluted from the column with an organic solvent (Erickson, 1997). However, because PCBs are lipophilic compounds, several studies showed that after the extraction, a clean-up procedure has to be used such that it excludes macromolecules present in the extracts which can interfere with the PCB congeners of interest in the gas chromatography procedure (Björklund et al., 2002; Van Emon et al., 2013).

3.2.2 Clean-up methods

The chromatographic materials used to isolate organic compounds such as high-performance liquid chromatography (HPLC) and gas chromatography (GC) columns are highly sensitive to trace amounts of lipophilic material which affect the active surfaces of the stationary phase and degrade the resolving power of the column (Rossetti et al., 2012). The extracts may contain various components with a high molecular size such as lipids, pigments and resins when analysing sediments, fish or water from lakes, rivers and other aquatic areas (Ahmed, 2001). Therefore a clean-up procedure is necessary after the completion of the extraction to avoid poor chromatographic performance caused by these lipophilic substances in the extracts (Singh et al., 2014). The choice of the clean-up method to be used depends not only on the selectivity and sensitivity of the instrument, but also the extraction method employed. The elimination of lipids in the extracts can be accomplished by destructive or non-destructive methods.

3.2.2.1 Non-destructive methods for lipid removal

The clean-up procedure of hydrophobic compounds from organisms and other environmental matrices requires that the samples be dried to remove water

and improve the efficiency of the process by sulphur removal. The absence of water in the extract makes the sample matrix more accessible to the use of a wide variety of solvents which extract the PCB compounds (Smedes and de Boer, 1997). Therefore, the homogenisation and extraction are much easier when the samples are dry (Khan et al., 2005). Two different non-destructive techniques applied for lipid removal include gel permeation chromatography and adsorption chromatography.

3.2.2.1.1 Gel permeation chromatography (GPC)

GPC is a clean-up method applied for samples with high lipid content such as foods of animal origin with a high fat content in which the analysis requires many purification steps (Focant et al., 2001). The separation relies on the size of the macromolecules in solution. The larger molecules (including fats) move quickly through the column. The smaller analytes, including most of the organic contaminants that accumulate in biological tissue, can enter the pores of the stationary phase more easily and therefore spend more time in these pores, increasing their retention time and they take longer to pass the column. The method performance is improved and enhances or extends GC column life. GPC is advantageous in that it is readily automated, but it cannot exclude all lipids and requires the larger volumes of solvent (low pressure or gravity systems) to elute the more polar substances (Hess et al., 1995).

3.2.2.1.2 Adsorption column chromatography

Adsorption column chromatography is the most widely used technique for the clean-up of extracts prior to the analysis for organic compounds. This technique involves the use of various adsorbent columns containing stationary phases such as alumina, silica and Florisil with different particle sizes and column dimensions. The relative strength of adsorption of these materials is: silica gel < Florisil < alumina (de Voogt 1994). Florisil is the most popular sorbent employed and is particularly suited for fatty samples (Singh et al., 2014). While alumina is generally suitable for chromatography of less polar compounds, silica gel gives good results with compounds containing polar functional groups (Żwir-Ferenc and Biziuk, 2006). Adsorption chromatography

is suitable for the separation of relatively non-polar compounds. These materials (Florisil, silica gel and alumina) adsorb polar substances *via* interactions with the -OH and =O moieties present at their surfaces. Non-polar analytes are eluted earlier than polar analytes. Since the bulky lipid material present in environmental samples contains many polar functional groups, it will be retained on such columns. Both alumina and Florisil may be used to separate PCBs from polychlorinated dibenzofurans (PCDFs) and polychlorinated dibenzodioxins (PCDDs) and to separate non-*ortho* substituted PCBs from the other PCBs. Fractionation is an important method for PCB analysis because different compounds can be separated using a single column and co-elution problems are avoided.

3.2.2.2 Destructive methods for lipid removal

Alkaline treatment (saponification) or oxidative dehydration by sulphuric acid treatment is the method commonly used for the lipid removal in sample extracts when the analytes of interest are stable under acidic or basic conditions (Ahmed, 2003). The simplest approach consists of direct addition of concentrated sulphuric acid to a sample extract dissolved in n-hexane. This method is used for the degradation of most aliphatic and aromatic compounds in environmental samples (Duarte-Davidson, 1991). A disadvantage is that it requires several repetitions resulting in a time-consuming procedure. The use of saponification is limited due to high temperatures which may cause the degradation of certain highly chlorinated compounds (Muir and Sverko, 2006). Sulphuric acid treatment is suitable for PCBs analysis because these compounds are stable under strong acid conditions. However, some pesticides such as Aldrin may be affected by treatment with strong sulphuric acid (Lang 1992). The use of acidified silica avoids the emulsion complications of liquid-liquid extraction approach, and it reduces the sample handling and solvent consumption (Lacorte and Guillamon, 2008). The use of acidified silica in combination with Florisil, silica, or alumina in multilayer columns improves the purification of the samples. All the techniques provide similar results with respect to recoveries and reproducibility (Oliver and Bothen, 1982).

3.2.3 Determination of PCBs

The analysis of PCBs can be divided into two distinct categories: specific and non-specific methods (Kamba et al., 2014). Specific methods include the main techniques currently used for PCB analysis: gas chromatography (GC) and mass spectrometry (MS). Specific methods are most appropriate compared to non-specific techniques when other compounds containing chlorine are to be analysed. Non-specific methods identify classes of compounds, such as chlorinated hydrocarbons to which PCBs belong.

3.2.3.1 Non-Specific techniques

Non-specific methods are used for the determination of PCBs in matrices contaminated with transformer oil, e.g. diesel fuel oil and gasoline. Different non-specific techniques include X-ray fluorescence, microcoulemetry, colorimetric tests, enzyme-linked immunosorbent assays (ELISA) and electrochemical methods (Finch, 1990; Kamba et al., 2014).

3.2.3.1.1 X-ray fluorescence technique

X-ray fluorescence is the only non-specific method that is not destructive. In this method, a sample is placed in a vessel which is transparent to X-rays. The homogenised sample is irradiated with a particular wavelength. If chlorine is present in the sample, the radiation of a different, but specific, wavelength is emitted back to a detector, and the total chlorine content is quantified. The X-ray fluorescence offers some advantages and disadvantages. Their analysers are expensive and require trained personnel to operate but can be quite cheap and time efficient when large numbers of samples are processed on a daily basis.

3.2.3.1.2 Microcoulometry method

A sample to be analysed by microcoulorimety is entirely combusted at high temperature and then is subjected to the titration process where the resulting chloride is titrated electrically with a silver electrode. By the total amount of silver produced to neutralize the chloride, the total amount of chloride can be easily calculated and the PCB concentration in the sample can be determined

from this result. Like X-ray fluorescence, microcoulorimetric method is expensive and need to be operated by a trained technician. It is less time efficient than X-ray fluorescence.

3.2.3.1.3 Colorimetric tests

Colorimetric methods are the simplest and least expensive of the chloride detection techniques. Firstly, the sample reacts with a sodium compound to break down the PCBs into fragments of hydrocarbons and chloride. The resulting chloride is then extracted into an aqueous phase where it can be detected by a colorimetric reagent. The most predominant method involves adding a known amount of mercuric nitrate which complexes with chloride in a ratio of 1:2 diphenyl carbazone. A very sensitive indicator to free mercuric ions is then added to the solution. If all of the mercury is complexed with chloride, no colour will appear. By adding a precise amount of mercuric nitrate to the sample, the test can be engineered to generate a specific endpoint at any chosen action level. For PCB, this level is usually 50 ppm. The advantages of the colorimetric technique include efficiency, and ease of use. The main disadvantage is that the method had lower sensitivity and hence it is hardly used in the detection of trace substances and it is not quantitative (Eboka et al., 2005; Cheng et al., 2014).

3.2.3.1.4 Electrochemical methods

The electrochemical method is similar to the colorimetric test in that it requires an initial reaction with a sodium based compound to remove the chloride from the PCB backbone. After the completion of the reaction, an ion-selective chloride electrode containing a membrane is used to quantitatively measure the concentration of chloride ions in the sample. The typical instruments used to make the measurement can then translate the concentration of chloride ions that it senses directly into parts per million PCB. This is a quantitative method with a limit of detection of about 2 ppm. It is just as fast as colorimetric techniques, but requires a main electrical power source and involves significant capital cost.

3.2.3.1.5 Enzyme-linked immunosorbent assay (ELISA)

ELISA is used to quantify most of PCBs and OCPs in environmental samples due to its speed, sensitivity, and selectivity, has a long shelf life and is relatively simple to use (Arya et al., 2006). Immunoassay tests use antibodies to bind with a target compound and the concentrations of the PCB and the target analyte are identified through a colorimetric reaction. By comparing the color developed by a sample of unknown concentration with the color formed by the standard containing the analyte at a known concentration, the PCB can be determined. The quantification of the PCB is performed by the intensity of color in the sample and is measured with a spectrophotometer; the amount of analyte in the sample is interpolated from a calibration curve (Muir and Sverko, 2006). Commercial ELISA kits for detection of PCBs and most OCPs are available from Millipore Corp. (Billerica, MA, USA) and Strategic Diagnostics (Newark, DE, USA). The detection limits for PCBs are in the order of μg L^{-1} in water or 0.1–1 μg g^{-1} range in soil and plant extracts. These kits are meant to be used with relatively little sample preparation and, although semi quantitative, are ideal for screening samples and complement more elaborate techniques involving GC analysis. Galloway et al. (2002) used an ELISA to analyse PCBs in mussel samples from New Bedford Harbour (USA). The ELISA and GC–ECD results were highly correlated, while the GC-ECD results were about 20% lower than quantitation by GC/MS. Samples were extracted and lipids were partially removed by chromatography on Florisil prior to exchanging the sample into a phosphate buffer/methanol solution for the immunoassay. The use of the ELISA saved additional isolation steps and GC analysis. Skerritt et al. (2003) examined the application of ELISAs for DDT and cyclodiene insecticides, heptachlor and endosulfan in plant-derived foods. The results showed that the clean-up step was necessary for foods that yielded highly coloured extracts such as coffee and spinach and for oily products such as cottonseed.

3.2.3.2 Specific techniques

3.2.3.2.1 Gas chromatography (GC)

GC was first recognized as an efficient method of mixture separation by the Russian botanist Mikhail Semenovich during his research on chlorophyll in the 1900s. This technique was ignored until the late 1930s and early 1940s when Martin and Synge (1941) introduced liquid-liquid chromatography by supporting the stationary phase (water) to separate acetyl amino acids. Nowadays, GC is a powerful and widely used tool for the separation, identification and quantification of components in a complex mixture using a gaseous mobile phase flowing over a stationary phase contained in a column (Siddiqui et al., 2013). The mobile phase is an inert gas such as helium or unreactive gas while the stationary phase is layer of liquid or an inert solid support contained inside the column. The mobile phase moves the constituents of a sample through the column which is packed with stationary phase (Kondeti et al., 2014). The GC separation is based on the interactions of analytes in a mixture with the stationary phase and the mobile phase (Kupiec, 2004). The major instrumental components of a modern GC are shown in Figure 3.4. It consists of an injection port, a column, carrier gas flow control equipment, an inlet system, an oven and heaters for maintaining temperatures of the injection port (inlet system), the column, and the detector. A recorder responds to a signal from the detector. The time elapsed between injection and elution is called the “retention time” (RT) that is used to differentiate target compounds in the samples based on those of standards.

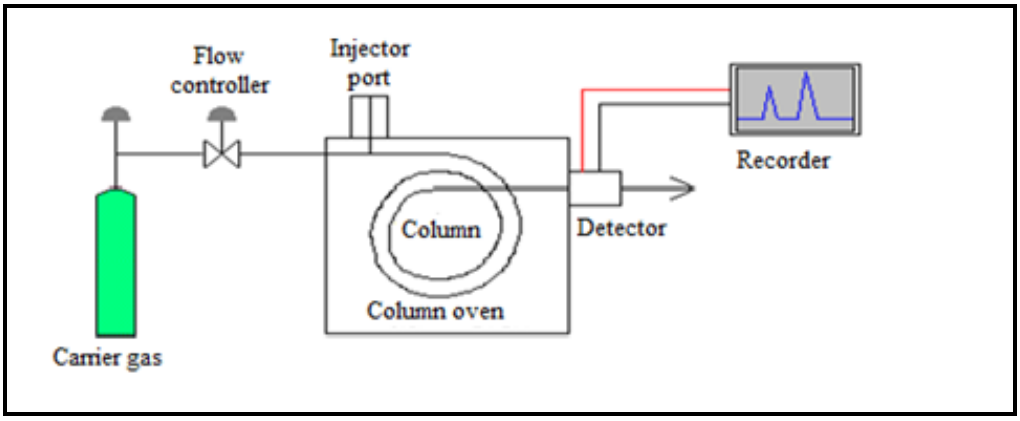


Figure 3.4: Schematic of a modern gas chromatograph.

3.2.3.2.2 Sample injection systems

Currently, capillary gas chromatography is the most popular technique used in the analysis of organic contaminants due to its separation efficiency and sensitivity (MaÐtouska and Krivµnkovµ, 2001). The GC analysis starts with the introduction of the sample onto the column. The injector provides a place for the sample introduction, its vaporization and splitting. There are many injection techniques used in the capillary GC. The most common techniques used in the analysis of PCBs or organochlorine pesticides (OCPs) include split injection, splitless injection and on-column injection (Bailey, 2005).

Split and splitless injection are both performed using the same inlet system, or the injector. A sample is introduced into the glass liner using a syringe. Splitless injection is used for dilute samples and most of the sample is introduced into the column. A small sample volume (1 - 2 µL) is injected into an injector at a high temperature (250 – 300 °C) and the heat of the injector rapidly volatilizes the sample. The carrier gas mixes with the vaporized sample and carries it into the column. Split injection is used for concentrated samples where only a small fraction of the sample volume (≤ 2%) is introduced into the column. The rest of the vaporized sample and a large flow of carrier gas pass out through a purge valve. Splitless injection offers the advantage of greater sensitivity for trace analysis rather than split injection which is limited since only a fraction of the sample enters the column.

On-column injection is a non-vaporizing technique which is mostly used for the analysis of high boiling compounds such as petroleum waxes, triglycerides and other thermally unstable compounds (Snow, 2004). An on-column injection starts with a cold injector and the sample reaches the column as a liquid and is vaporized *via* temperature programming of the column and/or the injector. The entire sample is directed into a capillary column. The injection of more than one to two microliters of solvent causes band broadening and/or insufficient quantitative performance of the GC system on-column, split and splitless injection (Mol et al., 1996; Buczyńska et al., 2014).

Programmed Temperature Vaporizing injection (PTV) is another technique of choice for the introduction of large sample volumes (up to 250 µL) to improve sensitivity to overcome these adverse effects caused by on-column and splitless injections. The sample is introduced into the liner at a controlled injection rate (Mol et al., 1996). The PTV injector is designed to allow the inlet to perform a pre-separation of target analytes from other components of the sample. PTV comprises the injection into the cold liner (temperature held below or near the solvent boiling point) and subsequent rising of temperature and transfer of analytes. This technique is of interest to avoid the discrimination of low volatile compounds and the degradation of thermally unstable analytes (Zrostlıkova et al., 2001). For most of the PTV application, a slow ramp rate can be applied to minimize the thermal decomposition of the labile analytes. The PTV inlet has the same basic functions as the split/splitless inlet except that it is temperature programmable (using CO_2 cooling) (García-Rodríguez et al., 2010).

3.2.3.2.3 Gas chromatography columns for PCB analysis

The column is one of the main components of a GC system. Mostly, gas chromatographs are equipped with ovens to keep the column at temperatures from 40 to 350 °C in order to evaporate the sample (Surendra et al., 2012). Two popular columns include the packed and capillary column. Packed chromatographic columns vary in length from 1 to 10 m and have an internal diameter of 2 to 4 mm. A capillary column can range from a few meters to over 100 m and internal diameters are typically a few tenths of a millimeter.

For the analysis and identification of PCB congeners, capillary columns are preferred. Fused silica open tubular capillary columns, generally coated with non-polar or medium-polarity chemically bonded liquid phases are generally used for separation of PCBs and organochlorine pesticides (Lang, 1992). Therefore, the separation of PCBs are commonly achieved using non-polar liquid phases such as 100 % dimethylpolysiloxane (type DB-1ms) or 5 % diphenyl-95% dimethylpolysiloxane (type DB-5ms) with column lengths of 25 to 60 m (Covaci et al., 2003). However, the choice of column-type largely depends on the possible co-elutions of target compounds and alternative

phases such as n-octyl or n-octyldecyl substituents (DB-XLB), (8%-phenyl-polycarborane-siloxane (HT 8) have been used (Careri et al., 2002; Ahmed, 2003). According to Muir and Sverko (2006), PCBs within a homolog group elute based to their number of ortho chlorines: 4 < 3 < 2 < 1 < 0 on non-polar stationary phases. Nguyen (2009) performed the separation of PCBs and organochlorine pesticides (OCPs) using capillary column ZB-5ms (5%-phenyl-Arylene-95% dimethylpolysiloxane) which is equivalent to DB-5ms, VF-5ms, CP-SIL 8 CB MS. However, in the study of Naert (2007) investigating the efficiency separation of PCB congeners using DB-5ms column, PCB 28 and PCB 31 were not sufficiently separated. Phenyl-Arylene bonded phase improves resolution of aromatic compounds and reduces activity for acidic and basic compounds. ZB-5ms is recommended for better quantitation of semi-volatile mixtures including PCBs and polycyclic aromatic hydrocarbons (PAHs) (Yamaguchi and Lee, 2010).

The basic technology for separation of PCB congeners described by Mullins et al. (1984) has not changed greatly over the years. These authors used a 5% phenyl methyl silicone phase to achieve the separation of PCB congeners. Improved routine separations of PCBs have been achieved using 60 m × 0.25 mm i.d. columns with hydrogen carrier gas. The Figure 3.5 depicts 100 % dimethylpolysiloxane (a), 5% diphenyl-95% dimethylpolysiloxane (b) and phenyl-polycarborane-siloxane (c) used as stationary phases.

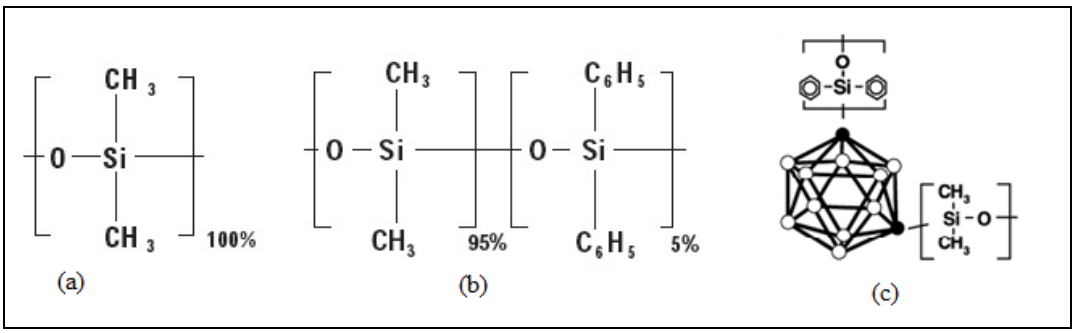


Figure 3.5: Structure of 100% dimethyl-polysiloxane (a), 5% phenyl-95% methyl-polysiloxane (b) and phenyl-polycarborane-siloxane (c) as stationary phases.

3.2.3.2.4 Gas chromatography detectors

The components of a mixture to be analysed elute from the column and produce a response at the detector. For each chromatographic determination, this response is converted by the detector into an electronic signal which is recorded in the data system where the components are separated, identified and measured. The magnitude of the detector response (signal) is plotted as a function of time generating a chromatogram. In general, GC detectors are very sensitive for trace analysis and find extensive use in environmental monitoring. The trendiest detectors used include the flame ionisation detector (FID), electron capture detector (ECD) and mass spectrometry detector (MSD). The FID detects most organic compounds when they are ionised and the eluting analytes cause a voltage drop across the collector electrodes as they are combusted in an air-H_2 flame (Surendra et al., 2012; Phonde and Magdum, 2015). The ECD is the most widely used detector for the quantification of POPs in environmental samples because it selectively responds to electrophilic compounds containing more halogen atoms (Tobiszewski and Namieśnik, 2011). In the ECD, a beta emitter such as radioactive tritium or ^{63}Ni is used to ionize the carrier gas. The radioactive source can produce beta particles quickly and collide with the molecules of the carrier gas. The identification of compounds using GC-ECD is based on the retention time in relation to the standard solutions under the same conditions (Webster et al., 2013). For PCB determination, the response of the ECD depends on the number and the position of the chlorine atoms in the PCB molecule. The main drawback of the GC/ECD method is the fact that the ECD does not yield a specific signal to individual compounds, all analytes will respond similarly (with varying magnitudes) in the detector. Generally, the sensitivity increases with the number of chlorine atoms (Liem, 1999). However, it has two major inconveniences: non-linear response behaviour across a relatively narrow range; and a wide variation in response even within a particular PCB-homologue group (Ahmed, 2003).

With respect to detection limits for organochlorine compounds such as PCBs and OCPs, lower values are obtained for GC/ECD than GC/MS. Plummer et

al. (2008) analysed halogenated volatile organic compounds in groundwater using GC/ECD and GC/MS. They found that the minimum detection levels (MDLs) identified in GC/ECD were two to more than four orders of magnitude below the GC-MS MDLs. In addition, Vidal et al., (2002) confirmed that limit of detection values obtained by use of GC-ECD were lower than those obtained by the use of GC-MS tandem mass spectroscopy. However, GC-MS offers advantages over GC/ECD for the analysis of PCBs. The work done by Duinker et al. (1988) showed that some problems associated with coeluting congeners are solved by GC-MS techniques in the case of Aroclor mixtures. Further, Pedersen-Bjergaard et al. (1996) indicated that the selectivity in the determination of PCBs may be improved by extensive sample preparation as well as the replacement of ECD by MS. In addition, Muir and Sverko (2006) evidenced that GC/MS avoids interferences of compounds which may arise from GC/ECD analysis such as those from sulphur, phthalate esters, and negative peaks generated by hydrocarbons.

3.2.4 Mass spectrometric detection

3.2.4.1 Introduction

In the early 1970s, with the establishment of the U.S. EPA, GC/MS started to be commercialized especially for the analysis of POPs such as PCBs, dioxins, and DDT (Gudzinowicz et al., 1976). Currently, MS is one of the most frequently employed techniques used to perform quantitative analysis due to its specificity, selectivity and typical low limit of detection (LOD). Some researchers displayed that mass spectrometers use the difference in mass-to-charge ratio (m/z) of ionized atoms or molecules providing information on the spectra that may be used for identification and quantification of target analytes (Aebersold and Mann, 2003; Strathmann and Hoofnagle, 2011).

Three major instrumental components are essential to perform mass spectrometry: (i) an ion source; (ii) a mass analyser for resolving the ions into their characteristics mass components according to their mass-to-charge ratio; and (iii) a detector for detecting the ions and recording the relative abundance of each of the resolved ionic species (Figure 3.6). The

performances of these three components influence the quality of both quantitative and qualitative data. In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum requirements (~10^{-6} to 10^{-8} mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries (Uggerud et al., 2003).

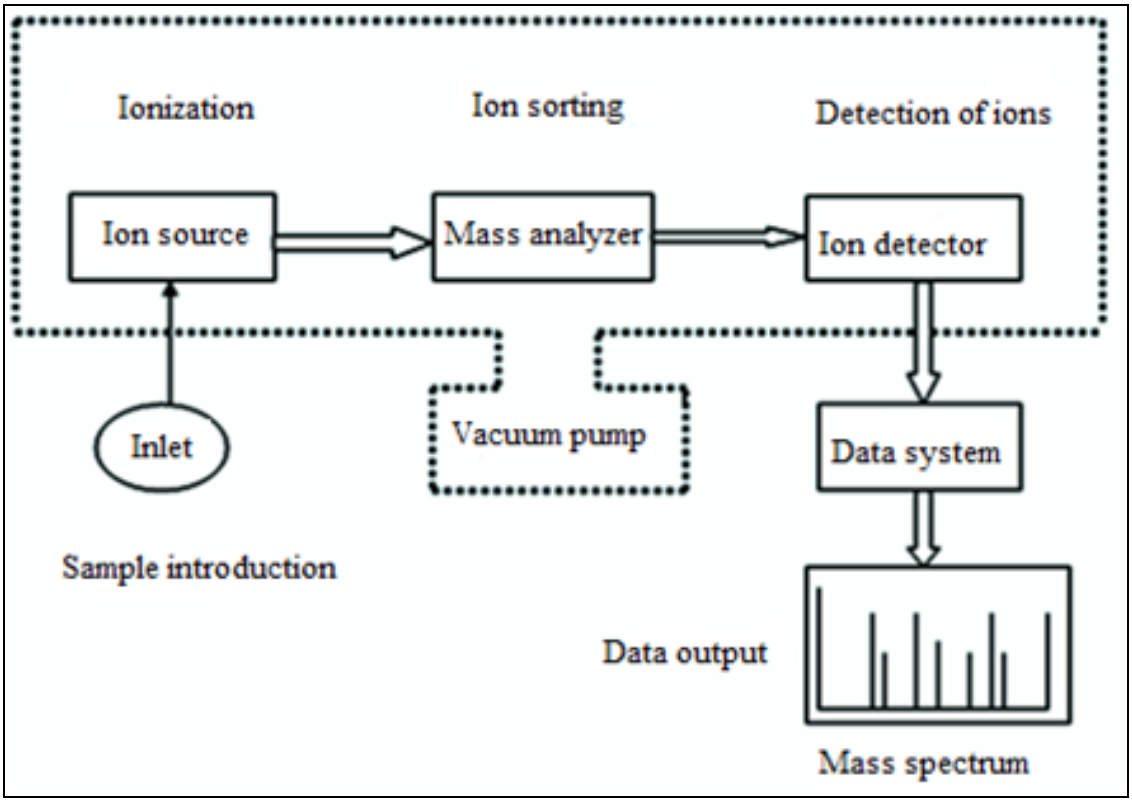


Figure 3.6: Basic components of a mass spectrometer

3.2.4.2 Ionization sources of GC/MS

There are numerous operation modes which can enhance the specificity of MS making it an extremely flexible tool for PCB analyses. The MS detector receives the eluate of the GC column and each eluting compound is analysed as it elutes. A MS employs magnetic and electric fields to exert forces on charged particles (ions) in a vacuum. Therefore, for a compound to be analysed must be ionized in the ion source, typically by electron bombardment (Siuzdak, 2004). The resulting radical cations and anions are accelerated out of the ion source and focused by electrostatic “lenses” towards the mass filter.

Several ionization techniques are used in GC-MS such as electron impact ionization (EI) and chemical ionization (CI) (Guo and Lankmayr 2012). Chemical ionisation can generate cations as well as anions, and therefore a differentiation is made between “positive chemical ionisation” (PCI) and “negative chemical ionisation” (NCI).

3.2.4.2.1 Electron impact ionization (EI)

For the analysis of environmental samples using GC/MS, EI is the most frequently used because it often produces both molecular and fragment ions. EI is based on the interaction of a beam of energetic electrons (70 eV) with the sample vapour (at a pressure in the range 10^{-7} –10^{-5} Torr). A unique series of ions are produced due to this interaction which depends on the chemical characteristics of the analyte (Traldi et al., 2006). When an electron that collides with a neutral analyte molecule, it can lose one electron, resulting in a charged molecular ion ($M^{+\cdot}$):

$$M(g) + e^{-}(70\ eV) \rightarrow M^{+\cdot} + 2e^{-} \qquad \text{Equation 3.1}$$

The molecular ion tends to decompose and series of fragment ions are formed by direct cleavage (e.g. direct cleavage of a C-C bond) or through rearrangement processes. This type of fragmentation provides structural information for structure of an unknown analyte (Covaci et al., 2003; Naert, 2007). During the electron ionization process, in most of cases, intense fragment ion peaks are observed rather than molecular ion peaks. A study of EI response for all 209 PCBs reported that molecular ion response decreased with increasing chlorine number (Cochran and Frame, 1999). EI leads to fine reproducible mass spectra, hence spectrum libraries based on EI data (Traldi et al., 2006).

3.2.4.2.2 Chemical ionization (CI)

For the analysis of trace organic compounds with GC/MS (EI mode), in some cases the molecular ion is not observed in the mass spectrum. Therefore chemical ionization may be used to obtain information related to the molecular

ion. In contrast to EI, chemical ionization (CI) produces ions with little excess energy and yields spectra in which the molecular ion is simply identified due to lesser number of fragments obtained (Naert, 2007). The chemical ionization process uses a reagent gas (R) such as methane, isobutane, or ammonia. This reduces the amount of sample molecules (hence analyte too) in the ion source compared to the amount of reagent gas molecules. In CI, a reagent gas is ionized by electron impact ionization then subsequently reacts with analyte molecules (M) to produce analyte ions. Depending on the setup of the instrument (source voltages, detector, etc.) and the type of reagent gas, positive or negative ions are recorded (Zhang et al., 2011). In CI two reaction steps are always necessary. In the primary reaction a stable cluster of reagent ions is produced from the reagent gas such as methane, isobutane, or ammonia through electron bombardment (Siuzdat, 2004). In positive chemical ionization, four types of reaction contribute to the formation of positive ions: protonation, hydride abstraction, charge exchange and adduct formation. Both protonation and hydride abstraction are observed when the most frequently reagent gases (methane, iso-butane and ammonia) are used (Ospina et al., 2003).

- **Protonation**

The most frequently used reaction in positive chemical ionization is protonation. The electrons from the filament ionize the reagent gas to produce reagent ions (RH^+) which interacts with the analyte molecules (M) to produce ionization often with a proton transfer. The results leads to the formation of quasimolecular ion $(M + H)^+$, which can then undergo fragmentation (Hübschmann, 2009).

$$M + RH^+ \rightarrow (M + H)^+ + R \qquad \text{Equation 3.2}$$

- **Hydride abstraction**

In this reaction, a hydride ion (H^-) is transferred from the quasimolecular ion to the reagent ion:

$$M + R^+ \rightarrow RH + (M - H)^+$$ Equation 3.3

- **Charge exchange**

This type of reaction produces a charged molecular ion with an odd number of electrons as in electron impact ionisation.

$$M + R^+ \rightarrow R + M^+$$ Equation 3.4

The charge exchange is observed when methane is used as a reagent gas.

- **Adduct formation**

Adduct is visible when the three steps above are not dissociated (Siuzdak, 2004).

$$M + R^+ \rightarrow (M + R)^+$$ Equation 3.5

In chemical ionization, negative ions can also be produced. Negative chemical ionization involves either the capture of an electron, abstraction of a proton or addition of a negatively charged species (Biemann, 1991). The tendency to negative ion is closely related to the electron affinity of the sample molecule. In this process, a reagent gas usually methane, is introduced into the ion source and negative ions are recorded (Muir and Svelko, 2006). In negative chemical ionization, compounds with high electron affinity such as halogenated chemicals are easily detected using negative chemical ionization (Cantrell et al, 2003). The electron capture negative ion (ECNI) mode has a limited linear range compared to the electron ionization. Another disadvantage is that it is more difficult to operate normally due to the higher sensitivity to temperature variations. ECNI is most sensitive for molecules with electron affinities greater than 0.5 eV (e.g., highly halogenated compounds). Fragmentation is compound specific and depends on the stereochemistry (Gurprasad et al., 2002).

3.2.5 Mass analysers

The mass analysers are applied to the sorting of ions based on their mass-to-charge ratios, and to focus ions onto the detector. The mass analysers include: magnetic sector, quadrupole mass filters, quadrupole ion traps, time-of-flight and ion cyclotron-resonance (van Galen, 2005). The magnetic sector and quadrupole mass filters are the mostly used currently in organic chemistry (Freidhoff et al., 1999).

3.2.5.1 Quadrupole mass analyser

Since the 1970s, quadrupole mass filters have been the most widely used mass analyser in mass spectrometry (Picó et al., 2004). A quadrupole mass analyser consists of an ionizer, an ion accelerator, and a mass filter consisting of a set of two pairs of metallic rods that serve as electrodes with a space down the middle (Figure 3.7). One set is at a positive electrical potential, and the other is at a negative potential. The rods are electrically connected to each other in opposite pairs (U+Vcos (ωt)) and - (U+Vcos (ωt)) where U is a constant (dc) voltage and Vcos (ωt) a variable (ac) voltage (radio-frequency, RF) applied to the two pairs of electrodes (Jordaan and Laurens, 2008). RF and dc potentials are applied between the opposite pairs of rods enabling ions with a specific m/z to have a stable trajectory and pass through to the detector. The pair of rods connected to the positive dc terminal acts as a high-pass mass filter compared to the low-pass mass filter (positively pair of rods) (Gilany et al., 2010).

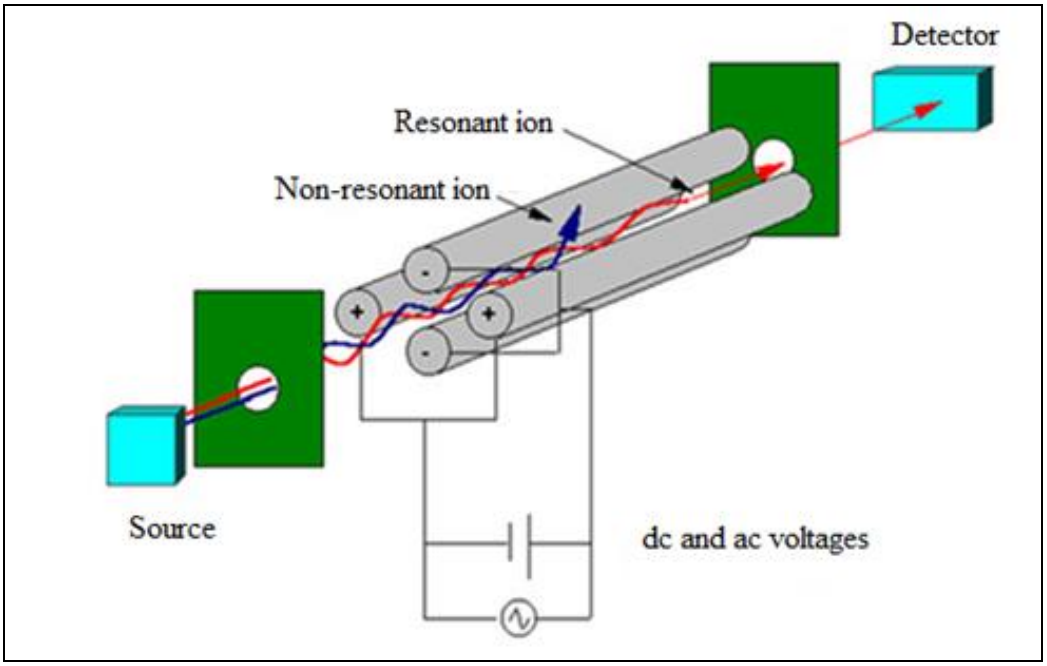


Figure 3.7: A quadrupole mass spectrometer

The alternating electric field diverts the ions go the central part in spirals as they pass down the quadrupole. The constant voltage drags them in one constant direction, towards one pair of electrodes. An ion with the right size drifts slightly in the constant part of the field, but is always dragged back by the alternating part.

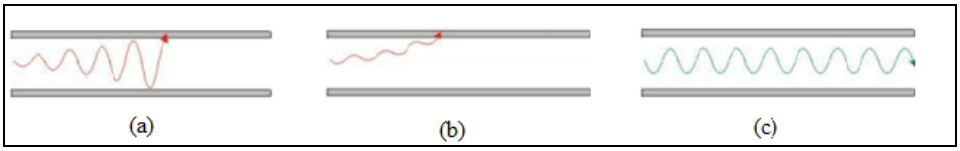


Figure 3.8: Mechanism of a quadrupole mass filter.

An ion with low mass will be dragged a large distance by the alternating field towards itself in stronger regions of field. Rapidly, it will collide with an electrode and disappear (Figure 3.8a).

An ion with high mass will not be affected by the alternating field, but will slowly drift in the constant part of the field (the dc part). The alternating field is not sufficiently strong to slog it back; it will also collide with an electrode, and is lost (Figure 3.8b).

For a given amplitude of the dc and RF voltages, only the ions of given m/z ratio will resonate and have a stable trajectory to enter and pass through the quadrupole and be detected. An ion with the right size (m/z) drifts slightly in the constant part of the field; it is always dragged back by the alternating part which is not somewhat strong enough to make it out of control into an electrode. An ion with the right size (m/z) is stable and reaches the end where it can be measured (c) (Li et al., 2006). The stability of an ion in a quadrupole mass analyser depends on the magnitudes of the alternating and constant fields. Quadrupole mass spectrometers offer the advantages of low scan times (< 100 ms), which is particularly useful for real time scanning of chromatographic peaks (Uggerud et al., 2003).

3.2.5.2 Magnetic sector analyser

The magnetic sector analysers rely on the interaction of ionized sample molecules and a magnetic field in order to filter and measure individual atomic mass of components which contribute to the total sample composition. Magnetic sector analysers utilise a combination of an electromagnet and an electrostatic focussing the device to generate an ion beam from the ion source. These ions from the ion source are accelerated to high velocity through a magnetic sector, in which a magnetic field is applied perpendicularly to the direction of ion motion (Figure 3.9). Ions in the magnetic field experience two equal forces; the Lorentz force due to the magnetic field (F_B) and centripetal force (F_C):

$$F_B = Bzev \qquad \text{Equation 3.6}$$

Where B is the strength of the magnetic field, z the number of charges, e is the charge of one electron and v is the velocity.

$$F_C = \frac{mv^2}{r} \qquad \text{Equation 3.7}$$

Where: r is the radius of the orbit

The kinetic energy of the ion is independent of its mass and is expressed as follows:

$$KE = zeU = \frac{1}{2}mv^2 \qquad \text{Equation 3.8}$$

Where U is the accelerating voltage, m is the mass of the ion and v is the magnitude of its velocity after its acceleration in an electric field.

For an ion that reaches the detector, $F_B = F_C$

$$BZev = \frac{mv^2}{r} \qquad \text{Equation 3.9}$$

With rearrangement and substitution of (4) in (3), the equation gives:

$$\frac{m}{z} = \frac{B^2 r^2 e}{2U} \qquad \text{Equation 3.10}$$

(Van Galen, 2005; Brondz, 2013).

Where: r is the radius of orbit (cm); m is the mass of the particle; U is the voltage applied to the particle (in Volts); B is the magnetic field in Tesla (1 tesla = 10^4 gauss) and e is unit charge of one electron.

Basically, the ions are sorted according to their mass-to-charge ratios by holding constant the voltage applied to the particle (U) and the radius (r) of the orbit while varying the magnetic field B (Uggerud et al., 2003; van Galen, 2005).

When similar ions pass through the magnetic field, they are deflected to the same degree and follow the same trajectory track. Those ions which are not selected by U and B values collide with either side of the magnet wall or will not pass through the slit to the detector. Magnetic sector analysers are used for mass focusing; they focus the angular dispersions in the plane perpendicular to the magnetic field (Bluck and Volmer, 2009). The ions

passing through the exit slit fall on a collector electrode, resulting in an ion current that is amplified and recorded.

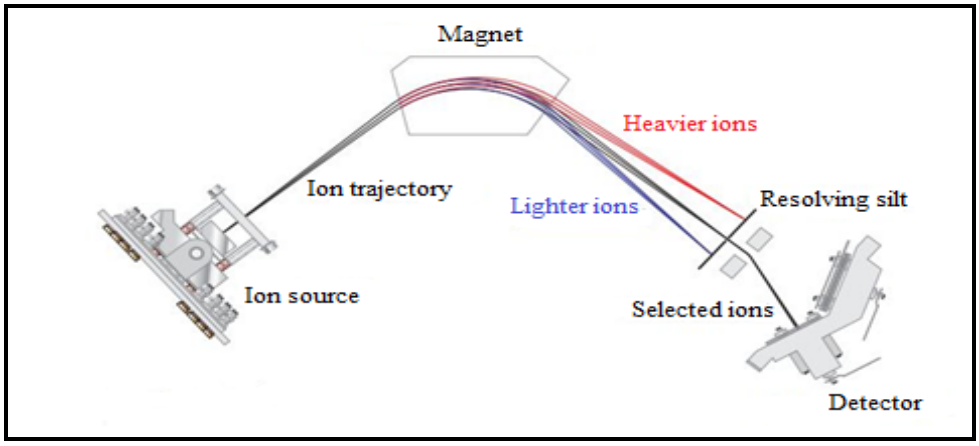


Figure 3.9: Basic components of a magnetic sector analyser (Diaz et al., 2001; Van Galen, 2005).

3.2.6 Retention times (RT)

Retention time is the fundamental qualitative measurement of chromatography. The separation of sample components on a GC column depends on the time taken by each individual component to move along and elute from the column. Most peak identification is performed by comparing the retention time of the unknown peak with that of a standard. The validation of the methods is easier if there is no variation (or minimal) in the retention time of each analyte (Giarrocco et al., 1997). The gas chromatographic retention time can be used as a property to characterise the compound, because under constant chromatographic conditions, the retention time of a compound is reproducible. Retention time depends on properties of the capillary column used for the analysis and on the operating conditions of the analytical instruments (GC/ECD or GC/MS).

3.2.7 GC/MS full scan *versus* selected ion monitoring (SIM)

In general, GC is used to separate individual components from mixture of compounds. Once separated, the individual components are identified and quantified by the MS. A MS is typically utilized in one of two ways: full scan

mode or SIM mode. The choice depends on the matrices to be analysed. If qualitative and quantitative analyses are involved to identify the pollutants in sample components using a mass spectrum, full scan mode is essential. If the quantitative analysis corresponds to trace components, SIM mode is suitable for analysis when the mass spectra of the trace components are known (Alasalvar et al., 2011).

