MONITORING AND RISK ASSESSMENT OF POLYCHLORINATED BIPHENYLS (PCBs) LEVELS IN SOIL CONTAMINATED BY OIL SPILLAGES FROM TRANSFORMERS IN SOUTH AFRICA

by

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Declaration

I declare that this research report is my own, unaided work. It is being submitted for the degree of Master of Science to the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination at any other university.

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Abstract

PCBs are chemical compounds which were synthesised in the laboratory in the 1920s. They are classified under the category of POPs. They were mainly used in electrical equipments and transformers as the insulating material. PCBs were released to the environment in the form of oil spillages, combustion of PCB-containing equipments and others. Its production was banned in the 1970s after its toxic, persistent, bioaccumulative and carcinogenic behaviour was discovered. This study was conducted to determine and monitor the level of PCB in soil contaminated by oil spillages from pole mounted transformers in Polokwane, Limpopo, South Africa after sites have been remediated. Seventy eight soil samples were collected from five sites. The QuEChERS extraction method and GC-MS was used to extract and analyse PCBs. The PCB congeners targeted in this study are PCB-180, PCB-158 and PCB-101. The concentration of PCB-180 ranges between 10.02 and 78.30 µg kg $^{-1}$, PCB-158 ranges between 3.89 and 45.36 µg kg $^{-1}$ and PCB-101 ranges between 2.42 and 39.12 µg kg $^{-1}$. The PCB congener with the highest concentration at all sites is PCB-180 followed by PCB-158 while PCB-101 has the least concentration; this order is consistent in all sampling sites. PCB concentrations after bioremediation were found to be extremely higher than concentrations before bioremediation which suggest that the bioremediation process was not efficient including actual analytical methods used. In comparison to the range of the PCB levels reported in literature, the PCB concentration determined from this study is found within a higher range. The PCBs concentration at all five sites was found to be below the legal limits.

Dedication

I am dedicating this research to my mother. She has been my pillar of strength and taught me that I should never let my circumstances determine who I become as an individual.

“Your present circumstances don’t determine where you can go; they merely determine where you start-Nido Qubein”

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DEFINITION OF TERMS

BIODEGRADATION/BIOREMEDIATION- The use of microorganisms such as bacteria and fungi to degrade and remove pollutants in the environment.

HYDROPHOBIC-Does not easily dissolve in water.

LIPOPHILIC-Dissolve in lipids, fats and oils.

PCB- a group of chlorinated chemical compounds which are formed when two benzene rings are linked by a single bond of carbon-carbon.

QuEChERS METHOD- acronym for Quick, Easy, Cheap, Effective, Rugged and Safe method. It is a technique developed by Department of Agriculture in the United States to extract pesticides from food.

LIST OF ABBREVIATIONS

ACN- Acetonitrile

ANZECC- Australian and New Zealand Environment and Conservation Council

ATDSR- Agency for Toxic Substances and Disease Registry

CBAs- Chlorobenzoates

CRM-Certified reference material

EPA-Environmental Protection Agency

GC-MS-Gas Chromatography Mass spectrometry

IARC- International Agency for Research on Cancer

IUPAC-International Union of Pure and Applied Chemistry

LOD- Limit of detection

LOQ- Limit of quantification

NAVFAC EXWC- Naval Facilities Engineering command Engineering and Expeditionary Warfare Center

NEMA-National Environmental Management Act

NOAA -National Oceanic and Atmospheric Administration

PCB-Polychlorinated biphenyls

POPs- Prevalent Organic Pollutants

PSA- primary second amine

PTFE- Polytetrafluoroethylene

RSD-relative standard deviation

SRM- Standard reference material

TCLP-Toxicity Characteristics Leaching Procedures

TSCA-Toxic Control Substance Act

UNEP- United Nations Environment Programme

USDHHS-United States Department of Health and Human Services

USEPA-United States Environmental Protection Agency

WHO-World Health Organisation

CHAPTER ONE-INTRODUCTION

1.1 Background

Polychlorinated biphenyls (PCBs) are synthetic compounds (Martinez et al., 2005) which are categorised as Persistent Organic Pollutants (POPs) (Phylicia, 2013). PCB oil was used as cooling liquid in electrical equipment and transformers (Ahlborg et al., 1992; Gray 2004). The use of PCB in transformer oil has contributed to PCB contamination in the environment (NOAA, 1991). PCBs are harmful to human health and the environment (Nwinyi, 2010; EPA, 2012); they are carcinogenic (Darážová et al., 2016) and bioaccumulate throughout the food chain (Martinez et al., 2005; UNEP, 2007).

Eskom as a power utility in South Africa makes use of transformers which are found mounted on poles as well as within the substations. Some of these transformers contain PCB oil which is used as insulating material (Amdany et al., 2014). There are thousands of pole mounted transformers throughout Limpopo province, and due to electrical faults, lightning, vandalism and explosions, PCB oil spillages occurs on bare ground.

The bioremediation company collect soil samples from the oil contaminated sites and conduct the laboratory test for PCB level. Whenever PCB concentration is 20 µg kg^{-1} or less, in-situ treatment of the contaminated site is conducted, and when the PCB level is more, the contaminated soil is excavated and disposed of at registered disposal site for further treatment. Based on the oil spill investigation reports compiled by the bioremediation company; two sites were found to have PCB concentration of more than 20 µg kg^{-1} and in-situ treatment was conducted. This study focuses on sites where in-situ treatment was conducted.

Monitoring of PCBs in the environment is critical due to their toxic, carcinogenic and bioaccumulative nature. In situ-remediation was conducted on sites contaminated with PCB oil using Spill-Sorb, however no studies were done to

determine if the bioremediation method used was effective. Furthermore, there are no studies done to conclude if remediation has decomposed or immobilised all PCBs and if some low levels could still be contaminating the surrounding environment.

CHAPTER TWO-LITERATURE REVIEW

2.1 Nomenclature and Properties of PCBs

Pereira (2004) defines PCBs as a group of chlorinated chemical compounds which are formed when two benzene rings are joined by a single bond of carbon-carbon. PCBs can also be defined as a substance consisting of two phenyl molecules bonded with two or more hydrogen atoms which are substituted by chlorine atoms (Gray, 2004). The chemical formula is shown as: $C_{12}H_{10}$-nCl when n is 1-10 (Australian and New Zealand Environment and Conservation Council [ANZECC] 2003; Gray, 2004; Shiu et al., 1986). The degree at which the chlorine atoms are substituted for the hydrogen around the biphenyl structure is varied (Crine, 1988). The placement of chlorine atoms at the different corners of the carbon rings create up to 209 individual chlorinated biphenyl compounds called congeners (Pereira, 2004; Shiu et al., 1986; USEPA, 2011).

The chemical structure of PCB indicates the combination of various congeners whereby PCB consists of definite number of chlorine molecules (Gray, 2004).
Gray (2004) and Agency for Toxic Substances and Disease Registry [ATDSR] (2000) indicated that PCB composes of carbon, hydrogen and chlorine atoms. Aroclor is used to describe four digit numbers such as Aroclor 1242 or 1260; the number of carbon atoms is presented by the first two digits while the last two represent the weight percentage of chlorine atoms (EPA, 2012).

PCBs are man-made compounds which were produced by chlorinating the biphenyl molecule (Naval Facilities Engineering command Engineering and Expeditionary Warfare Center [NAVFAC EXWC], 2012). During the production of PCBs, 1-10 chlorine atoms are placed on any available substitution place on the biphenyl structure which has six sides as presented on figure 2.1. The 209 possible congeners are differentiated by making use of a system proposed by Ballschmiter and Zell in 1980 (Pereira, 2004). Congeners refers to any chemical compound within the PCBs class, the congener name is determined by the number of chlorine

substituents and the position of each chlorine atom (NAVFAC EXWC, 2012). These congeners have different chemical and physical characteristics which depend on the position and extent of chlorination (USEPA, 2011).