In full scan mode, the MS monitors a wide range of masses of ions (50 - 550 m/z) generated from the ion source with known masses as mass-to-charge ratio (m/z). The full scan mode is used in environmental applications since samples may be complex mixtures and ion spectra are needed to identify compounds. The consistency and uniqueness of the mass spectra acquired by full-scan EI mode at 70 eV enable comparable GC-MS spectra on all relevant GC-MS instruments (Gathungu et al., 2014). As a result, identification of unknown compounds may be further simplified by comparing the similarity of their full-scan EI mass spectra with large mass spectral libraries of known compounds which contains that of the unknown (Stein and Heller, 2006).

In SIM mode, the mass spectrometers record only a few preselected ions, generally those most indicative of the compounds of interest. Because there are more scans (cycles) on each ion within the specified ion interval, i.e., the dwell time per ion is increased and sensitivity is significantly increased. SIM-mode can eliminate much of the noise inherent in the full-scan mode (Jia et al., 2006). Depending on the number of ions selected for monitoring, the sensitivity of the analyses is greater than the full-scan mode (Michalski et al., 2011). The SIM mode is predominantly suitable for target analytes at low concentrations in moderate-to-highly complex media when full-scan mode is unsuccessful. Hence, lower limits of detection can be achieved with SIM than the full-scan mode. For example, Fernandez-Martinez et al. (1999) developed a method for 15 volatile organic compounds in urban and rural atmospheres and obtained method detection limits (MDLs) from 0.001 to 0.008 μg m^{-3} (GC/MS, SIM mode), and 0.004 to 0.08 μg m^{-3} (GC/MS, full scan mode).

In full scan EI mode, the molecular ion is the most intense and specific in a mass spectrum which is not common in SIM mode due to its limited abundance in many molecules, or because not many compounds will have this mass in their spectra (Taudte et al., 2014).

A major advantage of the full-scan mode over the SIM mode is the simultaneous identification of other eluted compounds that could be of interest. However a major disadvantage will be reduced sensitivity (Soliman et al., 2004). Since there are many ions to be detected in full scan mode within the mass range, the use of SIM will probably yield more accurate results (Saba, 2013) for trace analysis. Typically two to four ions are monitored per compound and the ratios of those ions will be unique to the analyte of interest. The sensitivity is a function of the scanned mass range, scan rate, and resolution (Hoker et al., 2015). During instrument method development it is desirable to first test the solutions in full scan mode to determine the retention time and the mass fragments before moving to a SIM method. Modern mass spectrometers have extensive computer libraries containing mass-spectra of many different compounds to compare to the unknown analyte spectrum.

Generally, SIM mode is mostly used for the quantification of individual target compounds since it offers several advantages in comparison to scan mode: (i) SIM only records a fewer selected mass fragments per scan, resulting in a much longer dwell time for each monitored ion than in the full scan mode; (ii) the detection limits for target analytes are generally lower by almost an order of magnitude; (iii) the use of SIM mode is often less noisy and the linear quantification range is increased for trace analytes (Wang and Stout, 2010).

3.3. ANALYTICAL METHODS APPLIED IN THIS STUDY FOR THE DETERMINATION OF PCBs

3.3.1 Introduction

Analytical procedures used to determine six indicator PCB congeners in organs of fish, sediments, water and mussels were developed based on the

materials and equipment possessed by the Chemistry Department of the Nelson Mandela Metropolitan University. The determination of these analytes in complex matrices represents a challenging task because the concentrations in environmental matrices are naturally at ultra-trace levels. In practice, the methods used for the analysis of PCBs in this study consist of four major steps: (1) sampling and sample preparation; (2) isolation of target analytes from a representative sample (extraction step); (3) separation of PCBs from bulk co-extracted matrix components (clean-up step); (4) separation of the compounds of interest; identification and quantification.

3.3.2 Laboratory chemicals used

3.3.2.1 PCB Standards

All common laboratory chemicals used were of analytical grade and were used without further purification.

Individual PCB standards, IUPAC Nos. 28 (2,4,4'-trichlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), 153 (2,2',4,4'5,5'-hexachlorobiphenyl) and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) together with PCB mix solution 6 (No. 28, 52, 101, 138, 153, 180) (10 ng μL^{-1} of each congener) in isooctane were purchased from Sigma Aldrich (South Africa). Surrogate standard, 2,4, 5,6-tetrachloro-*meta*-xylene (TC*m*X) and injection standard PCB 209 (2,2',3,3',4,4',5,5',6,6'-decachlorobiphenyl) were also purchased from Sigma Aldrich (South Africa). The purity of the standards was greater than 99%. A standard stock solution containing all six PCB congeners was prepared at a concentration of 1 ng μL^{-1} in isooctane. Individual standard stock solutions in isooctane were prepared at 200 ng mL^{-1}. A solution of internal standard (PCB 209) was prepared at a concentration of 100 ng mL^{-1} in isooctane. Stock solutions were stored between 2 and 8°C, protected from light. Suitable PCB working solutions were prepared daily by dilution in isooctane. Reagent and solvent used are summarized in the Table 3.1.

Table 3.1: Reagents and solvent used

Reagent	Grade	Sources
Hexane Suprasolv® ($CH_3(CH_2)_4CH_3$)	> 98%	Merck (South Africa)
Isooctane ($(CH_3)_3CCH_2CH(CH_3)_2$)	> 99%	Merck (South Africa)
Dichloromethane (CH_2Cl_2)	> 99%	Merck (South Africa)
Acetone (CH_3COCH_3)	> 99.5%	Merck (South Africa)
Sulphuric acid (H_2SO_4)	98 – 100%	Merck (South Africa)
Florisil® (MgO_3Si)	60-100 mesh	Sigma Aldrich (South Africa)
Copper powder (trace metal basis)	99.99%	Sigma Aldrich (South Africa)
Nitrogen gas (Ni)	Baseline 5.0 > 99%	AFROX
Helium (He)	Baseline 5.0 > 99%	AFROX
Amyl alcohol ($C_5H_{11}OH$)	> 99%	Sigma Aldrich (South Africa)
Anhydrous sodium sulphate (Na_2SO_4)	≥ 99%	Merck (South Africa)
Sodium hexametaphosphate ($(NaPO_3)_6$)	65-70% P_2O_5 basis	Sigma Aldrich (South Africa)
Glass wool	-	Sigma Aldrich (South Africa)

Prior to the use of Florisil® to drive off any moisture adsorbed during storage and handling is necessary. Activation of Florisil® was achieved by heating at 130 °C for 12 h in a glass container lightly covered with aluminium foil in an oven overnight. It was then cooled in a dessicator before use.

3.3.3 Extraction and clean-up of sediment samples

In this study, Soxhlet extraction was used for the extraction of solid samples. All the solid samples were analysed using a congener-specific method described by the SW-846 method 8082 (USEPA, 1996). Extraction of PCBs in water samples was performed by liquid-liquid extraction (LLE), SW-846 method 3510 (USEPA, 1997) using a glass separatory funnel. The clean-up procedures were performed in two steps: Firstly, extracts were treated with concentrated sulphuric acid to remove hydrocarbons and other organic compounds which are co-extracted with the PCB residues. Secondly, a Florisil

chromatographic column was used to purify the extracts by removing co-extractable polar compounds, residual water, and residual acid.

3.3.3.1 Sampling and sample preparation of sediment samples

The primary objective of any sampling program is to obtain representative sample for the whole population under the study. Sampling procedures, locations, equipment, and sample preservation as well as handling requirements are identified in a sampling plan. The sampling methodology undertaken for this study including sample collection, treatment and storage, analytical procedures are summarized in this section. According to USEPA (2011), samples must be collected in glass containers with a Teflon-lined cap for PCB analyses. In this study, all the materials used were washed with warm water, and phosphate-free soap, rinsed in tap water, followed with de-ionised water and rinsed with acetone before to be dried in a muffle furnace for 12 h at 50 °C.

3.3.3.1.1 Sampling of sediments

Sediment samples were collected from the North End Lake (NEL) and Port Elizabeth Harbour (PEH). In the NEL sites were selected based to their close proximity to potential sources of contamination (e.g., industrial areas, storm water runoff, municipal discharges, therefore were expected to be contaminated by PCBs for this study (Table 3.2) while in the PEH, sediments were collected randomly (Table 3.3). Sediment samples were collected using a stainless steel grab sampler (Van Veen Grab) (Figure 3.10). The sampler covers an area of 0.1 m^2 corresponding to 15 kg. Grab samplers have jaws that close by a mechanism upon influence with the bottom surface. Van Veen grab sampler has long supports attached to each bucket, which are held together in an open position during lowering. When the grab reaches the bottom, the lowering wire relaxes, thus initiating the release hook which enables the buckets to close together before the grab leaves the sea/lake floor on moving. Grab sampler offers the advantage of being able to collect a large amount of material in one sample. Due to its weight and size, it is

commonly preferred to collect sediments in the aquatic environment where water is deep and currents are strong.

Table 3.2: The location and characteristics of the NEL sediment sampling sites

Station code	Coordinates		Depth (m)	Comments on location
	S	E		
NELS-1	33°56.294’	25°35.731’	2.7	Close proximity to NMBS
NELS-2	33°56.066’	25°35.426’	1.8	Near stormwater inflow
NELS-3	33°56.033’	25°35.849’	2.1	Close proximity of industries
NELS-4	33˚55.926’	25°35.539’	2.6	Near inflow
NELS-5	33˚55.069’	25°35.587’	2.6	Near industry
NELS-6	33˚55.880’	25°35.613’	3.2	Near lake inflow pipes
NELS-7	33˚55.991’	25°35.635’	3.3	Mid-lake
NELS-8	33˚56.109’	25°35.564’	2.3	Near industry
NELS-9	33˚56.131’	25°35.661’	2.9	Mid-lake
NELS-10	33˚56.200’	25°35.679’	2.5	Near NMBS and powerboat club
NELS-11	33˚56.130’	25°35.757’	3.0	Near residential area
NELS-12	33˚56.027’	25°35.744’	3.0	Near residential area
NELS-13	33˚56.284’	25°35.804’	2.6	Near NMBS

NELS: North End Lake sediments; NMBS: Nelson Mandela Bay Stadium

Table 3.3: Sampling location of sediments from Port Elizabeth Harbour

Station code	Coordinates	
	Latitude (S)	Longitude (E)
PEHS 1	33˚57’41.25”	25˚37’42.02”
PEHS 2	33˚57’47.31”	25˚37’50.01”
PEHS 3	33˚57’38.55”	25˚38’8.38”
PEHS 4	33˚57’54.98”	25˚37’47.13”
PEHS 5	33˚57’48.41”	25˚38’11.27”
PEHS 6	33˚58’8.42”	25˚38’8.02”
PEHS 7	33˚58’2.15”	25˚37’53.97”
PEHS 8	33˚57’51.10”	25˚38’36.49”
PEHS 9	33˚57’33.77”	25˚38’22.07”

PEHS: Port Elizabeth Harbour sediments

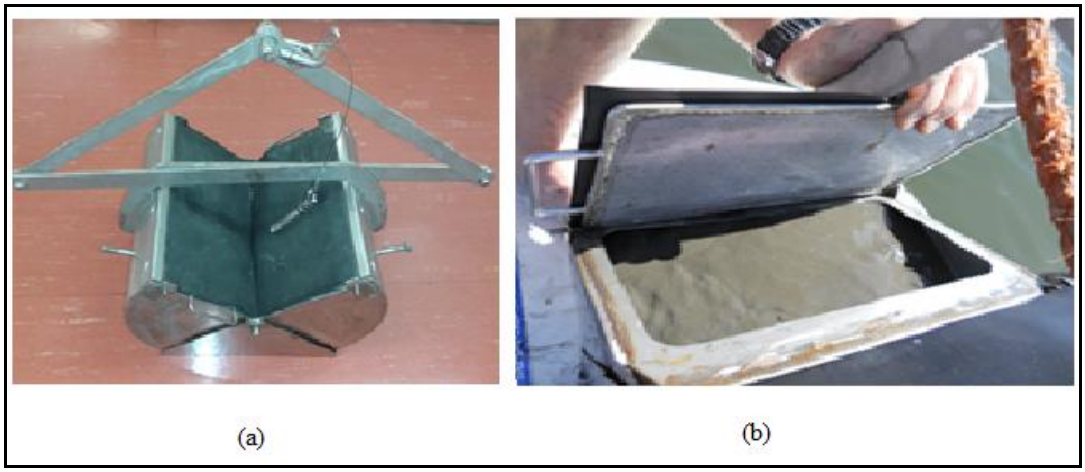


Figure 3.10: Picture of grab sampler used (a) and sampler containing sediment sample (b).

3.3.3.1.2 Sediment sample preparation

The sediment samples were dried at room temperature between six and ten days (Figure 3.11) depending on the moisture content and the characteristics of sediments (sand or mud). The air-dried sediments were gently crushed using a mortar and pestle to break up desiccated clumps and were sieved using a stainless steel sieve shaker (Madison Test Sieve, SABS) (< 1 mm) for about 20 minutes to obtain homogeneous samples. The powder was kept into glass bottle sealed with Teflon-lined lid, labelled and stored at 4 ^{o}C prior to the extraction procedure.

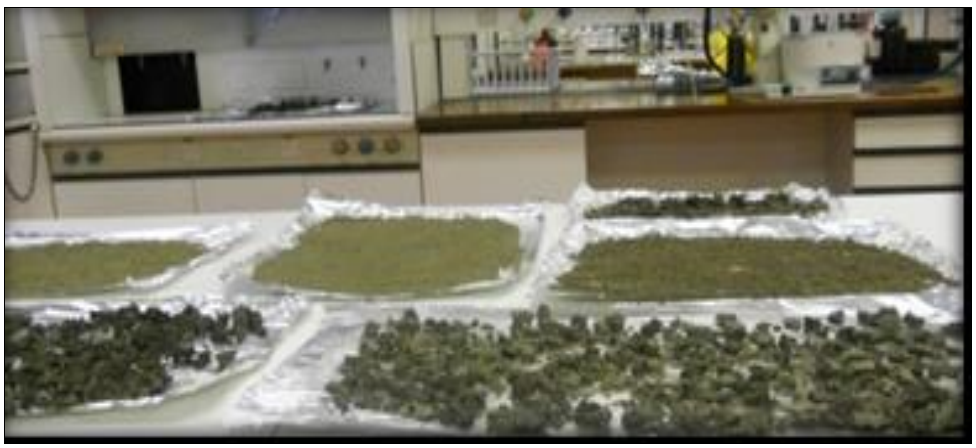


Figure 3.11: Sediment samples dried at room temperature.

3.3.3.1.3 Sediment moisture content determination

Water content in sediment sample is the ratio of the weight of water to the weight of the sediment in a given volume of sediment. The moisture content in sediment samples was determined using direct method according to Black (1965).

- An empty cleaned crucible was placed in the drying oven at 105 °C for 30 minutes, and then it was cooled in a desiccator. After cooling, the crucible was weighed to the nearest 1 mg and the mass recorded (X_0).
- Accurately, 10 g of the moist sediment were weighted in the crucible (X_0) and the mass (moisture sediment and crucible) was recorded (X_1) in g.
- The crucible with its content was dried in an oven at 105 °C for 48 hours. After cooling in desiccator, the weight was recorded in gram (X_2).

It was assumed that the mass loss was due to water loss only. The moisture content was calculated as follows:

$$Moisture\ content\ (\%) = \left(\frac{X_1 - X_2}{X_1 - X_0}\right) * 100$$

Where:

X_1 is the mass of the crucible containing the sample and X_2 is the mass of the crucible containing the dry matter in g.

3.3.3.1.4 Determination of organic matter in sediments

The organic matter is usually a component of sedimentary material; it provides an understanding of sediment cohesion. A simple estimate of the organic content of the sediments can be derived from the mass loss on ignition (LOI) in a muffle furnace. The organic matter in sediment samples was determined based on the method of Briggs (1977).

- The beakers (Pyrex Berzelius) (50 mL) containing 10 g of dried sediment at room temperature (X_d) were placed in a muffle furnace (ashing oven) for 8 hours at 550 °C.
- The beakers were removed from the ashing oven and placed in desiccators to cool down and the final mass (X_a) recorded.
- The percentage of the organic matter was calculated as a loss of mass during ashing from the initial mass using the following equation:

$$\left(\frac{X_d - X_a}{X_d}\right) * 100$$

Where:

X_d is the initial dry mass and X_a is the mass after ashing.

3.3.3.1.5 Determination of sediment particle size

Sediment particle size is the most fundamental property of sediment particles. Grain size analysis therefore provides important evidences to the sediment provenance, transport history and depositional conditions (Blott and Pye, 2001). The particle size was determined using hydrometer method (Gee and Bauder, 1986) and was classified according to the United States Department of Agriculture (USDA) as follows: clay (< 0.002 mm), silt (0.002 – 0.05 mm), very fine sand (0.05 – 0.10 mm), fine sand (0.10 – 0.25 mm), medium sand (0.25 – 0.5 mm), coarse sand (0.5 – 1 mm), very coarse sand (1 – 2 mm).

A mass of 40 g of air dried sediment was weighed out in a flask (500 mL) and allowed to equilibrate with the atmosphere overnight. 100 mL of a 5% solution of sodium hexametaphosphate ($(NaPO_3)_6$ and 250 mL distilled water were added to the dried sediment. The vials were shaken using a mechanical shaker for 1 hour prior the experiment. The mixture was then placed in a 1 L measuring cylinder and the volume was increased to 1000 mL with distilled water. The cylinder was closed and shaken by hand at least for 1 minute. Two drops of amyl alcohol were added to remove the foam. A hydrometer was inserted after 30 seconds, 60 seconds, 3 minutes, 1.5 hours and 24 hours. A blank sample containing a similar solution without sediment sample was also

prepared. The temperature of the solutions was recorded using a thermometer. The readings were used to calculate the percentage size fractions in the samples according to the following equations.

The concentration of sediment (C) was determined in suspension (g L^{-1}) (Gee and Bauder, 1986).

$C = R - RL$ Equation 3.11

Where:

R is the uncorrected hydrometer reading (in g L^{-1}) and RL is the hydrometer reading of the blank solution.

The summation percentage (P), for the given time interval was determined using:

$$P = \left(\frac{C}{C_0}\right) * 100$$ Equation 3.12

Where:

Co is the dried weight of the sample.

The mean particle diameter (X) in solution at each time *t* was determined using:

$$X = \theta t^{-1/2}$$ Equation 3.13

Where:

X = mean particle diameter in suspension, µm at time *t*

θ = the sedimentation parameter (µm min ^{½}) and is a function of the hydrometer settling depth, solution viscosity and particle and solution density (Gee and Bauder, 1986).

t = time in minutes

$$\Theta = \left(\frac{18\eta h'}{[g(p_s - p_l)]^{1/2}}\right)$$ Equation 3.14

Where:

h' is the hydrometer effective depth (cm), p_s = sediment particle density (g cm^{-3}), p_l= solution density (g cm^{-3}), *g* = gravitational constant (cm s^2) and η= fluid viscosity in poise (g cm^{-1} s^{-1}).

The relationship of the settling depth to the hydrometer dimensions were approximated by:

$$h' = -0.164R + 16.3$$ Equation 3.15

Where:

R = the uncorrected hydrometer reading (g L^{-1}).

The summation percentage was calculated as follows:

$$P_{2\mu m} = m \ln\left(\frac{2}{X_{24}}\right) + P_{24}$$ Equation 3.16

Where:

X_{24} is the mean particle diameter in suspension at 24 hours, P_{24} is the summation percentage at 24 hours, and m was determined using the following equation:

$$m = \frac{P_{1.5} - P_{24}}{\ln(X_{1.5} - X_{24})}$$ Equation 3.17

Where:

m is the slope of the summation percentage curve between *X* at 1.5 hours and *X* at 24 hours. $X_{1.5}$ is the particle diameter in suspension at 1.5 hours, and $P_{1.5}$

is the summation percentage at 1.5 hours. This procedure was repeated for the 30 second and 60 second readings.

Derivations and sources for these equations can be found in Gee and Bauder (1986). The detail of the sediment particle size determination is shown in Appendix 3.

3.3.3.2 Extraction of sediment samples

Sediment samples were extracted by Soxhlet apparatus which is the recommended procedure for aquatic sediments (U.S.EPA, 1997). A mass of 20 g of air-dried sediments were homogenized and mixed with 20 g anhydrous sodium sulphate to form a free flowing powder. The mixture was introduced into the cellulose thimble (41 x 123 mm) and 5 g of activated copper powder was added for removal of elemental sulphur (Smedes and de Boer, 1997), copper can react with the sulphur to form CuS. Soxhlet extraction was performed on sediment samples using 250 mL of methylene chloride and hexane (1:1, v/v). A Surrogate recovery standard, tetrachloro-*meta*-xylene (TC-*m*-X, 100 µL, 1 ng μL^{-1}) was added to each sample aliquot and blank prior to the extraction to monitor analytical recoveries of PCB congeners. The Soxhlet extracts were allowed to reflux for 24 h (Figure 3.12). The resulting extraction solution was allowed to cool, filtered through anhydrous sodium sulphate to remove any trace of water and then evaporated by rotary evaporator (Büchi Rotavapor R-205) to 1 mL and was subjected to the clean-up procedure.

3.3.3.3 Clean-up procedure of sediment samples

Most PCBs are stable under acid conditions; therefore, sulphuric acid (98%) was used for the removal of potential chromatographic interferences. Briefly, the concentrated extract (1 mL) was dissolved into 2 mL of n-hexane and transferred into a separatory funnel. 2 mL of sulphuric acid (98%) were added slowly to the extract and shaken vigorously for 2 min. The inorganic layer was decanted off. Several portions of sulphuric acid were used until the acid layer remained colourless. The organic extracts were passed through anhydrous

sodium sulphate and concentrated to 1 mL. However, an acid clean-up alone does not usually remove all of the substances that could interfere with PCB analysis from the samples. An additional clean-up by adsorption chromatography was also used to combat interferences from other interfering organic and polar species by fractionation on Florisil (60 – 100 mesh). A chromatographic column (400 mm long x 10 mm ID; with Pyrex® glass wool at bottom and a PTFE stopcock) (Figure 3.13) was packed with 10 g of florisil and a layer of 1 cm of anhydrous sodium sulphate was added on the top. The column was pre-eluted with 40 mL of n-hexane to remove any impurities and the eluate was discarded. After removal of any impurities from the column, the extract (1 mL) was transferred to the column using a disposable Pasteur pipette and the elution was performed with 80 mL n-hexane. The eluted extract was concentrated using rotary evaporator (Büchi Rotavapor R-205) at 40 °C to 2 mL and under a gentle stream of pure nitrogen. The residue fraction was dissolved into 1 mL of n-hexane and the content transferred into 2 mL Screw cap vials with TTFE/red rubber septa supplied by Chemetrix (Pty) (South Africa). Internal standard (PCB 209, 20 µL, 0.1 ng μL^{-1}) was added to each sample before the GC/MS.

Figure 3.12: Soxhlet extraction procedure.

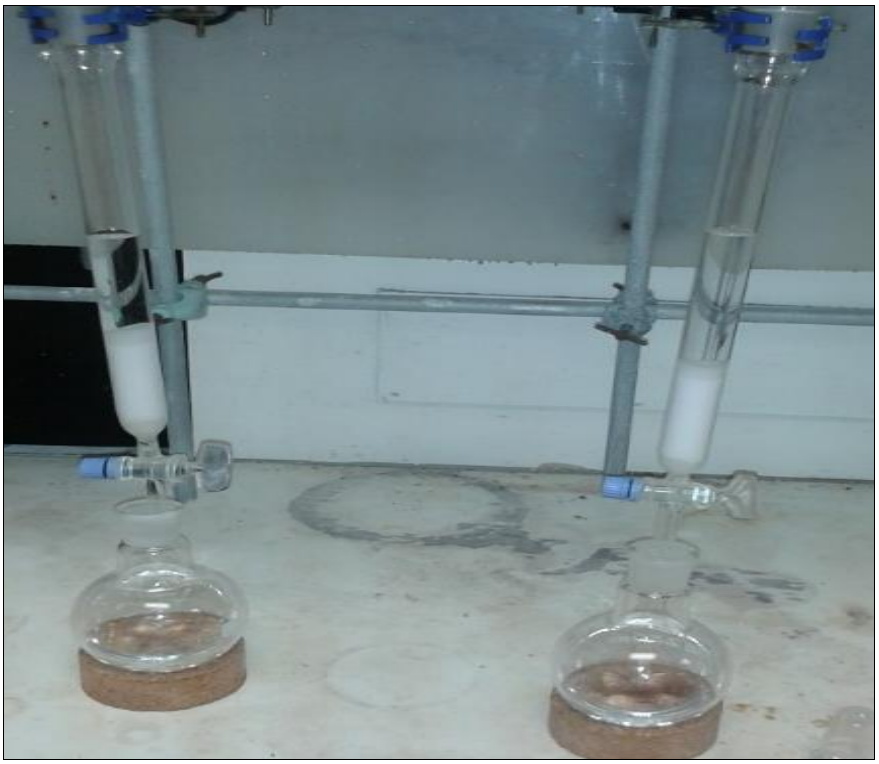


Figure 3.13: Chromatographic column clean-up procedure.

3.3.4 Extraction and clean-up of biota samples

In this study, biota samples including two fish species and two mussel species were analysed. Ethics clearance was obtained from the Nelson Mandela Metropolitan University, Ethics Clearance Committee (Appendix 1).

3.3.4.1 Sampling and sample preparation of fish

Fish samples were collected from the NEL to assess the extent of PCB contamination in the Nelson Mandela Bay Metropolitan Municipality. *Oreochromis mossambicus* and *Cyprinus carpio* were the two fish species analysed (Figure 3.14). The biometric data of all the fish analysed are indicated in the appendix 4.

The preparation of fish samples followed USEPA (2000a) guidance. Before dissection, fish were thawed to remove the aluminium foil, and then rinsed with tap water followed by deionized water. Fish were dissected and sample organs (livers, gills, muscle and gonads) were taken from each fish, weighed, and kept separately. The fillet was taken from the left side of the fish above the lateral line and below the dorsal area. The samples were labelled and were stored at - 20 °C in a freezer until chemical analysis. The condition factor which shows the degree of health of the fish in their habitat was expressed by the ratio between the body weight of fish and the body length power three (g cm^{-3}). When condition factor value is higher it means that the fish has attained a better condition. The condition factor of fish can be affected by numerous factors such as stress, sex, season, availability of feeds, and other water quality parameters (Nehemia et al., 2012).

Sample of gills, gonads and liver were ground using a mortar and pestle with four times more anhydrous sodium sulphate than the sample mass until homogenous form appeared. Muscle samples were cut and homogenized using a commercial stick blender (detachable Shaft for easy cleaning) with stainless steel blades (Game). The result was mixed with anhydrous sodium sulphate in a mortar for dehydration and homogenization of the samples. Briefly, muscle (10 g), gonad/liver (1 g), and gill (3 g) were dried before the extraction with sodium sulphate; (1:3 muscle tissues: sodium sulphate; 1:4

gonad, liver or gill organs: sodium sulphate). *O. mossambicus* were coded ELM while *C. carpio* were coded ELC (Appendix 4). In total 236 samples from fish organs out of 240 were processed and analysed; one liver sample was missed while three fish were juveniles (no gonad).

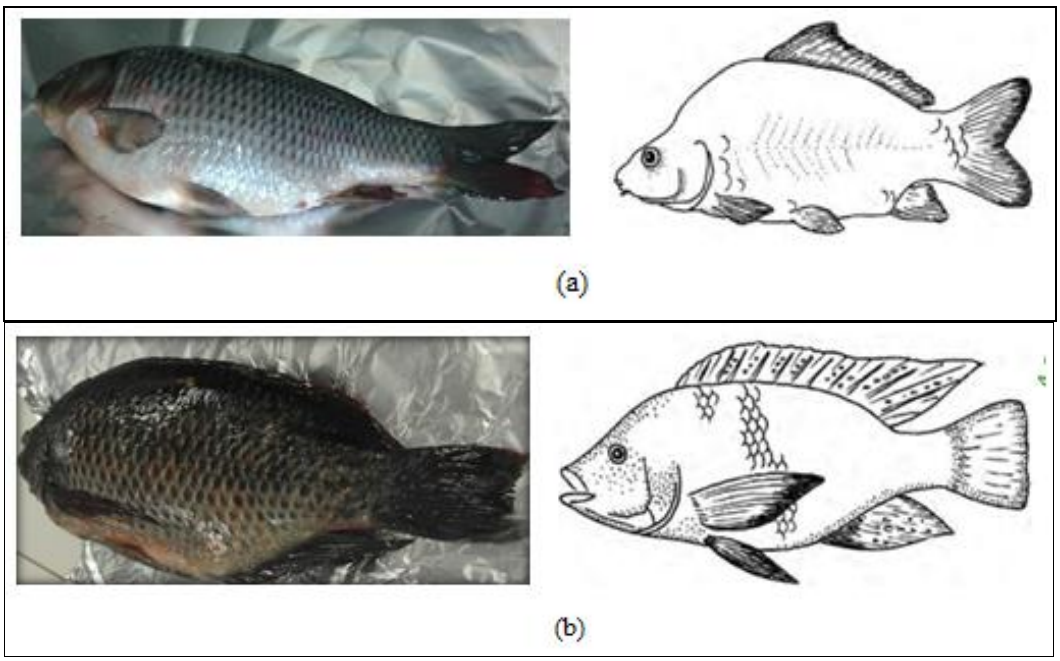
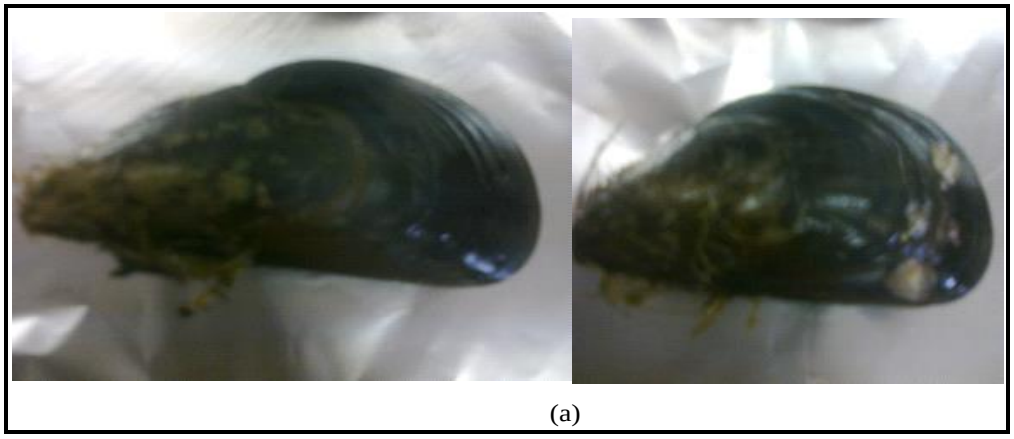


Figure 3.14: Pictures of *Cyprinus carpio* (a) *and Oreochromis mossambicus* (b).

3.3.4.2 Sampling of mussels and sample preparation

In total, 95 mussel samples were collected from PEH and in the vicinity of the point source of the NEL outflow into the sea. Blue mussels (*Mytilus galloprovincialis*) (Figure 3.15a) were hand-picked at five locations in the harbour while brown mussels (*Perna perna*) (Figure 3.15b) were sampled from six sites at the NEL outflow into the Indian Ocean. An electronic global positioning system (GPS 72 H Garmin) (Commercial Marine) was used to report the exact sampling locations provided in appendix 5.



(a)

(b)

Figure 3.15: Pictures of *Mytilus galloprovincialis* (a) and *Perna perna* (b) analysed.

The mussels were dissected and the soft tissue of the composite samples or individual sample was used for the analysis of PCBs. The mussel was opened carefully inserting a small knife (stainless steel) between the two valves and separating the muscles that hold the closed valves. Using a knife, tweezers and laboratory spatula, the soft tissue was removed from each valve. The knife was placed under the mantle and all of the soft tissue from the shell was taken. The soft tissue was weighed, homogenised using a mortar and pestle and placed in a labelled glass jar sealed with lid and were stored at – 20 ºC in a deep freezer to avoid the loss of water and to reduce most enzymatic and oxidative reactions until further analysis. The figure 3.16 showed different parts of mussels.

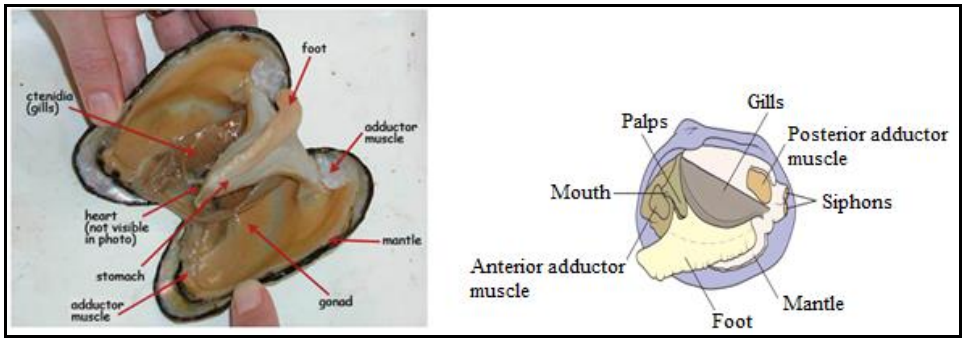


Figure 3.16: The external and internal anatomy of a mussel.

3.3.4.3. Extraction and clean-up of biota samples

PCBs in biological samples (organs of fish and mussels) were extracted using Soxhlet extraction with grade reagent hexane: acetone mixture (1:1 v/v). Firstly, the sample was mixed with anhydrous sodium sulphate to remove any moisture. A surrogate standard (TCmX, 100 µL, 1 ng μL^{-1}) was added to each sample prior the extraction. The clean-up procedure included acid treatment followed by adsorption chromatography. The samples were treated several times with concentrated sulphuric acid before the purification by Florisil column chromatography. The entire extraction and clean-up procedure for biota analysis is presented in Figure 3.17 on the next page.

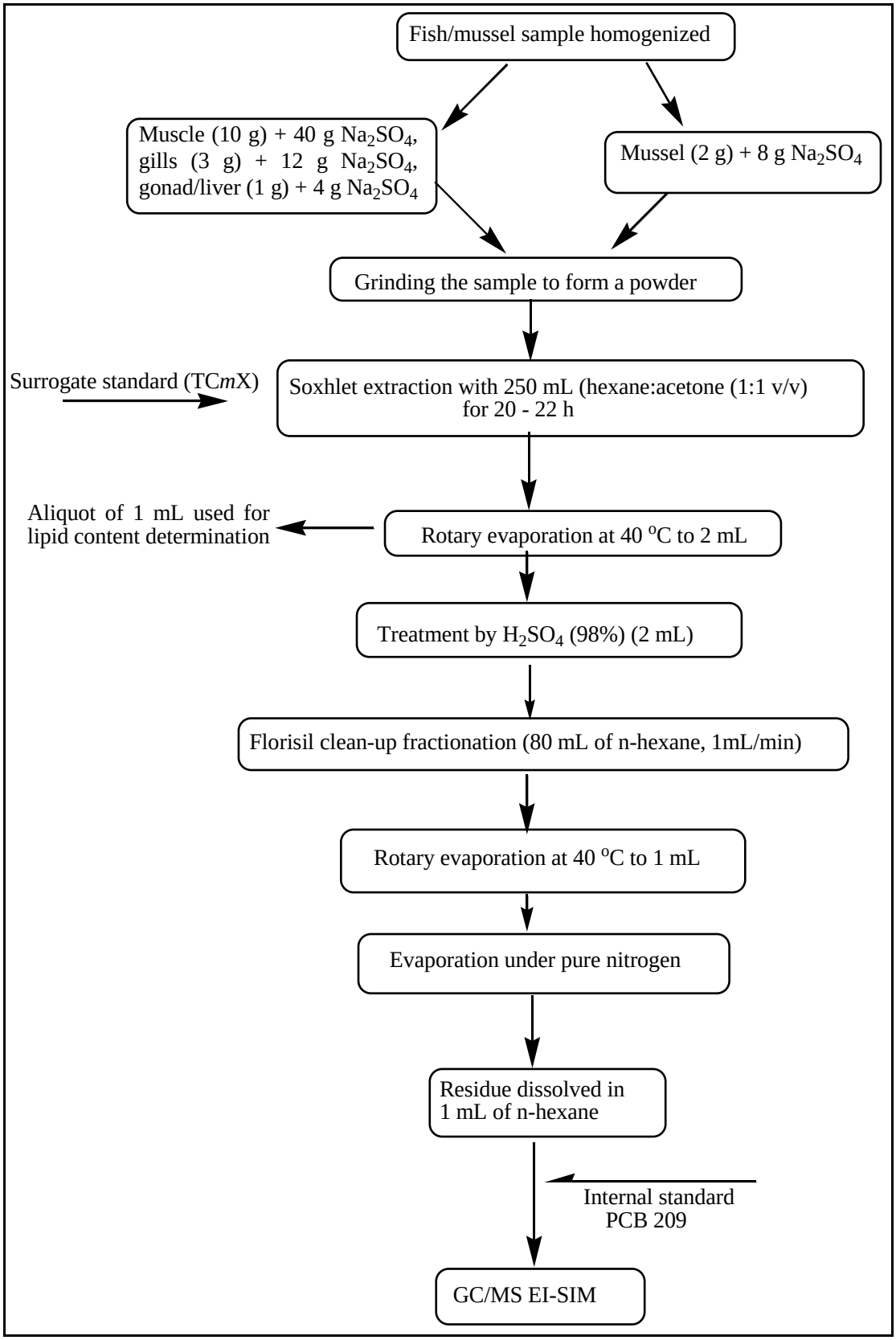


Figure 3.17: Flow diagram of the extraction and clean-up procedures of fish tissues and mussel samples.

3.3.4.4 Lipid content determination

Lipid contents of fish organs were determined during the analysis of PCBs. After the completion of the extraction procedure, extracts were evaporated and concentrated to obtain the lipids. The extractable lipid contents were determined gravimetrically by drying a fraction of the sample extract to a constant weight according to Muir and Sverko (2006). The lipid content was calculated as follows:

- An empty clean glass vial (20 mL) was dried at 105 °C for 3 hours, allowed to cool in desiccator and its weight, X_0 was determined and recorded.
- A 1 mL of the extract (5th step in Figure 3.17) was added to the vial X_0 and the weight was recorded, X_1.
- The sample was allowed to evaporate in the fume hood for 12 hours, and then the vial was reweighed, X_2.
- The lipid content was calculated using the formula:

$$\%\ lipid\ content = \frac{X_2 - X_0}{X_1 - X_0} * 100$$

It was assumed that the unevaporated mass was only due to lipids.

3.3.5 Extraction and clean-up of water samples

3.3.5.1 Sampling and sample preparation of water

Water samples were collected by hand in pre-cleaned 1 L brown glass bottles with Teflon-lined caps. Prior to use, the bottles were cleaned with tap water followed by deionized water and rinsed with acetone. Five of the sampling sites were in close proximity sites to stormwater inflow into the NEL, four sites were deeper out in the lake while two sites were in close proximity to the outflow from the lake (Appendix 6). In the water column, the organic contaminants tend to be bound to particulate matters. Before the extraction, water samples were filtered using Whatman® Glass microfiber filters (47 mm

Ø 100 circles GF/A, pore size of 1.0 µm) (from Sigma Aldrich, South Africa) to separate the particulate matter from the dissolved PCBs in the water.

3.3.5.2 Extraction of water and particulate matter samples

The dissolved PCBs were extracted by liquid–liquid extraction using a separatory funnel, 2000 mL with stopcock (glass or Teflon). The method was adapted from US EPA Method 3510C and as described by You et al. (2011) and Kumar et al. (2012) with some modifications. The particulate matter from the filters was extracted using Soxhlet extraction. Water samples were extracted within seven days after collection. Using a measuring cylinder, one-litre of water was measured and extracted three times with 60 mL of methylene chloride by shaking the funnel for two minutes, with periodic venting to release excess pressure and allowed to cool for 10 min. Volatile solvents such as hexane, benzene, ether, ethyl acetate, and dichloromethane are usually used for the extraction of semi-volatile compounds from water because their polarity is close to that of the target compound. The extracts were combined and were filtered through a funnel containing 4 g of anhydrous sodium sulphate (activated at 300 °C for 12 h before use) to take up water to become hydrated. The resulting extract was concentrated to 1 mL using rotary evaporator. The glass fiber filters containing particulates from each sample were Soxhlet extracted with 250 mL mixture of 50% methylene chloride/50% hexane for 18 hours. A Surrogate spike (100 µL, 1 ng μL^{-1}) was added to each sample before the extraction. The extracts were concentrated under vacuum to 1 mL and subjected to clean up.

3.3.5.3 Clean-up of water and particulate matter samples

The clean-up procedure was performed by removing the elemental sulphur with treatment of the extracts by concentrated sulphuric acid as it was done for solid samples as described in section 3.3.3.3. The purification was accomplished by Florisil (60-100 mesh). A glass column chromatography (400 mm long, 10 mm i.d) was packed with 2 g of activated anhydrous sodium sulphate, covered with 10 g of Florisil and topped with another 2 g of anhydrous sodium sulphate to avoid resuspension of the top layer when

pouring solvents into the column. The Florisil column mentioned above was rinsed with n-hexane (40 mL) to remove any impurities and the elution was accomplished by 80 mL of hexane (flow rate of 1 mL min^{-1}). The eluted extracts were concentrated using rotary evaporator and to dryness under a gentle stream of pure nitrogen. A 1 mL of hexane was added to the residue using a micropipette. The internal standard (PCB 209, 20 µL, 0.1 ng $µL^{-1}$) was added to each sample before the instrumental analysis.

3.3.6 Identification and quantification

3.3.6.1 GC separation

The separation occurs in the gas chromatographic column (such as capillary) when vaporized analytes are carried through by the inert heated mobile phase (so-called carrier gas such as helium). In this study, the analysis of PCBs in sediments, water, mussels and tissues of fish was performed on GC/MS because it was the best suited and the only available instrument at Nelson Mandela Metropolitan University that could be used for the detection of trace organic pollutants in environmental matrices including PCBs. Samples were identified and quantified using an Agilent GC-7890A coupled with Agilent 5975C mass spectrometer detector (GC/MSD). The capillary column used was a DB-1ms 30 m x 0.25 mm i.d. x 0.25 µm, maximum temperature: 340 °C (CJ &W Scientific, CA, USA). The injector used was splitless mode. Ultra-high purity helium was used as the carrier gas (flow rate of 1.2 mL^{-1} min). The temperature was programmed from an initial temperature 100 °C (1 min. hold) then increased by 15°C min^{-1} to 325 °C and held for 5 min. The mass spectrometer was operated in the electron impact (EI) ionization mode (70 eV) with ion source, quadrupole and transfer line temperatures of 230, 150 and 280 °C, respectively. The mass spectra of individual compounds were determined by separately injecting 1 µL of each sample analyte (standard) into the GC.

3.3.6.2 MS identification and quantification

The combination of the two vital components, gas chromatograph and mass spectrometer in a GC-MS, allows an accurate chemical identification. Currently, the most widely used method of identification of a chromatographic peak is its retention time or its relative retention time (RRTs), i.e., the adjusted retention time relative to the adjusted retention time of a selected reference compound. In the present study, the identification of the target analytes was performed by comparing the retention times (RT) in the chromatogram of the sample extract with those of each congener in the calibration standards and on intensity ratios of the monitored ions for quantification using GC/MS. The quantification was based on the base peak, and positive identification was confirmed by the presence of two confirming ions at the areas ratios within ± 20% variation (Ligor et al., 2007). The intensity ratios of the characteristic ions agree within ± 20% of the intensity ratios of these ions in the reference standard. (i.e. For an ion with an abundance of 80% in the reference spectrum of the standard, the corresponding abundance in a sample spectrum can range between 60% and 100%). A mixture of indicator congeners (PCBs 28, 52, 101, 138, 153 and 180) was used to produce the calibration curves with quantification based on internal standard method. Calibration curves were established in the range of 5 and 400 ng mL^{-1}. The six levels of calibration for each individual congener were used to quantify the indicator congeners in the sample. The response factor ratio of analyte and internal standard was plotted *versus* amount ratios of analyte and internal standard to determine the linear range of the detector for each component (Appendix 7). To compare the sensitivity of the two method options, standards were run in full scan and in SIM mode. In full scan mode no peaks were observed in the concentration ranges of 10 to 400 ng mL^{-1}. As expected the sensitivity for PCBs at low concentration was superior in SIM mode rather than full scan mode, so SIM analyses were used for all the samples. The characteristics of the gas chromatograph used for the analysis are indicated in the Table 3.4. The retention time of the analyte PCBs has been determined by injection of individual PCB, and subsequently injection of a mixture of PCB standard solutions all in SIM mode.

Table 3.4: Parameters of chromatographic determination of PCBs

Parameter	Description
Gas chromatography parameters	
Chromatograph type and Model	GC Hewlett Packard 7890
Column type	DB-1ms (100% dimethysiloxane)
Column dimensions	30 m x 0.25 mm x 0.25 µm (film thickness)
Injection port temperature	280 °C
Temperature program	100 °C (1 min) to 325 °C at 15 °C min^{-1}
Carrier gas	Helium
Constant column flow rate	1.2 mL min^{-1}
Injection volume	1µl
Injection mode	splitless
Average velocity	30 cm sec^{-1}
Mass spectrometer parameters	
Detector	HP 5975 MSD in SIM mode
Ion source temperature	230 °C
Quadrupole temperature	150 °C
Ionization mode	EI-SIM
Solvent cut time	3.60 min
Electron energy	70 eV
SIM width	0.500s

Based on these retention times of individual and combined standards, the exact acquisition time for SIM mode was set. To confirm the presence of a specific congener, the mass spectra of target analytes in the samples were compared to the mass spectra of analytes in the standards. The molecular mass and two additional confirming ions corresponding to the molecular ion cluster of each analyte $[M]^{+\cdot}$ and $[M\text{-}Cl_2]^{+\cdot}$ for loss of two chlorines (Table 3.5) established the presence of the analyte.

Table 3.5: Selected ions used for qualitative and quantitative analysis

IUPAC no.	Congeners	RT (min)	MW	Target ion (M^+)	Qualifier ion $(M-2Cl)^+$
PCB 28	2,4,4'-TriCB	10.115	258	256	186
PCB 52	2,2',5,5'-Tetra-CB	10.785	292	290	220
PCB 101	2,2',4,5,5'-pentaCB	11.722	326	324	256
PCB 138	2,2,3,4,4',5'-hexaCB	13.196	362	360	290
PCB 153	2,2'4,4',5,5'-hexaCB	12.794	362	360	290
PCB 180	2,2',3,4,4',5,5'-pentaCB	13.999	396	394	324
PCB 209	2,2',3,3',4,4',5,5',6,6'-decaCB	16.008	500	498	428

CB: chlorobiphenyl; MW: molecular weight (mass)

3.3.6.3 Sample amount determination

By calibration curve, the response factor (RF) for each component is calculated and then used to quantify the substances when unknown samples are run. The response factor is calculated using the area under the graph of a GC run and the concentrations of both the compound of interest and the internal standard. In this study, six indicator PCB congeners were identified and quantified. The calibration was made for each analyte of interest. The calculation of the sample amount was based on the internal standard method which uses the response factor (RF) from the calibration curve of all the target compounds. The RF is determined using the peak areas and the concentrations of both the compound of interest and the internal standard.

$$RF = \frac{A_s \times C_{IS}}{A_{IS} \times C_s}$$ Equation 3.18

Where:

- RF = Response factor the analyte
- A_s = Peak area of the analyte
- C_s = Concentration of the analyte in ng mL^{-1}
- A_{IS} = Peak area of the internal standard (PCB 209)
- C_{IS} = Concentration of the internal standard (PCB 209) used in the sample calibration in ng mL^{-1}

Linear calibration curves were plotted from the peak area results of the standards. The equation $y = mx + c$ was yielded.

Where:

- y is the ratio area, $y = \frac{Peak\ area\ of\ the\ sample}{peak\ area\ of\ the\ internal\ standard}$ Equation 3.19
- m is a constant that includes RF and Cis
- x is the sample concentration to be determined (C_i)
- c is the intercept of the regression line with the y-axis

After the determination of the response factor for each congener under the study, the unknown concentration of a component in a sample was quantified using the following equation:

$$Conc_i = \frac{[A_i * IS]/[A_{IS} * RF_i]}{SA}$$ Equation 3.20

Where:

- $Conc_i$ = the concentration of a component (i) in a sample in ng g^{-1}
- A_i = Peak area of the analyte (i) of interest to quantify
- IS = mass of internal standard in the sample in ng
- SA = mass of the sample analysed
- A_{IS} = Peak area of the internal standard in the sample
- RF_i = The response factor for a component (i) calculated from the most recent initial calibration

The total PCBs was obtained by summation of individual congener concentrations.

3.3.6.4 Limit of detection (LOD)

The LOD is the lowest amount of a substance that can be served to measure the sensitivity of the instrument. In this study, the LOD for the individual congeners was defined as the concentration of analyte that yields a signal to noise ratio of 3:1. The limit of quantification (LOQ) was calculated as the same as the LOD by a signal to noise ratio of 10:1. For this purpose, blank samples were spiked with a mixture containing PCB-28, 52, 101, 138, 153 and 180 at different levels prior to extraction.

After the identification, the signal was plotted *versus* the concentration of each congener. The limits of detection (LOD) for the target PCB congeners were calculated based on blank average concentration corresponding to m/z signal plus three times the standard deviation (Tasdemir et al., 2005). Currently, LOD can be determined in most laboratories on the basis of the standard deviation of replicate analyses at a single concentration. The LOD and LOQ of all the samples analysed are indicated in Table 3.6.

Table 3.6: Limit of detection and limit of quantification of the analysed samples

Analytes	LOD (ng g^{-1})	LOQ (ng g^{-1})
Fish		
PCB 28	0.48	1.59
PCB 52	0.16	0.54
PCB 101	0.02	0.05
PCB 138	0.09	0.31
PCB 153	0.07	0.24
PCB 180	0.12	0.39
North End Lake sediments		
PCB 28	0.01	0.04
PCB 52	0.02	0.07
PCB 101	0.20	0.67
PCB 138	0.06	0.21
PCB 153	0.02	0.05
PCB 180	0.05	0.17
Harbour sediments		
PCB 28	**0.01**	0.04
PCB 52	**0.02**	0.07
PCB 101	**0.06**	0.21
PCB 138	**0.12**	0.14
PCB 153	**0.07**	0.24
PCB 180	**0.15**	0.05
Blue mussels (*M. galloprovincialis*)		
PCB 28	0.01	0.04
PCB 52	0.01	0.05
PCB 101	0.12	0.24
PCB 138	0.15	0.50
PCB 153	0.01	0.03
PCB 180	0.28	0.94
Brown mussels (*Perna perna*)		
PCB 28	0.04	0.14
PCB 52	0.04	0.12
PCB 101	0.03	0.09
PCB 138	0.02	0.07
PCB 153	0.03	0.09
PCB 180	0.19	0.64
Water samples[a]		
PCB 28	0.07	0.21
PCB 52	0.04	0.15
PCB 101	0.04	0.11
PCB 138	0.11	0.30
PCB 153	0.06	0.20
PCB 180	0.08	0.26

[a]LOD are expressed in pg L^{-1}

3.3.6.5 Recovery percentage for the analytical method

The recovery is the term used to show of how much the original substance yielded up at the end of the experiment. To determine the extent of recovery, sample matrix blanks were spiked by adding a known amount of target analyte to a specified amount of matrix sample for which an independent estimate of target analyte concentration is available.

The spiking happens prior to sample preparation and analysis. A matrix spike is used to document the bias of a method in a given sample matrix. The recovery determination focused on the six PCB congeners (PCB-28, 52, 101, 153, 138 and 180), as potential target analytes. The samples with an initial PCB content below the detection limit were spiked with PCB standards at three levels (5, 10 and 20 ppb). The recovery of the matrix spike was calculated based on the ratio of the remaining surrogate/initial surrogate (added before Soxhlet extraction) as follows:

$$Recovery\ (\%) = \frac{Concentration\ added\ (ng\ g^{-1})}{Concentration\ added\ (ng\ g^{-1})}$$

The mean recoveries for every sample matrix are summarized in Table 3.7.

Table 3.7: Percentage recoveries of the analytes

Analytes	Recovery (n = 3 for every matrix except for fish where n = 6) (% ± Standard deviation)					
	Fish	NEL sediments	Blue mussels	Brown mussels	PEH sediments	Water
PCB 28	91.4 ± 3.84	78.42 ± 4.42	98.02 ± 4.60	87.88 ± 4.55	81.03 ± 3.84	80.63 ± 4.02
PCB 52	97.6 ± 2.08	97.63 ± 1.08	87.38 ± 4.42	91.89 ± 7.11	79.21 ± 8.65	83.32 ± 7.31
PCB 101	107 ± 4.83	78.85 ± 4.47	89.10 ± 4.32	88.96 ± 3.12	80.81 ± 7.27	79.44 ± 6.81
PCB 138	92.15 ± 5.65	92.15 ± 5.65	79.10 ± 6.32	88.47 ± 4.98	92.42 ± 2.70	91.49 ± 3.60
PCB 153	95.48 ± 2.65	95.48 ± 2.65	89.97 ± 5.27	89.41 ± 8.90	94.52 ± 1.83	96.22± 3.09
PCB 180	98.25 ± 3.25	98.28 ± 2.65	89.48 ± 3.35	92.53 ± 4.36	95.31 ± 1.80	93.84 ± 4.01
TC*m*X	95.13 ± 6.45	98.62 ± 5.38	93.0 ± 5.61	95.26 ± 8.68	98.26 ± 8.81	95.42 ± 7.26

3.3.6.6 Quality control of the analytical method

The quality control procedures are required to check the performance of the method. All of the procedures were controlled strictly by the analysis of procedural blank samples as well as the recoveries of surrogate standard in

each sample. For this purpose, internal standards (recovery and quantification standards) were added in a fixed amount to all the standards and samples. PCB 209 and a solution of 2,4,5,6-tetrachloro-*m*-xylene were used as internal/surrogate standard. The ideal internal standard is a PCB which is not found in the samples. PCB-209 is rarely present or undetectable in commercial mixtures and is a by-product in very few manufacturing processes. Procedural blanks were run with each batch of samples (5 or 8 samples per batch) and were taken through all the phases of the analytical procedure. The reagent/solvent was subjected to all the steps including, extraction, clean-up and analysis using GC/MS, SIM mode. Isooctane (1 µL) was run between the different calibrations to clean the syringe and to ensure no residue from the previous run remained in the column. The percentage recoveries were found to be in the range between 70 and 130%. In this study, the method used was accurate and the reference materials were not used. The linearity, accuracy and precision were good.

This chapter dealt with analytical method commonly used to determine organic contaminants such as PCBs in environmental matrices. The analytical method used in the present study was adapted from other studies based on the available equipment in the department of Chemistry at the Nelson Mandela Metropolitan University. The validation and method recovery was good ranging between 70 and 130% in all the types of matrices investigated.

CHAPTER 4

DISTRIBUTION OF POLYCHLORINATED BIPHENYL RESIDUES IN SEVERAL TISSUES OF FISH FROM THE NORTH END LAKE, PORT ELIZABETH, SOUTH AFRICA

The content of this chapter is reproduced from the paper published by *Water SA, 41*(4): 559-570 (2015), written by E. Kampire, G. Rubidge and J.B. Adams.

4.1 ABSTRACT

The concentrations and distribution of 6 PCB indicator congeners (IUPAC nos. 28, 52, 101, 138, 153, and 180) were measured in 236 organ samples of fish (*Cyprinus carpio* and *Oreochromis mossambicus*) from the North End Lake in Port Elizabeth, South Africa. Polychlorinated biphenyls (PCBs) were extracted from the fish muscles, gills, gonads and livers using USEPA method 8082, followed by a clean-up using concentrated sulphuric acid and florisil column chromatography. Analysis was achieved by gas chromatography–mass spectrometry (GC/MS) using the internal standard method. The concentrations of total PCBs in the liver, gonads, gills and muscle were 95.69, 57.49, 44.63, 34.14 ng g^{-1} lipid weight (lw) in *C. carpio* and 119.73, 59.21, 49.78, 34.63 ng g^{-1} (lw) in *O. mossambicus*, respectively. These values were relatively low compared to those reported in the literature. PCB levels were predictably highest in the lipid-rich livers. Individual congeners were not distributed homogeneously within the investigated organs. PCBs 153 and 138 were present at higher concentrations than other PCB congeners for both species. PCB contaminants in fish act as indicators of pollution in aquatic ecosystems and are a potential threat to human health when consumed.

4.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are synthetic chemicals made up of 209 isomers classified as persistent organic pollutants (POPs). They are

bioaccumulative substances, causing serious damage to the environment, and have been identified worldwide (Ahmed, 2003; Hu et al., 2010; Cimenci et al., 2013; Gdaniec-Pietryka et al., 2013).

PCBs were introduced into the environment primarily as a result of anthropogenic activities (Iwata et al., 1993). Due to their resistance to electrical, thermal, and chemical processes, PCBs have been used in a wide variety of applications since their commercial production in 1929 (Anyasi and Atagana, 2011).

Exposure to PCBs has been reported to cause adverse effects, including reproductive, immunological and neurological problems, while long-time exposure to some congeners affects liver functioning and may lead to developmental effects resulting in cancer (ATSDR, 2000). One of the main sources of these compounds to humans is dietary fish, which accumulate pollutants by direct absorption through the gills, exposure to contaminated sediments and by consumption of insects and smaller fish (Bush and Kadlec, 1995). In consequence, fish can accumulate hydrophobic compounds (e.g. PCBs) to concentrations considerably higher than those of the surrounding environment (OSPAR, 2004). The highest PCB concentrations are detected in lipid-rich tissues such as the liver and the muscle of fatty fish. Because of the persistence of these contaminants and the resulting harmful effects to organisms and human health, it is necessary to continue to monitor their distribution in the environment (Toaspern, 2003).

Fish consumption is an important constituent of human diets in that it increases the intake of omega-3 fatty acids which in turn lowers cholesterol, cancer risks, and blood pressure levels. However, despite the beneficial aspects of consumption, fish may also contain contaminants such as trace elements, PCBs or other pollutants.

Currently, very high resolution capillary columns are able to separate the 209 PCB congeners. However, the analysis of extracts of a biological matrix is still difficult because of co-elution. The six non-dioxin-like (NDL) PCBs (PCB nos. 28, 52, 101, 138, 153, 180) used as indicators are representative of the

congeners used in the PCB mixtures in the past and of the PCBs found in the environment at the present time (Aune et al., 1999). The sum of these congeners represents about 50% of the total non-dioxin-like PCBs in food (EFSA, 2005), and is recommended for regular monitoring (UNEP, 2003).

In aquatic organisms, most studies have reported on PCBs in the muscles of fish (Baeyens et al., 2007, Kočan et al., 2001). While industries are a key component of the country's economy, little research has been conducted on PCB contamination and no literature is available for PCB analysis in different organs of fish from the North End Lake. The purpose of the study was: to evaluate the levels of 6 indicator congeners of PCBs in organs of 2 fish species collected from the North End Lake, provide background information on the status of PCB contamination in the studied area, and evaluate the human health risks from consumption of fish muscle. There is increased environmental concern due to South Africa's growing chemical industry that has in turn led to increased industrial waste. The North End Lake is targeted for future recreational use; however, no literature is available regarding the current water quality status, particularly concerning persistent organic pollutants. In addition, the system is popular amongst local fishermen that catch, sell and consume the fish, even though it is potentially contaminated lake.

4.3 MATERIALS AND METHODS

4.3.1 Study area

The North End Lake (Figure 4.1) is an urban water body (33°56' S, 25°36' E) in the middle of a residential and industrial suburb, that of North End, Port Elizabeth, South Africa. The lake is situated adjacent to the new soccer stadium, which was built for the 2010 FIFA World Cup. The stadium was specially designed to have views of the lake on one side and the ocean on the other side. Water from the North End Lake is treated and utilized for irrigating green areas in the stadium. Repeated watering of the grass with PCB-contaminated water could result in PCB accumulation on the sports field

inside the enclosed stadium. The maximum depth of the lake is about 4 m (on average 3 m). The water level of the lake is controlled at a maximum height of 16.3 m amsl by means of an overflow leading to an underground concrete culvert, which flows into the sea. For several decades the lake was one of the main recreational areas of the city. As the city expanded, the lake was increasingly subjected to human impacts (Weichers et al., 1996), with an increased inflow from residential and industrial stormwater runoff drains (Figure 4.1).

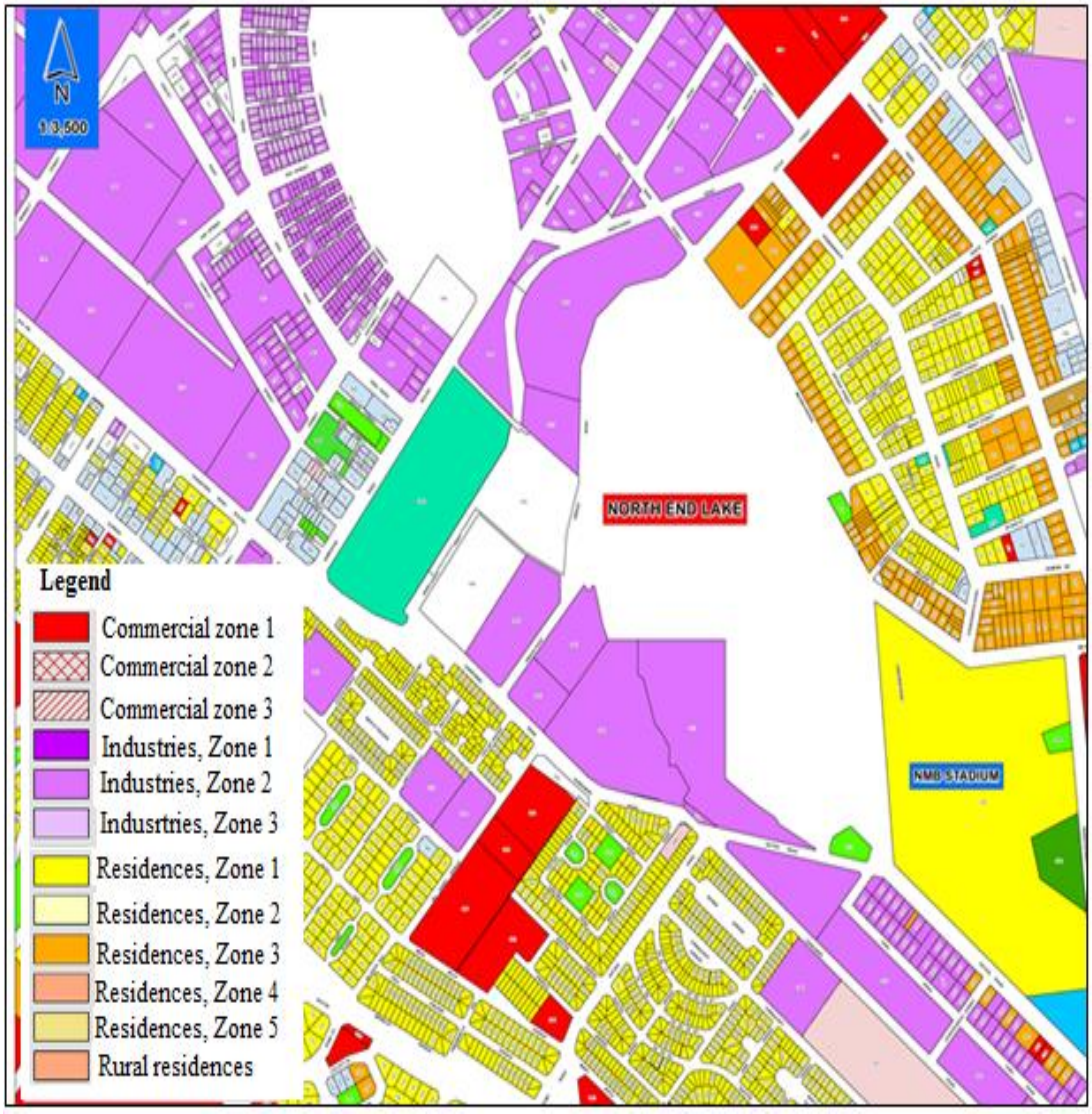


Figure 4.1: Study area: The North End Lake and land use of surrounded area.

4.3.2 Fish collection

During February, August and November of 2013, 60 fish from 2 species (*C. carpio* and *O. mossambicus*) were bought from the same fisherman at the lake. *C. carpio* was found to be the most abundant species caught in the lake (n = 51), while *O. mossambicus* (n = 9) originated from a single catch (23 February 2013). These two species may accumulate high levels of contaminants as they are omnivorous (*O. mossambicus*) and bottom-feeders (*C. carpio*). After sampling, fish were transported to the laboratory on ice, weighed and measured to record the fork lengths. The mean length (cm), weight (g) and the condition factor of the two fish species investigated are shown in Table 4.1. The raw data of each individual fish are indicated in the appendix 8. All fish were assigned an identification number and letter code. Fish were stored in a deep freezer at −20 °C until further analysis. The details for the analytical procedures are indicatied in the section 3.3.

Table 4.1: Biometric data of the two fish species analysed

Fish species	**Body length (cm)**			**Body weight (g)**			**Condition factor (CF) (g cm^{-3})**
	Min	Max	Mean ± SD	Min	Max	Mean ± SD	
***Oreochromis mossambicus* (*n* = 9)**	29	35	32 ± 2	550	1056	800 ± 156	2.3 (1.83–2.54)
***Cyprinus carpio* (*n* = 51)**	15	48	32 ± 7	211	1521	841 ± 332	1.53 (0.92–2.5)

CF = [(Body weight/(body length)^3) x 100] (Fernandes et al., 2008; Baptista et al., 2013)

4.4 DATA ANALYSIS

The data were analysed with Statistica 11.0 software. Statistical significant differences were determined using one-way analysis of variance (ANOVA) and Pearson test was used to assess correlations between contaminant concentrations and lipid content/size of fish. During statistical analysis, non-detectable (nd) data were assigned a half-value of the limit of quantification. All tests were considered statistically significant when the *p*-value < 0.05.

4.5 RESULTS

4.5.1 Levels and distribution of PCBs in fish tissues

The mean concentrations of individual congeners and total PCBs for both species are summarized in Tables 4.2 and 4.3. Total PCBs in *C. carpio* and *O. mossambicus* ranged from 34.14 ± 14.88 to 95.69 ± 24.56 and from 34.63 ± 18.44 to 119.73 ± 16.06 ng g^{-1} lipid weight (lw) with an average of 57.99 ± 26.89 and 65.84 ± 37.71 ng g^{-1} lw, respectively. Potential target organs for the accumulation of PCB congeners were identified. The liver represented the major target organ for PCBs, receiving the largest part (41% and 45%) of overall contaminant for *C. carpio* and *O. mossambicus*, respectively. Total PCBs in gills of both species were similar (49.78 ± 15.95 and 44.63 ± 21.23 ng g^{-1} lw, respectively, in *O. mossambicus* and *C. carpio*). Significant differences were observed between PCB levels of liver and other organs in each species (ANOVA $p < 0.05$). However, no significant differences were observed between the mean PCB concentrations in gills, muscles, livers and gonads of both fish species ($p > 0.05$).

Table 4.2: PCB levels in *C. carpio* tissues (n = 51, ng g^{-1} lw, mean ± standard deviation)

Analytes	Muscle (n = 51)	Gills (n = 51)	Gonads (n = 48)	Liver (n = 50)
PCB 28	3.66 ± 4.64	7.01 ± 7.66	7.31 ± 8.35	12.78 ± 8.47
PCB 52	3.44 ± 3.81	6.69 ± 7.11	7.00 ± 7.09	12.77± 7.67
PCB 101	5.44 ± 5.96	6.86 ± 6.43	9.14 ± 10.56	14.88 ± 7.70
PCB 138	7.45 ± 5.81	7.80 ± 5.80	12.24 ± 7.05	20.90 ± 10.12
PCB 153	8.57 ± 6.49	8.30 ± 7.22	12.79 ± 9.72	19.27 ± 8.34
PCB 180	5.59 ± 4.84	7.95 ± 5.27	9.01 ± 6.97	15.09 ± 9.58
Σ_6PCBs	34.14 ± 14.88	44.63 ± 21.23	57.49 ± 23.12	95.69 ± 24.56
% lipid content	2.26 ± 1.15	2.53 ± 1.48	2.77 ± 1.45	3.03 ± 1.73

Table 4.3: PCB levels in *O. mossambicus* tissues (n = 9, ng g^{-1} lw, mean ± standard deviation)

Analytes	Muscle (n = 9)	Gills (n = 9)	Gonads (n = 9)	Liver (n = 9)
PCB 28	6.47 ± 6.94	7.89 ± 5.48	9.45 ± 10.60	15.26 ± 6.57
PCB 52	3.42 ± 2.33	7.97 ± 4.93	7.97 ± 4.66	17.73 ± 6.34
PCB 101	6.38 ± 4.54	8.34 ± 6.77	8.50 ± 5.49	16.90 ± 3.13
PCB 138	5.54 ± 5.15	7.04 ± 4.14	9.67 ± 4.52	28.24 ± 5.98
PCB 153	6.50 ± 5.79	11.08 ± 3.90	12.87 ± 4.91	23.21 ± 6.04
PCB 180	6.32 ± 4.86	7.45 ± 4.29	10.75 ± 4.02	18.40 ± 7.05
Σ_6PCBs	34.63 ± 18.44	49.78 ± 15.95	59.21 ± 25.82	119.73 ± 16.06
% Lipid content	2.63 ± 0.91	2.83 ± 1.25	3.27 ± 1.70	3.44 ± 1.44

4.5.2 PCB congener's profiles

The percentage contributions of the six indicators to the total PCBs in organs of both species are shown in Figures 4.2a and b. In general, congeners with 5, 6 and 7 chlorine atoms were quantitatively more important in all organs, with predominance of hexachlorobiphenyls (138 and 153), which contributed to 35% and 45%, on average, of the total PCBs in both species.

PCB 28 was dominant in muscle of *O. mossambicus* (mean of 6.47 ± 6.94 ng g^{-1} lw) and contributed to the total PCBs equally as PCB 153 (19%). Similarly, PCBs 101 and 180 contributed 18% each to the total PCBs in muscle. PCB 153 contributed to the total PCBs in gills and gonads with the same amount (22%). With respect to bioamagnification, a higher occurrence of PCBs in *O. mossambicus* (100%) relative to *C. carpio* is expected due to its diet including algae, plant matter, sediment organic particles, small insects, invertebrates and other fish which would already have biomagnified the PCBs.

In *C. carpio*, the lower congeners (PCBs 28 and 52) as well as PCBs 101 and 180 contributed, on average, < 20% each to the total PCBs. Both hexachlorobiphenyls (PCBs 138 and 153) contributed, on average, > 20% to the total PCBs in liver, muscle and gonads.

Statistically significant differences were observed between individual congeners in tissues ($p < 0.05$). PCB 153 was significantly higher than PCBs 28, 52, 101 and 180 (ANOVA $p < 0.05$) in all the tissues. Similarly, PCB 138 was significantly higher than PCBs 28, 52, and 101 in liver. Furthermore, no significant difference was observed between congeners in gills and gonads (ANOVA $p > 0.05$).

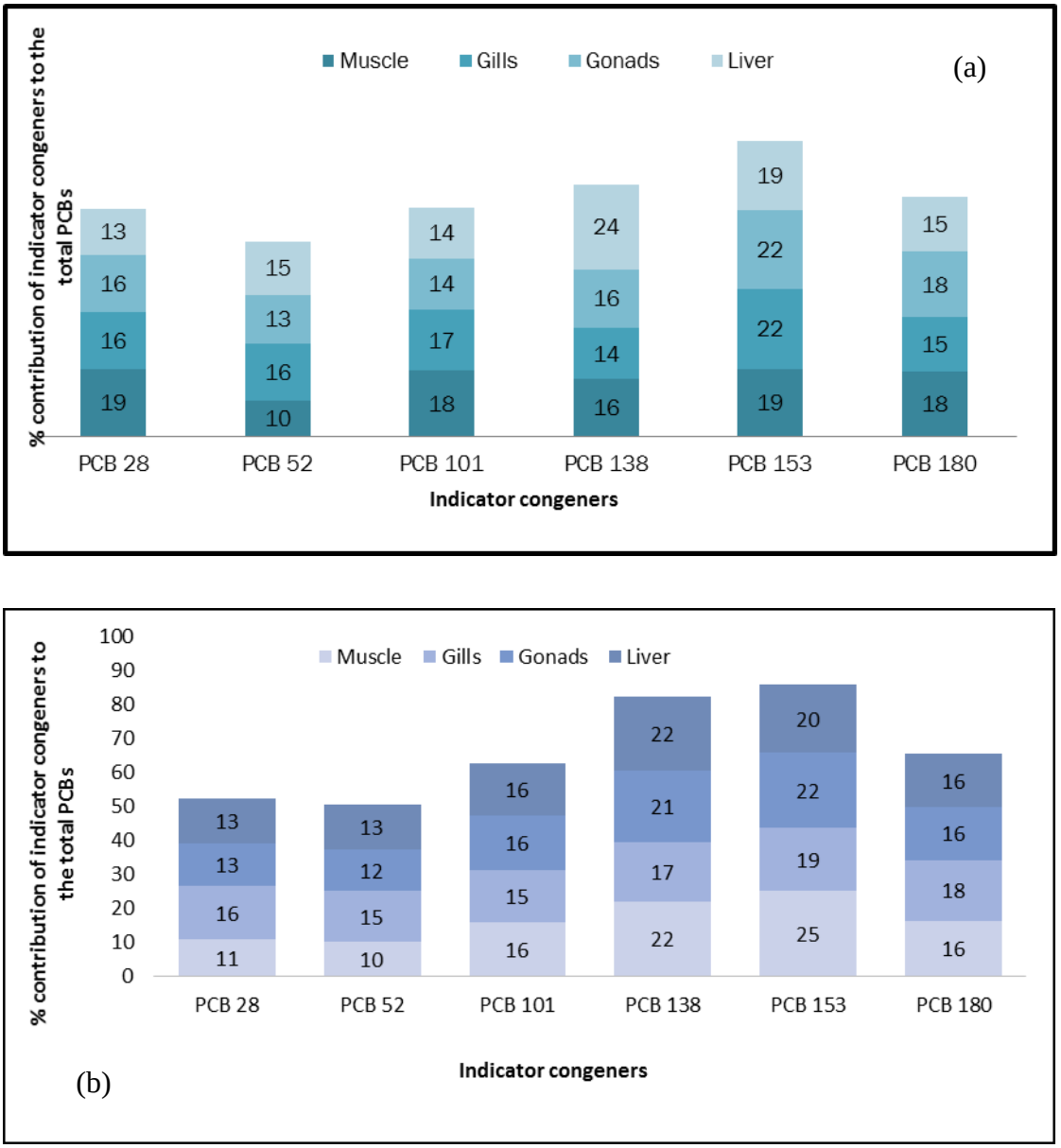
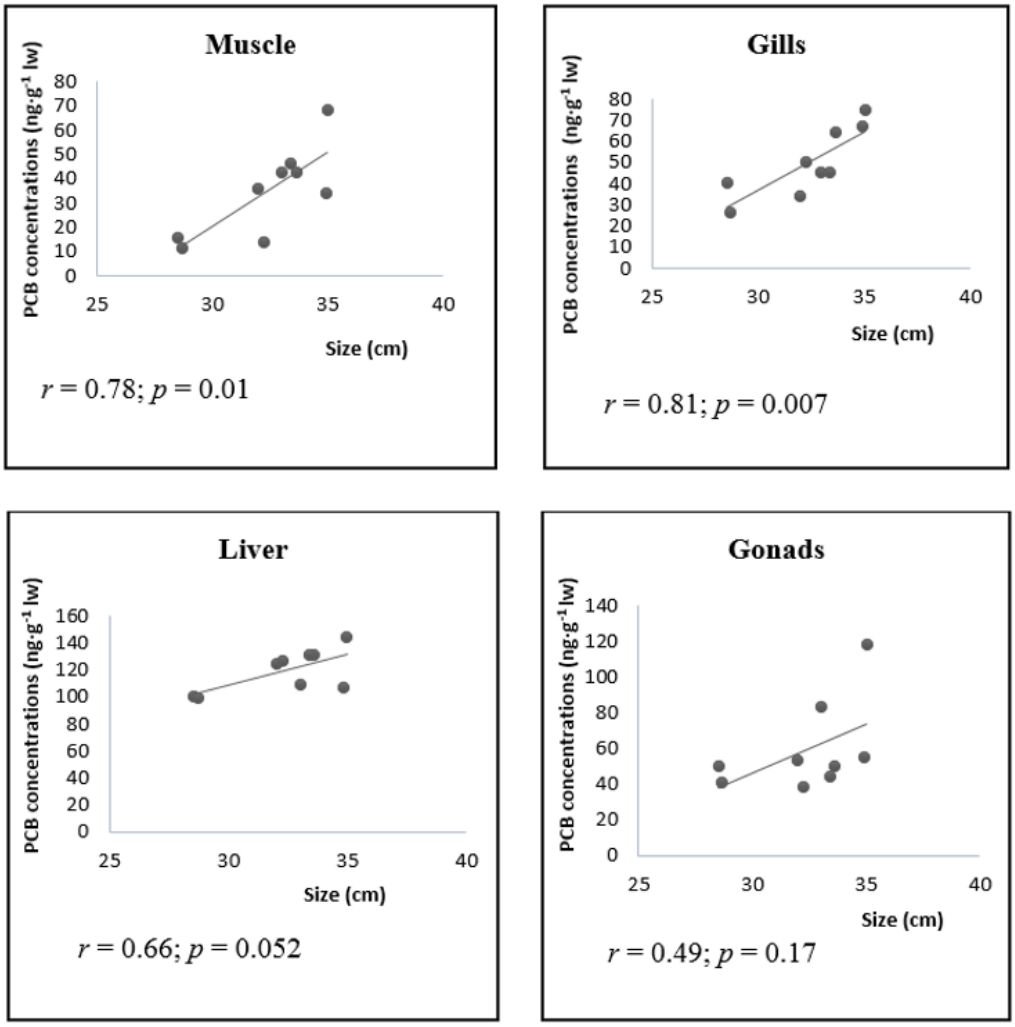


Figure 4.2: Contribution of indicator congeners to the total PCBs in (a) *O. mossambicus* and (b) in *C. carpio.*

4.5.3 Biological and biochemical parameters

The size of fish is dependent on the availability of food providing the energy needed for the fish to form body tissues and to tolerate adverse environmental conditions. The size of fish is used as an indicator of PCB contamination levels. The contaminant/length relationship occurs due to the older age of larger fish, which have a longer exposure period to contaminants (Gewurtz et al., 2011). The mean size and weight for the 51 *C. carpio* analysed was 32 ± 7 cm and 841 ± 332 g, respectively. For *O. mossambicus*, the mean size and weight of the 9 fish analysed was 32 ± 2 cm and 800 ± 147 g, respectively. The condition factor (CF) was calculated from the weight of the fish in relation to its size [CF = (body weight/(size)^3) x 100]. The condition factor serves as an indicator of growth, nutritional state and energy content of the fish. For both species, the CF showed good condition with means of 2.3 ± 0.2 g cm^{-3} and 1.53 ± 0.34 g cm^{-3}, respectively, in *O. mossambicus* and *C. carpio*. A significant positive relationship between body size (cm) and concentrations of PCBs was observed in the tissues of both species ($p < 0.05$), except in liver and gonads of *O. mossambicus* ($p > 0.05$) (Figures 4.3a and 4.3b).

(a)


Muscle
PCB concentrations (ng·g-1 lw)
Size (cm)
r = 0.78; p = 0.01
Gills
PCB concentrations (ng·g-1 lw)
Size (cm)
r = 0.81; p = 0.007
Liver
PCB concentrations (ng·g-1 lw)
Size (cm)
r = 0.66; p = 0.052
Gonads
PCB concentrations (ng·g-1 lw)
Size (cm)
r = 0.49; p = 0.17

(b)

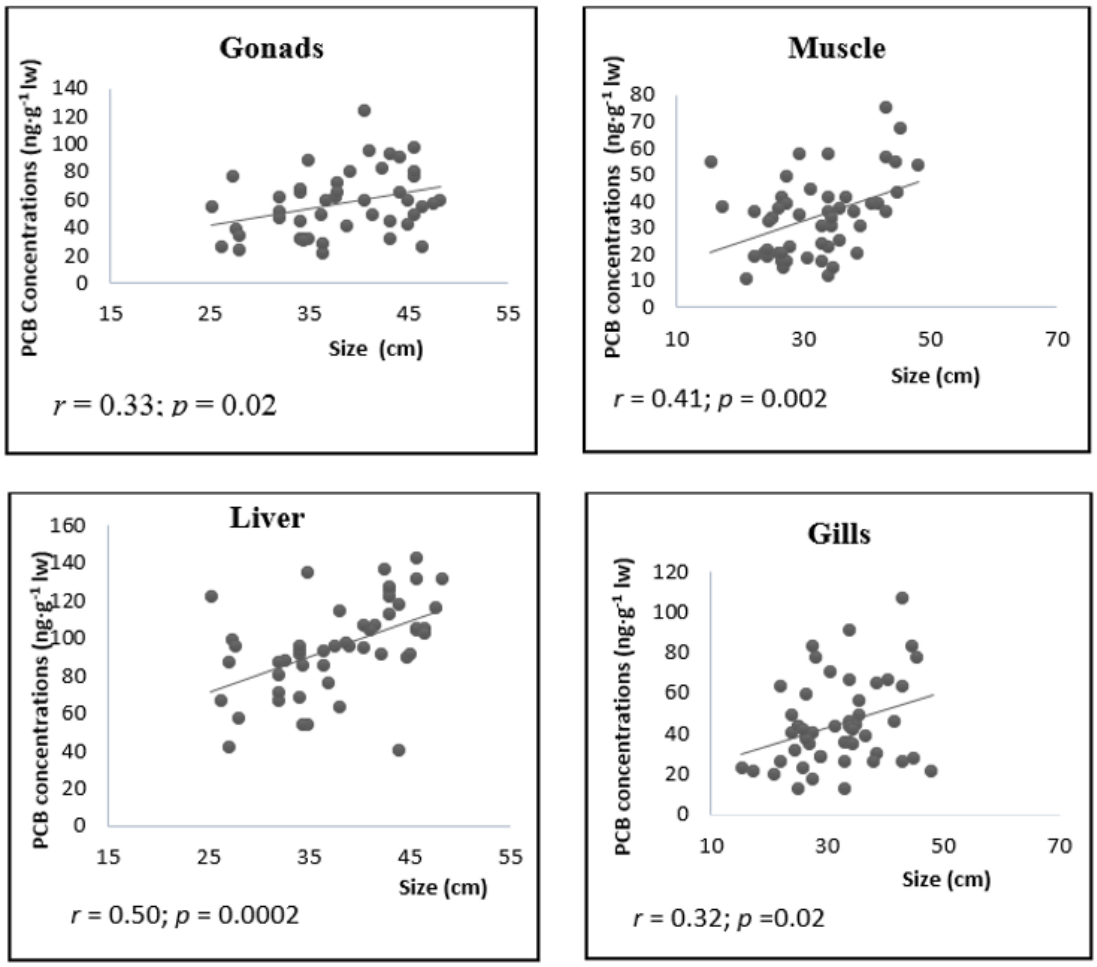
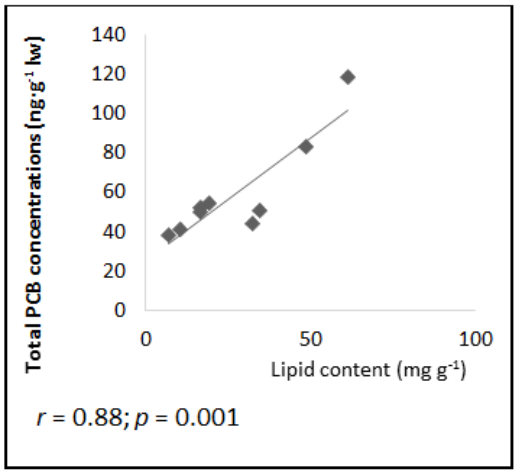


Figure 4.3: PCB concentrations in tissues of fish as function of size (a) in *O.mossambicus* and (b) in *C. carpio.*

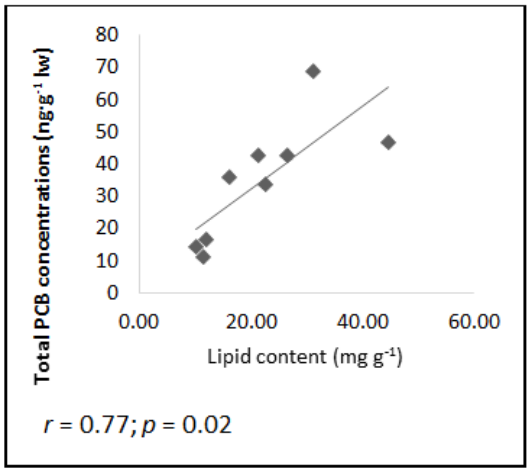
Since PCB compounds are hydrophobic (high Kow), their concentrations based on amounts of lipids are considered to be an important variable in their distribution in fish. The liver had the highest levels of lipid content in both species in comparison to the other tissues, followed by gonads, gills and muscle. Figures 4.4a and 4.4b showed the relationship between lipid content and PCB concentrations in *O. mossambicus* and *C. carpio*, respectively. A positive correlation between lipid content and contaminant levels was observed in tissues of both species, except in liver of *O. mossambicus* and in gills of *C. carpio* ($p > 0.05$). These results highlight the effect of size and lipid content on PCB levels and differences for different organs.