Previous methods differentiated congeners by positions where chlorine was substituted; six corners of biphenyl structures were numbered therefore identifying the congener by the number at which the substituted chlorine was positioned. Figure 2.1 shows 2,6,2',6' positions as ortho, 3,5,3',5' as meta and 4, 4'as para (NAVFAC EXWC, 2012)

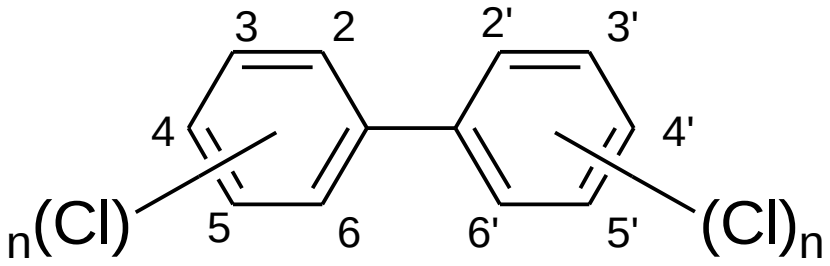

Figure 2.1: Chemical structure of PCB (Adapted from Grabowska, 2010)

NAVFAC EXWC (2012) specified that the naming of the congeners was simplified by convention called International Union of Pure and Applied Chemistry (IUPAC). The convention named the congeners as per the increase in chlorine content i.e. PCB 1 up to PCB 209. PCBs can be divided into homologues. Homologue refers to subgroups of PCB congener with the same number of chlorine substituents on biphenyl rings. For example monochlorobiphenyls consists of one chlorine atom while octachlorobiphenyls consist of eight chlorine molecules (table 2.1).

The homologues with different substitution patterns are called isomers, e.g heptachlorobiphenyl homologue consists of 24 isomers which has the same molecular weight of 395 (Kakareka and Kukharchyk, 2005). Table 2.1 shows congener with 1 to 10 chlorine substituents and are named as mono, di, tri, tetra,

penta, hexa, hepta, octa, nona, and deca-chlorobiphenyls. According to Winters (2003) Mono-, di- and tri-chlorinated PCBs are regarded as lighter and can volatize from the soil, heavier compounds remain in the soil; when the chlorine content and molecular weight increases, the compound solubility decreases and the mixture becomes more viscous.

The density, boiling point and hydrophobicity of PCB increase with the increase in chlorination (Darážová et al., 2016). PCBs have high molecular weight and boiling points; this causes PCB to be a viscous liquid which has low flammability properties (Darážová et al., 2016). These properties allow it to be more resistant to high temperature (Ahlborg et al., 1992; Darážová et al., 2016) resulting in insignificant chemical degradation. PCBs are highly resistant to chemical reaction because of their chemical structure and hydrophobic nature (Phylicia, 2013). The PCB solubility in water is very low and it has high octanol-water partitioning coefficients (Shiu et al., 1986).

Table 2.1: Homologous series of chloroderivatives of biphenyl (Grabowska, 2010).

Homologue	**Molecular Formula**	**Number of isomers**	**IUPAC number**
Monochlorobiphenyl	$C_{12}H_9Cl$	3	1 - 3
Dichlorobiphenyl	$C_{12}H_8Cl_2$	12	4 - 15
Trichlorobiphenyl	$C_{12}H_7Cl_3$	24	16 - 39
Tetrachlorobiphenyl	$C_{12}H_6Cl_4$	42	40 - 81
Pentachlorobiphenyl	$C_{12}H_5Cl_5$	46	82 - 127
Hexachlorobiphenyl	$C_{12}H_4Cl_6$	42	128 - 169
Heptachlorobiphenyl	$C_{12}H_3Cl_7$	24	170 - 193
Octachlorobiphenyl	$C_{12}H_2Cl_8$	12	194 - 205
Nonachlorobiphenyl	$C_{12}HCl_9$	3	206 - 208
Decachlorobiphenyl	$C_{12}Cl_{10}$	1	209

PCBs may be described as dense oily fluids, sticky resins or melting crystalline solids, all these properties depend on the chlorine amount (ATSDR, 1989). PCBs do not have odour or smell and their color range from colourless to light yellow or amber (ATSDR, 1989; WHO, 2003); they have low volatility at ambient temperature (ATSDR, 1989). PCBs are generally stable, relatively insoluble oily liquids which are able to be intensely absorbed to organic matter (Panero et al., 2005; USEPA, 1980).

The main reason why PCB was used in many applications is its ability to resist degradation at high temperatures, it can also resist breakdown caused by aging or oxidation (Washington State Department of Ecology (2015). PCBs have low vapour pressure (Ahlborg et al., 1992; Washington State Department of Ecology, 2015), but are volatile in water (Washington State Department of Ecology, 2015). One of the reasons why PCBs are distributed all over the planet is due to the long distance transportation of organic particles (ATSDR, 1989).

According to Ahlborg et al. (1992) and Mariñosa (2007) PCBs are very dense because of the chlorine atoms found in the molecule. PCBs form solid resins at low temperature conditions (Ahlborg et al., 1992; Mariñosa, 2007). PCBs have strong resistance to acids, bases and reduction (Ahlborg et al., 1992; Safe and Hutzinger, 1987). They are also compatible with organic materials (Safe and Hutzinger, 1987). PCBs can dissolve in hydrocarbons and some of the organic compounds; they can also be absorbed by fatty tissues (Ahlborg et al., 1992; World Health Organisation (WHO), 1993*)*. Ahlborg et al. (1992) stated that PCB lipophilicity is directly proportional to the degree of chlorination. PCB property responsible for its resistance to breakdown is hydrophobicity.

PCBs are vital due to their exceptional dielectric i.e. electrically insulating properties (Hutzinger et al., 1983). PCBs are not explosive and can form vapours which are more dense than air (Ahlborg et al., 1992; Central Pollution Control Board (CPCD), 2001); these compounds cannot conduct electricity therefore they

are ideal to be used as cooling liquids in electrical equipment (Ahlborg et al., 1992).

Table 2.2: Physical and chemical characteristic aroclors 1242, 1254 and 1260 (ATSDR, 1989 & 2000).

Aroclor	**1242**	**1254**	**1260**
Average molecular weight	232.2	328	357.7
Colour	Clear	Light yellow	Light yellow
Physical state	Oil	Viscous liquid	Sticky resin
Melting point ^{o}C	No data	No data	No data
Boiling point ^{o}C	325-366	365 - 390	385 - 420
Odor	No data	Mild hydrocarbon	No data
Organic solvents	Very soluble	Very soluble	Very soluble
Density g/cm^{3} at 25^{o}C	1.35	1.54	1.62
Partition coefficient Log Kow	5.6	6.5	6.8
Vapor pressure mm Hg at 25^{o}C	4.06 X 10^{-4}	7.71x10^{-5}	4.05x10^{-5}
Henry's law constant atm-m3/mol at 25^{o}C	5.2 X 10^{-4}	2.0x10^{-3}	4.6x10^{-3}
Flammability limits, ^{o}C	Unknown	None to boiling point	None to boiling point
Flash point ^{o}C (Cleveland open cup)	No data	No data	No data
Conversion factors Air (25°C)	1 mg/m^{3}= 0.092 ppm	1 mg/m^{3} = 0.075 ppm	1 mg/m^{3}= 0.065 ppm

2.2 Production and application

PCBs were named differently when they were manufactured in various countries (Phylicia, 2013). The production of PCB began in 1929 by the Monsanto chemical company in the Unites States (United States Department of Health and Human Services [USDHHS], 1995) and it was referred to as aroclors (NAVFAC EXWC, 2012). It was also produced in Europe and Japan (Eisler et al., 1986). Germany, France and Japan named PCB mixtures as Clophen, Prodolec and Phenoclor respectively (Fiedler, 2001).

It was reported that Monsanto Company in the US manufactured about 600,000 metric tons of Polychlorinated biphenyls in a period of 50 years; this figure represents half of the world wide total quantity (Holoubek, 2001). PCBs are not in use anymore however they are still contained in old equipment and have a potential to be discharged into our environment (NAVFAC EXWC, 2012). PCBs were manufactured as PCB congener mixtures in order to acquire certain chemical properties needed for a specific use (United Nations Environment Programme (UNEP), 1999; NAVFAC EXWC, 2012).