(a)

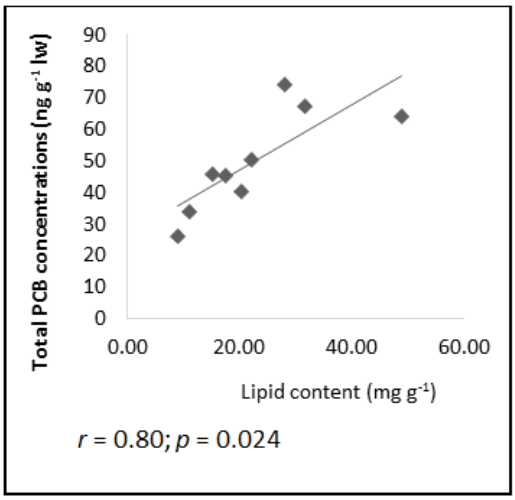
Gonads: *O. mossambicus*



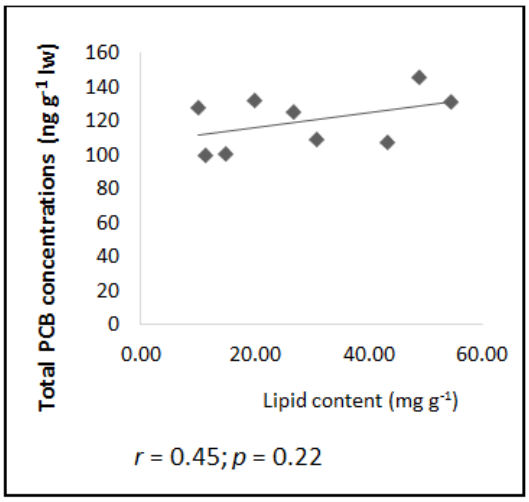
Muscle: *O. mossambicus*



Gills: *O. mossambicus*

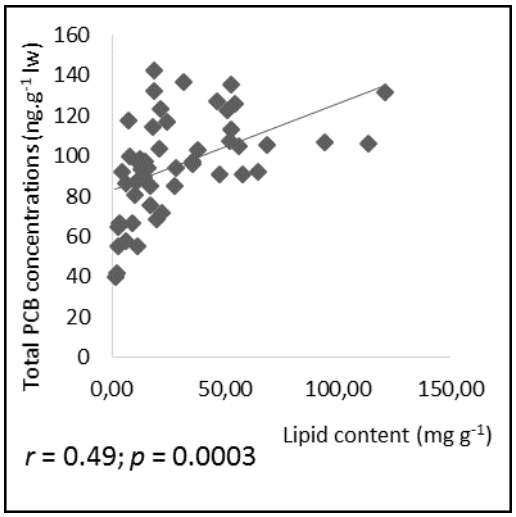


Liver: O. mossambicus

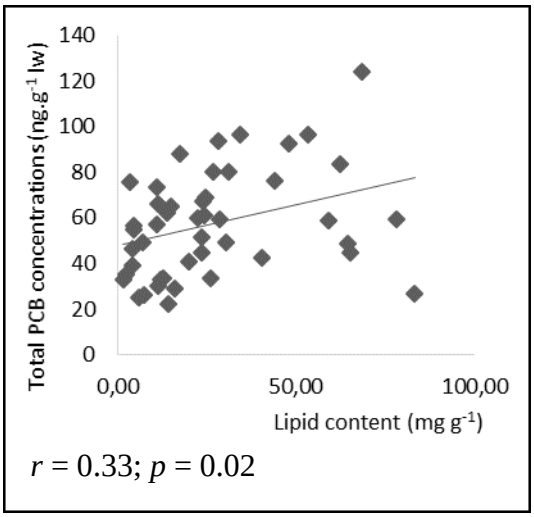


(b)

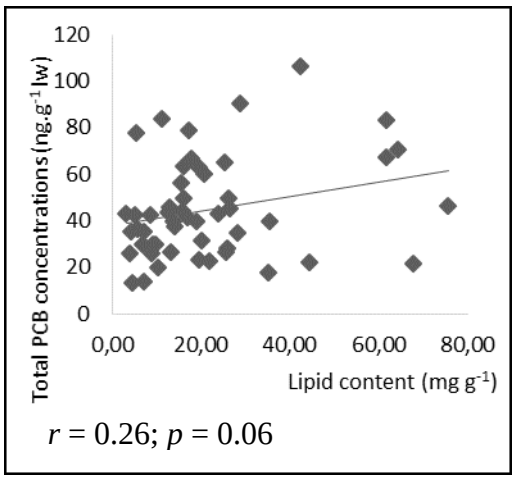
Liver: *C. carpio*



Gonads: *C. carpio*



Gills - *C. carpio*



Muscle - *C. carpio*

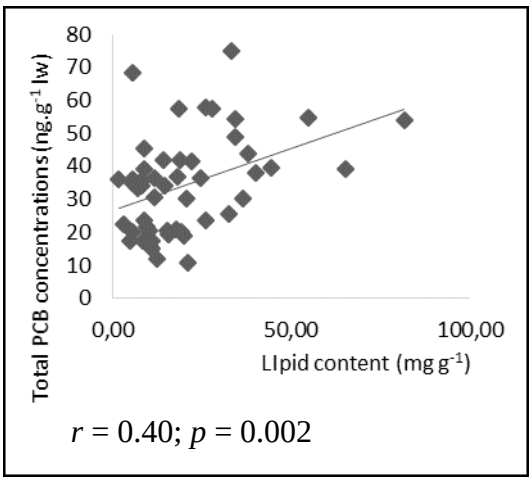


Figure 4.4: PCB concentrations versus lipid content (a) in *O. mossambicus* and (b) in *C. carpio.*

4.6 DISCUSSION

Fish act as indicators of PCB pollution in the aquatic ecosystems where they store chemical substances either directly from the surrounding environment or from their diet (Lavandier et al., 2013). These contaminants in fish are a threat to predators and humans when consumed due to the fact that PCBs are soluble in the lipids fraction, especially the higher chlorobiphenyls, thereby

increasing their bioaccumulation and biomagnification (Urbaniak, 2007; Beyer and Biziuk, 2009). Geyer et al. (1994) mentioned that fish lipid content varies according to species, age, sex, season and location. Mainly, PCBs enter into aquatic organisms via lipids of the ingested food (Hites et al., 2004), while their distribution in tissues is strongly influenced by their lipid content (Henshel and Sparks, 2006). The lipid plays a role in physiological processes of fish such as reproduction. The accumulation of organochlorine compounds shows that the reproductive cycle is associated with a large variation in lipid content (Antunes et al., 2007). Lipid content decreases during the spawning period by the elimination of lipid-bound hydrophobic compounds, such as POPs (Ondarza et al., 2014). Lipid content is among the most important factors that determine species body burden. A positive correlation was found between lipid content and PCB concentrations, which is in accordance with other studies. Previous investigations carried out on aquatic organisms showed that there is a correlation between the accumulation of PCB and the lipid content of tissues (Hebert and Keenleyside, 1995; Kočan et al., 2001).

These findings confirm that lipid content is one of the main factors involved in PCB accumulation in fish. However, a study by Nie et al. (2005) indicated that several factors such as physico-chemical properties of individual congeners and biological characteristics, such as sex, maturation state and, feeding habits, are all contributing factors to the accumulation of PCBs in fish. A correlation between lipid content and levels of PCB contaminants in liver and muscle of fish species, including eel (*Anguilla anguilla*), crucian carp (*Carassius carassius*) and catfish (*Ictalurus nebulosus*), was found by Roche et al. (2000). Even though it was infrequent, a negative correlation was observed in the fatty fish, eel, and a positive correlation in the non-fatty catfish. The negative correlation suggests that the youngest fish were the most contaminated. The positive relationship between lipid levels and organochlorine concentrations also tends to occur between species at the same trophic level, individuals of the same species, and within tissues in a single individual (Miller and Amrhein 1995). PCB contaminant levels have also been shown to be linked to the size of the fish samples (Boscher et al., 2010; Gewurtz et al., 2011). The condition factor (CF) values higher than 1 indicate

healthy fishes. Compared to smaller fish of the same species, larger fish tend to consume larger, more contaminated prey and to eat at higher trophic levels. Thus, the foods of large fish tend to be more contaminated than the foods of small fish (Amrhein, et al., 1999).

PCB levels detected in tissues of fish in this study showed a positive correlation between size of fish and body burdens of contaminants. The exception was found in liver and gonads of *O. mossambicus.* The size of fish can be reduced due to infections which can affect tissues. Fernandes et al. (2008) found that infected gonads showed a great reduction in size compared with non-infected ones. Mackay and Fraser (2000) proposed that the increase in concentration with size is due to the bioaccumulation properties of PCBs, their resistance to biotransformation and their slow depuration rates by organisms. Higher levels were expected in *O. mossambicus* in this study because of its predatory feeding habits compared to that of *C. carpio* (aquatic plants, benthic organisms, debris and detritus). However, no significant differences were observed between mean PCB concentrations of the same tissues of both fish species. In other studies on the tissues of fish, liver was found to be highly contaminated by the higher chlorinated congeners (i.e. PCBs 153 and 138) (Dabrowska et al., 2009; Wang et al., 2010; Brázová et al., 2012). With high lipid content, the liver appears to be the main organ for metabolism and storage of PCBs (Fernandes et al., 2008; Bodiguel et al., 2009).

In general, PCB 153 is one of the main contributors to the total PCB content in freshwater fish species worldwide due to its long half-life (Nicola et al., 2014). PCBs 153 and 138, detected at high levels in this study, have been shown to initiate histological and neurological damage in the liver (Duffy and Zelikoff, 2006). Vezina et al. (2004) showed that after exposure to PCB 153, rats, mice and monkeys exhibited liver damage. The estimation of hepatic sequestration showed that liver/muscle ratios for the sum of the six congeners were higher in *O. mossambicus* (4.92) compared to *C. carpio* (3.04). The bioaccumulation of PCBs in tissues is related to their logarithm octanol-water partition coefficient (log Kow). The lipophilicity and log Kow increase as the chlorine

content increases thereby decreasing the biodegradability of PCB compounds (Semple et al., 2003; Zhou et al., 2005). The higher chlorinated congeners are less soluble in water and are therefore detected at higher levels in aquatic organisms. The bioaccumulation of PCBs in aquatic organisms is directly linked to their degree of chlorination and lipophilicity (Karjalainen et al., 2006).

Tissue-specific and congener distribution patterns are considered when evaluating the fate and effects of PCBs in the environment. However, no studies have been done on organs of fish in the region (South Africa) for comparison. Recent studies have been conducted, in other countries on tissue-specific PCB distribution in fish (Monosson et al., 2003; Bodiguel et al., 2009; Ondarza et al., 2010; Wang et al., 2010; Brázová et al., 2012; Lavandier et al., 2013). The levels of PCBs detected in organs of fish in the present study were compared with these studies (Table 4.4). Wang et al. (2010) measured total PCBs in muscle, gonads and liver of *C. ursinus* and found higher levels than that detected in the present study. Ondarza et al. (2010) reported PCB levels (for the same species, *C. carpio*) but results were expressed on wet weight. Brázová et al. (2012) conducted a study on predatory and non-predatory fish and reported PCB concentrations three orders of magnitude greater than the present study. Other researchers (Monosson et al., 2003, Bodiguel et al., 2009, Mierzykowski, 2010) have reported higher concentrations for some organs. The studies of Mierzykowski (2010) and Bodiguel et al. (2009) on sea fishes revealed higher ratios of liver/muscle (7.23 and 15.2) than those found in this study (4.92 and 3.04). Lavandier et al. (2013) analysed PCBs in three fish species from Sepetiba Bay in Brazil and the mean levels detected in scabbard fish muscle were similar to the levels detected in muscle of *C. carpio* in this study. All these studies showed that the values reported for the fish in the North End Lake were relatively low. In natural environments, fishes are exposed to a wide range of organic contaminants which behave differently in response to physico-chemical properties.

Table 4.4: Comparison of total PCB levels in fish organs of the present study and some locations elsewhere (ng g^{-1} lw)[a] and (ng g^{-1} ww)

Location and year	Species	Liver	Gonads	Muscle	Gills	References
North End Lake, Port Elizabeth, South Africa	*O. mossambicus* (n = 9)	119.73[a]	59.21[a]	34.63[a]	49.78[a]	Present study
	C. carpio (n = 51)	95.98[a]	57.49[a]	34.14[a]	44.63[a]	
St. Paul Island, USA 2003-2004	*C. ursinus*	336.2[a]	214.8[a]	301.0[a]	-	Wang et al. (2010)
Negro River, Argentina	*C. carpio* (n = 15)	38.46	6.9	102.58	139.3	Ondarza et al. (2010)
Šíravský canal, Slovakia, 2009	Predator fish (*n* = 24)	120 000	79 400	99 300	-	Brázová et al. (2012)
	Non-predator fish (n = 8)	70 100	88 200	64 500	- -	
Rockall, Scotland, 2007	Monkfish and scabbard fish	892.5	1 891.4	123.3	- -	Mierzykowski (2010)
Gulf lions, N.W. Mediterranean, 2004-2005	European hake males	2 539.5	58.7	167	-	Bodiguel et al. (2009)
	Females	1 688.7	294.2	97.6	-	
Lower Hudson River Estuary, 1994	*F. heteroclitus* (40 females)[b]:					Monosson et al. (2003)
	Flax site[b]	234	227	47	-	
	SSM site[b]	150	227	37	-	
	Newark site[b]	1 312	1 596	209	-	
	Piermont site[b]	1 333	2 547	255	-	
	Iona site[b]	1 265	3 453	263	-	
Sepetiba Bay, Brazil	Scabbard fish[c]	4.54–22.71	-	3.97–11.04	-	Lavandier et al. (2013)
	Croaker[c]	3.41–34.22	-	2.29–25.88	-	
	Mullet[c]	3.70–24.53	-	2.37–27.60	-	

[a]Concentrations are expressed on lipid weight, NW: North-west; [b]Results are expressed as mean PCB concentrations at these sites

[c]Results are given in range of PCB concentrations

Major human exposure to PCBs occurs through food intake (WHO, 2000), and fish consumption is considered a significant potential route for this (Binelli and Provini, 2004; Ribeiro et al., 2008). Biomagnification of persistent contaminants is often observed as their elimination from tissues is slower than their uptake from food. Therefore, fish and animals at the top of the food chain are more contaminated than those feeding at lower trophic levels. Trimming the fat and skinning the fish prior to cooking may reduce the risk of exposure (USEPA, 1999a). Table 4.5 indicates maximum residue limits recommended by key international agencies.

Table 4.5: Tolerance maximum residue limit (MRL) (ng g^{-1} fat basis)

Types of food	USFDA	Health Canada	EPA
Milk (fat basis)	1500	200	-
Dairy products(fat basis)	1500	200	-
Poultry (fat basis)	3000	500	-
Eggs	300	100	300
Meat, beef (fat basis)	-	200	-
Fish and shellfish (edible portions)	2000	2000	2000
Infant and junior foods	200	-	-
Drinking water	-	200	500

Sources: (ATSDR, 2000)

Human consumption rates are based on the edible part of fish (muscle), but some ethnic groups may also eat various organs of fish. For example, Office of Environmental Health Hazard Assessment (OEHHA)'s California fish advisories has recommended that consumers not eat the liver and other organs of fish as they accumulate higher levels of organic contaminants relative to the muscle tissue (OEHHA, 2003). The USEPA has set a reference dose (RfD), which is defined as the daily exposure likely to be without significant risk of adverse effects during a lifetime. For total PCBs, the RfD is $2x10^{-5}$ milligrams per kilogram of consumer body weight per day and cancer slope factor (CSF) of 2 $(mg/[kg\text{-}d])^{-1}$ (USEPA, 2000). CSF is based on the

cancer potency of the chemical used to estimate the probabilities of risk of developing cancer (U.S. EPA, 1999).

Based on the methodology developed by the USEPA (2000), the assessment of adult human health risks was evaluated by the determination of fish consumption limits expressed as number of meals per month of edible parts in both species (*C. Carpio* and *O. mossambicus*). For both species, based on the non-cancer health endpoint, four 8-oz (0.227 kg) meals per month are recommended and, based on the cancer health endpoint; one 8-oz meal/month is recommended. Fish are nutritious, providing a good source of protein and other nutrients, and are recommended as part of a healthy balanced diet. However, considering contamination by PCBs and other lipophilic substances, it is advisable to consume fish in moderation and to make informed choices about which fish are safe to eat. For example, The American Heart Association recommends that healthy adults eat at least 2 servings of fish per week (Schwarzenegger et al., 2004).

This study showed that individual congeners were not distributed homogeneously within the investigated organs. Liver presented higher PCB concentrations than other tissues because liver is the main organ for PCB storage and has higher lipid content, therefore PCBs are more likely to accumulate in liver. The contamination levels of PCBs are the result of multiple factors affecting the fate of PCB congeners entering the aquatic environment. These factors include: the different degree of persistence for each congener, depending on the number of chlorine substitutions on the biphenyl rings, the physico-chemical properties of the PCB congeners (Kow, vapour pressure), and the prevailing percentage composition of the PCB commercial mixtures employed. The congeners detected in this study are dominant PCBs in technical PCB mixtures (Schulz-Bull et al., 1998).

4.7 CONCLUSION

This study is the first to report PCB contamination in the fishes of this lake, which receives stormwater inflow from industries and residential areas. The lipid contents of fish were found to be one of the main factors determining

PCB levels in fish. Fish size, which is a proxy for fish age, is another important factor influencing the PCB levels in tissues. Based on the total PCB concentrations, *O. mossambicus* was more contaminated as it is a semi-predator while *C. carpio* is a bottom-feeder. *C. carpio* are omnivorous fish and they eat any food which can be digested. They dig and burrow into the sediment in search of organic matter, such as larvae of insects, worms, molluscs and decayed matter containing bottom-dwelling organisms, pieces of plants and the young shoots of aquatic weeds.

Measurement of six indicator PCBs in tissues of *Cyprinus carpio* and *Oreochromis mossambicus* from the North End Lake have shown total PCB levels to decrease in the order: liver >gonads >gills >muscles, in both species. The concentrations of total PCBs in the liver, gonads, gills and muscle were 95.69, 57.49, 44.63, 34.14 ng g^{-1} lipid weight (lw) in *C. carpio* and 119.73, 59.21, 49.78, 34.63 ng g^{-1} (lw) in *O. mossambicus*, respectively. The presence of PCBs in fish of the North End Lake could be harmful since they may be biomagnified through the food chain, i.e., with humans being the end consumer. Fatty foods of animal origin constitute a potential source of PCB exposure. Based on the non-cancer and on the cancer health endpoint, four 8-oz (0.227 kg) meals/month and one 8-oz meal/month were recommended for both species, respectively. Therefore, regulatory implementations for monitoring of wastewater emissions into this lake need to be implemented, as this is suspected to be the primary source of PCBs in the North End Lake.

CHAPTER 5

CHARACTERIZATION OF POLYCHLORINATED BIPHENYLS (PCBs) IN SURFACE SEDIMENTS OF THE NORTH END LAKE, PORT ELIZABETH IN SOUTH AFRICA

5.1 ABSTRACT

The distribution and concentration of six indicator Polychlorinated biphenyl (PCB) congeners nos. 28, 52, 101, 138, 153 and 180 were determined in surface sediments from the North End Lake in Port Elizabeth, South Africa using gas chromatography-mass spectrometry (GC-MS). The total PCB concentrations in the samples ranged from 1.60 to 3.06 ng g^{-1}, dry weight (dw). The concentrations of congener profiles showed significant differences. Generally the highest PCB concentrations were associated with higher organic matter contents and small grain size. The highly chlorinated PCBs dominated with regards to levels in sediments. PCB 138 was the major contributor to total PCBs and was detected at 100% of sites. This study provided a snapshot of the PCB contamination status in the North End Lake sediments, and allowed for a comparison between the investigated system and other systems worldwide.

5.2 INTRODUCTION

PCBs are persistent organic compounds associated with a broad spectrum of negative human health effects due to their bioaccumulation and biomagnification in the food chain (Salem et al., 2014; Grimm et al., 2015). PCBs are classified as 209 congeners that are similar in structure but differing in the position and/or number of chlorine atoms (EFSA, 2010). Despite their ban in the late 1970s, PCBs continue to be recorded as one of the major contaminants worldwide due to their persistence (USEPA, 1999), release from atmospheric deposition, surface runoff from industrial wastewater discharge, and from municipal waste sites in the environment (Lana et al., 2008). Aquatic sediments contaminated by PCBs may pose potential risks to fish, and in turn humans and wildlife that consume fish (Sorell and McEvoy, 2013). Fish from

the North End Lake are eaten and constitute a source of PCB exposure in humans. PCBs were mainly used worldwide between 1930s and 1970s as complex mixtures, such as Aroclor in electrical transformers and capacitors, heat transfer systems, paints, coatings, and flame retardants because of their relatively low flammability and excellent dielectric properties (Erickson and Kaley, 2011).

PCBs are among the organochlorine compounds that typically reside in soils and sediments where they mainly partition into organic matter (Ge et al., 2013). When PCBs are discharged into the aquatic environment, either they are adsorbed onto suspended particulate matter in the water column or deposited onto the surface sediment (Mechlińska et al., 2010). Sediment-bound contaminants are transported in an ecosystem by means of trophic transfer such as consumption of benthic organisms by fish (Clark et al., 1990). These pollutants tend to bind to sediments for a long period of time, hence sediments act as a natural sink for a variety of organic as well as inorganic contaminants (Alkhatib and Weigand, 2002). High levels of PCB have been detected in sediment samples from various parts of the world (Kocan et al., 2001; Tam and Tashiro et al., 2004). DiPinto et al. (1993) showed that 97% of PCBs released into the water column are retained by sediments. Accumulation of sediment-sorbed PCBs by benthic organisms is derived from sediment ingestion and/or diffusion from pore water (Zhou and Wong, 2000). The analysis of sediment samples constitutes an important step to assess the environment and impact of anthropogenic activities on aquatic systems. In developing countries, the source of PCBs has been attributed to the use of transformers and capacitors containing PCB oils (Bentum et al., 2012). These oils enter the environment through poor handling of damaged electrical equipment, leakages, spillage during retro filling, and illegal dumping of PCB containing waste into the aquatic environment.

In South Africa, Grobler (1994) suggested that PCBs are mainly attributed to the industrial waste and possible dumping of products containing or contaminated by PCBs. Except for the health care waste incinerators, no other facilities exist in South Africa to destroy the hazardous PCBs. PCB

processing and distribution have been prohibited in most developed countries since the late 1980s (EFSA, 2010). However, large quantities are still in use or await destruction, while an unknown amount is still present in the environment (Kanzari et al., 2012). The contamination of the environment due to residential and industrial activities is a problem recognized in many African countries (Mansour, 2009). The NEL is located in the city of Port Elizabeth where numerous industries are situated. Because of the high level of industrialisation in the surrounding areas, the relatively low lying North End Lake is subject to runoff and possibly some waste from these industries and nearby residential areas. Due to the persistence of PCBs and the resulting harmful effects to organisms and human health, the objective of this study was to characterize PCBs in the sediments, to determine the extent of PCB contamination within the NEL in order to evaluate the environmental quality of this aquatic system. Furthermore, this study provides the preliminary baseline quantitative information on PCB contamination in surface sediments for better management of the environment. The resulting data will be useful as a reference on PCB levels for subsequent comparative research.

5.3 MATERIAL AND METHODS

Two sampling trips were undertaken in August 2013 and March 2014 to determine the levels of PCBs in the North End Lake surface sediments. Thirteen sampling sites were located along the lake (Figure 5.1). Some of the sites were selected because of their proximity to industrial areas and inflow drains. Sites 1, 10 and 13 were located near the Nelson Mandela Soccer stadium, Sites 2 and 3 were located close to stormwater drains into the lake; Sites 4 and 6 were adjacent to municipal and industrial site drainage pipes into the lake. Sites 5, 7 and 9 were located at the mid-lake while Site 8 was close to powerboat club while sites 11 and 12 were close to residential areas. An electronic global positioning system (GPS 72 H Garmin) (Commercial Marine) was employed to identify the precise location of each site and the depth was recorded using Garmin 100 depth sounder Commercial Marine. A total of 42 surface sediment (0-10 cm in depth) samples were collected using a stainless steel Van Veen grab sampler. Three independent subsamples per

station were collected except at Site 2 where an additional three samples were taken due to the close proximity of this site with runoff. After collection, the sediment samples were thoroughly homogenized using a clean stainless spoon and transferred into polyethylene bags (about 1 to 2 kg). Samples were then transported to the laboratory where they were stored at 4º C prior to further processing. Solid samples (soil/sediments) should be stored in the dark at 4 °C to retain state with no algae growth in pre-cleaned glass materials (See section 3.3.3.1.2).

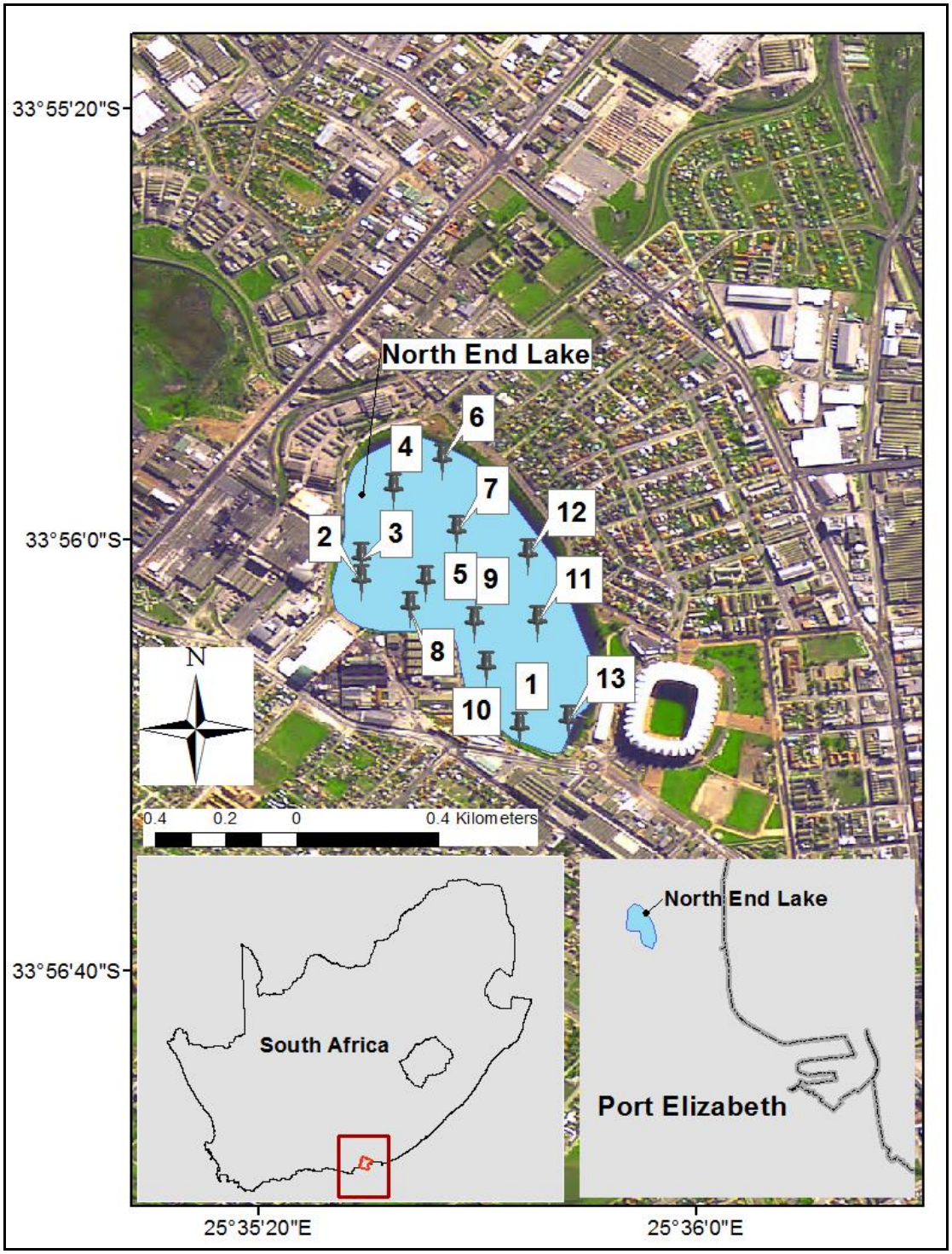


Figure 5.1: Map of South Africa, Port Elizabeth showing the sampling sites in the North End Lake.

5.4 DATA ANALYSIS

The data were analysed with Statistica software (Version 11.0). Statistically significant differences were determined using one-way analysis of variance (ANOVA) and the *t*-test. All tests were considered statistically significant at *p*-value < 0.05. 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. For the comparison of the means of two groups, Scheffè's test was used to calculate the effect size using the following formula:

$d = |\overline{X_1} - \overline{X_2}|/S_{max}$ (Ellis and Steyn, 2003).

Where: *d* = Cohen's *d* effect size

$\bar{X}_1 and \bar{X}_2$ = averages of the two groups compared and S_{max} is the largest standard deviation of the two groups. If *d* < 0.5, the effect size is small; *d*: 0.5 – 0.79, the effect size is medium and an effect size is large when *d* > 0.8. Data with *d* > 0.8 is considered practically significant, since it is the result of a difference having a large effect (Ellis and Steyn, 2003).

5.5 RESULTS

5.5.1 Particle size distribution and sediment organic matter content

The sediment characteristics (particle size, organic carbon) are known to influence PCB distribution between sites due to the sorption process. Sediment particles were composed of sand, silt and clay (Table 5.1). Sediment grain size fractions are classified as clay (< 0.002 mm), silt (0.002 - 0.05 mm) and sand (0.05 - 2.0 mm). The total organic matter content in the sediment samples varied between 5.76 and 18.85% with average of 12% overall. High organic matter content of over 10% was found at the majority of the sites. High organic matter content was found at the Sites 8 and 6 (18.85 and 18.37%), respectively. The textural class of sediments collected at these sites was silt loam. A significant difference was observed between grain-sizes

(F = 16.07, p < 0.05). The low organic matter content was found at the sites S1 and S13. The grain fraction at these sites was mostly sandy. Sediment grain-size distributions varied among sampling sites.

Table 5.1: Organic matter content (%), particle sizes (%) in the sediment samples

Sites	Organic matter (%)	Grain size (%)			Textural class
		Sand	Clay	Silt	
S1	5.76	58.63	27.77	13.58	Sandy clay loam
S2	16.63	21.73	10.12	68.11	Silt clay loam
S3	9.53	39.16	15.98	44.86	Loam
S4	16.14	39.16	8.16	52.66	Silt loam
S5	8.74	45.03	21.72	33.23	Loam
S6	18.37	25.59	17.89	56.51	Silt loam
S7	11.75	43.08	27.77	29.14	Sandy clay loam
S8	18.85	29.45	17.77	52.76	Silt loam
S9	13.93	72.18	17.89	9.93	Sandy loam
S10	9.24	85.65	12.05	2.28	Loamy fine sand
S11	13.73	56.64	25.84	17.51	Sandy clay loam
S12	10.21	56.65	17.89	25.46	Sandy loam
S13	3.54	58.64	19.81	21.56	Sandy loam

5.5.2 PCB levels in sediments

The concentrations of PCB congeners in sediments from the North End Lake are presented in Table 5.2. All PCB congeners were observed in most of the investigated sediment samples. The PCB concentrations in surface sediments ranged from 1.60 to 3.06 ng g^{-1} dry weight (dw). The concentrations of measured PCBs were not highly variable at the different stations. The highest concentration (3.06 ng g^{-1}) was observed in sediments collected from Site 6 with predominance of PCB 153 (1.26 ng g^{-1} dw) and 138 (1.01 ng g^{-1} dw) (Table 5.3). On the other hand, the lower PCB concentration was detected at station 13. Among the studied PCB congeners, PCB 138 was the most abundant in the investigated samples with an average concentration (0.78 ± 0.16 ng g^{-1}), followed by PCB 153 (0.67 ± 0.22 ng g^{-1}) and PCB-101 (0.28 ± 0.06 ng g^{-1}). The hexachlorinated biphenyl congeners (PCBs 138 and 153) contributed more than 60% to the total PCBs in the sediments analysed. PCB

138 showed predominance at Sites 3, 11 and 12 (> 40%) while PCB 153 was dominant at Sites 6, 10 and 13 (41% at each of these three sites) (Figure 5.2).

Table 5.2: PCB concentrations (ng g^{-1} dw) in sediments samples (mean ± standard deviation, n = 3)

Sites	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCBs
S1	0.06 ± 0.19	0.18 ± 0.39	0.26 ± 0.18	0.74 ± 0.22	0.44 ± 0.34	0.20 ± 0.26	1.88 ± 0.24
S2	0.22 ± 0.16	0.18 ± 0.14	0.38 ± 0.18	0.91 ± 0.34	0.61 ± 0.19	0.34 ± 0.08	2.64 ± 0.27
S3	nd	0.09 ± 0.24	0.32 ± 0.27	0.88 ± 0.23	0.58 ± 0.38	0.15 ± 0.03	2.03 ± 0.34
S4	0.26 ± 0.18	0.23 ± 0.13	0.22 ± 0.14	0.80 ± 0.25	0.72 ± 0.43	0.21 ± 0.02	2.44 ± 0.24
S5	nd	0.14 ± 0.06	0.33 ± 0.25	0.78 ± 0.34	0.54 ± 0.32	0.14 ± 0.07	1.93 ± 0.29
S6	0.13 ± 0.16	0.20 ± 0.26	0.26 ± 0.14	1.01 ± 0.26	1.26 ± 0.19	0.20 ± 0.04	3.06 ± 0.31
S7	0.43 ± 0.32	0.27 ± 0.42	0.31 ± 0.07	0.47 ± 0.13	0.57 ± 0.21	0.18 ± 0.05	2.22 ± 0.26
S8	0.13 ± 0.18	0.13 ± 0.03	0.32 ± 0.03	0.96 ± 0.13	0.57 ± 0.08	0.29 ± 0.01	2.39 ± 0.32
S9	0.35 ± 0.23	0.30 ± 0.34	0.17 ± 0.08	0.44 ± 0.09	0.70 ± 0.06	0.24 ± 0.29	2.20 ± 0.25
S10	0.34 ± 0.12	0.21 ± 0.32	0.30 ± 0.07	0.60 ± 0.20	0.94 ± 0.11	0.24 ± 0.02	2.63 ± 0.23
S11	nd	nd	0.30 ± 0.04	0.94 ± 0.15	0.70 ± 0.18	0.30 ± 0.02	2.25 ± 0.38
S12	nd	0.04 ± 0.09	0.25 ± 0.10	0.83 ± 0.24	0.50 ± 0.16	0.13 ± 0.04	1.75 ± 0.31
S13	0.09 ± 0.06	nd	0.15 ± 0.24	0.56 ± 0.08	0.65 ± 0.18	0.15 ± 0.10	1.60 ± 0.25

nd: not detected

A significant difference was observed between the six indicator congeners ($F = 43.5$, $p < 0.05$). Similarly, a significance difference was observed between mean concentrations of the indicator PCB congeners ($d > 0.8$). Mean concentration of PCB 138 was significantly higher than mean concentrations of PCBs 28, 52, 101 and 180 ($p < 0.05$) (d = 3.38, 4.42, 3.99 and 4.51), respectively. Similarly, PCB 153 was significantly higher in concentration than PCBs 28, 52, 101 and 180 ($p < 0.05$) (d = 2.42, 2.95, 2.40 and 2.81), respectively. However, no statistically significant difference was observed between concentrations of congeners nos. 28, 52, 101 and 180 ($p > 0.05$) as well as between PCB 138 and PCB 153 ($p > 0.05$). The distribution of PCBs in sediments was: PCB 138 > 153 > PCB 101 > PCB 180 > PCB 28 > PCB 52.

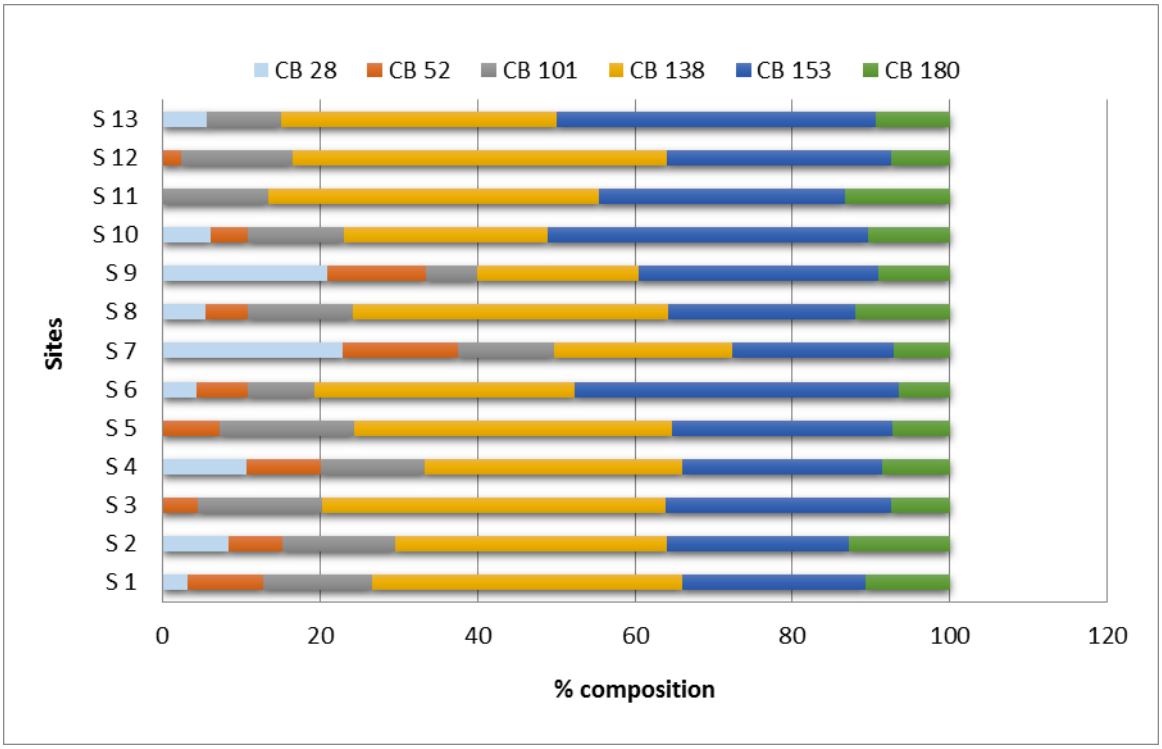


Figure 5.2: Composition (%) of indicator PCB congeners in sediment samples.

PCBs are known to sorb to organics, organic matter content and sediment grain size can be used to explain the distribution of PCBs in sediments. The analysis showed that a positive correlation exists between sediment organic content and

total PCB concentrations in sediment samples (Figure 5.3) ($p < 0.05$). There was no correlation of contaminant content to particle fraction (sand and clay). However there was a correlation with silt fraction with a statistical significant difference ($r = 0.35$, $p < 0.05$).

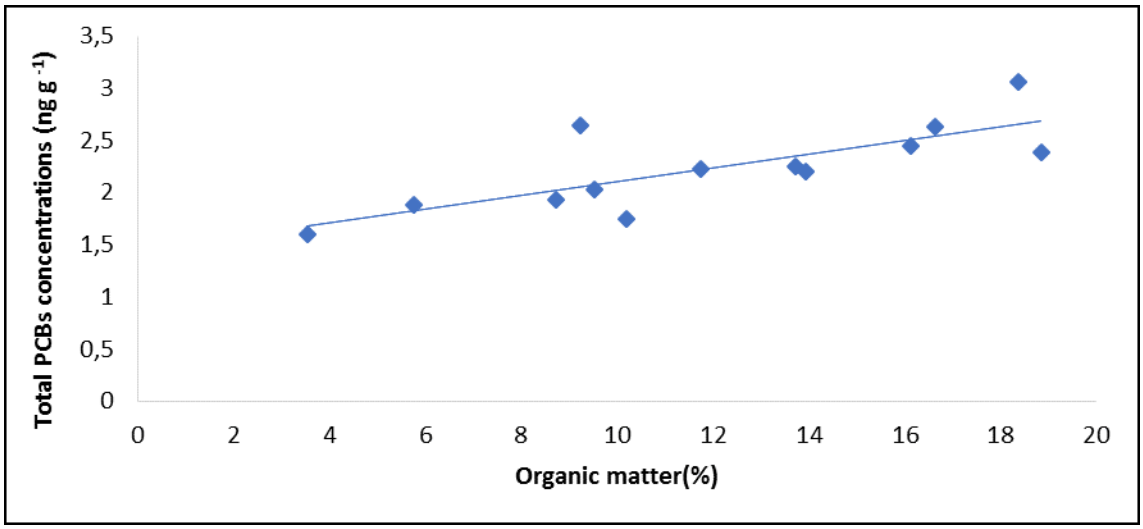


Figure 5.3: Total PCB levels versus organic matter content of sediments.

5.6 DISCUSSION

The six PCB congeners detected in the present study are considered environmentally persistent and predominantly present in most PCB mixtures and in environmental samples such as soils and sediments (Aune et al., 1999). The North End Lake receives domestic sewage and industrial waste, which may be contaminated by a variety of chemicals. The distribution of PCBs indicates that areas of the lake affected by stormwater and inflow discharge are more subjected to PCB contamination than other sampling locations (Sites 6, 2 and 4). The highest total PCB level was detected at the Site 6 (3.06 ng g^{-1}), persistence of PCBs in this area may be linked to the high content of sediment organic matter and the particle size (silt) (Table 5.1) and due to the location of this site which is near the inflow water pipes from the surrounding areas, therefore suspecting a possible localized pollutant discharge source. The lowest concentration was detected at Site 13 (1.60 ng/g dw) which is in the proximity of Nelson Mandela

soccer stadium, i.e. no runoff or stormwater drains were observed around this site. The differences in concentrations of PCBs in sediments are not only due to anthropogenic impacts, but also to the organic content and grain size of the sediment. It is well-known that sediments having a high organic matter content but also a high amount of fine grain size fraction (< 2 μm), accumulate by far higher amounts of pollutants than sandy sediments (Baeyens et al., 1991). The findings from this study revealed that higher concentrations of PCB congeners were related to organic matter, the silt and fine sand. The observed positive correlation of contaminant concentrations with organic matter confirms a well-known strong affinity to organic matter for organic chemicals such as PCBs due to the strength of their hydrophobic character (Prokeš et al., 2014). Similarly, other authors found that PCB levels in sediments are subjective to the affinity between organic matter and grain size. Delbeke et al. (1990) showed that PCB levels in the sediment reveal a higher affinity for the sediment size fraction (< 63 μm). A study by Evans et al. (1990) suggested that the organic matter content of sediment increases with decreasing particle size. Doyle et al. (2003) indicated that anthropogenic organic compounds tend to sorb and concentrate in finer grained sediments such as fine silt and fine sand. A significant sorption takes place if sediment is mainly composed of clay due to its small particle size, high surface area, and high surface charge (Carey et al., 1998). These findings confirmed that grain size of sediments play an important factor governing the accumulation of PCB contaminants in sediments. In the present study, the fraction of sediment samples at Sites 1 and 13 was moderately fine (sandy loam) with a lower content of organic matter (5.76 and 3.54%), respectively. The results showed that the high sand proportion implies that the capacity of sediments to adsorb organic pollutants (PCBs) is low. However, Davies et al. (2010) found that a high sand fraction favours abiotic processes and increases diffusion of oxygen in the sediment making pollutants more bioavailable due to faster oxidation of organic matter and simultaneous release of associated persistent organic micro-pollutants such as PCBs.

The industrialization of Port Elizabeth may account for PCBs in the sediments of the NEL, where numerous industries are situated. PCBs found major use in industries as a dielectric in the electrical power distribution industry especially in infrastructures (NIP, 2011). A power station, using water from the North End Lake for cooling had begun to generate considerably more electricity at the beginning of the Second World War to satisfy the increased demand at the time (García-Rodríguez et al., 2007). Considering that the cooling water was returned to the lake, it may have received wastes or PCB leakage from that power station which operated until 1974.

The indicator PCBs 28, 52, 101, 138, 153, and 180 are major congeners detected in environmental samples (Hsieh et al., 2011). The prevalence of high levels of PCBs 138 and 153 detected in the present study are in keeping with other findings reflecting the high persistence among the indicator PCB congeners commonly present in various sample matrices (Carro et al., 2000; EFSA, 2010). The higher congeners were generally more prevalent and it is well known that the biodegradability of PCBs decreases as the number of chlorine atoms increases (Abramowicz et al., 1993; Rhee et al., 1993). The higher chlorinated PCB congeners are more likely to be adsorbed to particulate material resulting in accumulation and deposition in the sediment. They have relatively low mobility and hence tend to remain closer to the contamination source, whereas lighter PCB congeners may be subject to microbial degradation and volatilisation (Piñeiro et al., 1996; Zhou et al., 2014). The hydrophobicity which increases with molecular weight, seems to be responsible for the stronger sorption on the organic matter of the sediment size fractions. In aquatic environments, PCBs are usually detected at much higher concentrations in sediments than in overlying water. This distribution between sediment and lake water has also been observed in the North End Lake (Section 6.4).

No data on PCB contamination in the NEL are available to compare trends of concentrations in sediment samples. In South Africa, literature showed little research on PCB content in sediments. Greichus et al. (1977) reported on PCBs

measured in sediments of water bodies from South Africa in the Voelvlei dam (Western Cape): 0.06 mg kg^{-1} and the Hartbeespoort dam (North-West Province): 0.32 mg kg^{-1} dw. Grobler (1994) also checked the presence of PCBs (Aroclor 1254 and 1260) in the sediment of the Olifants River in Mpumalanga, but levels were below the detection limit. The levels reported by Greichus et al. (1977) were two-fold higher magnitude compared to the levels detected in the present study. Compared to concentrations reported from other developing and industrially developed countries around the world, PCB concentrations measured in the present study were similar to those reported by Guzzella et al. (2005), Rajendran et al. (2005), He et al. (2006), El-Khady et al. (2007), Li et al. (2008), and Verhaert et al. (2013) (Table 5.3). All of these studies were conducted in developing countries, namely India, China, Egypt, Singapore and Democratic Republic of Congo, respectively. However, the levels of total PCBs in this study were higher than the total PCBs of similar congeners reported by Ssebugere et al. (2014) in sediments from the Napoleon Gulf in Uganda but lower than the ones from Lakes Maryut, Manzala, and Qarum in Egypt (3.06 to 388; 2.53 to 76.37; 1.48 to 137.2 ng g^{-1} dw; Barakat et al., 2012a, 2012b, 2013); rivers in Tianjin in China (44 to 154 ng g^{-1} dw; Liu et al., 2007). The levels of total PCBs reported in sediment from developed countries (France, Spain, USA and Canada) were generally higher than the PCB residues reported in the studied area suggesting greater industrial activities, and various waste discharges in the environment. Kannan et al. (1995) indicated that the history of PCBs is longer and more extensive in developed countries than in developing nations, and the contamination is also relatively more serious in developed countries. A study by Feng et al. (1997) reported sediment concentrations varying from 80 to 1410 ng g^{-1} dw in Hudson River (USA), despite PCB production ending in the late 1970s in America. Syakti et al. (2012) analysed seven indicator PCBs in marine sediments directly exposed to wastewater from Cortiou, Marseille in France and found levels of PCBs ranging from 11.5 to 791.5 ng g^{-1} dw while Marvin et al. (2004) reported PCB levels ranging from 2.6 to 255 ng g^{-1} dw in sediments from Ontario Lake in Canada.

Table 5.3: PCB concentrations in the North End Lake sediments and other worldwide areas (ng g^{-1} dw)

Location	Country	PCB extent	References
North End Lake	South Africa	1.60 – 3.06	Present study
Napoleon Gulf, Lake Victoria	Uganda	0.362 – 0.848	Ssebugere et al. (2014)
North-east India	India	0.18 – 2.33	Guzzella et al. (2005)
Bay of Bengal	India	0.02 – 6.57	Rajendran et al. (2005)
Nile River	Egypt	1.5 – 2.2	El–Khady et al. (2007)
Gaobeidian wastewater	Singapore	0.91 – 3.70	Li et al. (2008)
Congo River Basin	Democratic Republic of Congo	< 0.05 – 1.4	Verhaert et al. (2013)
Yellow River	China	1.4 – 5.3	He et al. (2006)
Hudson River	USA	80 – 1410	Feng et al. (1997)
Mediterranean basin	France	11.5 – 751.5	Syakti et al. (2012)
Thau Lagoon	France	0 – 28.3	Leaute (2008)
Ebro River	Spain	5.3 – 17.7	Fernández et al. (1999)
Ontario Lake	Canada	2.6 – 255	Marvin et al. (2004)
Rivers in Tianjin	China	44 – 154	Liu et al. (2007)
Yangtze Estuary	China	0.19 – 18.95	Liu et al. (2003, 2008)
Lake Maryut	Egypt	3.06 – 388	Barakat et al. (2012a)
Lake Manzala	Egypt	2.53 – 76.37	Barakat et al. (2012b)
Lake Qarum	Egypt	1.48 – 137.2	Barakat et al. (2013)

Sediments are heterogeneous in composition and the different components exhibit different interactions with contaminants. To evaluate and compare PCB contaminants in sediments from the North End Lake, PCB levels detected in the present study were compared to the Interim freshwater Sediment Quality

Guidelines (ISQGs) of 34.1 ng g^{-1} (CCME, 2001) and the South African recommended sediment guidelines (21.6 ng g^{-1}) (Taljaard, 2006). The levels of PCBs detected in the present study were low compared to the limits of the above-mentioned.

5.7 CONCLUSION

The present study was the first of its kind focussing on the levels of PCB pollutants in the sediments of the North End Lake in South Africa. The levels of PCB congeners in the NEL sediment decreased in the order: PCB 138 > PCB 153 > PCB 101 > PCB 180 > PCB 28 > PCB 52. PCB 138 was found to be the most abundant congener present in the lake sediments. The PCB residues detected in this study were likely the results of accidental, unintentional waste disposal and possible illegal dumping into the lake. It frequently happens that wastewater input by stormwater outlets contributes to higher contamination because some recycled paper and laundry detergents may contain small amounts of PCBs.

Contamination by PCBs was moderate in relation to similar areas worldwide and lower than the Canadian and South African sediment quality guidelines. Measurable levels of these compounds in the sediments indicate an environment impacted by anthropogenic activity that could negatively affect the aquatic biota of the North End Lake over time. Consequently, human health may be affected through consumption of fish through informal fishing practice at the lake. Source control measures could be considered for reducing point source inflow of PCBs, protecting, or restoring, sediment quality, particularly in areas that support a diversity of anthropogenic activities (e.g. fishing and tourism).

CHAPTER 6

INFLOW AND OUTFLOW WATER CONCENTRATIONS AND CONGENER PROFILES OF POLYCHLORINATED BIPHENYLS AND THE EFFECT ON MARINE MUSSELS AT AN OUTFALL SITE, PORT ELIZABETH, SOUTH AFRICA

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6.1 ABSTRACT

Inflowing and outflowing water from the North End Lake were collected to determine the levels of polychlorinated biphenyl (PCB) contamination. Mussels at the outflow to the sea were also sampled. This study investigates the effect of a freshwater point source contributing PCBs' to a marine outfall region. The samples were analysed by an internal standard method for six indicator PCB congeners using gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode. The total PCB concentrations in the water (dissolved plus particulate phases) ranged from 0.180 to 0.355 ng L^{-1} and from 20.84 to 31.34 ng g^{-1} wet weight (ww) in mussels. Lighter PCB congeners exhibited highest concentrations in the water samples while heavier PCBs were dominant in mussels. PCB 52 was the most abundant in the water samples while PCB 153 was abundant in mussels. To protect human health from the possible effects of eating shellfish that are contaminated with PCBs, the Environmental Protection Agency in the USA regulates that the level of PCBs in water be no greater than 0.17 ng L^{-1} of water.

6.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are one of the most widespread, persistent and risky pollutants of marine ecosystems (Moore et al., 2002). Coastal zones

particularly areas near urban and industrial centers are exposed to the largest concentrations of these contaminants. The persistent nature of PCBs along with their mobility influences their presence in almost all water bodies, making them both a potential environmental concern and a human health concern (Breivik et al., 2004). PCBs are transported easily by air and water and, therefore, disperse widely from their sources. The release of various organic pollutants from different sources such as runoff or effluent discharges into the environment is an issue of great concern in many countries. Lakes, seas, rivers, and dams have become the immediate environmental reservoirs for all possible organic pollutants (Chee et al., 1996) coming from aerial deposition, surface runoff, domestic wastewater from households, and industrial effluents (Stevens et al., 2003). Water reservoirs are places of sediment accumulation and serve as storage tanks for both dissolved and solid matter (Wolska et al., 2003). In South Africa, contaminated stormwater from urban areas and runoff from rivers are sources of hydrocarbon loads into the marine environment (Taljaard et al., 2000).

PCB congeners largely vary in their chemical and physical properties which affects their environmental distribution. When released into the environment, PCBs tend to partition to the organic component of soil, water and sediment. Within an aquatic system, PCBs can either freely dissolved in water; associated with dissolved organic carbon in the water column; or sorbed to particles (Mechlińska et al. 2010). They can be converted into a dissolved form, or undergo the process of bioaccumulation at the higher levels of trophic chains (Nasr et al., 2010). The differences in congener profiles can lead to differences in uptake, metabolism and bioaccumulation of PCB in an ecosystem. Therefore organisms that live in aquatic environments like mussels are fit for assessing the levels of marine contamination. The use of bivalves as bioindicator organisms is particularly beneficial due to the similarity in pollutant exposure and uptake mechanisms and as such the species differences in uptake a negligible. The brown mussel (*Perna perna*) is prolific on rocky reefs along the east coast of South Africa (Berry and Schleyer, 1983) but is absent along the west coast of

South Africa (Hammond and Griffiths, 2006). This mussel has a lower growth rate and tolerance to desiccation than the invasive Mediterranean mussel (*Mytilus galloprovincialis*), making it less competitive than the alien species. It has been found that mussels, especially the brown mussels live in the rocky intertidal shore where discharges of waste take place (Regoli and Principato, 1995).

Bioaccumulation of pollutants depends on the species, habitat and the type of PCB congeners (WHO, 2003). Due to their hydrophobic nature and high partition coefficients, PCBs tend to partition out of the water column and accumulate in sediments and biota in the aquatic environment (Lakshmanan et al., 2010). Humans and wildlife that consume contaminated organisms can also accumulate PCBs in their tissues which lead to body burdens (Beyer and Biziuk, 2009). In South Africa, research concerning PCB contamination in water and mussels is sparse. There is no reported literature on PCB levels in water of the North End Lake in Port Elizabeth. Therefore, the present study aimed to provide data on the contamination levels of PCBs in the North End Lake water and mussels collected around the vicinity of the point-source at the outflow of this lake into the Indian Ocean. The study focused only on six indicator PCBs since they are known to persist and bioaccumulate in the food chain. The North End Lake was selected as it is surrounded by industries and receives waste discharges and stormwater from the surrounding areas. Data from this study provides an important baseline for future pollutant monitoring programmes.

6.3 MATERIALS AND METHODS

Water and brown mussel samples were collected in September and October 2014. Water samples were collected from eleven locations including the surface water and wastewater inflow into the lake (S1- S10), as well as outfall (M1) (Figure 6.1). A total of 36 water samples were collected with three samples per site except at Site 9 where three additional samples were collected. Water samples were collected by hand in pre-cleaned 1 L brown amber glass bottles with Teflon-lined caps. Prior to use, the bottles were cleaned with tap water

followed by deionized water and rinsed with acetone. Five of the sampling sites S6, S7, S8, S9 and S10) were in close proximity sites to stormwater inflow into the NEL, four sites (S1, S2, S4 and S5) were deeper out in the lake while two sites (S3 and M1) were in close proximity to the outflow from the lake. Samples were transported to the laboratory where they were stored in a cold room at 4°C until analysis. Because water is a heterogeneous matrix (contains suspended solid particles such as sediment or algae) (Alvarez, 2010), the most common method to distinguish the dissolved and particulate fractions of a sample is by filtration. In the laboratory, before the extraction the particulate and dissolved phases were separated through filtration using the glass fibre filter, pore size of 1.0 µm, diameter 47 mm supplied by Sigma Aldrich (South Africa). Materials retained on the filters were defined as the particulate phase. The filters with suspended solids and associated particulate chemical fractions were closed in aluminium foil for preservation and stored at 4°C prior to analysis.

Sixty brown mussels (*Perna perna*) were handpicked from rocks at six locations namely M1–M6 around the point source of the NEL outflow into the sea. An electronic global positioning system (GPS 72 H Garmin) (Commercial Marine) was used to report the exact sampling locations provided in appendix 5.

M1 was considered as the point source of reference. Three sites (M2, M3 and M4) were located to the north and Sites M5 and M6 on the south of M1 (Figure 6.1). Individual mussels (12) were collected at Sites 1, 3, 4 and 6 while 18 samples were collected at Sites 2 and 5 but due to the small size of mussels, some individuals were pooled to form a composite sample. Mussels collected at each site were placed in polyethylene bags and transported to the laboratory on ice. Upon arrival in the lab, the mussels were scrubbed with a brush (stainless steel) to remove adhering epibionts after which the weight and the shell length of each mussel was recorded before being stored in a deep freezer at – 20 °C. Two or three individual mussels of similar shell length (45-84 mm) from the same site were pooled together to obtain the representative samples (35 samples).

Typically six samples from each site were analysed except at Site 6 where five samples were analysed.

The mussels were dissected and the soft tissue of the sample was used for the analysis of PCBs. The mussel was opened carefully inserting a small knife (stainless steel) between the two valves and separating the muscles that hold the closed valves. Using a knife, tweezers and laboratory spatula, the soft tissue was removed from each valve. The knife was placed under the mantle and all of the soft tissue from the shell was taken. The soft tissue was weighed, homogenised using a mortar and pestle. The homogenised tissue was kept in a labelled glass jar sealed with lid and was stored at – 20 ºC in a deep freezer to avoid the loss of water and to reduce most enzymatic and oxidative reactions until further analysis.

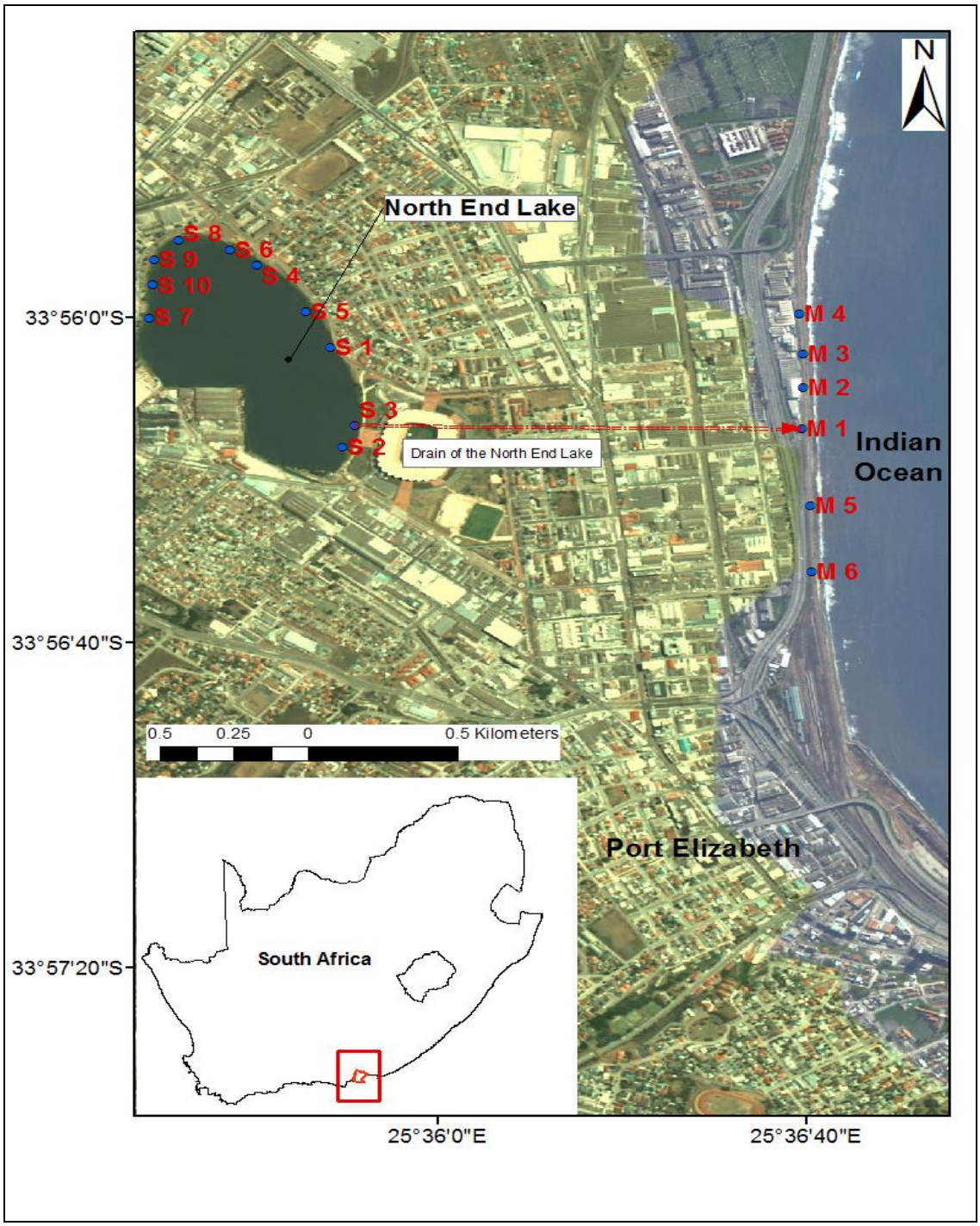

Figure 6.1: Map of South Africa, Port Elizabeth showing the sampling sites in the North End Lake and in the Indian Ocean.

6.4 STATISTICAL ANALYSIS

The data were analysed with STATISTICA 11.0 software. Significant differences of the means and variances were determined using one-way analysis of variance (ANOVA). Concentrations below the limit of detection were assigned a half value of the detection limit for statistical analysis (Helsel and Hirsch, 2002). All the tests were considered statistically significant when the *p*-value < 0.05.

6.5 RESULTS

6.5.1 PCB levels in water samples

Concentrations of the total PCBs in water (dissolved plus particulate phases) are presented in Table 6.1. All PCB congeners were present in most of the water samples. Total PCB concentrations ranged from 0.180 ± 0.09 to 0.355 ± 0.017 ng L^{-1}. The average concentrations of PCBs in the dissolved and particulate phases were 0.121 ± 0.025 and 0.144 ± 0.06 ng L^{-1}, respectively. The dissolved phase represented 46% of PCBs and the suspended phase represented an average 54% of PCBs in all the water samples. The highest concentration of total PCBs in the water samples (0.355 ng L^{-1}) was detected at Site 7. In the particulate phase, significant differences were observed between Sites S3, S8, S9 and S11 ($F = 5.53$, $p < 0.05$). However, similar results were obtained from Sites 1, 2, 5 and 10 ($F = 0.08$, $p > 0.05$). No statistically significant difference was observed between different sites in the dissolved phases ($F = 0.69$, $p > 0.05$). Furthermore no significant difference was observed between the dissolved and particulate phases ($F = 1.46$, $p > 0.05$). S3 is the lake outflow and M1 is the outflow into the sea from S3. When comparing these two sites it was found that there was no significant difference in PCB concentrations ($p > 0.05$).

Table 6.1: Mean concentrations of indicator PCB congeners and total PCBs in the water samples (ng L^{-1})

	PCB 28		PCB 52		PCB 101		PCB 138		PCB 153		PCB 180		Total PCBs		ΣPCBs	
Sites	DP	PP	DP	PP	DP	PP	DP	PP	DP	PP	DP	PP	DP	PP	DP + PP	SD
S1	0.025	0.015	0.039	0.019	0.006	0.008	0.013	0.037	0.018	0.024	0.014	0.010	0.115	0.114	0.230	0.011
S2	0.018	0.015	0.053	0.013	0.010	0.006	0.028	0.041	0.022	0.040	0.021	0.009	0.153	0.123	0.276	0.015
S3	0.030	0.038	0.026	0.049	0.003	0.019	0.017	0.049	0.014	0.045	0.021	0.013	0.102	0.221	0.324	0.015
S4	0.023	0.023	0.023	0.020	0.003	0.008	0.013	0.042	0.010	0.054	0.008	0.010	0.080	0.157	0.237	0.015
S5	0.044	0.012	0.056	0.014	0.015	0.007	0.013	0.025	0.020	0.040	0.014	0.008	0.163	0.106	0.269	0.016
S6	0.033	0.015	0.052	0.026	0.009	0.012	0.014	0.023	0.025	0.045	0.017	0.018	0.150	0.140	0.290	0.013
S7	0.021	0.035	0.034	0.043	0.006	0.021	0.023	0.057	0.018	0.058	0.011	0.028	0.112	0.243	0.355	0.017
S8	0.011	0.019	0.030	0.012	0.006	0.011	0.015	0.010	0.031	0.021	0.010	0.005	0.102	0.078	0.180	0.009
S9	0.023	0.017	0.038	0.019	0.007	0.011	0.028	0.011	0.023	0.013	0.009	0.005	0.128	0.075	0.203	0.010
S10	0.026	0.010	0.031	0.013	0.004	0.008	0.018	0.027	0.016	0.033	0.009	0.013	0.104	0.104	0.207	0.010
M1	0.030	0.023	0.037	0.048	0.005	0.012	0.020	0.056	0.016	0.072	0.009	0.014	0.118	0.225	0.343	0.021

DP: dissolved phase, PP: particulate phase, SD: standard deviation. All sites had three replicates except for Sites 9 which had six. Lake water are sampled at sites S1, S2, S4 and S5, inflow water at sites S6, S7, S8, S9 and S10 and outflow water from the lake into the sea corresponds to both sites S3 and M1.

In terms of congener profiles, the lower chlorinated congeners (PCBs 52 and 28) were predominant in the water samples and represented 49% and 35% of PCBs in the dissolved and particulate phases, respectively (Figure 6.2).

PCB 52 represented 32% of the dissolved phase. PCB-101 was detected at lower levels in all the samples with an average percentage < 10%. PCB 180 represented an average of 10% in the water samples. Both hexa-CBs (138 and 153) were detected each at higher levels in the particulate phase (28 and 24%), respectively while their composition was < 20 % in the dissolved phase. Statistically significant differences were observed between individual congeners ($F = 22.6$, $p < 0.05$). PCB 153 was significantly higher than PCB 101 and 180 in the particulate phase ($p < 0.05$). Similarly, PCB 52 was significantly higher than PCB 101, 138, 153 and 180 ($p < 0.05$).

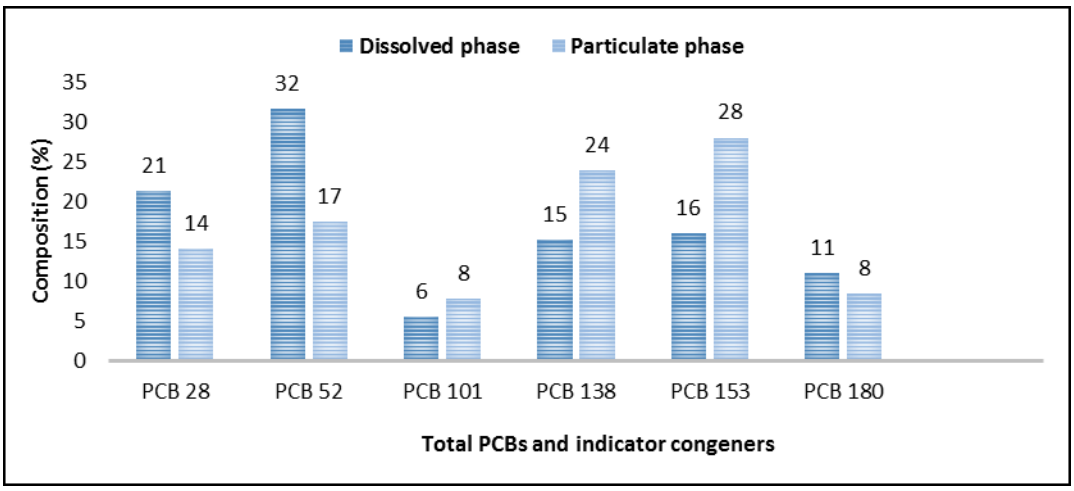

Figure 6.2: Percentage composition of indicator congeners in water samples.

Water samples were grouped into three categories (Figure 6.3): lake water including Sites 1, 2, 4, and 5; inflow water including Sites 6, 7, 8, 9, and 10 and outflow water corresponding to Sites S3 and M1. Specifically, tetra-CB (PCB 52) was present at higher concentrations in the dissolved phase with concentrations of 0.035, 0.029 and 0.031 ng L^{-1} in lake water, inflow and outflow water, respectively. The hexa-CB (PCBs 138 and 153) were the most abundant

congeners in the particulate phase. They were detected at an average of 0.038 ng L^{-1} in both lake water and inflow water and 0.055 ng L^{-1} in the outflow water. All the congeners were found to be significantly higher in the outflow water than inflow and lake water ($p < 0.05$) (Figure 6.3). This implies a net release of PCBs into lake water, either from sediment or atmospheric deposition. The distribution of total PCBs in water samples was: PCB 52 > PCB 153 > PCB 138 > PCB 28 > PCB 180 > PCB 101.

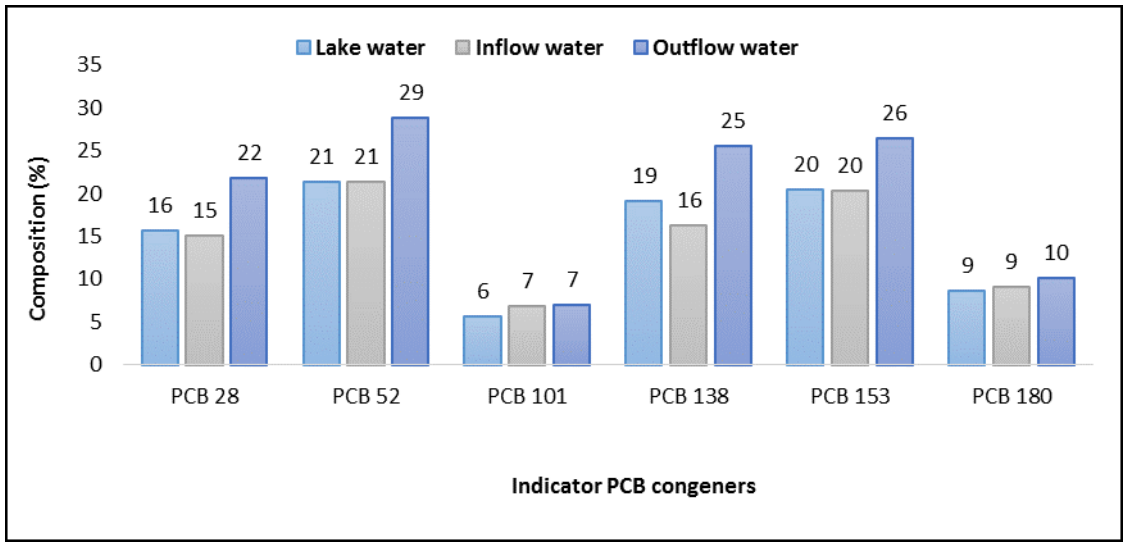

Figure 6.3: Composition (%) of indicator congeners in lake water, inflow and outflow water samples.

6.5.2 PCB levels in mussels

Brown mussels in the vicinity of the North End lake outflow (the sites are breakwater rocks with significant wave action) were found to contain PCBs. Table 6.2 summarizes the concentrations of the individual and total PCB congeners in mussel samples. The total PCB concentrations ranged from 20.85 ± 2.57 to 31.34 ± 4.62 ng g^{-1} wet weight (ww). M1 (point source into the ocean) had a significantly higher total PCB concentrations compared to mussels from Sites M3, M4 and M6 ($F = 10.0$, $p < 0.05$) while M2 and M5 had higher total PCB concentrations compared to mussels from Site M4 ($p < 0.05$) only.

The PCB pattern found in mussels showed a predominance of PCB-153 followed by PCB-138 and PCB-52 for indicator PCBs. PCBs 153 and 138 were significantly higher than PCBs 101 and 180 at Site M1 and M2 ($p < 0.05$). The average contributions of indicator congeners to the total PCBs were < 20% for the lower congeners (PCBs 28 and 52) including PCBs101 and 180. The hexachlorobiphenyls (PCBs 153 and 138) contributed > 20% to the total PCBs. The distribution of PCBs in mussels was: PCB 153 > PCB 138 > PCB 52 > PCB 28 > PCB 180 > PCB 101.

Table 6.2: Mean concentrations of individual and total PCBs ± standard deviation (ng g^{-1} ww) in mussels (n = 6 except for Site 6 where n = 5)

Sites	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs
M1	5.13 ± 1.91	6.98 ± 2.60	3.01 ± 1.03	6.36 ± 1.18	6.71 ± 2.02	3.16 ± 0.30	31.34 ± 4.62
M2	4.08 ± 1.02	5.05 ± 1.28	2.89 ± 1.07	6.07 ± 1.2	7.15 ± 1.4	3.14 ± 0.91	28.37 ± 3.86
M3	3.91 ± 2.58	3.52 ± 2.09	3.12 ± 1.26	3.92 ± 1.41	4.25 ± 1.27	3.15 ± 1.14	21.85 ± 4.46
M4	3.41 ± 1.47	3.86 ± 1.65	1.96 ± 0.46	4.80 ± 1.40	4.50 ± 1.42	2.32 ± 0.96	20.85 ± 2.57
M5	4.16 ± 0.57	5.10 ± 0.92	2.78 ± 0.48	6.31± 0.74	7.12 ± 1.02	3.40 ± 0.94	28.86 ± 4.67
M6	3.37 ± 0.66	5.12 ± 1.43	2.47 ± 0.70	4.87 ± 1.22	6.66 ± 0.55	2.85 ± 0.85	25.34 ± 3.01

The raw data of all the mussels analysed are found in the Appendix 10.

6.6 DISCUSSION

The indicator PCB congener nos. 28, 52, 101, 138, 153 and 180 were detected in most of the samples. In the water samples, Sites 1 and 10 exhibited similar total concentrations of PCBs in the dissolved and particulate phases. The Sites S3, S7 and M1 exhibited higher levels of PCBs in the particulate phase. The lower congeners were more concentrated in the dissolved phase while higher congeners dominated the particulate phase due to the greater solubility of lower congeners in water than the more chlorinated ones. This trend is in agreement with the work of other researchers (Schulz-Bull et al., 1998; Kurt and Ozkoc., 2004). Higher levels of total PCBs found in the water flowing out of the lake could be explained by the contamination loads from municipal and industrial

wastewater discharges, atmospheric deposition and other sources of contaminants within the lake. The inflow water at Site 7 was the most contaminated, and seems to be the main source of pollution to the North End Lake. Water from this site had the highest particulate component. Sites S3 and M1 contained similar total PCB concentrations: 0.324 and 0.343 ng L^{-1}, respectively. Samples at these sites corresponded to the lake's outflow into the sea. S3 is a tunnel drain located in the proximity of the Nelson Mandela Bay soccer stadium, and the Site M1 is the end of this drain into the sea. S2 which was just adjacent to the drain outlet (S3) also had high PCB levels (0.276 ng L^{-1}). The water flowing out from the lake had higher levels of PCBs in the particulate phase than that in the dissolved phase whereas the transport of PCBs to the North End Lake took place both with the aqueous phase and the suspended matter. In water samples, tetra-CB (PCB 52) was higher in the dissolved phase while the hexa-CBs (PCBs 138 and 153) were the most abundant congeners in the particulate phase. Similar findings have been reported by Moret et al. (2005), Manodori et al. (2006), Martinez et al. (2010), and Zhang et al. (2011a). In Ghana, Afful et al. (2013) and Kuranchie-Mensah et al. (2011) reported PCB 52 as one of the predominant contaminants in water samples collected from Lake Bosomtwe and Lake Weija. Generally, the less-chlorinated congeners are more water soluble and volatile while higher chlorinated PCBs resist the degradation and volatilization and sorb more strongly to particulate matter (Kurt and Ozkoc, 2004).

No prior research on PCB concentrations has been carried out previously on the North End Lake water and sea outfall. Therefore, no data are available to compare the trend of contamination in this area. In comparison to other studies, Amdany et al. (2014) reported lower levels of PCBs (0.038 – 0.150 ng L^{-1}) in the Hartbeespoort Dam (South Africa). Grobler (1994) analysed PCBs (Aroclors 1254 and 1260) in water from the Oliphants River (South Africa) but no PCBs were detected. Table 6.3 shows a comparison of PCB levels in water from elsewhere. The levels of PCBs detected in water in the present study (0.180 –

0.355 ng L^{-1}) were higher than the reported levels ranging from < 0.001 to 0.006 ng L^{-1} by Chen et al. (2008) in Three Gorges Reservoir, China but were in the range of the PCB levels detected by Wang et al. (2009) (0.08 – 0.51 ng L^{-1}) in the same area as Chen et al. (2008). Moreover, other studies reported higher levels of PCBs in water than the present study. Nasr et al. (2009) reported PCB levels in water from Egypt varying from 6.04 to 67.89 ng L^{-1} and Zhang et al. (2002) reported 33.4 to 1064 ng L^{-1} in the Pearl River Estuary. Vogelsang et al. (2006) monitored five wastewater treatment plants in Norway for seven individual PCBs and found that the total PCB concentrations varied from non-detected to 4.1 ng L^{-1}. Egyptian and Ghanaian studies by Eissa et al. (2013) and Afful et al. (2013) detected higher PCB levels than the present study (Table 6.3).

Table 6.3: Comparison of PCB concentrations in water between this study and other studies worldwide

Locations	PCBs (ng L^{-1})	Year	References
Three Gorges Reservoir, China	< 0.001 – 0.006 0.08 – 0.51	2005 2008	Chen et al. (2008) Wang et al. (2009)
Baltimore Harbour, USA	0.10 –1.52	1996/97	Bamford et al. (2002)
Lake Michigan, USA	0.34 – 1.74	1991	Pearson et al. (1996)
Yangtze River, China	0.29 – 2.0	1998/1999	Sun et al. (2002)
Okinawa Island, Japan	1.59 – 2.48	2002	Sheikh et al. (2007)
Campeche, Mexico	0.07 – 3.40	2000	Carvalho et al. (2009)
Bay of Bengal, India	1.93 – 4.46	1998	Rajendran et al. (2005)
Venice Lagoon, Italy	0.45 – 10.5	2003	Manodori et al. (2006)
Hudson River Estuary, USA	0.86 – 6.0	2001	Yan et al. (2008)
Alexandria Governorate, Egypt	0.02 – 6.11[b] 0.0 5 – 12.51[a]	2011/2012	Eissa et al. (2013)
Houston Ship Channel, USA	0.49 – 12.5	2003	Howell et al. (2008)
Songhua River, China	1.1 – 14	2007	You et al. (2011)
Yangtze River Delta, China	1.23 – 16.6	2009	Zhang et al. (2011)
El Menofiya Governorate, Egypt	6.036 – 67.89	2007/2008	Nasr et al. (2009)
Lake Bosomtwe, Ghana	1090 – 7190	2012/2013	Afful et al. (2013)

Locations	PCBs (ng L^{-1})	Year	References
North End Lake, Port Elizabeth, South Africa	0.16 – 0.36	2014	Present study

[a, b] Results are expressed in µg L^{-1}; [a] surface water; [b] drinking water

Mussels, being filter feeders and sedentary organisms, have been widely used for monitoring of contaminants. In agreement with the findings of Okay et al. (2009), the concentrations of PCBs in this research were predictably found to be higher in mussels compared to that of water due to the fact that the mussels have the ability to bioconcentrate organic contaminants in their tissues to very high levels. The contamination levels in mussels are related to the chemical content of the water in which they reside (Potrykus et al., 2003). Figure 6.1 shows the location of the mussel sampling sites in the vicinity of the North End Lake outflow into the ocean. Site 1 was considered to be the point source because the North End Lake's water drains into the ocean through this site. A change in total PCB levels was observed further away from the site M1. The variation of total PCB contamination levels was in the order M4 < M3 < M2 < M1 > M5 > M6. This showed a drop on either side of the point source as expected. At these sites the prevailing east and west wind directions drive longshore currents.