PCBs were synthesised artificially via cadogan coupling laboratory conditions by substituting hydrogen atoms by chlorine on a biphenyl molecule (Martinez et al. (2005). PCBs were commercially produced in the 1920s and used in electrical transformers as insulating material (Gray, 2004). PCB is said to be a group of compounds which are used for different purposes commercially and industrially (Janik et al., 2005).

Askarel, chlorectol, elemex, inerteen and pyranol are usual brands of which PCB is well known (Gray, 2004). The other end-uses of PCBs include rubbers, synthetic resins, ink, adhesives, carbonless paper, sealants, caulking materials, hydraulic fluids and pesticide extenders (ATSDR, 2000). Dielectric fluids, plasticizers in paint, heat transfer agents and others form part of the PCB applications (Eisler et al., 1986). PCBs were also used in printing inks and capacitors (De Voogt, 1989). PCB has been mostly used in thermal oil and

transformers (Takada et al., 2001). In transformers there are three forms of PCBs which are normally used, namely Aroclor 1242, 1254 and 1260

Table 2.3: Applications of A1242, A1254 and A1260 (IARC, 1979)

Application	**Aroclors**		
	1242	1254	1260
Capacitors		X	
Transformers	X	X	X
Heat transfer	X		
Hydraulics/lubricants:			
Hydraulic fluids	X	X	X
Vacuum pumps		X	
Gas-transmission turbines	X		
Plasticizers:			
Rubbers	X	X	
Synthetic resins		X	X
Carbonless paper	X		
Miscellaneous:			
Adhesives	X	X	
Wax extenders	X	X	
Dedusting agents		X	
Inks		X	X
Cutting oils		X	
Pesticide extenders		X	
Sealants and caulking compounds		X	

(Gray, 2004; Hutzinger et al., 1974). The former application of the most common Aroclors is shown in table 2.3 and the composition of aroclor 1254 and 1260 is indicated in table 2.4. PCB use and sales were reduced in the United States during the 1970s and were only restricted to capacitor and transformer manufacturers.

PCB use was phased out in 1976 (U.S. National Oceanic and Atmospheric Administration [NOAA], 1991).

Table 2.4: Molecular composition and percentage of congeners, for Aroclors 1254 and 1260 (Hutzinger et al, 1974).

Aroclor	**Chemical composition**	**Percentage**
1254	$C_{12}H_6Cl_4$	11 %
	$C_{12}H_5Cl_5$	49%
	$C_{12}H_4Cl_6$	34%
	$C_{12}H_3Cl_7$	6%
1260	$C_{12}H_5Cl_5$	12%
	$C_{12}H_4Cl_6$	38%
	$C_{12}H_3Cl_7$	41%
	$C_{12}H_2Cl_8$	8%
	$C_{12}HCl_9$	1%

2.3 Environmental concerns and regulatory requirements

PCBs have a unique combination of physical and chemical characteristics which made them preferable, and have a huge variety in terms of industrial application (WHO, 1993). However some of these properties make PCBs harmful to the environment (WHO, 1993).

In 2013, International Agency for Research on Cancer (IARC) categorised PCB as a pollutant which causes cancer to humans ((Darážová et al., 2016). Scientific studies done by researchers proved that PCBs have harmful or toxic characteristics (Blais et al., 1998, EPA, 1999). Hurtzinger et al. (1974) and Safe

(1990) highlighted that PCBs have vastly accumulated within our environment due to their persistence and lipophility, and were further identified as prevalent organic pollutants (POPs).

PCBs are recorded to be detrimental to the immune and nervous system; they also damage the reproduction of humans and animals (Gray, 2004). PCBs are lipophilic meaning that they dissolve in lipids of organisms, they bio accumulate, bio magnify and persist throughout the food chain (Ahlborg et al., 1992; Gray 2004; Janik et al., 2005; Martinez et al., 2005; Takada et al., 2001; UNEP, 2007, USEPA, 1980; Wu et al., 2004).

PCBs simulate oestrogen in women of child bearing age and infants and causing reproductive disorders (Gray, 2004). Effects of PCBs on human include fatigue, nervousness, poor infant inhabitation, thyroid dysfunction, low birth rate, headaches, dizziness etc. (Gray, 2004). UNEP (2008) states that there are recorded cases whereby people use oils in electrical equipment to cook and as a hand cleaner. Small quantities of PCB can damage the liver, neurological and immune systems in case of constant exposure. These substances have also been proven to be endocrine disruptors and they severely affect wildlife at extremely low concentrations (Bergeron et al., 1994; Soto et al., 1995).

PCBs are among the widespread contaminants with the ecosystem globally (Ahlborg, 1992; WHO, 1993). It has been detected in air, soil, sediments, fish, plants, animals and in human blood, tissue and milk (Bench, 2003; Crine, 1988; WHO, 1993). Residuals of PCBs were detected in snow deposit coming from regions such as Antarctic where no industrial activity has occurred (Safe and Hutzinger, 1987). The marine and estuarine sediments are global sinks for PCBs which are sorbed onto particulate matter (Berkaw et al., 1996). PCBs were discovered as environmental pollutant in 1979 (Bench, 2003). This discovery resulted from the improved capability of analytical equipment in PCB detection (Bench, 2003).

PCBs were declared as persistent organic pollutant (POPs) during the Stockholm Convention in 2001 which took place in the United Kingdom (Phylicia, 2013) because they tend to persist in the environment (UNEP, 2007; Washington State Department of Ecology, 2015). The agreement was made internationally to decrease and eradicate POPs which include PCBs (Phylicia, 2013). PCBs are still found in our environment today regardless of the fact that their production was stopped in the US.

PCBs have low water solubility and they are able to resist chemical reactions therefore allowing them to persist and move through the water, atmosphere and soils environments (EPA, 2002). For example, PCBs were released from the manufacturing processes into waste water streams and were adsorbed onto soil and sediments (Phylicia, 2013).

PCBs are considered to be serious contaminants due to their environmental persistence, effects of bioaccumulation and threats they pose to the people and environment (EPA, 2012). The sources of PCB on the environment are transformer fires, municipal waste discharges, precipitation, runoff, current and historic industrial discharges, concealed dumping, combustion products and accidental spills among others (NOAA, 1991). According to UNEP (1998) there are substantial magnitudes of PCB contaminated transformers which are still used and packaged for various social and economic motives.

National Environmental Management Act 107 of 1998 (NEMA) regulates the phase-out of PCB materials as well as materials contaminated by PCBs in South Africa. According to the NEMA regulations, PCB contaminated material is defined as an article which was free of PCB before but end up with a PCB concentration of more than 50 mg kg^{-1} but less 500 mg kg^{-1}. Any material with a PCB concentration of less than 50 mg kg^{-1} is referred to as non-PCB material (NEMA). PCB material refers to oil or materials with a PCB concentration of more than 500 mg kg^{-1} (NEMA).

2.4 The science and significance of PCB microbial degradation

The removal of harmful compounds such as PCB is a very significant and difficult task, due to the fact that these compounds can get adsorbed into organic material found within the environment. This makes it difficult to decontaminate the soil using traditional methods (Nwinyi, 2010). PCBs have a potential to cause harm to the humans and the environmental well-being; it is therefore essential to urgently develop bioremediation strategies which can remove PCBs from the soil (Nwinyi, 2010).

Environmental pollution resulting from PCBs is an increasing concern, microbes are playing a huge role in removing POPs from the environment (Seeger et al., 2010) and are used to transform the pollutants into non-toxic products (Nwinyi, 2010). Anaerobic group of microbes and aerobic bacteria can bioremediate PCB in soil by making use of different mechanisms (Liu, 2004). Seeger et al. (2010) indicated that PCBs can be bio transformed by anaerobic and aerobic bacteria, this happens when highly chlorinated PCB undergo reductive dehalogenation by anaerobic microbes. This process includes selective dechlorination from para and meta positions as presented by figure 2.2 (Seeger et al., 2010).

It is suggested in literature that, as part of the natural chlorine cycle, chlorinated aromatic compounds will go through biodegradation on the environment (Field and Siera-Avarez, 2007). Therefore, it cannot be assumed that chlorinated aromatic compounds will persist on the environmental indefinitely (Field and Siera-Avarez, 2007). Abramowitz (1990) and Field and Siera-Avarez (2007) pointed out that biphenyls with higher chlorine are reduced to biphenyls with lesser chlorine due to dehalogenation process occurring under anaerobic conditions. The chlorinated biphenyls with less chlorine are prone to be oxidised by the aerobes (Field and Siera-Avarez, 2007; Seeger et al., 2010).

Some of the bacteria used for the microbial degradation of PCBs are *Pseudomonas, Micrococcus, Corynebacteria, Bacillus, Achromobacter* and

Arthrobacter species (Nwinyi, 2010). According to Furukawa (2000) and Pieper (2005), *Pseudomonas, Alcaligenes, Achromobacter, Burkholderia, Comamonas, Sphingomonas, Ralstonia, Acinetobacter* are among the gram negative genera bacteria while *Rhodococcus, Corynebacterium* and *Bacillus* are among gram-positive bacteria which can biodegrade PCB (Borja et al., 2005; Furukawa, 2000; Pieper, 2005*).* Bedard et al. (1986) and Bopp (1986) highlighted that all these bacteria have enzymes which has the ability to attack the double ortho-substituted congeners; these enzymes are called biphenyl 3, 4- dioxygenase.

The chlorination degree and isomeric substitution pattern determines the biodegradation potential of a biphenyl molecule (Nwinyi, 2010). In theory, the degradation of PCB releases carbon dioxide, chlorine and water (Nwinyi, 2010; Anyasi and Atagana, 2011). During the degradation process, chlorine is removed from a biphenyl ring; the resultant compound undergoes cleavage and gets oxidised (Bedard and Haberl, 1990; Boyle et al., 1992; Nwinyi, 2010).

PCBs can be readily adsorbed by soil constituents; PCBs with lower levels of chlorine are less absorbed and are therefore slightly mobile in soil (Anyasi and Atagana, 2011). Seeger et al. (1997) stated that PCB molecule binds strongly to organic matter in solids, therefore bioaccumulate through the food chain.

PCBs are resistant to biodegradation by microorganisms due to their chemical and thermal stability (Singh and Wards, 2004; Furukawa and Fujihara, 2008). Highly chlorinated PCB congeners are more chemically stable and less soluble in water, therefore more resistant to bioremediation (Anyasi and Atagana, 2011).
PCB availability, incomplete breakdown, low expression of catabolic genes, PCB toxicity and metabolic intermediates are some of the factors which are responsible for limiting PCB bioremediation in soil (Vasilyeva and Strijakova, 2007; Pieper and Seeger, 2008).

Flores et al. (2009) highlighted that POPs tend to bind tightly to soil; this limits the efficacy of bioremediation. Bioavailability of PCB is increased when various

surfactants are used (Seeger et al., 2010). Biosurfactants are most effective in comparison to the synthetic surfactants because they are less toxic and more biodegradable (Makkar and Rockne, 2003).

The key factor for PCB biodegradation in polluted soils is the expression of the catabolic genes of PCB degrading microbes (Seeger et al., 2010). Biphenyl can be used to induce the biphenyl dioxygenase genes of PCB degrading strains (Singer et al., 2003; Vasilyeva and Strijakova, 2007). Biphenyl can be toxic to the bacteria (Cámara et al., 2004); so the natural substrates are used to induce the catabolic biphenyl dioxygenase genes instead of biphenyl (Ohtsubo et al., 2004; Pieper, 2005). PCB degradation in soil is increased by plant terpenes (Singer et al., 2000). Rhizoremediation can also be used to remedy the PCB contaminated soil (Vasilyeva and Strijakova, 2007; Macková et al., 2009). Some plants are able to enhance in situ degradation of PCBs (Villacieros et al., 2005).

The herbicides and pesticides are the main sources of chlorobenzoates (CBAs) (Yuroff et al., 2003). CBAs are also released when aromatic compounds with high chlorine content such as PCBs are degraded (Yun et al., 2007). CBAs cannot be degraded by native PCB degrading bacteria (Martínez et al. 2007). Toxic metabolites such as antibiotic protoanemonin can accumulate as a result of CBAs degradation by microbes (Skiba et al., 2002), and this results in decreased overall PCB degradation. Pieper (2005) stated that PCB bioremediation can be increased by the use of microbial groups of PCB-degrading and CBA mineralizing bacteria. PCBs have low biodegradation rate (Chuang et al., 1998). Seeger et al. (2010) revealed that the improvement of biocatalyst responsible for the bioremediation of PCB polluted sites is one of the new developments which have been reported.

The half-life of PCBs with high chlorine content ranges between 9 and 16 years as per geochronological studies which were conducted in sediments (Field and Siera-Avarez, 2007). The meta- and para-chlorinated compounds are prone to be reduced than ortho-chlorines (Field and Siera-Avarez, 2007). CBAs can accumulate as a product of the reaction and cannot be metabolised by aerobic

PCB degrading bacteria; however they can be metabolised into carbon dioxide and chlorine by other bacteria (Field and Siera-Avarez, 2007). Extensive PCB degradation can be accomplished by combining chlorobenzoates and PCB degrading genes into one organism (Field and Siera-Avarez, 2007).

In situ bioremediation is one of the preferred alternative techniques of remedying PCB-polluted soils (Ruiz-Aguilar et al., 2002). The rates of in-situ bioremediation can be limited by low concentration of microbes which are meant to remove PCBs; it is beneficial to add exogenous microbes and nutrients for their modification (Ruiz-Aguilar et al., 2002). Bumpus et al (1985) specified that PCBs and other aromatic compounds can be oxidised and mineralised by White-rot fungi. PCB transformation by White-rot fungi is also restricted by its low bioavailability (Ruiz-Aguilar et al., 2002). The solubility and availability of the hydrophobic compounds can be enhanced by adding surfactants, therefore promoting microbial degradation (Aronstein et al., 1991).

There are two metabolic pathways through which PCB biodegradation occurs, namely aerobic and anaerobic biodegradation (Huang et al., 2004). The extent of chlorination of PCB congeners, type of microorganisms and redox conditions determine which pathway the congener will undergo (Borja et al., 2005: Aken et al., 2010). Aerobic and anaerobic bacteria can biodegrade PCBs (Joutey et al., 2013); the use of these microorganisms is the only known technique which has the ability to biodegrade PCBs in soil and water (Mackova et al., 2007).

There are bacteria which are known to feed on hydrocarbons exclusively (Yakimov, 2007). These bacteria are able to degrade hydrocarbons and are referred to as hydrocarbon degrading bacteria (Joutey et al., 2013). Most bacteria can degrade organic contaminants, however single organism do not have enzymatic capabilities to metabolize most organic compounds in contaminated soil (Joutey et al., 2013). The great biodegradation of organic compounds in contaminated soil can be achieved by a mixture of microbial communities (Fritsche and Hofrichter, 2005). Seeger et al. (2001) emphasised that highly

chlorinated PCBs undergoes reductive dehalogenation by anaerobes while PCB with lower chlorine content undergoes oxidation by aerobes.

Factors affecting microbial degradation:

Microbes are able to degrade several organic contaminants; therefore they play a critical role in bioremediation (Joutey et al., 2013). The efficiency of remediation by microbes depends on several factors such as chemical characteristic and concentration of the contaminant, bioavailability of pollutants to microbes and the physiochemical properties of the environmental media (El Fantroussi and Agathos, 2005). Generally the rate of contaminant degradation is influenced by biological factors such as microorganisms, their nutritional requirement as well as the environmental factors (Joutey et al., 2013)

Biological factors:

The metabolic ability of microbes is referred to as biotic factor (Joutey et al., 2013). The proliferation process of degrading microbes is one of the biotic factors which affect the microbial degradation of the organic pollutants (Riser-Roberts, 1998). Environmental Response Division (1998) specified that the degrading microorganisms can be inhibited when the carbon sources are limited due to competition amongst microbes as well as predation of microbes by protozoa and bacteriophages.