It was observed that mussels were contaminated by higher chlorinated congeners (hexa-CBs 153 and 138) and the lower PCB congener no. 52 which had the highest levels at Site M1. This result can be attributed to the high octanol water coefficient (K_{OW}) of PCBs which relates to less aquatic solubility, high toxicity, and bioaccumulation (Eqani et al., 2013). The log Kow values of PCB congeners ranges from an average of 4.5 for mono-CBs to 8.1 for hepta-CBs (Rapaport and Eisenreich, 1984). The higher the value of Kow, the greater is the bioaccumulation either through direct partitioning or food ingestion (Patil, 1991).

The predominance of PCBs 153 and 138 reflects their persistence among the PCB congeners. Carro et al. (2000) suggested that PCBs 153 and 138 are the most dominant in biota samples. PCB 180 was detected at lower levels in both

water and mussel samples. Boon et al. (1984) attributed the lower uptake of hepta-CB to their unfavorable stereochemistry. PCB 180 has no vicinal-H atoms and is therefore non-metabolizable. Their unfavorable stereochemistry restricts their passage through membranes (very low uptake) (Colombo et al., 1997).

In comparison with other studies on different mussel species (Table 6.4), levels of PCBs in *Mytilus galloprovincialis* detected in the Port Elizabeth Harbour (14.48 - 21.37 ng g^{-1} ww) were slightly lower than levels detected in *Perna perna* in the present study (20.84 - 31.40 ng g^{-1} ww). Degger et al. (2011) analysed brown mussels *(Perna perna)* and semi-permeable membrane devices (SPMDs) from the South African marine environment. The results showed PCB concentrations in the South African harbours (Cape Town, Port Elizabeth, Richards Bay and Saldanha Bay) ranging between 34 -131 µg g^{-1} lipid weight in the mussels and 29 -158 µg g^{-1} lipid weight in the SPMDs. SPMDs exposed at Port Elizabeth Harbour in 2009 contained levels of 105 µg g^{-1} of congeners with 63% represented by three indicator congeners (PCB 28 = 19, PCB 52= 4 and PCB 138 = 43). The Levels of PCBs are expressed on lipid weight basis. Similar to this study, PCB 138 exhibited higher concentrations in the Port Elizabeth Harbour.

Similar total PCB levels were reported by other authors: 15.13 to 37.49 ng g^{-1} ww (Salem et al., 2014) in mussels from the Mediterranean Coast in Egypt; 1.93 to 22.31 ng g^{-1} ww (Potrykus et al., 2003) of seven indicator PCBs in *Mytilus trossulus* from the Baltic Sea; 0.77 to 22.99 ng g^{-1} ww (Okay et al., 2009) of six indicator congeners in mussels from the Istanbul Strait, Turkey. However, other studies revealed higher levels than this study. Khaled et al. (2004) reported levels of 6.75 to 66.4 ng g^{-1} wet weight in mussels collected along the Egyptian Red Sea coast. Scarpato et al. (2010) detected total PCBs in mussels from the coasts of the western Mediterranean Sea ranging from 5 to 60 ng g^{-1}. El-Nemr et al. (2003) reported levels ranging from 8 to 437 ng g^{-1} ww in mussels from Mediterranean Coast, Egypt. Chouikhi et al. (1989) found levels of PCBs in brown mussels (*Perna perna*) in the Bay of Algiers varying from 17.66 to 386.52 ng g^{-1} fresh weight. The presence of PCBs in mussels may be attributed to illegal

waste dumping, runoff from urban and industrial areas and industrial discharges into the North End Lake. This study reports higher levels in brown mussels than in blue mussels reported in the Port Elizabeth Harbour.

Table 6.4: Comparison of PCB levels in mussels from the present study and other worldwide studies

Locations	Mussel species	Range of total PCB levels (ng g^{-1} ww)	References
North End Lake, Port Elizabeth, South Africa	*Perna perna*	20.54 – 31.40	Present study
South African Harbours (Cape Town, Port Elizabeth, Richards Bay and Saldanha Bay)	*Perna perna*	34 - 131[a]	Degger et al. (2011)
Central Adriatic Sea	*Mytilus galloprovincialis*	3.43 – 9.81	Perugini et al. (2004)
Port Elizabeth Harbour, South Africa	*Mytilus galloprovincialis*	14.48 – 21.37	This study
Vistula and Odra estuaries, Baltic Sea	*Mytilus trossulus*	1.93 – 22.31	Potrykus et al. (2003)
Istanbul Strait, Turkey	*Mytilus galloprovincialis*	0.77 – 22.99	Okay et al. (2009)
Red Sea Coast, Egypt	*Brachiodontes sp.*	6.7 – 66.4	Khaled et al. (2004)
Mediterranean Coast, Egypt	*Lutraria elliptica* and *Donax trunculus*	15.13 – 37.49	Salem et al. (2014)
Mediterranean Coast, Egypt	*Donax* sp.	29 – 37.6	Abd-allah et al. (1998)
Coasts of West Mediterranean	*Mytilus galloprovincialis*	5.0 – 60.0	Scarpato et al. (2010)
Bay of Algiers, Algeria	*Mytilus galloprovincialis*	64.2 – 185.8[b]	Fouial-Djebbar et al. (2011)
Bay of Algiers, Algeria	*Perna perna*	17.66 – 386.52	Chouikhi et al. (1989)
Mediterranean Coast, Egypt	*Modiolus auriculatus* and *Donax* sp.	8 – 437	El-Nemr et al. (2003)

[a]Results are expressed in µg g^{-1} lipid weight; [b]Results are expressed in ng g^{-1} dry weight

6.7 CONCLUSION

Water and mussels analyzed were found to be contaminated by PCBs. PCBs concentrations were higher in mussels than in water, because bivalves are filter feeding organisms, and can concentrate contaminants to levels well above those present in water. With the accumulation potential of these compounds in the food chain, humans are the most affected. Due to the lipophilic nature of PCBs, foods of animal origin are an important source of exposure. In drinking water, PCB levels reported typically range between 0.1 and 0.5 ng L^{-1}. A person drinking 2 litres of water a day containing 0.5 ng L^{-1} is exposed to a daily dose of 0.01–0.02 ng kg^{-1} (body weight 100–50 kg) (WHO, 2000). Food and Drug Administration (FDA) in the USA has set residue limits of PCBs of 2 ppm in the edible portion of mussels. The levels of PCBs detected in mussels of this study were found below the set limits of FDA. The South African regulatory limit (maximum allowable concentration) to protect human health from consumption of contaminated shellfish is set to be less than 0.02 mg kg^{-1} (Shellfish Monitoring Programme Annual Report, 2011). The sources of PCBs in the North End Lake could be attributed to municipal and industrial discharges into the lake since it is surrounded by many industries including metal recycling, plastic industry, Firestone, tyre manufacturer, ASPEN pharmaceutical manufacturer and Fresenius Kabi and Coca-Cola. The results indicated that the accumulation of PCBs depends not only on local pollution sources, but also on biological characteristics of the organism and phase (particulate matter *versus* dissolved). Lower PCB congeners were the most ubiquitous in the water samples while the higher congeners were dominant in mussels. PCBs are still expected to be detected in water due to the environmental recycling of this refractory type of compound. Most of the PCBs are bound to the soil and sediments and may be released to the water slowly over a long period of time.

CHAPTER 7

DISTRIBUTION OF POLYCHLORINATED BIPHENYL RESIDUES IN SEDIMENTS AND BLUE MUSSELS (*MYTILUS GALLOPROVINCIALIS*) FROM PORT ELIZABETH HARBOUR, SOUTH AFRICA

The content of this chapter is reproduced from the paper published in *Marine Pollution Bulletin 91*(1), 173–179(2015), written by E. Kampire, G. Rubidge & J.B. Adams.

7.1 ABSTRACT

Sediment and *Mytilus galloprovincialis* samples collected from the Port Elizabeth Harbour were analysed for six indicator PCB congeners to assess their contamination status. The concentrations of total PCBs in sediments and *M. galloprovincialis* ranged from 0.56 to 2.35 ng g^{-1} dry weight and 14.48 to 21.37 ng g^{-1} wet weight, respectively. Congeners 138 and 153 were dominant and accounted for an average of 29% and 24% of total PCBs in *M. galloprovincialis*; 32% and 30% in sediments, respectively. Sediments are home to a wide variety of aquatic life. None of the sediments analysed exceeded the PCB limits recommended by the Canadian interim sediment quality guideline and the South African recommended sediment guidelines (21.5 ng g^{-1}). These baseline data are important as both humans and aquatic life are sensitive to the toxic effects of PCBs.

7.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are environmental contaminants regulated by the Stockholm Convention of Persistent Organic Pollutants (POPs). POPs are chemicals that have high persistence in the environment, high bioaccumulation through the food chain and pose risks and adverse effects to humans and wildlife (Viet, 2005). The synthesis of PCBs was described for the first time in 1881 and its commercial production began at the end of 1920 (UNEP, 2002), specifically between 1929 until their ban in the late 1970s. Total global production has been estimated at 1.3 million tons (Breivik et al.,

2002). PCBs were used worldwide in industrial and commercial applications mainly in electrical equipment (transformers and capacitors), hydraulic equipment, plasticizers in paints, plastics, and rubber products and many other industrial applications (Breivik et al., 2002, 2004). PCBs are introduced into the environment via point sources due to inappropriate disposal practices, leakage from industrial facilities or chemical waste disposal (Meijer et al., 2003). Runoff or atmospheric transport is thought to be one of the major sources of PCBs to the aquatic environment. Because of low water solubility, PCBs are commonly accumulated by the marine biota (Chouksey et al., 2004; Venkatachalapathy et al., 2011). According to O'Donoghue and Marshall (2003), more studies were conducted in the past on marine environmental contamination than currently. However with increasing urbanisation, contamination is a real threat. Marine ecosystems may be stressed by point source discharges (sewage effluents, industrial wastes) and non-point source contamination (Harbour activities, storm drainage, agricultural runoff) which lead to the introduction of pollutants in the aquatic environment (Dabrowski et al., 2002).

The major threats to the health and biodiversity of the marine environment result from human activities; 80% of the contamination load in the oceans originates from municipal, industrial and agricultural runoff, as well as atmospheric deposition (UNEP, 2006). In South Africa, hydrocarbons are loaded into the marine environment from Harbour activities and oil refineries; contaminated storm water from urban areas and runoff from rivers (Taljaard et al., 2000). In 1991, 247 tonnes/year of hydrocarbons, 19.420 tonnes/year of suspended solids and 601 trace metals were estimated to be loaded in the environment of Port Elizabeth from storm water runoff (CSIR, 1991). PCBs are usually associated with sediment rich in organic matter. Therefore sediments and organisms that live in aquatic environments like mussels are suitable representative samples for assessing the levels of marine contamination.

The Mediterranean blue mussel (*Mytilus galloprovincialis* Lamarck*)* is an invasive species that has been introduced to many parts of the world. *M.*

galloprovincialis was first discovered in South Africa in 1979, in Saldanha Bay (Branch and Steffani, 2004) as a result of shipping. *M. galloprovincialis* first appeared on the south coast of the country in 1988 (McQuaid and Phillips, 2000) following its introduction to Port Elizabeth Harbour for aquaculture. Mussels offer the advantage of a wide geographic distribution, abundance, sedentary behaviour, and have a great capacity to accumulate organic contaminants over long periods at the same site (Ponnusamy et al., 2014). Mussels live in the rocky intertidal shore, where generally discharges of waste take place (Viarengo and Canesi, 1991; Regoli and Principato, 1995). Mussels can accumulate chemical concentrations 10^2 - 10^5 times higher than their surrounding water (Villeneuve et al., 1999) due to the large volumes of water they filter (between 1 and 250 L) (Kryger and Riisgard, 1988), taking up and removing all suspended particles. Mussels provide a powerful tool for detecting long term trends in bioavailable pollutants at defined locations. Although, there is a South African mussel watch programme it is limited to metals. Organic compounds are not well studied in the South African environment (Wepener and Degger, 2012). Because some previous studies indicated that there is PCB contamination in Port Elizabeth Harbour (Degger et al., 2011), the aim of the present study was to investigate and assess PCB contaminants in sediments and *M. galloprovincialis* from the Harbour of Port Elizabeth in order to obtain current information on the status of contamination of these compounds. The results obtained will serve as a guide to monitor trends of contamination from PCBs and as comparative reference for further researchers.

7.3 MATERIALS AND METHODS

7.3.1 Study area

Port Elizabeth Harbour (Figure 7.1) is situated on the western side of Algoa Bay on the south east coast of South Africa. Port Elizabeth Harbour has been important on the South Africa east coast since 1820. The Port handles many products including petroleum products that are imported from other South African ports. The motor industry has been an important industrial activity for

the Eastern Cape and the port plays a leading role in this regard and boasts a large open area car terminal. The fishing industry also makes extensive use of the port.

7.3.2 Sampling and sample preparation

Surface sediments (0-20 cm) and blue mussels (*M. galloprovincialis* Lamarck) of approximately the same size (53-84 mm) were collected in September 2012 and March 2013, respectively from the Port Elizabeth Harbour. Eleven marine sediment samples were collected using a grab sampler from nine sampling sites (1-9) while *M. galloprovincialis* were collected from five sampling stations (3, 4, 6, 7 and 8) (Figure 7.1).

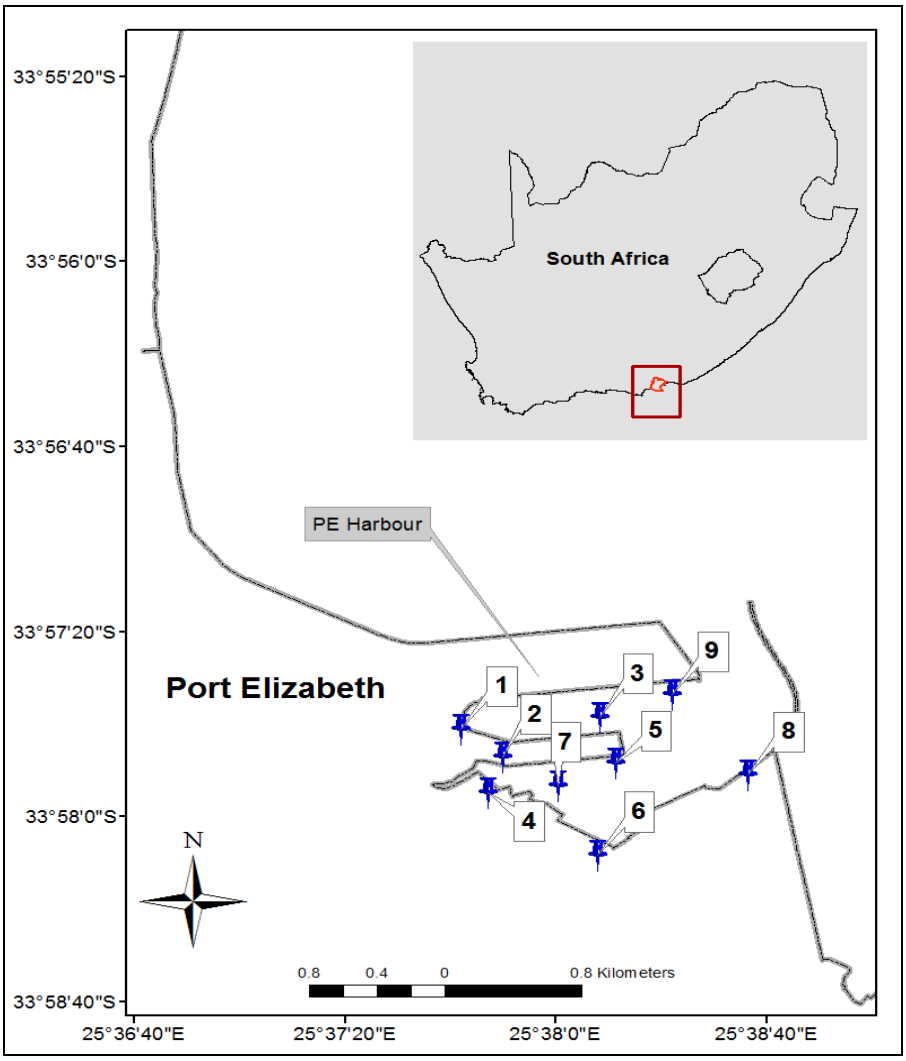

Figure 7.1: Map of South Africa, Port Elizabeth showing the position of the sampling sites in the PEH (Port Elizabeth Harbour).

The top 0-20 cm sediment layer was thoroughly mixed by stirring with a cleaned stainless steel scoop and transferred in a polyethylene bag, then kept on ice and transported to the laboratory where they were dried at room temperature. The dried sediments were ground using a mortar and pestle and sieved (300 µm stainless steel) removing stones and other debris resulting in a fine powder. The fine sediment was transferred into a glass jar and sealed with a Teflon-lined lid and was then refrigerated at 4 °C from four to seven days while performing the extraction and were analysed within two weeks. Twelve individual mussels of similar shell length (62) were selected from each site. They were wrapped in aluminium foil and transported live to the laboratory on ice. In the laboratory, the mussels were scrubbed with a brush to remove adhering epibionts, after which the weight and length of each mussel was recorded before being stored in a deep freezer that prevented the loss of water from the organism. The shell length varied from 62 to 84 mm and the wet weight varied between 14.28 and 44.37 g. The mussels were dissected and the soft tissue of the sample was used for the analysis of PCBs. The soft tissue was weighed, homogenised using a blender and placed in a sealed glass jar and kept frozen (- 20) until further analysis. The weight of dissected tissues varied between 2.51 and 7.23 g (section 3.3.4.2).

7.4 DATA ANALYSIS

The data were analysed using STATISTICA Version 11.0 software. Statistical significant differences of the means and variances were determined using one-way analysis of variance (ANOVA). All tests were considered statistically significant when the *p*-value < 0.05.

7.5 RESULTS

The six indicator congeners (PCBs 28, 52, 101, 138, 153 and 180) recommended by the European Union for assessing environmental contamination (Commission of the EC, 1999) were analysed to determine their levels and distribution in *M. galloprovincialis* and marine sediment samples. These congeners were also chosen by Environmental Protection

Agency (EPA) to cover a wide range of chlorination considering their relatively high levels in environmental samples (Rayne and Ikonomou, 2002).

The mean and total PCB concentrations in *M. galloprovincialis* and sediment samples are summarised in Tables 7.1 and 7.2, respectively. The concentrations are reported on wet weight (ww) and dry weight (dw) basis for *M. galloprovincialis* and sediments, respectively. Total PCBs were detected at concentrations ranging from 14.48 to 21.37 ng g^{-1} ww in *M. galloprovincialis* with an average of 18.58 ± 8.69 ng g^{-1} and 0.56 - 2.35 ng g^{-1} dw in sediments with an average of 1.67 ± 0.50 ng g^{-1}. Among the studied PCB congeners, hexa-CBs showed predominance in all the samples. Statistically significant differences were observed between hexa-CBs ($p < 0.05$) and lower chlorinated biphenyls such as tri-CB (PCB 28), tetra-CB (PCB 52) and penta-CB (PCB 101). Both hexa-CBs (PCBs 138 and 153) contributed more than 60% and 54% to the total PCBs in the analysed sediments and mussels, respectively. The average contribution of PCB 153 was 29% and 20% in sediments and in *M. galloprovincialis,* respectively. The accumulation of PCBs in sediment and mussel samples increased with the degree of chlorination. Congeners nos. 28 and 52 were detected at low levels in all the samples. In the sediment samples, they were detected only at four sites with levels ranging between 0.05 and 0.45 ng g^{-1} dw. The frequencies of detection of both PCBs 138 and 153 were 100% in all the sediment samples and varied from 67% to 92% in mussels. PCBs 180 and 101 were detected in 89% of the sediment samples. PCB 28 was present in < 10 - 25% in the samples while the frequency of PCB 52 ranged from 8% to 50%. No significant difference was observed between total PCB concentrations detected from the different sites ($p = 0.226 > 0.05$).

Table 7.1: Mean, range and total PCB concentrations (ng g^{-1} ww) in *M. galloprovincialis*

Analytes	Site 3	Site 4	Site 6	Site 7	Site 8
PCB 28	0.86 (nd-10.34)	1.66 (nd-10.0)	2.14 (nd-8.68)	1.40 (nd-16.80)	1.45 (nd-8.44)
PCB 52	1.72 (nd-7.63)	2.26 (nd-7.65)	3.66 (nd-9.10)	0.54 (nd-6.46)	2.28 (nd-14.73)
PCB 101	2.53 (nd-12.56)	0.37 (nd-1.78)	1.40 (nd-3.10)	2.08 (nd-5.99)	2.78 (nd-10.73)
PCB 138	4.64 (nd-9.12)	5.11 (nd-12.6)	5.88 (3.0-14.45)	5.85 (nd-11.6)	5.90 (3.7-12.93)
PCB 153	4.21 (nd-9.81)	2.56 (nd-8.85)	4.46 (1.5-9.69)	5.65 (nd-13.84)	5.45 (nd-8.36)
PCB 180	2.77 (1.3-5.71)	2.52 (nd-6.56)	3.76 (0.8-11.12)	3.48 (nd-8.95)	3.51 (1.97-6.04)
ΣPCBs ± SD	16.73 ± 8.57	14.48 ± 7.23	21.31 ± 7.00	19.00 ± 10.52	21.37 ± 8.97

nd: not detectable; SD: standard deviation; ww: wet weight; the raw data of all the samples are found in Appendix 9.

Table 7.2: Mean and total PCB concentrations (ng g^{-1} dw) in sediment samples

Analytes	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9
PCB 28	nd	nd	nd	0.07	0.2	nd	0.43	0.22	nd
PCB 52	nd	nd	0.23	0.06	nd	nd	0.31	0.15	nd
PCB 101	nd	0.26	0.28	0.12	0.28	0.33	0.24	0.27	0.38
PCB 138	0.78	0.72	0.40	0.15	0.78	0.64	0.39	0.46	0.71
PCB 153	0.60	0.60	0.38	0.16	0.79	0.41	0.55	0.37	0.45
PCB 180	0.35	0.40	0.13	nd	0.30	0.13	0.13	0.19	0.27
ΣPCBs ± SD	1.73 ± 0.34	1.98 ± 0.20	1.42 ± 0.15	0.56 ± 0.06	2.35 ± 0.32	1.51 ± 0.25	2.05 ± 0.15	1.66 ± 0.27	1.81 ± 0.27

dw: dry weight; SD: standard deviation

The contributions to the total PCB accumulation given by each congener in *M. galloprovincialis* and sediment samples are shown by Figures 7.2 and 7.3, respectively.

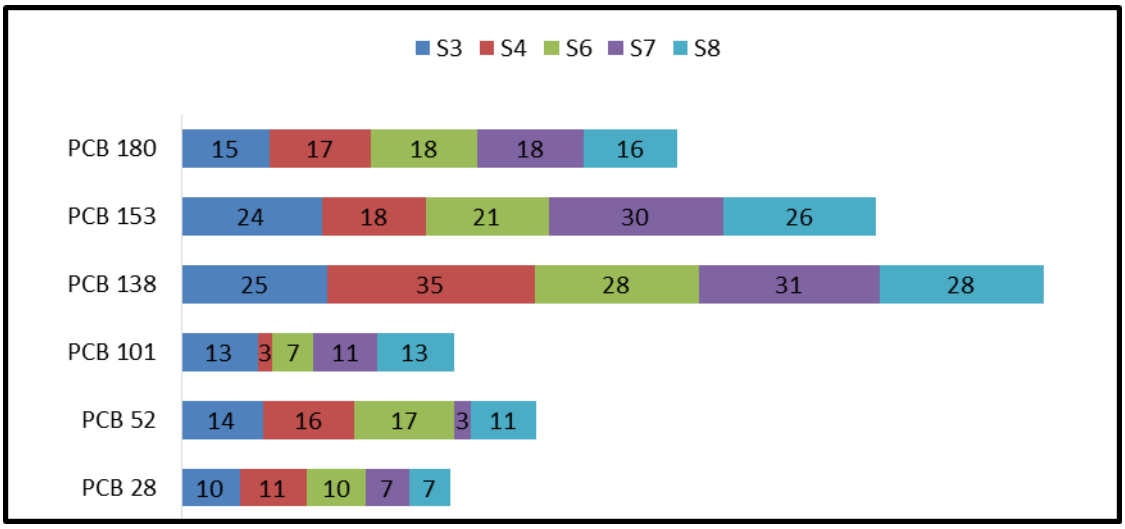

Figure 7.2: Percent contribution of indicator congeners to the total PCBs in *M. galloprovincialis* at five different sites

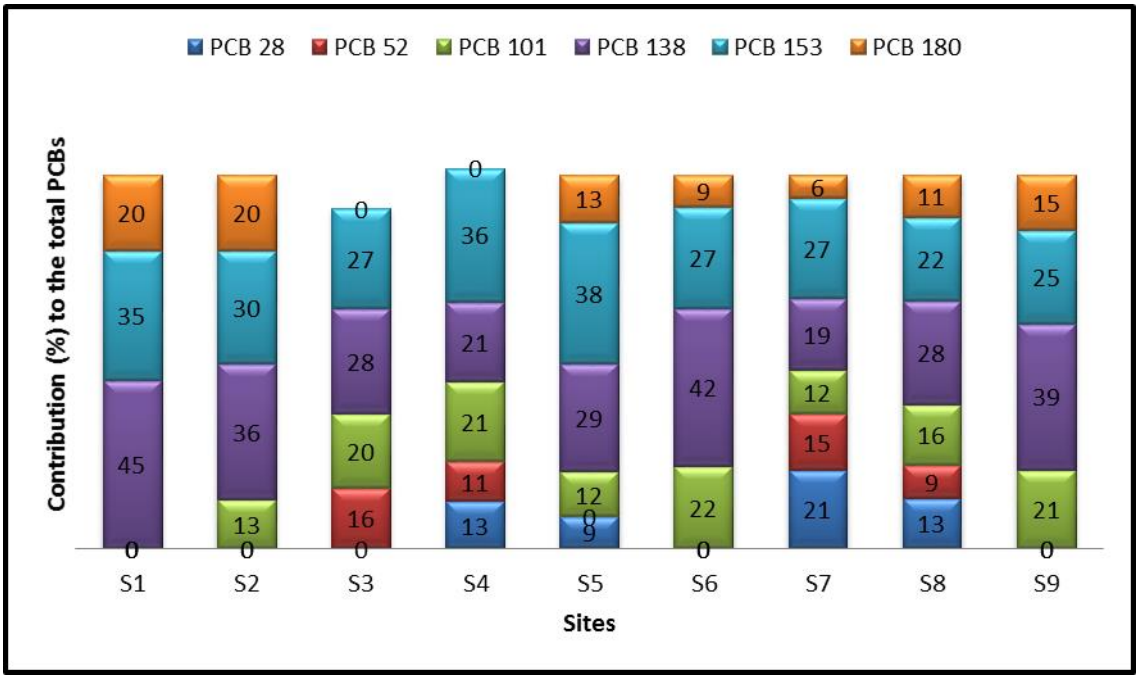

Figure 7.3: Contribution (%) of indicator congeners to the total PCBs in sediments at nine different sites (S1 to S9)

7.6 DISCUSSION

Mytilus galloprovincialis and sediments collected from the Port Elizabeth Harbour were contaminated by PCBs reflecting the influence of sources of contamination in the Port Elizabeth Harbour. According to Kennish (1997), PCBs enter the marine environment through the release of industrial discharge and runoff of waste water. The use of PCBs in South Africa has been attributed mainly to oils in transformers and electrical equipment. The total PCB burden of each sample was enriched in both hexa-CB (Figures 7.2 and 7.3). PCB 138 was dominant in sediment at all the sites and contributed to the total PCBs with an average of 34%. Similar to this study, Barakat et al. (2013) and Ssebugere et al. (2014) reported the predominance of PCB 138 in sediments collected from the Lake Maryut in Egypt and the Napoleon Gulf in Uganda, respectively. PCBs 138 and 153 are found dominant in environmental samples due to their high lipophilicity, low volatility, resistance to biodegradation, stability and persistence in the aquatic ecosystem (Nhan et al., 2001). The primary transport routes of PCBs into marine and coastal environments include atmospheric deposition and surface runoff from industries and other disposal wastes (Valavanidis and Vlachogianni, 2011). The degradation of a PCB molecule depends upon the degree of chlorination, vaporisation, solubility and adsorption to particles (Abramowicz et al., 1993; Rhee et al., 1993). The lower occurrence of the lower chlorinated congeners can be explained by their slow transformation while the highly chlorinated congeners resist degradation by microorganisms (WHO, 1993). Persistence of higher chlorinated congeners in the aquatic sediments is due to their low water solubility and partitioning to particles (Kennish, 1992; Salem et al., 2014). PCB congeners identified in sediments of this study were predominantly penta-CB to hepta-CB (Figure 7.3) which are in accordance with Kennish (1992).

Bottom sediments are the primary sinks for PCBs (Kang et al., 2000). PCBs in marine sediments arise from both natural and man-made sources such as wastewater discharges from urban areas and industries which are located around the Port Elizabeth Harbour and from vessels using the Port Elizabeth

Harbour. The concentrations of PCBs in sediment depend upon the characteristics of the sediments and their proximity to the source. The higher concentrations of PCBs in sediment samples were found at Site 5 (2.35 ng g^{-1} dw) which is near the closed end of the Harbour. The lowest concentration (0.56 ng g^{-1} dw) was found at Site 4 and this was attributed to the nature of the sediment collected which was mostly sandy and consequently not rich in organic carbon which increase the affinity for PCBs. Barakat et al. (2002) analysed PCBs in sediments from the Alexandria Harbour in Egypt and reported PCB concentrations ranging from 0.9 to 1210 ng g^{-1} dry weight with a median of 260 ng g^{-1} dw. Sapozhnikova et al. (2004) analysed PCBs in sediments from the Salton Sea in USA and found higher concentrations (116 - 304 ng g^{-1} dw). The concentrations found in the present study (0.56 - 2.35 ng g^{-1} dw) were much lower compared to the levels reported by the aforementioned authors. Table 7.3 compares PCB levels of the present study to PCB levels reported elsewhere. The concentrations of PCBs reported by Tolosa et al. (1995), Zhou et al. (2000) and Minh et al. (2007) were similar to the levels detected in the present study. However, other studies reported higher levels of PCBs in sediments.

Table 7.3: Comparison of PCB concentrations in Port Elizabeth Harbour sediments with other locations

Locations	Range of PCBs (ng g^{-1} dw)	References
Victoria harbour, Hong Kong	3.2 - 27	Connell et al. (1998)
Osaka Bay, Japan	2.5 - 240	Tanabe et al. (1991)
Suva harbour, Fiji	<0.17 - 69	Morrison et al. (1996)
Port Moresby, Papua New Guinea	3.3 - 24	Iwata et al. (1994)
Xiamen Harbour, China	45 - 311	Hong et al. (1995)
Xiamen Harbour, China	0.01 - 0.32	Zhou et al. (2000a)
Istanbul Strait, Turkey	0.013 - 969.9	Okay et al. (2009)
Guanabara Bay, Brazil	17.83 - 116.04	de Souza et al. (2008)

Locations	Range of PCBs (ng g^{-1} dw)	References
Ho Chi Minh, Vietnam	2.3-8.9	Iwata et al. (1993)
Mekong River delta, South Vietnam	0.039-9.2	Minh et al. (2007)
NW Basin in Mediterranean Sea	1.4 - 5.8	Tolosa et al. (1995)
Port Elizabeth Harbour, South Africa	0.56 - 2.35	Present study

NW: North-west

Contaminated sediments can affect aquatic organisms and wildlife by contributing to the bioaccumulation and biomagnification in the food chain. Numerous toxic effects such as tumour, developmental, and reproductive impacts, neurological and decreased biodiversity in aquatic ecosystems are attributed to contaminated sediments (U.S. EPA, 1998). The contaminated sediments pose a threat when the pollutants bioaccumulate in edible tissue of aquatic organisms (Scarpato et al., 2010). Some of the indicator congeners are known to pose a threat to human health such as neurological (PCBs 180 and 138), neuroendocrinological (PCBs 101, 138, and 153), immunological (PCBs 101, 153, 180) and carcinogenic effects (PCBs 180, 153) (Cimenci et al. 2013) through consumption of contaminated fatty foods (fish or mussels). The levels of total PCBs detected in sediments were lower than the Canadian interim sediment quality guideline value of 21.5 ng g^{-1} for marine sediments (CCME, 2001) and 21.6 ng g^{-1} recommended sediment guidelines in South Africa for the protection of marine aquatic ecosystems (Taljaard, 2006).

Concentrations of contaminants in mussels are related to the levels of chemicals in the water that they reside in and the food that they filter from the water (NOAA, 1998, Potrykus et al., 2003). Compared to other aquatic organisms, mussels have limited ability to undertake Phase I biotransformation to metabolize organic contaminants (Boon et al., 1989, Stegeman and Lech, 1991). Most of the mussels found along the coast or inshore areas are directly impacted by major industrial and urban effluents and/or river discharges. Investigations carried out on the bioaccumulation mechanisms on mussels showed that PCBs with a higher number of chlorines

are accumulated in a larger extent (Thompson et al., 1999). Degger et al. (2011) analysed mussels and semi-permeable membrane devices (SPMDs) from the South African marine environment. The results showed PCB concentrations in the South African harbours (Cape Town, Port Elizabeth, Richards Bay and Saldanha Bay) ranging between 34-131 µg g^{-1} lipid weight in the mussels and 29-158 µg g^{-1} lipid weight in the SPMDs. SPMDs exposed at Port Elizabeth Harbour in 2009 contained levels of 105 µg g^{-1} of congeners with 63% represented by three indicator congeners (PCBs 28 = 19, CB-52 = 4 and CB-138 = 43). These findings showed that South African harbours including the area of the present study is contaminated by PCBs.

The presence of PCBs in Port Elizabeth Harbour is suggested to originate from recent industrial activities linked to major port developments at Coega near Port Elizabeth (Ryan et al., 2012). In order to know the magnitude of contamination in mussels, concentration of total PCBs in the present study was compared with those reported from other countries (Table 7.4).

The concentrations of indicator PCB congeners in *M. galloprovincialis* varied from 14.48 to 21.37 ng g^{-1} ww with a predominance of higher chlorinated congeners (153 and 138). This is similar to the range of 15.13 - 37.49 ng g^{-1} ww reported by Salem et al. (2014) for the mussels collected from the Mediterranean Coast in Egypt. Further evidence to support this was presented by Potrykus et al. (2003) who analysed seven indicator PCBs in the blue mussel *Mytilus trossulus*, and reported that the total PCBs ranged from 1.93 to 22.31 ng g^{-1} ww in the Baltic Sea. Among the PCB congeners detected by Potrykus et al. (2003), hexa-CBs (PCB 153 and 138) were found at the highest levels. The levels detected in this study are compared also to the detected levels of Khaled et al. (2004) who analysed seven indicator congeners in mussels collected along the Egyptian Red Sea Coast and reported the concentrations of total PCBs ranging from 6.75 to 66.4 ng g^{-1} wet weight similar to Scarpato et al. (2010) who detected total PCBs in mussels from the coasts of the western Mediterranean Sea with a range of 5 - 60 ng g^{-1}. The levels of PCBs in *M. galloprovincialis* may be attributed to the waste

dumped, intensive maritime traffic and industrial activities from the Port Elizabeth Harbour.

According to Villeneuve et al. (1999); Piersanti et al. (2014); Salem et al. (2014), the predominance of PCB 153 in *M. galloprovincialis* is due to the presence of chlorine atoms at the *ortho*, *meta* and *para* positions (2-4 or 5) in one or both rings. Other authors, (Endicott et al., 1998; Villeneuve et al., 1999; Khim et al., 2000; Mikoszewski and Lubelska, 2010; Mendoza et al., 2006) analysed mussels from different areas and found higher levels of PCBs compared to the present study (Table 7.4).

PCBs 153 and 138 assume the role of predominant contaminants in marine organisms' tissues and sediments (Turrio Baldassarri et al., 1993; Thompson et al., 1999; Bayarri et al., 2001; de Souza et al., 2008; Ssebugere et al., 2014). Sediments are home to a wide variety of aquatic life. Therefore, the contaminated sediments may adversely affect the environment through bioaccumulation and biomagnification of lipophilic compounds such as PCBs.

Table 7.4: Comparison of PCB concentrations (ng g^{-1} ww) in mussels from Port Elizabeth Harbour with other studies

Location	Total PCBs	References
Harbour, Port Elizabeth	14.48 - 21.37	Present study
South African Harbours (Cape Town, Port Elizabeth, Richards Bay and Saldanha Bay)	34 - 131[a]	Degger et al. (2011)
Coastal areas of South Korea	4.4 - 422.0	Khim et al. (2000)
Mediterranean Coast, Egypt	15.13 - 37.49	Salem et al. (2014)
Danish Straits and South-eastern Baltic Sea	8.0 - 289.0	Mikoszewski and Lubelska (2010)
Red Sea Coast, Egypt	6.7 - 66.4	Khaled et al. (2004)
Vistula and Odra estuaries, Baltic Sea	1.93 – 22.31	Potrykus et al. (2003)
Coasts of West Mediterranean	5.0 - 60.0	Scarpato et al. (2010)
Chilean Coast	10 - 298.0	Mendoza et al. (2006)
Pearl River Delta, South China	41 - 729.2	Fang (2004)
Izmit Bay, North-eastern Marmara Sea	4.2 - 140.7	Ergül et al. (2010)
Saginaw Bay, Lake Huron, Michigan	76 - 1200	Endicott et al. (1998)

[a]Results are expressed in µg g^{-1} lipid weight

7.7 CONCLUSION

The study provided information on the current status of PCB levels in sediments and mussels from Port Elizabeth Harbour and from the levels obtained; it would appear that Port Elizabeth Harbour does show levels of contamination. The highest PCB concentrations of 21.37 ng g^{-1} wet weight and 2.35 ng g^{-1} dry weight, respectively in *M. galloprovincialis* and sediments were detected. In all the analysed samples, PCB 138 was detected at highest concentrations. The distribution of PCB congeners varied in the order: CB 138 >153 >101>180 >28 >52 and CB 138 >153 >180 >52 >101>28, respectively in sediments and *M. galloprovincialis*. The sources of these compounds in the Port Elizabeth Harbour may be attributed to human activities and industrial wastes. The levels of PCBs detected in sediments and *M. galloprovincialis* were low compared to other studies and the limits recommended by international organizations.

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

“Life expectancy has gone up, and we are now living in a soup of carcinogenic influences; clearly the longer you live, the more likely these are to have an effect. … we have changed our environment by the introduction of novel chemicals and radio nuclides. We see now that some man-made chemicals are able to interfere with the cell-signaling mechanisms in the human body. The fact that these are not just single chemicals, but PCB and POP compounds in huge variation also seems to be a factor. If you disrupt the cell-signalling molecules such as hormones which control gene expression, then you can cause dysgenesis (tissue damage) at much, much lower doses". By Prof Vyvyan Howard (www.canceractive.com/cancer-active-page-link.aspx?n=1052, accessed on 24th June 2015).

8.1 CONCLUSIONS

The present study was designed to identify and quantify polychlorinated biphenyls (PCBs) in the Nelson Mandela Bay Municipality. The general hypothesis was that the NEL and PEH are contaminated by PCBs. The data from Chapters 4, 5 and 6 (fish, sediments, water, brown mussels) showed that the NEL is contaminated by PCBs while data provided in Chapter 7 (sediments and blue mussels) indicated that the PEH is contaminated by PCBs. These findings highlight the importance of examining PCBs from local contaminated environments.

The findings indicate that fish from the NEL are contaminated by PCBs but that concentrations are low compared to international standards. In *C. carpio*, the sum of PCBs (28, 52, 101, 138, 153 and 180) were 95.89, 57.49, 44.63 and 34.14 ng g^{-1} lipid weight (lw) respectively in liver, gonads, gills and muscles. In *O. mossambicus*, the sum of PCBs (28, 52, 101, 138, 153 and 180) were 119.73, 59.21, 49.78 and 34.63 ng g^{-1} lipid weight (lw) respectively

in liver, gonads, gills and muscles. Liver was the most contaminated organ of fish due to its high lipid content.

Results showed that sediments are the primary sink for PCBs and the total PCB concentrations in the NEL surface sediments ranged from 1.60 to 3.06 ng g^{-1}, dry weight (dw). The contamination levels measured in the sediments of the NEL were comparable to other lakes in developing countries but were lower compared to the lakes from the developed countries. However, the PCB levels in sediments were lower compared to Canadian and South African sediment guidelines (34.1 and 21.5 ng g^{-1}). Sediments are loose substances that settle at the bottom of a water body and may be a source of contamination to water. Water samples including inflow, lake water and outflow from the NEL were contaminated by PCBs. The total PCB concentrations (both the dissolved and particulate phases) ranged from 0.16 to 0.36 ng L^{-1}. Higher average PCB concentrations were detected in the water flowing out of the lake to the sea (0.33 ng L^{-1}). The inflow water (0.25 ng L^{-1}) and the lake water (0.24 ng L^{-1}) had similar PCB concentrations. PCB levels detected in brown mussels (*Perna perna*) collected around the vicinity of the outfall of the NEL into the Indian Ocean ranged from 20.85 to 31.34 ng g^{-1} wet weight (ww). Higher PCB levels were detected at the outfall point of the NEL into the Indian Ocean with predominance of PCB 52 at the site.

Sediments and blue mussels (*Mytilus galloprovincialis*) from PEH were contaminated by PCB pollutants. The concentrations of total PCBs in sediments and *M. galloprovincialis* ranged from 0.56 to 2.35 ng g^{-1} dry weight and 14.48 to 21.37 ng g^{-1} wet weight, respectively. The levels of PCBs detected in sediments and *M. galloprovincialis* were low compared to other studies and the limits recommended by international organizations.