The degree at which the pollutant is degraded depends on the concentration of the pollutant as well as the quantity of catalysts available (Joutey et al., 2013). The catalyst in this context refers to the number of microorganisms which are able to metabolise the pollutants and the enzyme amount produced by each cell (Joutey et al., 2013). The sufficient amount of nutrients and oxygen must be available in a usable form and correct amounts to promote unlimited development of microbes (Environmental Response Division, 1998).

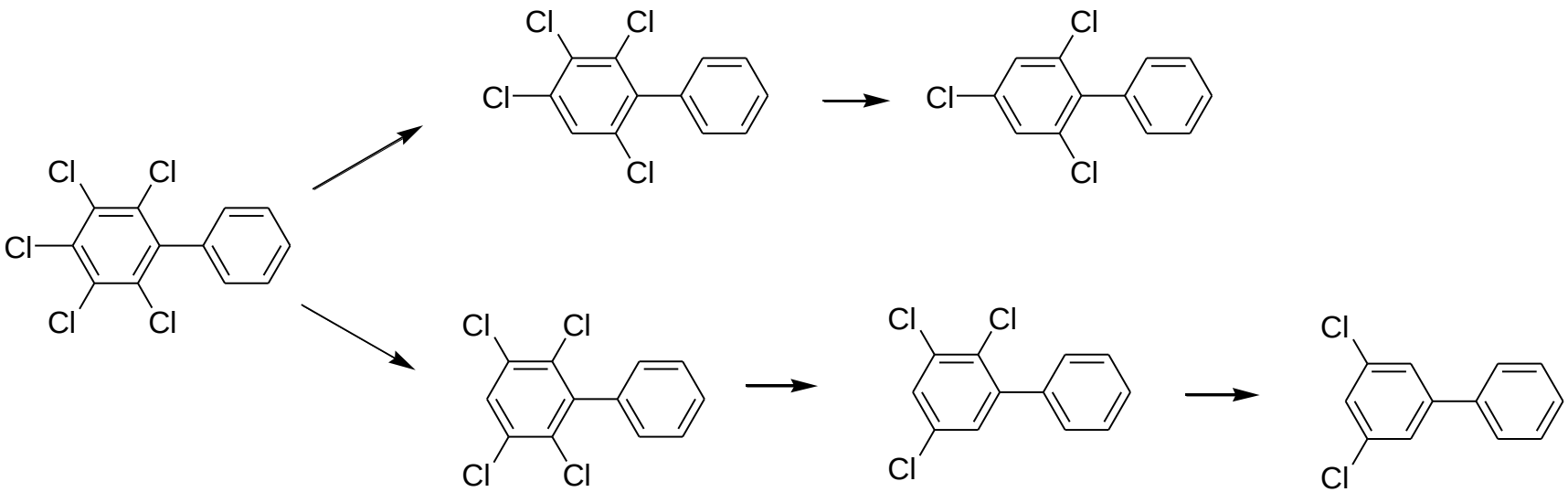

Figure 2.2: Dechlorination of double-flanked chlorines of 2, 3, 4, 5, 6-pentachlorobiphenyl by Dehalococcoides ethenogenes strain 195 and ortho dechlorination of 2, 3, 5, 6-chlorobiphenyl by bacterial strain 0-17 (Adapted from Pieper and Seeger, 2008).

Environmental factors:

Joutey et al. (2013) said that adsorption potential of an organic pollutant to the solid surface is influenced by the soil type and organic content in the soil. The availability of the pollutant to microbes is reduced by adsorption and absorption consequently reducing the rate of metabolism (Environmental Response Division, 1998).

Anaerobic PCB-dechlorination:

Congeners with four or more chlorine content go through anaerobic reductive dechlorination (Aken et al., 2010). In this process the PCB accepts electrons for the oxidation of organic substrates (Anyasi and Atagana, 2011). Anaerobic environment favours reductive alteration, whereby chlorine is displaced by hydrogen (Brazil et al., 1995). Anyasi and Atagana (2011) emphasised that aerobic bacteria grow at a faster rate than anaerobic bacteria and can maintain high degree of degradation, therefore mineralizing the contaminants.

Anaerobic transformation of chlorinated organic compounds comprises of reductive dehalogenation; whereby halogenated organic compounds function as electron acceptor (Borja et al., 2005). The aspect which limits metabolism in the anaerobic condition is electron acceptor (Anyasi and Atagana, 2011). It is therefore an advantage to have microbes which can use PCBs as terminal electron acceptors (Brown et al., 1987). Chlorinated aliphatic and aromatic hydrocarbons can be attacked through the dechlorination process in the absence of oxygen.

Disulfiro bacterium, Dehalobacter restricus, Dehalococcoides ethenogenes, Enterobacter agglomeraus are some of the bacteria isolated from the dechlorination reaction (Anyasi and Atagana, 2011). According to (Anyasi and Atagana, 2011), PCB undergoes reductive dechlorination under anaerobic environment in soil and sediments. Anyasi and Atagana (2011) indicated that the makeup of the active microbial community determines rate, route and extent of dechlorination process. The environmental aspects which include temperature, pH, carbon source, hydrogen, other electron donors, the availability and lack of

electron acceptors besides PCBs influence the active microbial activity (Mackova et al., 2010).

Aerobic biodegradation of PCBs:

PCB congeners with low chlorine content are the products of dechlorination of high chlorine congeners; and they act as substrates for aerobic microorganism (Komancova et al., 2003). The important factor of aerobic biodegradation is chlorine numbers per molecule and its placement (Furukawa et al., 2004). PCB congeners which are easily biodegradable are those with three or less chlorine atoms per molecule (Anyasi and Atagana, 2011).

PCB congeners with more than three chlorine atoms per molecule are resistant and require reductive dechlorination before oxidative mineralization (Aken et al., 2010). There are two gene clusters involved in the aerobic PCB degradation (Borja et al., 2005). The first gene is responsible for transforming PCB congener to chlorobenzoates, the second one degrade chlorobenzoic acid (Anyasi and Atagana, 2011).

2.5 Remedial measures of PCB

2.5.1 In-situ treatment methods

Biological treatment:

Furukawa (2008) defines bioremediation as the use of microorganisms to remove toxins like PCBs and it follows two stages, namely anaerobic and aerobic biodegradation. Aerobic biodegradation occurs with the presence of oxygen whereby a toxin becomes oxidised and anaerobic biodegradation occurs in oxygen deprived environments (USEPA, 2013). During anaerobic degradation chlorine in PCB is replaced by hydrogen atom, therefore reducing the number of chlorine on the PCB structure (Furukawa, 2008).

Furthermore the aerobic bacteria degrade a PCB structure with less chlorine into chlorobenzoic acid. Another method that uses plants is called phytoremediation

and it uses plants to eradicate the pollutants from soil and water (Meagher, 2000). Natural processes such as adsorption transport and translocations, hyper accumulation, transformation and mineralisation takes place in plants and can therefore remedy the contaminated environmental media. For example carrot, corn, cabbage and some of grass species can be used to degrade of PCB from PCB contaminated soil (Chekol et al., 2004).

Natural attenuation:

Natural attenuation as stated by Declercq et al. (2012) happens when the mass, toxicity, movement, volume, concentration of pollutant specifically in soil and water is reduced by different physical, chemical and biological processes acting together without human interference. Such processes are biodegradation, dispersion, dilution, sorption, volatilisation and biochemical stabilization (Declercq et al., 2012).

Physical method: Capping

Contaminated sediment is covered with a clean layer of silt, soil, gravel, or crushed rock debris to reduce the bioavailability of pollutant by separating the sediments from water, therefore limiting the mixing of soils/sediments by organisms (Eek et al., 2008).

Bioventing:

Anyasi and Atagana (2011) describe bioventing as a bioremediation method which allows the treatment of unsaturated soil. This technique makes use of indigenous microorganisms to degrade organic pollutants adsorbed in the unsaturated zones of the soil (USEPA, 2004a). Bioventing induces the airflow to provide oxygen, this promote the biodegradation of contaminants (Anyasi and Atagana, 2011). Bioventing technique makes use of vacuum enhanced soil vapour extraction system, whereby a flow of oxygen into the subsurface is caused by soil pressure gradient (Anyasi and Atagana, 2011). This triggers the aerobic decomposition of the contaminant. The design of the bioventing system should involve sufficient

airflow; the effectiveness of bioventing is hindered by low permeability and temperature (Anyasi and Atagana, 2011).

The following site characteristics promote the feasibility of bioventing method (Anyasi and Atagana, 2011):

Intrinsic permeability which means that enough oxygen should be supplied to the system

Sufficient microorganisms should be present.

The energy sources should be supplied depending on whether the microorganisms in the system are heterotrophic, autotrophic, aerobic and anaerobic.

The optimal pH of the soil should be ranging from 6 to 8. The optimal humidity should be 40-60 % and the temperature of the soil should be 10-45 ^{o}C.

Advantages and disadvantages of bioventing (Anyasi and Atagana, 2011):

This method is less expensive and it can be combined with other techniques.

The equipment used in this system is readily available, easy to install, the disturbance to the operation site is minimal and the treatment period is short. Bioventing is not effective when the water table is closer to the surface and it requires specific site conditions.

Biosparging

It is a method which uses indigenous microorganisms to biodegrade organic pollutants in the soil (Anyasi and Atagana, 2011). The technique includes the injection of atmospheric air onto the aquifers to accelerate the oxygen dissolution (USEPA, 2004b). The presence of oxygen stimulates the microbe's activity and enhances degradation of pollutants in the soil (USEPA, 2004b). Biosparging can be applied in saturated and unsaturated soil zones (Held and Dorr, 2000). When the air is injected into the aquifer, small passages are formed. These passages allow the air to move into the unsaturated zone of the soil, transporting the volatile pollutants into the unsaturated zone (Anyasi and Atagana, 2011).

Volatile vapours are then collected with the soil vapour extraction and get treated at the surface (Anyasi and Atagana, 2011). For biosparging to be effective, it is significant for the sparge point to be located below the polluted zone because the air flows in an upward direction (EPA, 1994). The shortcoming of biosparging process is that it can only be complete when it is in combination with physical techniques and it is expensive. This method is only effective for treating homogeneous soil (Anyasi and Atagana, 2011).

2.5.2 Ex-situ treatment methods

Biological treatment: Land farming

Biological treatment in terms of land farming entails a process of twisting over the mixture of contaminated media and soil surface occasionally, with the aim of exposing it to air (Gomes et al., 2013). Furthermore Tang et al. (2002) emphasise that the combination of photolysis, volatilization and biodegradation are techniques through which land farming treatment occurs.

Thermal treatment: Thermal desorption

This process involves the application of high temperatures on contaminated soil, sludge or filter cake to remove the toxin by increasing its volatility (Norris et al., 1999), then collection and/or thermal destruction of volatile toxins follows. The applied heat should be high enough to evaporate or combust the toxin as a way of removing it from the soil.

2.5.3 Treatment methods: both in and ex-situ

Chemical treatment involving chlorination, solvent extraction as well as oxidation can treat PCB polluted sites on and out of site (Gomes et al., 2013).

Dechlorination:

Chlorine is consecutively removed from PCB structure by making use of catalysts and reducing agents such as zero valent iron (Wu et al., 2012). During reductive dechlorination process, chlorine is removed from the biphenyl structure and replaced with hydrogen, this decontaminate PCBs (Mousa et al., 1998). For

example Sodium/Ammonia ($Na/NH_{3)}$ mineralizes chlorine to form sodium chloride; removing PCBs from the soil (Pittman Jr & He, 2002).

Solvent extraction:
The toxins are isolated from soil and sediments by mixing organic solvents with polluted soil (Gomes et al., 2013).

2.6 The challenges involving the degradation of PCBs

There has been considerable work done to achieve better alternative technology necessary for the destruction of PCBs found within our environment (Anyasi and Atagana, 2011). Incineration is highly effective however it is costly; incomplete combustion produces polychlorinated dibenzo furans/dioxins (PCDF/Ds) which are not desirable on the environment (Borja et al., 2005).

There have been many PCB remediation technologies discovered in the past two decades, some of them are already in use; however none of these methods have been accepted like conventional method (Anyasi and Atagana, 2011). This might be because the alternative methods are not certified to be applicable to all the media polluted by PCBs (Anyasi and Atagana, 2011). Other reason might be that the by-products are not certainly known. These technologies are also expensive, therefore preventing them from being commercialised (Borja et al., 2005).

The factors stated above posed threat to the government agencies as well as researchers who were trying to come up with the alternative methods of PCB remediation besides incineration (Anyasi and Atagana, 2011). It was also suggested that countries should extensively review the extent of PCB challenges so that suitable technology that will suffice for each country can be determined (Borja et al., 2005). Anyasi and Atagana (2011) said that there is a need to develop PCB degrading technologies which are friendly on the environment.

CHAPTER THREE-GENERAL AND SPECIFIC OBJECTIVES

3.1 General objectives

The general objective of this study is to determine and monitor the level of PCBs in soil contaminated by oil spillages from pole mounted transformers in Polokwane, Limpopo, South Africa after sites have been remediated. This is conducted to assess the effectiveness of the remediation technique and check whether PCBs on these sites still pose an environmental problem.

3.2 Specific objectives

The specific objectives are:

- To obtain data specifying pole mounted transformers with PCB oil which had previously spilled.
- To obtain the laboratory test results specifying the sites which had PCB concentration of 20 μg kg^{-1} or less and has been treated on site by the Bioremediation company.
- To collect soil samples from sites where PCB oil spilled and in-situ bioremediation was conducted.
- To do the laboratory analyses to determine the concentration of PCB in soil samples with QuEChERS extraction method followed by GC-MS analysis.
- To compare the PCB level before and after bioremediation and determine the effectiveness of the bioremediation method used.
- To compare the PCB concentration (results from the analysis) with the national and international PCB standards.

3.3 Research questions

- Is the method of bioremediation used to treat sites contaminated by PCB oil spillage from Eskom pole mounted transformers in Limpopo effective?

- Is the PCB concentration determined at Eskom sites after bioremediation in Limpopo within the national and international acceptable PCB standards in soil?

3.4 Hypothesis

The method of bioremediation used to treat PCB contaminated sites is not effective.

3.5 Justification of the research project

PCBs are significant pollutants in the environment due to their toxic and persistent characteristics. PCBs are phased out, however they are contained in several equipments world-wide and have a potential to be released into the environment. The release of PCBs into the environment poses a threat to humans and ecosystems. There are regulations in place to control how PCB-contaminated material should be used, disposed, decontaminated, stored etc. These regulations specify the concentration of PCB which is regarded to be acceptable in the environment.

It is critical to ensure that PCBs are properly managed to prevent them from being released to the environment. There are measures in place such as bioremediation which are used to treat PCB-contaminated sites when it is released into the environment. In order to be certain that South Africa or any other country is complying with PCB legal requirements, studies should be conducted to determine and monitor PCB concentrations in water, soils, air, sediments, fish and plants.

Information with regard to PCBs on the environment is limited in Africa (Batterman et al., 2007); therefore this investigation will add to the existing studies. PCB studies are critical in determining the extent of PCB contamination

at various sites/countries. The availability of data on PCB concentration is essential to evaluate if the existing control measures put in place are efficient.

This investigation will determine if the in-situ bioremediation method used was effective in degrading PCBs. This can influence the development of more effective bioremediation methods, ultimately impact on decision-making by various organisations in terms of selecting the appropriate bioremediation method.