The results from this research are significant in that they provide evidence on the PCB contamination levels in fish, sediments, mussels and water samples. Contaminated foods such as fish are considered as the main route of PCB exposure to humans. The PCB levels detected in fish were low compared to the international standards but revealed that fish from the NEL were not safe

for human consumption over a long period of time due to the bioaccumulative property of PCBs. Populations continue to eat fish from the NEL which contain PCBs and a wide variety of adverse health effects are associated with consumption of large amounts of fish through the excessive exposure to PCBs. PCB concentrations in fish/mussels are a function of the levels in water and sediment. PCB residues detected in sediment revealed that there could be transfer of PCB pollutants to the fish species such as *C. carpio* which is known to be a bottom-feeder and *O. mossambicus* which is a semi-predator that also harvests plankton from the water and feeds on benthos and detritus. Mussels could also be affected by contaminated sediments and water. In all the sediment samples analysed, the predominant congeners were both hexachlorobiphenyls (PCBs 138 and 153) and were found to be dominant in the organs of fish and mussels.

The findings indicated that the NEL water is and continues to be a significant industrial point source of PCB contamination in mussels around the outfall of the lake into the Indian Ocean. The levels detected in fish and mussels are related to the transfer of PCB contaminants from water/or sediments. Humans at the top of the food chain are exposed at high risk through consumption of contaminated fish/mussels from contaminated areas such as NEL and PEH.

The overall hypothesis of this research was confirmed. The results obtained from this study contributed to the understanding of PCB contamination in the NEL and PEH and provided baseline data on the potential impacts on human health. The dissemination of the results may be used to alert the public and the relevant municipal authorities to the presence of PCB contaminants in these bodies of water and the associated risks in fish/mussel consumption.

8.2 RECOMMENDATIONS

The harmful health effects caused by persistent organic pollutants such as PCBs in the environment has led to developed countries setting and implementing regulations for environmental protection. However, all over the world and particularly in many African countries, there is a remarkable growing population accompanied by intense urbanization, industrial activity

and consequently an increase of waste discharge into the environment. Pollution can limit uses of aquatic resources; for example the NEL is used for recreational activities such as water skiing, but swimming no longer take place due to high *E. coli* levels. Based on the findings from this study, the following recommendations were made:

- There is a need to formulate and implement pollution control and monitoring policies to regulate the contaminant discharges into the NEL and PEH.
- Due to the long half-lives and wide distribution of POP compounds throughout food chains, there is need to establish baseline contaminant PCB levels in other local aquatic ecosystems such as rivers, estuaries, lakes, and ponds for the evaluation of long-term monitoring of health risks which can indicate if significant declines have occurred in the environment.
- The Nelson Mandela Metropolitan University should seek to expand analytical capabilities for POP analyses and monitoring in order to allow different researchers at different levels to conduct POP analysis at the same time. Equipment which uses less solvent and are less time consuming is to be of priority.

8.3 SHORTCOMINGS OF THE RESEARCH

This study has made an important contribution to the body of knowledge on PCB contamination in the environment of the Nelson Mandela Bay Municipality. However it is equally important to note that there were certain limitations. This study was the first conducted in the Chemistry Department of NMMU, therefore, some of the equipment was limiting as only two Soxhlet extraction apparatus was available. Soxhlet extraction uses a lot of solvent and is time consuming which limited the number of samples that could be analysed.

The analysis of PCBs in fish from PEH was initially planned in order to make a comparison with the NEL fishes. However, the fish from PEH were not

analysed due to problems with sampling and access that were out of direct control in the PEH. In the initial research proposal, PCBs in selected aquatic organisms including fish, crabs and mussels were supposed to be sampled and quantified for PCB levels of contamination in the aquatic food web. However, crabs were not analysed due to their non-availability in the areas of this study during the sampling trips

8.4 RECOMMENDED FUTURE RESEARCH DIRECTIONS

PCBs are chemicals that remain intact in the environment and cause harmful ecosystem effects. The objective of the Stockholm Convention is to protect human health and the environment from POP chemicals. At the moment, the Stockholm Convention has listed 23 POPs that need to be eliminated or restricted for the production and use in order to achieve a better and cleaner environment. Based on the findings from this research, the investigations have identified some interesting directions for future research.

Given that to date this project was the first report on PCB contamination in the NEL and PEH (fish, mussels, water and sediments), there is a need to broaden the scope of research to include other pollutants (PAHs, dioxin-like PCBs, dioxins, furans, and pesticides). Several of the PCB congeners are highly toxic to terrestrial and aquatic animals. Research on congener-specific toxicity, bioaccumulation, and environmental levels is needed for better characterisation of the sources of PCBs in order to reduce the release of these compounds, but also to better understand the exposure and risk scenarios particularly, if humans are to be in close contact with these sources as in the case in the NEL. This project reports only on two fish species from the NEL, further work may be conducted on a greater variety of biota (including fish, crabs, mussels, snails) and other organs of fish. The kidney, brain, and bone should be analysed for PCB contamination levels especially the brain which is associated with neurotoxicity caused by effects of PCBs. The distribution of PCBs in sediments is dependent upon a number of factors. Grain size and organic matter content are the main characteristics of sediments and the important factors that control the sorption of hydrophobic

organic contaminants such as PCBs. Clay and silt have properties that cause preferential sorption of PCBs due to the high surface area. The sorption of these contaminants onto sediments controls their transport, degradation, and eventual fate. Thus, it is very important for future work to differentiate the sedimentary environment in terms of sediment transport and the analysis of grain size. Also, in the present study, only impacted areas, i.e. NEL and PEH, were analysed. Future work should be conducted to assess whether there are significant differences in polychlorinated biphenyl residues between impacted and control/reference areas. The samples should be taken within the NEL underground concrete culvert to study the transport of PCBs within this area. As the sedimentation process is often found in the deepest section of small lakes and harbours, it is recommended that future work analyses underwater samples because the littoral samples often display lower values than deeper samples due to the resuspension processes.Research on seasonal and temporal trends of PCB levels in the NEL and PEH require analysis to better understand and model the factors which exert a dominant influence over ambient PCB levels. Finally, the determination of PCB levels between blue mussels and brown mussels in the same water would be of interest as well as the study of PCB distribution in the different organs of mussels.

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APPENDIX 1: ETHICS CLEARANCE

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Nelson Mandela Metropolitan University
for tomorrow

Chairperson of the Research Ethics Committee (Animal)
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Ref: [A12-SCI-CHE-001/Approval]

Contact person: Mrs U Spies

21 September 2012

Dr G Rubidge
NMMU
Faculty of Science
Department of Chemistry
A Block, Room 201b
North Campus

Dear Dr Rubidge

CHARACTERIZATION AND FATE OF POLYCHLORINATED BIPHENYL RESIDUES IN SELECTED AQUATIC ORGANISMS AND SEDIMENTS FROM THE NORTH END LAKE AND PORT ELIZABETH HARBOUR, SOUTH AFRICA

PRP: Dr G Rubidge
PI: Ms E Kampire

We take pleasure in informing you that the above-mentioned application submitted to the Research Ethics Committee (Animal) for ethics approval, was approved by the Committee.

The ethics clearance reference number is **A12-SCI-CHE-001**.

Ethics approval remains valid for three years, provided that the approved protocols and conditions remain unchanged and that the applicant agrees to regular monitoring (video recordings if necessary where field work is involved) by the RECA for the duration of the project. At the end of the third year, you will have to affirm that the project is complete, or reapply for ethics approval. You will receive the appropriate reminder and documentation each year well in time for any applicable deadline.

Please inform your co-investigators of the outcome. We wish you well with the project.

Yours sincerely

G S Dealtry

Dr G Dealtry
Chairperson: Research Ethics Committee (Animal)

cc: Department of Research Capacity Development
Faculty Officer: Science

/us

APPENDIX 2: NOMENCLATURE OF PCBs

1. PCB nomenclature: mixtures and homologs

PCB mixtures: Aroclor		PCB homolog group	
IUPAC Name	CASRN	IUPAC Name	CASRN
Aroclor 1016	12674-11-2	Monochlorobiphenyl	27323-18-8
Aroclor 1210	14760-87-4	Dichlorobiphenyl	25512-42-9
Aroclor 1216	151820-27-8	Trichlorobiphenyl	25323-68-6
Aroclor 1221	11104-28-2	Tetrachlorobiphenyl	26914-33-0
Aroclor 1231	37234-40-5	Pentachlorobiphenyl	25429-29-2
Aroclor 1232	11141-16-5	Hexachlorobiphenyl	26601-64-9
Aroclor 1240	71328-89-7	Heptachlorobiphenyl	28655-71-2
Aroclor 1242	53469-21-9	Octachlorobiphenyl	55722-26-4
Aroclor 1248	12672-29-6	Nonachlorobiphenyl	53742-07-7
Aroclor 1250	165245-51-2	Decachlorobiphenyl	2051-24-3
Aroclor 1252	89577-778-6		
Aroclor 1254	11097-69-1		
Aroclor 1260	11096-82-5		
Aroclor 1262	37324-23-5		
Aroclor 1268	11100-14-4		

CASRN: Chemical Abstracts Service (CAS) Registry Number

2. PCB congener's nomenclature and Log K_{OW}

IUPAC #	IUPAC Name	CASRN	Log Kow
MONOCHLOROBIPHENYLS			
1	2- Chlorobiphenyl	2051-60-7	4.46
2	3- Chlorobiphenyl	2051-61-8	4.69
3	2-Chlorobiphenyl	2051-62-9	4.69
DICHLOROBIPHENYLS			
4	2,2'-Dichlorobiphenyl	13029-08-8	4.65
5	2,3- Dichlorobiphenyl	16605-91-7	4.97
6	2,3'- Dichlorobiphenyl	25569-80-6	5.06
7	2,4- Dichlorobiphenyl	33284-50-3	5.07
8	2,4'- Dichlorobiphenyl	34883-39-1	5.07
9	2,5- Dichlorobiphenyl	34883-39-1	5.06
10	2,6- Dichlorobiphenyl	33146-35.1	4.84
11	3,3'- Dichlorobiphenyl	2050-67-1	5.28
12	3,4- Dichlorobiphenyl	2974-92-7	5.22
13	3,4'- Dichlorobiphenyl	2974-90-5	5.29
14	3,5- Dichlorobiphenyl	34883-41-5	5.28
15	4,4'- Dichlorobiphenyl	2050-68-2	5.30
TRICHLOROBIPHENYLS			
16	2.2',3-Trichlorobiphenyl	38444-78-9	5.16
17	2,2',4-Trichlorobiphenyl	37680-66-3	5.25
18	2,2',5-Trichlorobiphenyl	37680-65-2	5.24
19	2,2'6-Trichlorobiphenyl	38444-73-4	5.02
20	2,3,3'-Trichlorobiphenyl	38444-84-7	5.57
21	2,3,4-Trichlorobiphenyl	55702-46-0	5.51
22	2,3,4'-Trichlorobiphenyl	38444-85-8	5.58
23	2,3,5-Trichlorobiphenyl	55720-44-0	5.57
24	2,3,6-Trichlorobiphenyl	55702-45-9	5.35
25	2,3',4-Trichlorobiphenyl	55712-37-3	5.67
26	2,3',5-Trichlorobiphenyl	38444-81-4	5.66
27	2,3',6-Trichlorobiphenyl	38444-76-7	5.44

28	2,4,4'-Trichlorobiphenyl	7012-37-5	5.67
29	2,4,5-Trichlorobiphenyl	15862-07-4	5.60
30	2,4,6-Trichlorobiphenyl	35693-92-6	5.44
31	2,4',5-Trichlorobiphenyl	16606-02-3	5.67
32	2,4',6-Trichlorobiphenyl	38444-77-8	5.44
33	2,3'4-Trichlorobiphenyl	38444-86-9	5.60
34	2,3'5'- Trichlorobiphenyl	37680-68-5	5.66
35	3,3'4- Trichlorobiphenyl	37680-69-6	5.82
36	3,3',5- Trichlorobiphenyl	38444-87-0	5.88
37	3,4,4'- Trichlorobiphenyl	38444-90-5	5.83
38	3,4,5- Trichlorobiphenyl	53555-66-1	5.76
39	3,4',5- Trichlorobiphenyl	38444-88-1	5.89
TETRACHLOROBIPHENYLS			
40	2,2',3,3'-Tetrachlorobiphenyl	38444-93-8	5.66
41	2,2',3,4- Tetrachlorobiphenyl	52663-59-9	5.69
42	2,2',3,4'- Tetrachlorobiphenyl	36559-22-5	5.76
43	2,2',3,5- Tetrachlorobiphenyl	70362-46-8	5.75
44	2,2',3,5'- Tetrachlorobiphenyl	41464-39-5	5.75
45	2,2',3,6- Tetrachlorobiphenyl	70362-45-7	5.53
46	2,2',3,6'-Tetrachlorobiphenyl	41464-47-5	5.53
47	2,2',4,4'- Tetrachlorobiphenyl	2437-79-8	5.85
48	2,2',4,5- Tetrachlorobiphenyl	70362-47-9	5.78
49	2,2',4,5'- Tetrachlorobiphenyl	41464-40-8	5.85
50	2,2',4,6- Tetrachlorobiphenyl	62796-65-0	5.63
51	2,2',4,6'- Tetrachlorobiphenyl	68194-04-7	5.61
52	2,2',5,5'- Tetrachlorobiphenyl	35693-99-3	5.84
53	2,2',5,6'- Tetrachlorobiphenyl	41464-41-9	5.62
54	2,2',6,6'- Tetrachlorobiphenyl	15968-05-5	5.21
55	2,3,3',4- Tetrachlorobiphenyl	74338-24-2	5.21
56	2,3,3',4'- Tetrachlorobiphenyl	41464-43-1	6.11
57	2,3,3',5- Tetrachlorobiphenyl	70424-67-8	6.17
58	2,3,3',5'- Tetrachlorobiphenyl	41464-49-7	6.17

59	2,3,3',6- Tetrachlorobiphenyl	74472-33-6	5.95
60	2,3,4,4'- Tetrachlorobiphenyl	33025-41-1	6.11
61	2,3,4,5- Tetrachlorobiphenyl	33284-53-6	6.04
62	2,3,4,6- Tetrachlorobiphenyl	54230-22-7	5.89
63	2,3,4',5- Tetrachlorobiphenyl	74472-34-7	6.17
64	2,3,4',6- Tetrachlorobiphenyl	52663-58-8	5.95
65	2,3',5,6- Tetrachlorobiphenyl	33284-54-7	5.86
66	2,3',4,4'- Tetrachlorobiphenyl	32598-10-0	6.20
67	2,3',4,5-Tetrachlorobiphenyl	73575-53-8	6.20
68	2,3',4,5'- Tetrachlorobiphenyl	73575-52-7	6.26
69	2,3',4,6- Tetrachlorobiphenyl	60233-24-1	6.04
70	2,3',4',5- Tetrachlorobiphenyl	32598-11-1	6.20
71	2,3',4',6- Tetrachlorobiphenyl	41464-46-4	5.98
72	2,3',5,5'- Tetrachlorobiphenyl	41464-42-0	6.26
73	2,3',5,6- Tetrachlorobiphenyl	74338-23-1	6.04
74	2,4,4',5- Tetrachlorobiphenyl	32690-93-0	6.20
75	2,4,4',6- Tetrachlorobiphenyl	32598-12-2	6.05
76	2,3',4',5'- Tetrachlorobiphenyl	70362-48-0	6.13
77	3,3',4,4'- Tetrachlorobiphenyl	32598-13-3	6.36
78	3,3',4,5- Tetrachlorobiphenyl	70362-49-1	6.35
79	3,3',4,5'- Tetrachlorobiphenyl	41464-48-6	6.42
80	3,3',5,5'- Tetrachlorobiphenyl	33284-52-5	6.48
81	3,4,4',5- Tetrachlorobiphenyl	70362-50-4	6.36
PENTACHLOROBIPHENYLS			
82	22'33'4-Pentachlorobiphenyl	52663-62-4	6.20
83	22'33'5- Pentachlorobiphenyl	60145-20-2	6.26
84	22'33'6- Pentachlorobiphenyl	52663-60-2	6.04
85	22'344'- Pentachlorobiphenyl	65510-45-4	6.10
86	22'345- Pentachlorobiphenyl	55312-69-1	6.23
87	22'345'- Pentachlorobiphenyl	38380-0-2	6.29
88	22'346- Pentachlorobiphenyl	55215-17-3	6.07
89	22'346'- Pentachlorobiphenyl	73575-57-2	6.07

90	22’34’5- Pentachlorobiphenyl	68194-05-0	6.36
91	22’34’5- Pentachlorobiphenyl	68194-05-8	6.13
92	22’355’- Pentachlorobiphenyl	52663-61-3	6.35
93	22’356- Pentachlorobiphenyl	73575-56-1	6.04
94	22’356’- Pentachlorobiphenyl	73575-55-0	6.13
95	22’35’6- Pentachlorobiphenyl	38379-99-6	6.13
96	22’366’- Pentachlorobiphenyl	73575-54-9	5.71
97	22’3’45- Pentachlorobiphenyl	41464-51-1	6.29
98	22’3’46- Pentachlorobiphenyl	60233-25-2	6.13
99	22’344’5- Pentachlorobiphenyl	38380-01-7	6.39
100	22’44’5- Pentachlorobiphenyl	39485-83-1	6.23
101	22’455’-Pentachlorobiphenyl	37680-73-2	6.38
102	22’456’-Pentachlorobiphenyl	68194-06-9	6.16
103	22’45’6-Pentachlorobiphenyl	60145-21-3	6.22
104	22’466’-Pentachlorobiphenyl	56558-16-8	5.81
105	233’44’-Pentachlorobiphenyl	32598-14-4	6.65
106	233’44’-Pentachlorobiphenyl	70424-69-0	6.64
107	233’4’5-Pentachlorobiphenyl	70424-68-9	6.71
108	233’45’-Pentachlorobiphenyl	70362-41-3	6.71
109	233’46-Pentachlorobiphenyl	74472-35-8	6.48
110	233’4’6-Pentachlorobiphenyl	38380-03-9	6.48
111	233’55’-Pentachlorobiphenyl	39635-32-0	6.76
112	233’56-Pentachlorobiphenyl	74472-36-9	6.45
113	233’5’6-Pentachlorobiphenyl	68194-10-5	6.54
114	2344’5-Pentachlorobiphenyl	74472-37-0	6.65
115	2344’6-Pentachlorobiphenyl	74472-38-1	6.49
116	23456-Pentachlorobiphenyl	18259-05-7	6.33
117	234’56-Pentachlorobiphenyl	68194-11-6	6.46
118	23’44’5-Pentachlorobiphenyl	31508-00-6	6.74
119	23’44’6-Pentachlorobiphenyl	56558-17-9	6.58
120	23’455’-Pentachlorobiphenyl	68194-12-7	6.58
121	23’45’6-Pentachlorobiphenyl	56558-18-0	6.64

122	2’33’45-Pentachlorobiphenyl	76842-07-4	6.64
123	2’344’5-Pentachlorobiphenyl	65510-44-3	6.74
124	2’3455’-Pentachlorobiphenyl	70424-70-3	6.73
125	2’3456’-Pentachlorobiphenyl	74472-39-2	6.51
126	33’44’5-Pentachlorobiphenyl	57465-28-8	6.89
127	33’455’-Pentachlorobiphenyl	39635-33-1	6.95
HEXACHLOROBIPHENYLS			
128	22’33’44’-Hexachlorobiphenyl	38380-07-3	6.74
129	22’33’45’- Hexachlorobiphenyl	55215-18-4	6.73
130	22’33’46- Hexachlorobiphenyl	52663-66-8	6.80
131	22’33’46’- Hexachlorobiphenyl	61798-70-7	6.58
132	22’33’55’- Hexachlorobiphenyl	38380-05-1	6.58
133	22’33’56- Hexachlorobiphenyl	35694-04-3	6.86
134	22’33’56’- Hexachlorobiphenyl	52704-70-8	6.55
135	22’33’56’-Hexachlorobiphenyl	52744-13-5	6.64
136	22’33’66’-Hexachlorobiphenyl	38411-22-2	6.22
137	22’344’5-Hexachlorobiphenyl	35694-06-5	6.83
138	22’344’5’-Hexachlorobiphenyl	35065-28-2	6.83
139	22’344’6-Hexachlorobiphenyl	56030-56-9	6.67
140	22’344’6’-Hexachlorobiphenyl	59291-64-4	6.67
141	22’3455’-Hexachlorobiphenyl	52712-04-6	6.82
142	22’3456-Hexachlorobiphenyl	41411-61-4	6.51
143	22’3456’-Hexachlorobiphenyl	68194-15-0	6.60
144	22’345’6-Hexachlorobiphenyl	68194-14-9	6.67
145	22’3466’-Hexachlorobiphenyl	74472-40-5	6.25
146	22’3466’-Hexachlorobiphenyl	51908-16-8	6.89
147	22’34’56-Hexachlorobiphenyl	68194-13-8	6.64
148	22’34’56’-Hexachlorobiphenyl	74472-41-6	6.73
149	22’34’56’-Hexachlorobiphenyl	38380-04-0	6.67
150	22’34’66’-Hexachlorobiphenyl	68194-08-1	6.32
151	22’355’6-Hexachlorobiphenyl	52663-63-5	6.64
152	22’3566’-Hexachlorobiphenyl	68194-09-2	6.22

153	22’44’55’-Hexachlorobiphenyl	35065-27-1	6.92
154	22’44’56’-Hexachlorobiphenyl	60145-22-4	6.76
155	22’44’66’-Hexachlorobiphenyl	33979-03-2	6.41
156	233’44’5-Hexachlorobiphenyl	38380-08-4	7.18
157	233’44’5’-Hexachlorobiphenyl	68782-90-7	7.18
158	233’44’6-Hexachlorobiphenyl	74472-42-7	7.02
159	233’455’-Hexachlorobiphenyl	39635-35-3	7.24
160	233’456-Hexachlorobiphenyl	41411-62-5	6.93
161	233’4’56-Hexachlorobiphenyl	74472-43-8	7.08
162	233’4’5’6-Hexachlorobiphenyl	39635-34-2	7.24
163	233’4’56-Hexachlorobiphenyl	74472-44-9	6.99
164	233’4’5’6-Hexachlorobiphenyl	74472-45-0	7.02
165	233’55’6-Hexachlorobiphenyl	74472-46-1	7.05
166	2344’56-Hexachlorobiphenyl	41411-63-6	6.93
167	23’44’55’-Hexachlorobiphenyl	52663-72-6	7.27
168	23’44’5’6- Hexachlorobiphenyl	59291-65-5	7.11
169	33’44’55’- Hexachlorobiphenyl	32774-16-6	7.42
HEPTACHLOROBIPHENYLS			
170	22’33’44’5-Heptachlorobiphenyl	35065-30-6	7.27
171	22’33’44’6- Heptachlorobiphenyl	52663-71-5	7.11
172	22’33’455’- Heptachlorobiphenyl	52663-74-8	7.33
173	22’33’456- Heptachlorobiphenyl	68194-16-1	7.02
174	22’33’45’6- Heptachlorobiphenyl	38411-25-5	7.11
175	22’33’456’- Heptachlorobiphenyl	40186-70-7	7.17
176	22’33’466’- Heptachlorobiphenyl	52663-65-7	6.76
177	22’33’4’56- Heptachlorobiphenyl	52663-70-4	7.08
178	22’33’55’6- Heptachlorobiphenyl	52663-67-9	7.14
179	22’33’566’- Heptachlorobiphenyl	52663-64-6	6.73
180	22’344’55’- Heptachlorobiphenyl	35065-29-3	7.36
181	22’344’5’6- Heptachlorobiphenyl	74472-47-2	7.11
182	22’344’56- Heptachlorobiphenyl	60145-23-5	7.20
183	22’344’5’6-Heptachlorobiphenyl	52663-69-1	7.20

184	22’344’66’- Heptachlorobiphenyl	74472-48-3	6.85
185	22’3455’6- Heptachlorobiphenyl	52712-05-7	7.11
186	22’34566’- Heptachlorobiphenyl	74472-49-4	6.99
187	22’34’55’6- Heptachlorobiphenyl	52663-68-0	7.17
188	22’34’566’- Heptachlorobiphenyl	74487-85-7	6.82
189	233’44’55’- Heptachlorobiphenyl	39635-31-9	7.71
190	233’44’56- Heptachlorobiphenyl	41411-64-7	7.46
191	233’44’5’6- Heptachlorobiphenyl	74472-50-7	7.55
192	233’455’6- Heptachlorobiphenyl	74472-51-8	7.52
193	233’4’55’6- Heptachlorobiphenyl	69782-91-8	7.52
OCTACHLOROBIPHENYLS			
194	22’33’44’55’-Octachlorobiphenyl	35694-08-7	7.80
195	22’33’44’56-Octachlorobiphenyl	52663-78-2	7.56
196	22’33’44’56’-Octachlorobiphenyl	42740-50-1	7.65
197	22’33’44’66’-Octachlorobiphenyl	33091-17-7	7.30
198	22’33’455’6-Octachlorobiphenyl	68194-17-2	7.62
199	22’33’4566’-Octachlorobiphenyl	52663-75-9	7.62
200	22’33’45’66’-Octachlorobiphenyl	52663-73-7	7.20
201	22’33’455’6’-Octachlorobiphenyl	40186-71-8	7.27
202	22’33’55’66’-Octachlorobiphenyl	2136-99-4	7.24
203	22’344’55’6-Octachlorobiphenyl	52663-76-0	7.65
204	22’344’566’-Octachlorobiphenyl	74472-52-9	7.30
205	233’44’55’6-Octachlorobiphenyl	74472-53-0	8.00
NONACHLOROBIPHENYLS			
206	22’33’44’55’6-Nonachlorobiphenyl	40186-72-9	8.09
207	22’33’44’566’-Nonachlorobiphenyl	52663-79-3	7.74
208	22’33’455’66’-Nonachlorobiphenyl	52663-77-1	7.71
DECACHLOROBIPHENYL			
209	22’33’44’55’66’-Decachlorobiphenyl	2051-24-3	8.18

APPENDIX 3. PARTICLE SIZE DETERMINATION OF THE NORTH END SEDIMENTS

SAMPLE	Distilled Water	30s	60s	180s	1.5h	24h	T30s	T60s	T180s	T1.5h	T24h	η	ρ_l
ESL 4	-2,5	11,8	9,8	8,8	1,8	1,8	14,3	12,3	11,3	4,3	4,3	1,104709	1,0315
ESL 2	-2,5	7,8	6,8	4,8	1,8	0,8	10,3	9,3	7,3	4,3	3,3	1,104709	1,0315
ESL 3	-2,5	23,8	21,8	14,8	3,8	1,8	26,3	24,3	17,3	6,3	4,3	1,104709	1,0315
ESL 6	-2,5	19,8	18,8	11,8	4,8	2,8	22,3	21,3	14,3	7,3	5,3	1,104709	1,0315
ESL 10	-2,5	13,8	8,8	7,8	2,8	1,8	16,3	11,3	10,3	5,3	4,3	1,104709	1,0315
ESL 8	-2,5	14,8	13,8	9,8	5,8	4,8	17,3	16,3	12,3	8,3	7,3	1,104709	1,0315
ESL 7	-2,5	28,8	26,8	22,8	7,8	3,8	31,3	29,3	25,3	10,3	6,3	1,104709	1,0315
ESL 5	-2,5	27,8	25,8	18,8	6,8	4,8	30,3	28,3	21,3	9,3	7,3	1,104709	1,0315
ESL 9	-2,5	19,8	15,8	11,8	4,8	2,8	22,3	18,3	14,3	7,3	5,3	1,104709	1,0315
ESL 1	-2,5	24,8	21,8	16,8	7,8	3,8	27,3	24,3	19,3	10,3	6,3	1,104709	1,0315
ESL 11	-2,5	23,8	20,8	17,8	6,8	2,8	26,3	23,3	20,3	9,3	5,3	1,104709	1,0315
ESL 12	-2,5	15,8	12,8	8,8	4,8	2,8	18,3	15,3	11,3	7,3	5,3	1,104709	1,0315
ESL 13	-2,5	24,8	21,8	17,8	5,8	3,8	27,3	24,3	20,3	8,3	6,3	1,104709	1,0315

h'	*h'60s*	*h'180s*	*h'1.5h*	*h'24h*	q*30s*	q*60s*	q*180s*	q*1.5h*	q*24h*	c*30s*	c*60s*	c*180s*
13,9548	14,2828	14,4468	15,5948	15,5948	3,999309	3,999309	3,999309	4,489245	4,489245	5,655877	3,999309	3,265422
14,6108	14,7748	15,1028	15,5948	15,7588	4,092231	4,092231	4,092231	4,489245	4,512788	5,787288	4,092231	3,341292
11,9868	12,3148	13,4628	15,2668	15,5948	3,706592	3,706592	3,706592	4,441784	4,489245	5,241912	3,706592	3,026419
12,6428	12,8068	13,9548	15,1028	15,4308	3,806666	3,806666	3,806666	4,417862	4,465577	5,383438	3,806666	3,10813
13,6268	14,4468	14,6108	15,4308	15,5948	3,952028	3,952028	3,952028	4,465577	4,489245	5,589012	3,952028	3,226818
13,4628	13,6268	14,2828	14,9388	15,1028	3,928175	3,928175	3,928175	4,39381	4,417862	5,555278	3,928175	3,207341
11,1668	11,4948	12,1508	14,6108	15,2668	3,577565	3,577565	3,577565	4,345306	4,441784	5,05944	3,577565	2,921069
11,3308	11,6588	12,8068	14,7748	15,1028	3,60374	3,60374	3,60374	4,369625	4,417862	5,096457	3,60374	2,942441
12,6428	13,2988	13,9548	15,1028	15,4308	3,806666	3,806666	3,806666	4,417862	4,465577	5,383438	3,806666	3,10813
11,8228	12,3148	13,1348	14,6108	15,2668	3,681148	3,681148	3,681148	4,345306	4,441784	5,20593	3,681148	3,005645
11,9868	12,4788	12,9708	14,7748	15,4308	3,706592	3,706592	3,706592	4,369625	4,465577	5,241912	3,706592	3,026419
13,2988	13,7908	14,4468	15,1028	15,4308	3,904176	3,904176	3,904176	4,417862	4,465577	5,521338	3,904176	3,187746
11,8228	12,3148	12,9708	14,9388	15,2668	3,681148	3,681148	3,681148	4,39381	4,441784	5,20593	3,681148	3,005645

c*1.5h*	c*24h*	Co	P_{30s}	P_{60s}	P_{90s}	$P_{1.5h}$	P_{24h}	Msand	Mclay	$P_{50um\ (Sand)}$	$P_{2um\ (Clay)}$	P_{silt}
0,473208	0,118302	52,644	27,16359	23,36449	21,46493	8,168072	8,168072	10,9619	0	39,16461	8,168072	52,66732
0,473208	0,118922	52,644	19,56538	17,66583	13,86673	8,168072	6,268521	5,480948	1,375427	21,73812	10,15057	68,11131
0,468205	0,118302	52,644	49,95821	46,15911	32,86224	11,96718	8,168072	10,9619	2,761647	39,16461	15,97708	44,85832
0,465684	0,117678	52,644	42,36	40,46045	27,16359	13,86673	10,06762	5,480948	2,761876	25,59486	17,89188	56,51326
0,470713	0,118302	52,644	30,96269	21,46493	19,56538	10,06762	8,168072	27,40474	1,375482	85,65941	12,05747	2,283124
0,463148	0,116421	52,644	32,86224	30,96269	23,36449	15,76628	13,86673	5,480948	1,375654	29,45285	17,77866	52,76849
0,458035	0,117051	52,644	59,45597	55,65686	48,05866	19,56538	11,96718	10,9619	5,569167	43,08022	27,77409	29,14569
0,460599	0,116421	52,644	57,55642	53,75731	40,46045	17,66583	13,86673	10,9619	2,76235	45,03897	21,722	33,23904
0,465684	0,117678	52,644	42,36	34,7618	27,16359	13,86673	10,06762	21,92379	2,761876	72,17658	17,89188	9,931544
0,458035	0,117051	52,644	51,85776	46,15911	36,66135	19,56538	11,96718	16,44284	5,569167	58,63674	27,77409	13,58917
0,460599	0,117678	52,644	49,95821	44,25955	38,5609	17,66583	10,06762	16,44284	5,568193	56,64934	25,84203	17,50863
0,465684	0,117678	52,644	34,7618	29,06314	21,46493	13,86673	10,06762	16,44284	2,761876	56,64934	17,89188	25,45878
0,463148	0,117051	52,644	51,85776	46,15911	38,5609	15,76628	11,96718	16,44284	2,76211	58,63674	19,80685	21,55641
									Total % for all samples	**48,58787**	**18,51742**	**32,8947**

APPENDIX 4. BIOMETRIC DATA OF FISH ANALYSED

Code	Length (cm)	Weight (g)	CF (g cm^{-3})	Code	Weight (g)	Length (cm)	CF (g cm^{-3})
***Cyprinus carpio* (n = 51)**							
ELC 1	42	1416	1.98	**ELC 35**	1399	48	1.30
ELC 2	48	1521	1.34	**ELC 38**	870	46	0.92
ELC 3	34	628	1.59	**ELC 39**	211	25	1.31
ELC 4	33	587	1.67	**ELC 40**	1211	44	1.42
ELC 5	34	769	1.95	**ELC 41**	770	38	1.40
ELC 6	32	495	1.51	**ELC 42**	920	44	1.07
ELC 7	45	1399	1.55	**ELC 43**	802	39	1.35
ELC 8	32	592	1.80	**ELC 44**	920	41	1.33
ELC 9	34	680	1.73	**ELC 45**	802	39	1.35
ELC 10	37	837	1.67	**ELC 46**	1399	43	1.75
ELC 11	43	1204	1.51	**ELC 47**	903	38	1.71
ELC 12	32	531	1.60	**ELC 48**	1093	42	1.45
ELC 13	34	629	1.57	**ELC 49**	459	26	2.58
ELC 20	32	542	1.65	**ELC 50**	350	28	1.59
ELC 23	27	407	2.06	**ELC 51**	459	26	2.28
ELC 24	43	892	1.11	**ELC 52**	427	35	1.01
ELC 26	43	1152	1.44	**ELC 53**	313	27	1.55
ELC 27	45	1310	1.43	**ELC 54**	938	39	1.61
ELC 29	46	1105	1.17	**ELC 55**	727	36	1.50
ELC 30	46	1519	1.52	**ELC 56**	431	28	2.07
ELC 31	34	674	1.71	**ELC 57**	834	36	1.74
ELC 32	46	1143	1.17	**ELC 58**	895	41	1.34
ELC 33	36	786	1.68	**ELC 59**	988	46	0.98
ELC 36	43	783	1.02	**ELC 60**	535	28	2.48
ELC 37	41	767	1.15	**ELC 61**	613	34	1.51
ELC 34	46	1101	1.16				

APPENDIX 5. THE SAMPLING LOCATIONS OF BLUE MUSSELS IN THE PORT ELIZABETH HARBOUR AND BROW MUSSELS AT THE NORTH END LAKE OUTFALL INTO THE INDIAN OCEAN

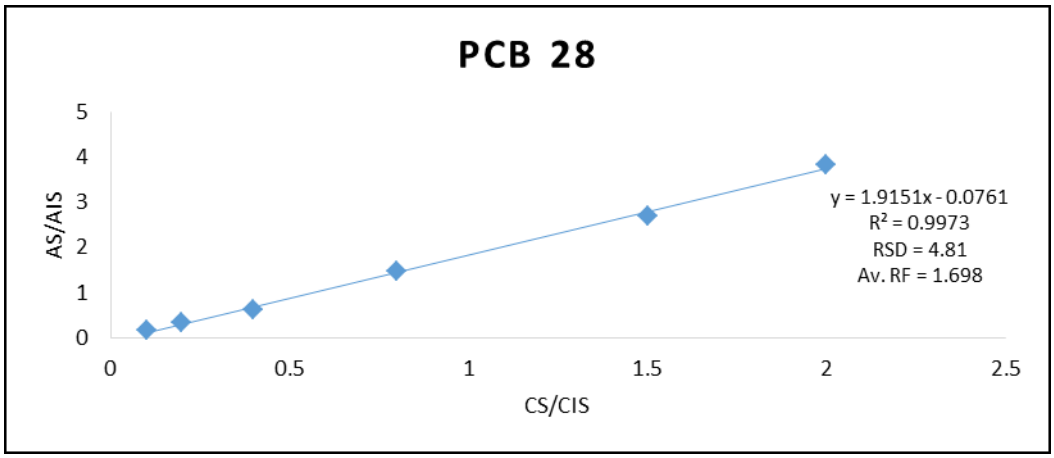
Station code	Sampling location of brown mussels		Station code	Sampling location of blue mussels	
	Latitude	Longitude		Latitude	Longitude
Site M1	33°56.250’	25°36.684’	**Site 3**	33˚57’38.55”	25˚38’8.38”
Site M2	33°56.238’	25°36.687’	**Site 4**	33˚57’54.98”	25˚37’47.13”
Site M3	33°56.200’	25°36.650’	**Site 6**	33˚58’8.42”	25˚38’8.02”
Site M4	33˚56.215’	25°36.700’	**Site 7**	33˚58’2.15”	25˚37’53.97”
Site M5	33˚56.264’	25°36.787’	**Site 8**	33˚57’51.10”	25˚38’36.49”
Site M6	33˚56.288’	25°36.696’			

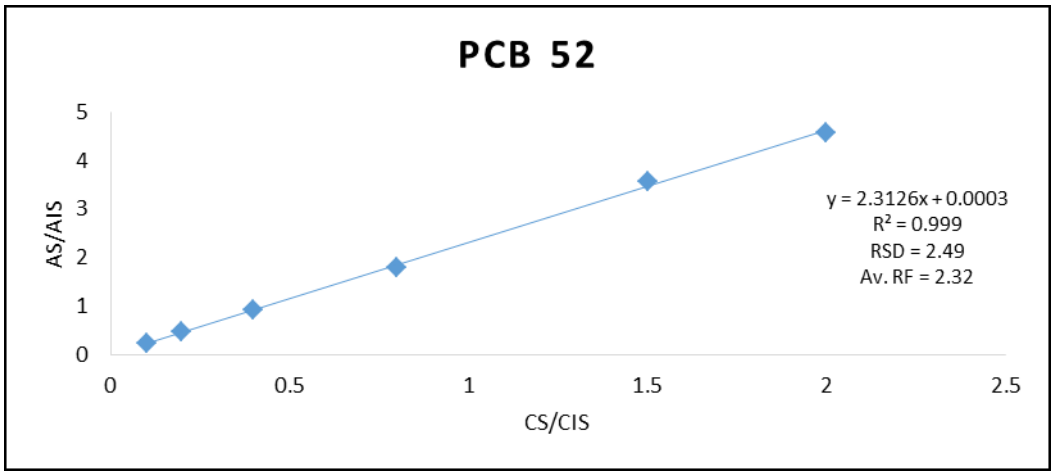
APPENDIX 6. THE SAMPLING LOCATIONS OF WATER

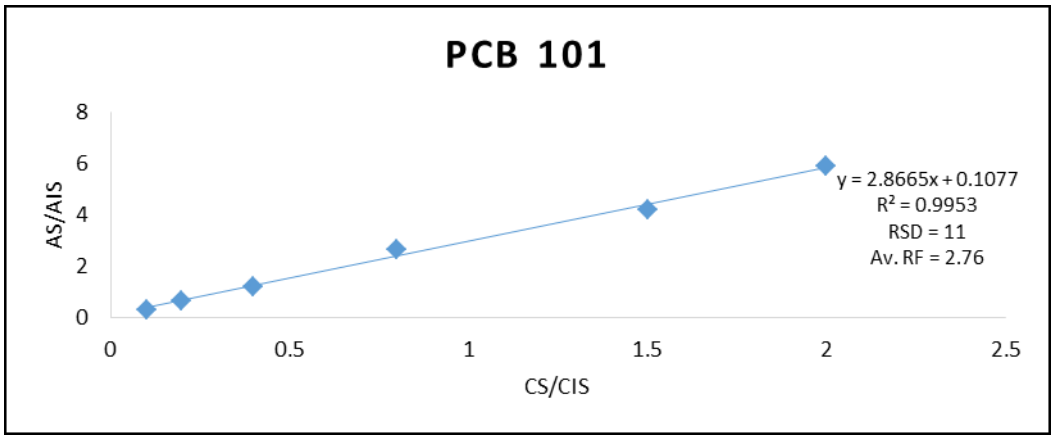
Site code	Latitude (S)	Longitude (E)	Comments
S1	33º56.172’	25º35.858’	Lake water
S2	33º56.297’	25º35.827’	Lake water
S3	33º56.298’	25º35.837’	Outflow water
S4	33º55.979’	25º35.773’	Lake water
S5	33º55.866’	25º35.493’	Lake water
S6	33º55.899’	25º35.475’	Inflow water
S7	33º56.029’	25º35.459’	Inflow water
S8	33º55.947’	25º35.472’	Inflow water
S9	33º56.001’	25º35.468’	Inflow water
S10	33º56.085’	25º35.466’	Inflow water
M1	33º56.250’	25º36.684’	Outflow water