CHAPTER FOUR: RESEARCH METHODOLOGY

4.1 Introduction

The methods and experimental procedure are presented in this chapter. The chapter covers sample collection, materials and reagents, quality assurance as well as sample extraction by QuEChERS methods followed by analysis using GC-MS.

4.2 Sample collection

Five sampling sites were chosen and a total of seventy eight soil samples were collected from these sites. These sites represent the poles mounted with transformers and are numbered as DBK177/4, JGC 106/13/4, JSK112/5/4, MBR 46/2 and MHG 204 (figure 4.1). The sampling sites are also labelled according to the pole numbers. The study area is in Polokwane, Limpopo province.

The location of the sampling sites is reflected on figure 4.2. Table 4.1 indicates the coordinates of the sampling sites. The sample collection was conducted on 03rd, 8th and 9th October 2015.

The samples were randomly collected using the grid sampling technique at a depth of 20 cm. Samples at pole number JGC 106/3/14 were collected at a depth of 12 cm due to the presence of electrical cables (figure 4.3). The samples were collected from all pink-shaded grids (table 4.2), and they were collected in triplicates.

Figure 4.1: Labels on poles mounted with transformers (Rampjapedi, 2015)

Table 4.1: Coordinates of the sampling sites

Sampling sites	**Latitudes**	**Longitudes**
DBK177/4	23 27 13.7	29 20 16.1
JGC 106/3/14	24 47 13.9	29 49 36.7
JSK112/5/4	24 42 55.9	29 48 07.0
MBR 46/2	23 50 35.9	29 19 36.2
MHG 204	24 18 08.6	29 59 51.5

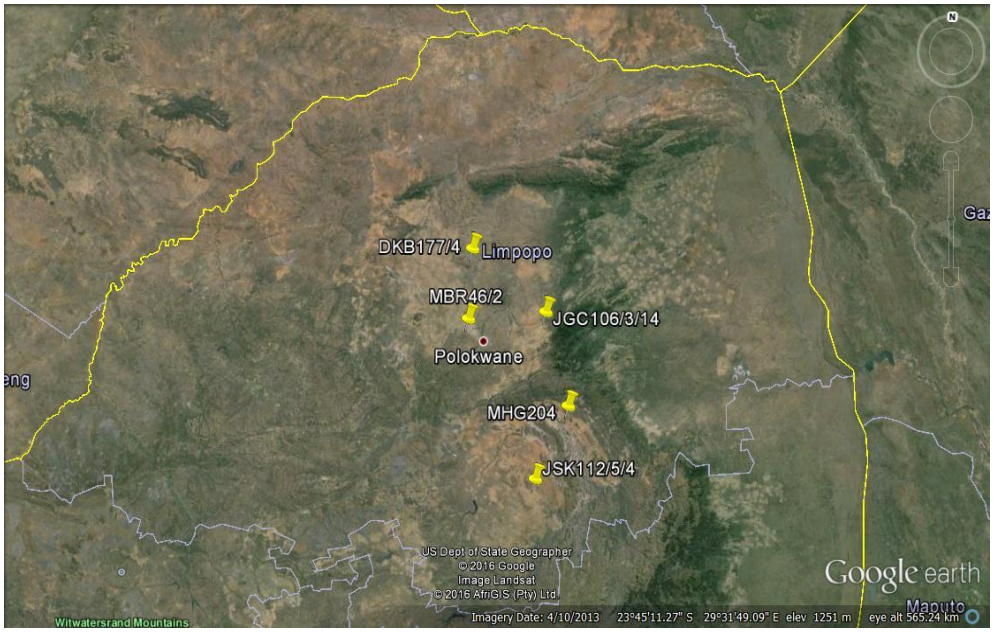

Figure 4.2: Location of the sampling sites

Figure 4.3: Electrical cables exposed at pole no. JGC 106/3/14 during sampling collection (Rampjapedi, 2015)

The coding of A, B and C was used to identify and distinguish three samples collected from one sampling grid. All samples were stored in ziplogs plastic bags and labelled accordingly. The samples were further stored in a cooler bag and transported to the chemical laboratory at the School of Chemistry, University of

the Witwatersrand, where they were stored in a fridge. The details of the sampling sites are summarized in table 4.3.

Table 4.2: Sampling grids from 5 sampling sites

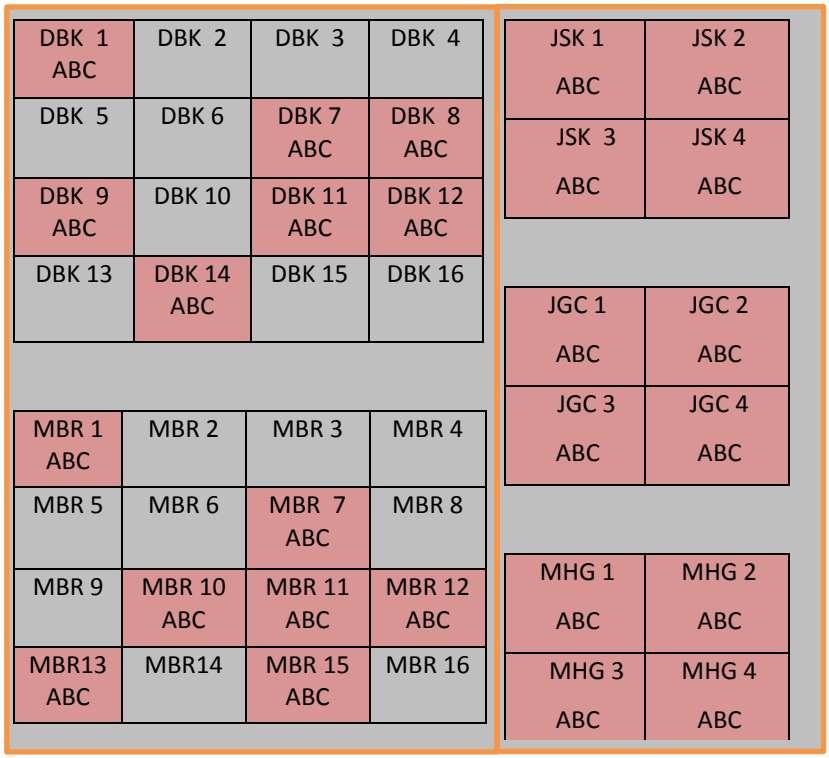

Table 4.3: Details of the sampling sites

Sampling sites	**Number of samples**	**Area of sampling sites in m^2**	**Area of sampling grids in m^2**	**Sampling Depth**
DBK177/4	21	2.56 m^2	0.16 m^2	0.2 m
JGC 106/3/14	12	0.36 m^2	0.09 m^2	0.12 m
JSK112/5/4	12	0.36 m^2	0.09 m^2	0.2 m
MBR 46/2	21	2.56 m^2	0.16 m^2	0.2 m
MHG 204	12	0.64 m^2	0.16 m^2	0.2 m

4.3 Materials and reagents

Aroclors A1254 and A1260 are composed of 79 PCB congener mixtures; appendix C indicates the 79 PCB congeners as well as their composition. Aroclors A1254 and A1260 standards with a concentration of 50 mg/mL were purchased from Sigma Aldrich Johannesburg-RSA. The stock solution of 500 μg/mL of each aroclor in hexane was prepared by mixing 1 mL of A1254 and 1 mL of A1260 in 100 mL volumetric flask. A1254 and A1260 were mixed because they are composed of different PCB congeners. The mixture of Aroclors (A1254:A1260) was used as a reference standard (RS) for calibration. Solutions of 100, 50, 30, 25, 20, 10 and 5 μg/mL were prepared from the mixture of A1254:A1260. All the solutions were prepared in hexane (HPLC). Acetonitrile (ACN), magnesium sulphate monohydrate, sodium chloride and bondesil primary/secondary amine (PSA) were from Sigma–Aldrich, Johannesburg, South Africa. Anhydrous sodium sulphate was from Merck, Johannesburg, South Africa. A1254 and A1260 contain all the three PCB congeners targeted in this study.