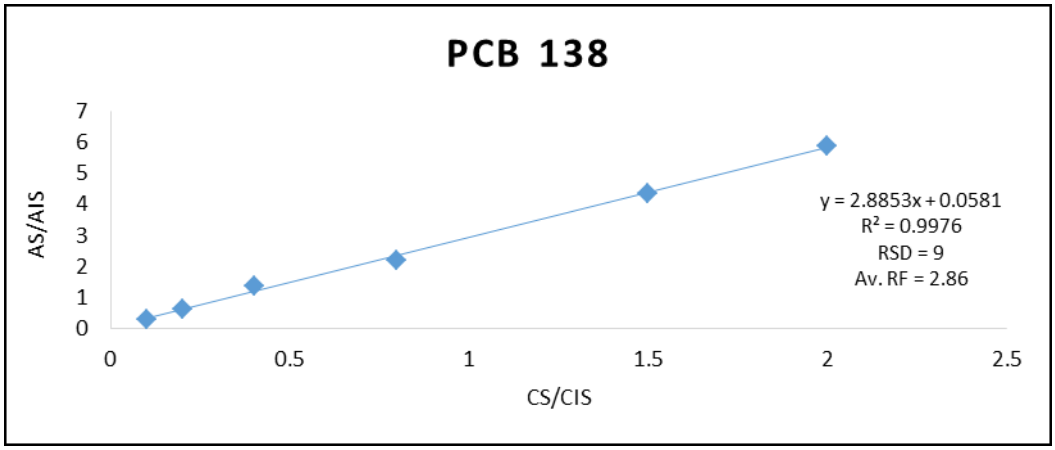
APPENDIX 7. LINEARITY OF THE SIX INDICATOR CONGENERS

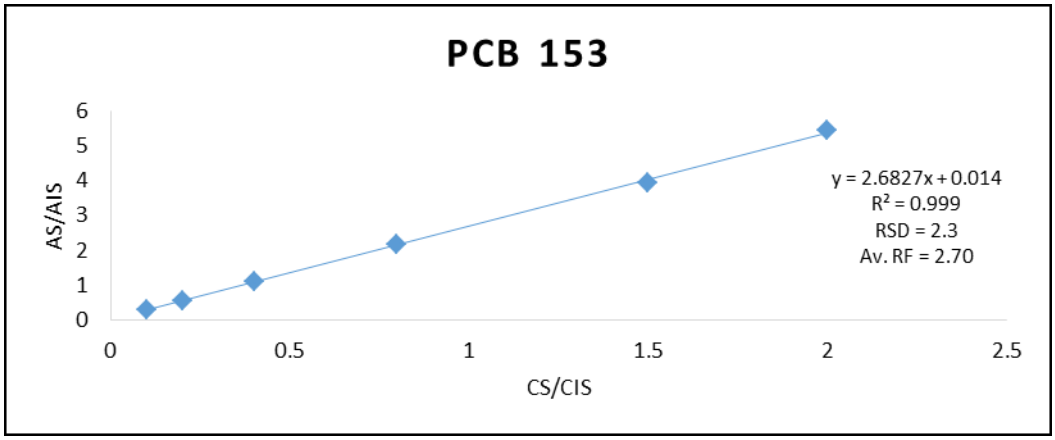
RSD = relative standard deviation, Av. RF =average response factor, (r^2 > 0.99 and RSD < 20%)

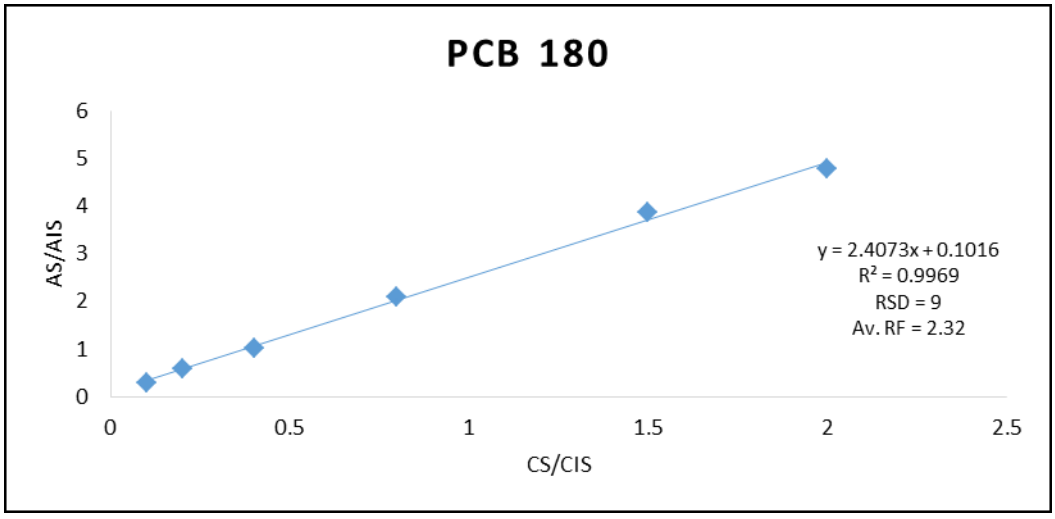







PCB 138
AS/AIS
CS/CIS
y = 2.8853x + 0.0581
R² = 0.9976
RSD = 9
Av. RF = 2.86


PCB 153
AS/AIS
CS/CIS
y = 2.6827x + 0.014
R² = 0.999
RSD = 2.3
Av. RF = 2.70


PCB 180
AS/AIS
CS/CIS
y = 2.4073x + 0.1016
R² = 0.9969
RSD = 9
Av. RF = 2.32

APPENDIX 8. PCB CONGENER CONCENTRATIONS FOR EACH OF THE TISSUES OF FISH

1. Concentrations of indicator PCB congeners and sum PCBs in *O. mossambicus* (n = 9)

Liver samples								
CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCBs ± SD
EFL 14	5.17	10.87	26.3	14.92	19.80	25.67	33.37	130.93 ± 8.24
EFL 15	4.64	13.26	14.55	16.16	28.01	23.51	12.13	107.62 ± 6.37
EFL 16	2.7	11.45	10.82	17.6	29.25	16.14	15.15	100.41 ± 6.68
EFL 17	3.56	14.84	17.89	15.23	26.37	22.32	12.14	108.79 ± 5.3
EFL 18	5.66	16.32	29.47	20.8	34.38	30.24	14.12	145.33 ± 8.28
EFL 19	3.47	12.5	13.77	22.49	31.18	28.33	16.97	125.24 ± 7.75
EFL 21	1.9	15.04	19.3	17.71	18.86	13.04	15.75	99.7 ± 2.43
EFL 22	1.3	32.02	12.68	12.7	16.85	27.59	25.72	127.56 ± 8.27
EFL 25	2.59	11.0	14.77	14.46	27.46	44.02	20.24	131.95 ± 12.23
Gill samples								
CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCBs ± SD
EFGi 14	4.63	14.32	8.32	8.3	13.32	6.67	13,28	64.21 ± 3.3
EFGi 15	0.96	8.28	2.78	4.33	2.91	2.58	5,36	26.24 ± 2.2
EFGi 16	3.68	5.21	3.6	5.23	13.23	11.05	2,14	40.46 ± 4.39
EFGi 17	1.75	7.06	7.44	8.24	6.69	6.1	10,27	45.8 ± 1.48
EFGi 18	2.02	10.51	5.52	2.01	6.68	9.3	11,17	45.19 ± 3.48
EFGi 19	1.84	1.01	6.01	4.31	8.08	7.29	7,38	34.08 ± 2.65
EFGi 21	4.08	15.16	9.19	10.43	8.42	15.06	9,09	67.35 ± 3.08
EFGi 22	3.6	7.42	12.35	15.04	15.67	13.66	10,01	74.15 ± 3.15
EFGi 25	2.88	10.44	8.07	7.18	6.39	10.05	8,39	50.52 ± 1.58
Gonad samples								
CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCBs ± SD
EFGo 14	5.82	33.48	17.34	21.08	8.56	19.74	18.37	100.2 ± 8.04
EFGo 15	3.73	12.84	8.25	7.42	9.55	4.46	8.31	42.52 ± 2.74
EFGo 16	3.03	6.91	4.58	11.0	3.89	9.82	13.67	36.2 ± 3.84
EFGo 17	5.59	17.22	14.03	15.82	15.86	6.77	13.67	69.7 ± 3.73
EFGo 18	3.74	2.32	3.4	5.34	9.74	14.0	9.51	34.8 ± 4.46
EFGo 19	2.13	5.15	3.44	7.58	15.67	9.98	10.68	41.82 ± 4.37
EFGo 21	3.15	4.03	4.54	6.52	16.35	13.68	9.27	45.12 ± 5.03
EFGo 22	1.34	5.44	5.52	7.81	2.97	15.03	4.16	36.77 ± 4.33

EFGo 25	0.9	3.15	4.14	4.92	10.48	6.32	9.15	29.01 ± 2.90
Muscle samples								
CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCBs ± SD
EFM 14	4.24	2.0	2.83	4.93	18.4	5.24	13.17	46.57 ± 6.55
EFM 15	2.3	17.12	6.8	3.74	5.6	6.61	2.88	42.75 ± 5.14
EFM 16	2.91	1.89	1.49	5.18	4.53	19.6	0.98	33.67 ± 7.06
EFM 17	3.06	9.32	4.45	7.95	6.53	6.0	8.29	42.54 ± 1.77
EFM 18	3.61	18.73	6.73	16.74	5.61	11.27	9.5	68.58 ± 5.31
EFM 19	2.09	2.64	4.04	9.55	1.36	4.58	13.48	35.65 ± 4.63
EFM 21	1.9	0.86	0.34	1.99	3.52	1.34	3.21	11.26 ± 1.28
EFM 22	2.2	2.88	3.1	4.35	1.78	1.81	2.49	16.41 ± 0.96
EFM 25	1.32	2.8	0.99	3.02	2.53	2.06	2.85	14.25 ± 0.76

2. Concentrations of indicator PCB congeners and sum PCBs in *C. carpio* (n = 51)

i. Muscle samples

Code	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
EFM 1	4.62	3.88	4.05	2.88	9.64	14.65	3.96	39.06 ± 4.66
EFM 2	5.37	7.77	4.69	2.73	11.52	23.12	4.31	54.14 ± 7.58
EFM 3	1.4	4.9	3.14	5.1	11.18	17.74	3.34	45.4 ± 5.78
EFM 4	1.89	1.96	3.33	8.67	2.15	4.46	9.18	17.29 ± 2.45
EFM 5	1.6	1.96	0.94	5.36	0.4	1.16	2.0	11.82 ± 1.77
EFM 6	2.92	5.79	4.59	2.15	10.37	5.44	4.0	34.3 ± 8.67
EFM 7	0.78	nd	1.73	4.61	2.19	2.69	3.84	15.06 ± 1.63
EFM 8	0.95	nd	4.07	nd	13.68	12.05	4.96	34.76 ± 5.87
EFM 9	0.45	2.66	0.4	0.66	0.24	0.52	18.14	22.62 ± 7.10
EFM 10	0.98	nd	3.41	8.77	18.22	4.53	0.67	35.6 ± 6.79
EFM 11	2.18	4.49	3.73	4.51	3.34	2.98	4.63	23.68 ± 0.70
EFM 12	1.08	nd	nd	nd	9.86	10.06	16.23	36.15 ± 6.99
EFM 13	1.83	7.18	4.54	3.83	4.24	5.89	4.77	30.45 ± 1.24
EFM 20	3.56	0.98	0.58	4.96	1.58	4.52	7.65	20.27 ± 2.79
EFM 23	2.59	4.75	2.67	1.57	0.46	2.1	4.02	15.57 ± 1.58
EFM 24	2.3	11.76	3.57	4.24	2.49	4.46	3.84	30.36 ± 3.35
EFM 26	2.13	nd	nd	9.12	15.51	5.36	6.52	36.51 ± 5.88
EFM 27	2.88	12.31	0.94	2.45	4.54	9.87	13.66	43.77 ± 5.36
EFM 29	1.38	3.68	3.88	3.26	0.87	8.96	nd	20.65 ± 2.96

Code	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
EFM 30	0.55	2.12	3.45	2.97	1.13	2.22	5.54	17.43 ± 1.52
EFM 31	2.31	nd	2.21	6.0	0.64	2.45	8.12	19.42 ± 3.07
EFM 32	2.86	nd	nd	11.95	6.4	1.81	5.47	25.63 ± 4.19
EFM 33	1.32	nd	nd	nd	4.09	11.25	5.31	20.65 ± 3.83
EFM 36	1.49	4.38	4.14	5.5	3.77	5.85	13.0	36.64 ± 3.47
EFM 37	0.85	12.16	nd	2.8	3.94	6.65	8.7	34.25 ± 3.76
EFM 34	0.7	nd	14.99	30.85	16.11	5.58	0.89	68.42 ± 11.73
EFM 35	2.46	6.02	nd	20.61	8.56	14.35	4.95	54.49 ± 7.35
EFM 38	2.38	4.67	6.45	7.89	13.63	20.51	4.53	57.68 ± 6.29
EFM 39	0.82	3.98	nd	4.16	12.19	13.69	2.03	36.05 ± 5.32
EFM 40	1.63	nd	nd	nd	13.21	16.78	11.89	41.88 ± 2.53
EFM 41	1.96	nd	nd	nd	11.49	15.55	7.13	34.17 ± 4.21
EFM 42	2.74	5.42	14.78	nd	17.61	24.49	12.8	75.1 ± 6.96
EFM 43	3.64	nd	10.08	15.3	10.86	13.33	7.97	57.54 ± 2.86
EFM 44	2.03	nd	6.98	10.74	15.72	18.59	5.33	57.36 ± 5.64
EFM 45	4.85	4.1	3.59	9.18	12.67	15.87	9.25	54.66 ± 4.77
EFM 46	3.92	1.06	3.2	9.56	10.93	12.87	2.01	39.63 ± 5.10
EFM 47	3.81	1.77	9.85	11.38	12.44	12.17	1.45	49.06 ± 5.17
EFM 48	3.67	nd	4.91	10.81	6.73	11.3	4.09	37.84 ± 3.33
EFM 49	3.37	nd	5.64	0.41	0.54	0.55	3.56	10.7 ± 2.36
EFM 50	2.5	13.48	4.02	0.73	4.69	0.42	0.13	23.47 ± 5.08
EFM 51	1.22	9.28	6.62	1.15	1.68	0.65	0.59	19.97 ± 3.70
EFM 52	1.62	18.95	6.71	2.72	1.75	1.28	1.82	33.23 ± 6.86
EFM 53	1.58	7.82	nd	1.4	3.85	3.67	0.79	17.53 ± 2.76
EFM 54	3.89	6.19	8.0	2.56	9.91	2.18	1.58	30.42 ± 5.66
EFM 55	2.61	nd	nd	11.47	11.09	17.01	2.31	41.88 ± 6.08
EFM 56	2.02	nd	13.54	2.46	11.61	10.14	1.33	39.08 ± 5.55
EFM 57	2.15	6.41	nd	nd	5.07	3.18	6.06	20.72 ± 1.45
EFM 58	2.22	nd	4.35	nd	nd	13.59	1.12	19.06 ± 6.17
EFM 59	2.23	3.55	nd	11.76	14.82	10.02	1.24	41.39 ± 5.70
EFM 60	3.4	nd	4.45	12.76	5.87	7.12	6.65	36.85 ± 3.18
EFM 61	1.57	nd	nd	5.86	4.56	6.45	4.67	21.54 ± 2.54

ii. Gill samples

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
EFGi 1	5.34	9.64	6.43	10.19	3.16	2.67	14.39	46.48 ± 4.52
EFGi 2	4.46	3.36	1.7	3.71	3.93	3.78	4.91	21.39 ± 1.05
EFGi 3	2.01	9.35	13.95	6.11	4.2	5.29	4.77	43.67 ± 3.74
EFGi 4	0.72	6.15	2.99	5.43	7.08	5.1	8.81	35.56 ± 1.96
EFGi 5	1.68	10.04	6.12	6.96	4.53	3.85	14.42	45.92 ± 3.96
EFGi 6	3.21	10.22	4.5	7.01	5.45	6.49	10.35	44.02 ± 2.44
EFGi 7	1.89	10.44	6.03	7.13	4.07	6.72	10.75	45.14 ± 2.6
EFGi 8	1.18	nd	5.44	2.18	8.95	6.34	6.83	29.74 ± 3.28
EFGi 9	0.84	7.97	1.9	9.91	5.95	2.32	8.41	36.46 ± 3.33
EFGi 10	2.86	9.29	4.03	11.73	3.22	4.68	10.27	43.22 ± 3.65
EFGi 11	1.1	7.24	4.94	4.61	2.38	2.43	4.73	26.33 ± 1.81
EFGi 12	1.68	5.98	2.53	2.24	2.57	8.39	4.48	26.19 ± 2.45
EFGi 13	1.36	9.17	3.76	7.63	4.49	7.14	10.35	42.54 ± 2.57
EFGi 20	2.53	9.27	7.17	3.59	3.02	7.07	9.88	40.0 ± 2.84
EFGi 23	1.79	nd	5.81	10.22	4.46	6.17	8.59	35.25 ± 3.55
EFGi 24	0.82	nd	2.45	2.23	4.57	1.7	2.78	13.73 ± 1.49
EFGi 26	2.24	5.19	4.31	6.28	2.13	3.91	4.66	26.48 ± 1.39
EFGi 27	1.98	nd	4.32	5.58	3.72	5.86	8.66	28.14 ± 2.86
EFGi 29	0.88	nd	nd	9.93	2.89	7.66	9.42	29.9 ± 4.59
EFGi 30	1.25	6.34	7.54	4.03	8.63	5.56	7.6	39,7 ± 1.66
EFGi 31	2.4	7.3	4.67	4.11	11.37	14.09	8.29	49.83 ± 3.86
EFGi 32	2.29	nd	nd	18.43	7.77	14.67	8.65	49.52 ± 7.5
EFGi 33	2.8	nd	nd	1.88	5.23	9.15	6.45	22.71 ± 3.75
EFGi 36	1.98	9.19	3.31	15.63	13.44	8.42	6.39	56.38 ± 4.52
EFGi 37	3.67	8.63	3.26	4.41	6.78	6.16	5.63	34.87 ± 1.87
EFGi 38	0.67	nd	3.2	nd	17.7	30.3	26.6	77.8 ± 13.71
EFGi 39	1.41	nd	9.24	nd	4.76	3.37	5.82	23.19± 3.57
EFGi 40	2.63	12.2	13.11	16.39	14.01	20.65	13.84	90.2 ± 3.08
EFGi 41	1.82	nd	3.06	nd	4.76	8.91	9.44	26.17 ± 4.16
EFGi 42	2.04	7.2	3.02	9.08	28.21	6.2	12.87	66.58 ± 8.99
EFGi 43	0.76	nd	2.98	10.0	10.87	10.0	8.94	42.79 ± 3.98
EFGi 44	3.51	11.88	12.94	25.72	22.79	26.1	6.8	106.23 ± 8.2
EFGi 45	1.2	nd	2.91	nd	7.53	16.37	2.9	29.71 ± 6.24
EFGi 46	1.74	10.0	2.87	10.2	14.95	13.92	11.89	63.83 ± 4.29
EFGi 34	5.47	14.1	11.52	20.56	12.1	17.49	7.53	83.3 ± 9.34
EFGi 35	5.45	12.19	7.46	9.61	17.71	14.24	5.88	67.09 ± 4.41
EFGi 47	3.88	nd	8.4	1.74	2.01	1.77	3.71	17.63 ± 2.92

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
EFGi 48	4.07	9.1	3.28	4.47	4.76	0.48	nd	22.09 ± 3.32
EFGi 49	1.67	12.33	4.31	0.43	1.37	0.51	1.1	20.05 ± 4.63
EFGi 50	4.91	21.34	18.7	10.96	11.68	9.42	6.44	78.54 ± 5.72
EFGi 51	4.24	8.18	9.96	10.37	10.91	13.68	9.98	63.08 ± 1.8
EFGi 52	1.04	nd	nd	2.67	3.63	3.98	3.07	13.35 ± 1.78
EFGi 53	3.55	13.78	20.56	11.93	11.94	12.41	12.96	83.58 ± 3.32
EFGi 54	2.71	3.18	13.12	11.87	14.44	12.01	10.6	65.22 ± 3.98
EFGi 55	2.86	10.0	14.9	4.72	14.26	14.75	1.21	59.84 ± 5.83
EFGi 56	3.94	5.07	10.09	6.05	7.11	11.4	1.39	41.11 ± 9.57
EFGi 57	1.71	nd	nd	9.73	8.91	10.64	8.09	37.37 ± 4.9
EFGi 58	7.19	7.82	9.68	16.77	11.44	11.28	13.63	70.62 ± 3.12
EFGi 59	3.59	9.23	6.49	3.61	9.45	5.5	5.33	39.61 ± 2.32
EFGi 60	0.96	8.04	7.64	3.84	13.34	6.88	2.68	42.42 ± 3.75
EFGi 61	3.28	7.32	4.83	3.28	8.4	5.32	2.47	31.62 ± 2.28

iii. Gonad samples

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
Gonad 1	4.55	6.49	5.64	7.41	13.6	5.19	10.28	36.48 ± 3.24
Gonad 2	5.15	5.64	2.95	17.11	11.4	14.91	7.28	50.7 ± 5.53
Gonad 3	1.9	4.25	6.1	5.22	7.95	3.1	6.47	22.74 ± 1.71
Gonad 5	1.92	15.85	10.86	4.0	16.83	6.37	11.3	49.36 ± 5.06
Gonad 7	2.9	10.0	7.58	3.83	3.84	15.36	1.97	42.58 ± 4.99
Gonad 8	4.0	9.76	11.63	4.77	9.64	6.15	9.64	51.59 ± 2.58
Gonad 9	3.45	3.87	3.33	10.64	3.58	10.75	12.8	44.97 ± 4.35
Gonad 10	3.42	10.14	7.6	9.57	15.08	6.78	10.45	59.62 ± 2.91
Gonad 12	2.61	10.4	11.22	10.15	7.88	9.41	13.42	62.48 ± 1.85
Gonad 13	2.05	9.4	8.73	4.34	4.1	2.58	4.52	33.67 ± 2.77
Gonad 20	0.75	3.34	2.11	7.0	11.74	3.73	18.37	46.29 ± 6.27
Gonad 24	2.92	4.63	4.32	9.53	2.59	10.88	1.42	33.37 ± 3.81
Gonad 26	5.66	4.83	7.91	5.42	6.48	12.09	7.91	44.64 ± 2.60
Gonad 27	4.53	5.69	4.7	12.55	17.71	16.75	1.6	59.0 ± 6.76
Gonad 29	2.75	nd	nd	15.68	8.63	13.9	11.36	49.57 ± 6.83
Gonad 30	5.48	5.68	5.66	2.34	4.75	4.68	3.75	26.86 ± 1.27
Gonad 31	3.67	13.6	14.97	3.47	6.05	8.14	22.68	68.91 ± 7.03
Gonad 32	3.85	5.79	6.16	14.24	11.94	26.91	11.34	76.38 ± 7.71
Gonad 33	0.89	nd	nd	10.18	10.31	15.57	13.3	49.36 ± 6.68

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
Gonad 36	3.96	14.63	13.75	8.12	18.68	13.33	11.44	79.95 ± 3.50
Gonad 37	1.45	10.29	6.13	6.83	10.19	16.86	6.91	57.21 ± 4.01
Gonad 35	4.9	17.23	18.26	20.95	20.17	25.15	22.19	123.95 ± 2.84
Gonad 38	4.84	12.49	12.27	17.8	10.16	17.05	26.86	96.63 ± 6.04
Gonad 39	2.13	4.73	7.67	12.67	11.88	11.27	8.12	56.34 ± 3.06
Gonad 40	2.76	1.05	5.89	19.15	22.42	12.42	6.16	67.09 ± 8.33
Gonad 41	2.63	10.16	10.01	6.76	16.12	22.22	8.12	73.27 ± 5.85
Gonad 42	3.98	13.19	12.0	17.78	23.13	19.28	10.01	92.26 ± 4.99
Gonad 43	2.26	16.56	10.4	12.5	17.66	19.99	12.03	89.14 ± 3.76
Gonad 44	3.73	10.88	25.84	7.63	27.94	16.31	7.81	96.41 ± 8.98
Gonad 45	2.38	10.66	9.66	17.93	15.68	16.23	10.09	80.25 ± 3.64
Gonad 46	2.48	5.18	14.47	16.52	19.3	23.84	14.56	93.87 ± 6.22
Gonad 47	2.68	5.5	11.36	8.05	13.46	13.02	20.47	71.86 ± 5.16
Gonad 48	5.71	21.98	3.35	10.61	11.39	10.77	25.41	83.51 ± 8.20
Gonad 49	1.8	11.05	17.32	11.76	11.99	11.12	3.03	66.27 ± 4.58
Gonad 50	1.76	6.31	2.78	8.69	2.42	1.37	1.69	23.26 ± 2.95
Gonad 51	1.59	6.48	8.46	2.69	3.89	2.53	2.44	26.49 ± 2.50
Gonad 52	0.35	7.3	5.2	2.46	12.06	6.17	2.31	35.5 ± 3.61
Gonad 53	1.15	8.94	9.07	12.01	12.88	12.15	10.74	65.79 ± 1.67
Gonad 54	2.12	nd	nd	6.11	12.33	16.79	5.74	40.97 ± 6.70
Gonad 55	1.96	nd	nd	nd	9.3	8.0	3.16	22.46 ± 4.26
Gonad 56	0.93	7.26	3.84	7.14	9.82	6.87	1.92	39.01 ± 2.81
Gonad 57	1.93	2.68	2.1	6.2	11.72	8.7	2.94	34.34 ± 3.88
Gonad 58	2.52	13.41	8.01	8.99	10.02	14.9	6.29	61.62 ± 3.28
Gonad 59	0.46	8.83	7.76	4.98	6.33	11.2	5.6	44.7 ± 2.32
Gonad 60	0.42	5.7	4.3	5.31	5.9	11.13	2.91	35.25 ± 2.80
Gonad 61	1.87	3.18	3.12	7.12	10.19	6.62	1.68	32.01 ± 3.20

iv. Liver samples

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
Liver 1	6.67	5.11	10.80	15.06	37.29	29.11	9.42	106.79 ± 12.61
Liver 2	7.97	19.93	26.69	16.80	19.31	35.84	12.92	131.49 ± 8.18
Liver 3	0.71	14.02	7.03	22.74	13.65	12.02	22.73	92.19 ± 6.23
Liver 4	2.46	24.08	14.97	5.98	8.49	18.98	16.79	89.29 ± 6.71
Liver 5	2.08	26.04	19.79	5.95	4.56	14.4	23.01	93.75 ± 8.92
Liver 6	1.85	4.68	13.95	9.50	11.24	10.63	16.66	66.66 ± 4.08

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
Liver 7	4.17	13.27	23.26	8.72	5.77	13.64	13.75	78.41 ± 5.95
Liver 8	3.70	13.94	12.73	19.01	4.95	14.17	6.99	71.79 ± 5.16
Liver 9	2.94	10.79	10.98	19.08	14.30	5.98	7.61	68.74 ± 4.73
Liver 10	2.05	11.77	16.56	13.20	11.97	18.82	3.33	75.65 ± 5.32
Liver 11	4.55	12.57	14.96	26.42	20.2	27.63	24.22	126.00 ± 6.19
Liver 12	1.96	12.40	18.65	6.49	17.35	12.69	25.36	92.94 ± 6.47
Liver 13	2.72	28.47	18.35	8.52	7.62	18.92	2.97	84.85 ± 9.43
Liver 20	2.01	21.32	13.95	8.56	19.14	9.42	14.64	87.03 ± 5.09
Liver 23	1.56	15.99	12.01	27.77	5.94	22.3	2.27	86.58 ± 9.60
Liver 24	2.45	23.74	13.35	14.50	26.72	22.28	22.61	123.20 ± 5.37
Liver 26	4.58	18.25	23.27	15.05	19.13	24.59	12.97	113.26 ± 4.52
Liver 27	4.95	15.04	5.51	5.92	30.5	22.87	12.18	92.02 ± 9.82
Liver 29	5.08	6.18	10.21	21.44	19.18	22.29	25.48	104.78 ± 7.57
Liver 30	7.48	18.71	20.96	8.58	14.11	22.60	21.36	106.32 ± 5.39
Liver 31	1.73	14.75	15.98	24.81	4.81	11.23	24.78	96.36 ± 7.80
Liver 32	1.64	16.14	11.50	20.85	23.14	36.14	34.71	142.48 ± 9.90
Liver 36	2.42	10.74	10.00	20.67	34.22	21.14	29.54	126.31 ± 9.74
Liver 37	3.20	6.12	10.35	26.11	32.51	22.10	35.56	132.75 ± 11.82
Liver 34	4.0	10.08	10.01	30.01	27.74	26.55	22.08	126.47 ± 8.93
Liver 38	4.92	2.12	3.43	15.42	28.02	28.12	28.17	105.28 ± 12.46
Liver 39	4.65	9.62	2.11	11.12	38.93	31.74	29.26	122.78 ± 14.75
Liver 40	0.72	4.11	4.02	2.03	13.05	6.32	10.52	40.05 ± 4.26
Liver 41	2.11	10.22	11.78	5.67	43.25	36.04	27.35	114.31 ± 18.84
Liver 42	1.72	6.98	12.05	20.10	28.18	38.08	12.45	117.84 ± 11.69
Liver 43	4.39	12.34	18.00	18.01	19.16	29.15	18.47	135.13 ± 4.94
Liver 44	2.73	9.8	12.10	19.00	19.22	9.78	18.27	88.17 ± 4.62
Liver 45	3.88	17.84	12.34	13.15	14.54	15.46	22.45	95.78 ± 3.71
Liver 46	4.13	12.58	28.04	25.36	20.48	20.66	19.75	126.87 ± 5.32
Liver 35	4.62	10.26	20.10	26.68	28.82	21.49	15.42	122.77 ± 6.92
Liver 47	3.96	29.76	20.51	2.88	26.63	14.48	2.83	97.09 ± 11.58
Liver 48	4.39	13.56	10.01	17.68	18.59	15.38	2.87	78.09 ± 5.84
Liver 49	0.45	7.14	19.34	12.62	12.65	5.85	6.95	64.55 ± 5.15
Liver 50	1.77	10.00	9.89	8.73	11.29	9.26	8.38	57.55 ± 1.05
Liver 51	0.74	10.32	2.02	10.07	23.03	15.51	8.98	59.93 ± 8.47
Liver 52	0.72	7.32	8.31	8.31	15.74	9.76	5.80	55.24 ± 3.46
Liver 53	0.69	4.12	2.12	6.72	18.51	14.41	6.62	52.50 ± 6.35
Liver 54	1.31	10.42	14.70	13.19	29.79	17.66	8.30	94.06 ± 7.65
Liver 55	1.77	22.10	14.37	3.28	19.31	14.18	9.88	93.22 ± 9.73
Liver 56	1.91	10.00	17.09	11.33	29.26	22.99	9.06	99.73 ± 8.12

CODE	Lipid content (%)	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs ± SD
Liver 57	3.35	0.55	0.15	10.74	46.39	18.40	8.86	85.09 ± 17.19
Liver 58	3.17	10.00	13.02	27.79	23.47	23.78	9.16	107.22 ± 8.07
Liver 59	3.85	17.89	9.91	22.55	34.60	11.58	6.36	102.89 ± 10.34
Liver 60	2.8	15.48	7.05	17.75	24.62	26.70	5.51	97.11 ± 8.74
Liver 61	1.83	0.30	0.12	11.94	23.64	10.36	8.61	54.97 ± 8.71
Average	3.03	12.78	12.77	14.88	20.90	19.27	15.09	95.69 ± 3.39

APPENDIX 9: PCB CONGENER CONCENTRATIONS OF BLUE MUSSELS

(*Mytilus galloprovincialis*, n = 60) at five different sites from the Port Elizabeth Harbour

SITE 6							
Sample code	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCBs ± SD**
MS 1	8.68	5.96	1.34	3.03	1.5	8.52	29.03 ± 3.35
MS 2	nd	8.65	2.04	4.14	2.66	1.04	18.53 ± 3.03
MS 3	nd	nd	3.01	5.16	3.74	1.56	13.56 ± 2.10
MS 4	nd	nd	nd	14.46	6.86	3.73	25.05 ± 5.75
MS 5	nd	6.82	1.74	3.41	1.63	0.91	14.51 ± 2.43
MS 6	8.43	9.11	2.26	4.72	2.48	1.21	28.21 ± 3.36
MS 7	nd	7.29	1.41	3.29	9.7	0.81	22.50 ± 3.91
MS 8	nd	nd	3.38	9.29	3.7	11.12	27.49 ± 4.67
MS 9	8.58	6.12	1.53	4.09	1.53	5.48	27.33 ± 2.76
MS 10	nd	nd	nd	9.74	5.67	4.92	20.33 ± 4.06
MS 11	nd	nd	nd	nd	4.66	1.68	6.34 ± 1.89
MS 12	nd	nd	nd	9.23	9.68	4.19	22.8 ± 4.56
SITE 3							
Sample code	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCBs ± SD**
MS 13	nd	nd	nd	6.94	10.77	1.75	19.46 ± 4.56
MS 14	3.13	5.13	16.04	6.47	1.26	0.6	32.63 ± 5.65
MS 15	4.42	2.54	1.11	2.80	1.24	nd	12.11 ± 1.56
MS 16	0	nd	nd	9.97	8.85	1.88	20.7 ± 4.69
MS 17	2.82	6.08	10.26	4.16	1.52	0.49	25.33 ± 3.55
MS 18	nd	nd	nd	5.0	6.57	5.06	16.63 ± 3.09
MS 19	nd	6.86	1.49	2.66	11.12	0.87	23.00 ± 4.30
MS 20	nd	nd	nd	nd	nd	9.16	9.16 ± 3.74
MS 21	nd	nd	nd	nd	nd	3.48	3.48 ± 1.42
MS 22	nd	nd	nd	nd	nd	4.06	4.06 ± 1.66
MS 23	nd	nd	1.47	5.99	9.16	1.63	18.25 ± 3.72
MS 24	nd	nd	nd	11.64	nd	4.31	15.95 ± 4.73
SITE 7							
Sample code	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCBs ± SD**
MS 25	1.18	nd	nd	5.81	9.48	4.49	20.96 ± 3.79
MS 26	nd	nd	nd	9.19	nd	nd	9.19 ± 3.75
MS 27	1.89	1.18	1.89	7.26	9.62	2.66	24.5 ± 3.49
MS 28	nd	nd	3.92	6.99	11.04	1.33	23.28 ± 4.42
MS 29	1.98	1.35	2.87	3.97	7.34	3.75	21.26 ± 2.11
MS 30	nd	nd	nd	nd	nd	5.53	5.53 ± 2.26

MS 31	7.13	1.07	1.40	2.20	1.72	1.70	15.22 ± 2.28
MS 32	2.13	1.25	5.99	11.57	13.84	8.85	43.63 ± 5.07
MS 33	nd	nd	nd	7.87	5.41	3.41	16.69 ± 3.36
MS 34	1.93	1.08	nd	nd	nd	2.92	5.93 ± 1.23
MS 35	0.57	0.58	5.72	6.53	9.39	4.30	27.09 ± 3.47
MS 36	nd	nd	3.17	8.81	nd	2.69	14.67 ± 3.44
SITE 4							
Sample code	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCBs ± SD**
MS 37	nd	nd	nd	6.55	3.42	1.64	11.61 ± 2.64
MS 38	nd	8.38	1.22	2.13	2.15	1.61	15.49 ± 2.95
MS 39	nd	nd	nd	9.79	nd	4.76	14.55 ± 4.08
MS 40	9.35	5.97	0.74	1.43	6.28	1.52	25.29 ± 3.49
MS 41	nd	nd	nd	nd	nd	2.64	2.64 ± 1.08
MS 42	nd	nd	nd	7.92	5.01	2.88	15.81 ± 3.30
MS 43	nd	nd	0.88	nd	nd	2.78	3.66 ± 1.12
MS 44	nd	nd	nd	9.12	nd	2.18	11.3 ± 3.65
MS 45	nd	nd	nd	6.74	6.17	2.39	15.3 ± 3.17
MS 46	10.54	6.64	0.56	7.10	1.24	1.31	27.39 ± 4.10
MS 47	nd	nd	nd	7.69	5.28	4.72	17.69 ± 3.38
MS 48	nd	6.12	1.06	2.89	1.13	1.78	12.98 ± 2.16
SITE 8							
Sample code	**PCB 28**	**PCB 52**	**PCB 101**	**PCB 138**	**PCB 153**	**PCB 180**	**ΣPCBs ± SD**
MS 49	nd	nd	5.05	7.69	nd	3.36	16.2 ± 3.25
MS 50	nd	1.86	3.52	5.26	7.85	3.10	21.59 ± 2.72
MS 51	nd	nd	nd	6.06	8.46	3.08	17.60 ± 3.64
MS 52	nd	nd	10.74	4.82	6.14	5.18	26.88 ± 4.07
MS 53	nd	nd	nd	9.06	7.95	4.25	21.26 ± 4.20
MS 54	2.59	nd	nd	5.33	4.35	1.98	14.25 ± 2.20
MS 55	nd	10.85	2.58	5.75	3.58	2.53	25.29 ± 3.74
MS 56	8.45	nd	3.91	12.43	6.92	4.78	36.49 ± 4.24
MS 57	6.45	14.74	4.81	3.74	3.70	2.44	35.88 ± 4.50
MS 58	nd	nd	nd	3.69	6.47	2.62	12.78 ± 2.65
MS 59	nd	nd	nd	nd	nd	6.05	6.05 ± 2.47
MS 60	nd	nd	2.86	6.56	10.01	2.74	22.17 ± 3.92

APPENDIX 10: PCB CONGENER CONCENTRATIONS OF BROWN MUSSELS

(*Perna perna*, n = 35) at six different sites from the point source of the NEL outflow into the Indian Ocean.

Sites	PCB 28	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	ΣPCBs
SITE 1							
M 1	6.51	8.94	1.64	6.07	4.94	2.94	31.04
M 1a	7.36	8.45	3.20	4.85	5.49	2.82	34.17
M 1b	2.59	5.09	3.86	7.8	6.37	3.5	27.21
M 1c	3.46	5.48	2.02	5.44	5.11	3.52	25.03
M 1d	4.45	6.43	3.01	6.31	9.88	2.94	33.02
M 1e	6.42	7.51	4.32	7.67	8.44	3.22	37.58
Av. ± SD	5.13 ± 1.91	6.98 ± 2.60	3.01 ± 1.03	6.36 ± 1.18	6.71 ± 2.02	3.16 ± 0.30	31.34 ± 4.62
SITE 2							
M 2	5.31	4.53	3.4	7.3	8.69	1.55	30.78
M 2a	3.72	3.61	2.11	7.28	8.31	2.93	27.96
M 2b	2.72	6.04	1.49	6.25	5.53	2.96	24.99
M 2c	4.28	3.76	2.71	5.06	6.16	3.92	25.89
M2d	3.33	5.56	3.02	4.31	5.98	3.42	25.62
M 2e	5.14	6.77	4.58	6.19	8.22	4.05	34.95
Av. ± SD	**4.08 ± 1.02**	**5.05 ± 1.28**	**2.89 ± 1.07**	**6.07 ± 1.2**	**7.15 ± 1.4**	**3.14 ± 0.91**	**28.37 ± 3.86**
SITE 3							
M 3	8.93	2.91	4.53	2.34	5.79	4.1	28.60
M 3a	1.76	0.72	3.11	2.42	2.63	4.47	15.11
M 3b	3.77	2.29	4.57	5.62	4.88	2.23	23.36
M 3c	2.89	6.85	2.53	3.38	3.48	3.92	23.05

M 3d	2.41	3.87	1.32	4.56	5.37	2.28	19.81
M 3e	3.67	4.48	2.64	5.21	3.32	1.87	21.19
Av. ± SD	**3.91± 2.58**	**3.52 ± 2.09**	**3.12 ± 1.26**	**3.92 ± 1.41**	**4.25 ± 1.27**	**3.15 ± 1.14**	**21.85 ± 4.46**
SITE 4							
M 4	5.5	6.98	1.65	4.17	2.29	0.98	21.57
M 4a	4.2	3.93	2.31	3.1	5.3	1.28	20.12
M 4b	1.72	2.61	2.62	4.67	4.63	2.86	19.11
M 4c	1.92	2.46	1.41	5.62	3.8	2.49	17.7
M 4d	2.98	3.25	2.11	7.13	6.52	3.18	25.17
M 4e	4.16	3.91	1.67	4.12	4.44	3.11	21.41
Av. ± SD	**3.41 ± 1.47**	**3.86 ± 1.65**	**1.96 ± 0.46**	**4.80 ± 1.40**	**4.50 ± 1.42**	**2.32 ± 0.96**	**20.85 ± 2.57**
SITE 5							
M 5	**4.99**	**6.71**	**1.83**	**7.59**	**6.5**	**2.18**	**29.80**
M 5a	4.2	5.08	3.07	5.59	7.14	2.84	27.92
M 5b	3.85	4.29	2.75	5.82	6.14	2.83	25.68
M 5c	4.43	4.23	3.05	5.95	8.77	4.7	31.13
M 5d	3.28	5.46	3.03	6.77	7.82	3.92	30.27
M 5e	4.2	4.83	2.96	6.12	6.32	3.94	28.37
Av. ± SD	**4.16 ± 0.57**	**5.10 ± 0.92**	**2.78 ± 0.48**	**6.31± 0.74**	**7.12 ± 1.02**	**3.40 ± 0.94**	**28.86 ± 4.67**
SITE 6							
M 6	3.82	5.59	1.35	3.03	6.14	1.84	21.77
M 6a	4.04	7.26	3.05	5.27	6.74	2.87	29.23
M 6b	2.71	4.89	2.27	6.22	7.42	3.56	27.07
M 6c	3.67	4.44	3.02	5.45	6.87	2.17	25.62
M 6d	2.63	3.42	2.66	4.38	6.12	3.81	23.02

APPENDIX 11: TECHNICAL EDITOR CLEARANCE

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4 August 2015

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The thesis submitted by Edwige Kampire for the degree of Philosophiae Doctor in the Faculty of Science has been edited by me with regard to editing and layout of the document.

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