4.4 Methods

4.4.1 QuEChERS extraction method

The QuEChERS extraction method was done using the procedure reported by Raw et al. (2010). Ten grams of soil sample was homogenized using a mortar and pestle in 50 mL teflon tube, 10 mL of acetonitrile was added and sample was shaken strongly for 1 min. This was followed by salting-out step with 1.5 g sodium chlorine and 3 g of anhydrous magnesium sulphate into the tube and the mixture was shaken vigorously for 1 min and then placed in the centrifuge. After centrifuge, 6.5 mL of organic supernatant was transferred into the polypropylene centrifuge tube to clean-up with 1.65 g anhydrous magnesium sulphate and 27.5 g primary secondary amine (PSA). The solution was centrifuged for 5 min and filtered using a 0.45 μm PTFE and injected in the GC- MS for analysis. Blank homogenized soil samples with no pesticides detected were used for recovery

studies, and for the preparation of matrix-matched standards for calibration. The samples were spiked with 20 µg kg^{-1} of a standard mixture of A1254:A1260. The spiked samples were allowed to stand for 30 min. These were extracted as described above.

4.4.2 Analysis of PCBs

The analysis of PCBs was carried out by GCxGC/TOFMS (Time of Flight system-mass spectrophotometry), Agilent 5980-1472E. The separation of congeners was done in a 60 m x 0.25 mm x 0.25 µm Agilent HP-5MS column. Helium was used as the carrier gas at 18 psi pressure and 1.9 mL/min flow. The injector was kept at constant pressure and 250ºC temperature. The initial oven temperature was set at 70°C, held for 2 min, then increased to 150°C at a rate of 25°C/min, then increased to 200°C at a rate of 3°C/min, then increased to 280°C at a rate of 8°C/min. The temperatures of the injector and detector were 270°C. The injection volume was 1 µL in the splitless mode. Using mass range from 50 to 500 amu. The temperatures of the TOFMS, source and transference line were 150ºC, source 230ºC and 280ºC respectively.

The equipment was operated in Scan mode (EI) at 1.52 scan/s**.** The PCB congeners were identified qualitatively using their retention times, and their concentrations calculated on dry weight basis. The targeted PCB congeners in this study are PCB-101, PCB-158 and PCB-180. Appendix D-F indicates the mass spectrum for PCB-101, PCB-158 and PCB-180. These congeners were chosen as indicator PCBs which are commonly analysed. Appendix A shows the structure, IUPAC identification number and RT at which the PCBs congeners eluted.

4.4.3 Quality assurance

Quality assurance of applied analytical methods was achieved by preparation of blank samples and standard solutions. The sensitivity of the method is expressed by limit of detection (LOD), limit of quantification (LOQ) and linearity (R^2). LOD of the method were assessed based on the lowest concentrations of the residues in

each of the matrices that could be reproducibly measured at the operating conditions of the GCxGC/TOFMS. The precision values for the method was expressed as percentage relative standard deviation (%RSD) and linearity (R^2). The spiked concentrations 20 µg kg^{-1} of A1254:A1260 mixture standard were used for recovery calculation.

4.4.4 Data analysis

Description of data was performed using Minitab 16 statistical software. Anova one way was used to compare the results. The results obtained in this study were compared with some PCB regulation on soil in South Africa and other international organisations.

CHAPTER FIVE-RESULTS AND DISCUSSION

5.1 Quality assurance aspects

The recovery of 20 µg kg $^{-1}$ mix standards from the spiked replicate soil samples was 97%.The precision of the analysis calculated as the % relative standard deviation (%RSD) was 8% and was within the acceptable range of less than 15%. The detection limits ranged from 2 to 8 µg kg $^{-1}$ showing the higher sensitivity of the Gas Chromatography – MS at the operating conditions. The limit of quantification (LOQ) ranged from 1.01 to 8.33 µg kg $^{-1}$. Analysis of PCBs mixture of standards gave a linear calibration curves with a good regression line ($r > 0.999$). All calibration curves are shown in appendix B.

5.2 The trends of PCB-101, PCB-158 and PCB-180 concentration per sampling site after bioremediation

The concentrations of PCB-101, PCB-158 and PCB-180 were detected in all soil samples collected from five sites. The mean concentration of PCB-101 at site JSK112/5/4 was found to be 21.12 µg kg $^{-1}$**,** 30.71 µg kg $^{-1}$ for PCB-158 and 46.94 µg kg $^{-1}$for PCB-180 (table 5.1). PCB-180 was found to have the highest mean concentration, followed by PCB-158 and least PCB-101. At site MBR46/2, the mean concentration of PCB-101 was found to be 3.70 µg kg $^{-1}$, 6.54 µg kg $^{-1}$ for PCB-158 and 12 µg kg $^{-1}$ for PCB-180 (table 5.1). The order of PCB concentration here was found to be similar to the site mentioned above.

The determined mean concentrations of PCB-180 at JGC106/3/14 was found to be 3.49 µg kg $^{-1}$, 5.69 and 17.22 µg kg $^{-1}$ for PCBs 158 and 101, respectively (table 5.1). PCB-180 has the lowest mean concentration in comparison with PCB-158 and PCB-101 (figure 5.1).

The mean concentration of PCB-101 at MHG204 was found to be 5.03 µg kg $^{-1}$. PCB-158 mean concentration was found to be 9.56 µg kg $^{-1}$ while PCB-180 mean

concentration at this site was found to be 11.49 µg kg $^{-1}$, PCB-180 has the highest concentration and PCB-101 has the lowest concentration which is similar trend to other two sites mentioned above.

Site DBK177/2 has the mean concentrations of PCB-101, PCB-158 and PCB-180 as 31.14, 36.04 and 62.53 µg kg $^{-1}$. Figure 5.1 indicates that the highest concentration at this site is that of PCB-180 and the lowest is that of PCB-101. This trend is similar to most sites and shows that PCB-180 was the one with the highest concentration. This trend could reflect original source trend of these PCBs in the transformer oil.

Table 5.1: Summary of determined PCB concentrations (µg kg^{-1}) in soil samples after bioremediation

Sampling grid	**PCB-101**	**PCB-158**	**PCB-180**	**Sampling grid**	**PCB-101**	**PCB-158**	**PCB-180**	**Sampling grid**	**PCB-101**	**PCB-158**	**PCB-180**
JSK 1	29.53 ± 0.05	45.36±0.22	50.63± 0.05	MBR 1	3.11 ±1.30	5.60±0.88	10.39 ±0.12	JGC 1	4.44 ±3.46	7.11±0.32	27.61 ±0.09
JSK 2	19.91± 0.10	36.24±0.04	48.33± 0.01	MBR 7	3.12± 4.12	4.95±0.95	11.80± 0.10	JGC 2	2.42± 5.70	3.89±0.82	11.92± 0.07
JSK 3	24.51 ± 0.06	24.02±0.26	45.25± 0.03	MBR 10	3.52 ±2.22	7.36±0.67	13.58 ±0.09	JGC 3	3.94 ±0.84	6.70±0.16	15.54 ±0.41
JSK 4	10.34± 0.19	17.21±0.19	43.54±0.01	MBR 11	5.06± 0.60	8.26 ±1.12	12.24± 0.19	JGC 4	3.14± 2.95	5.06 ±2.24	13.80± 0.27
Mean	21.12	30.71	46.94	Mean	3.70	6.54	12.00	Mean	3.49	5.69	17.22
Sum	84.29	122.83	187,75	Sum	14.81	26.17	48.01	Sum	13.94	22.76	68.87
SD	8.16	12.55	3.16	SD	0.92	1.53	1.31	SD	0.89	1.49	7.08
% RSD	38.74	40.10	6.73	% RSD	24.98	23.42	10.95	%RSD	25.52	26.21	41.14
Sampling grid	**PC-101**	**PCB-158**	**PCB-180**	**Sampling grid**	**PCB-101**	**PCB-158**	**PCB-180**				
MHG 1	6.47 ±1.96	11.02±1.77	11.76± 0.42	DBK 7	23.55 ±4.40	34.21±0.78	78.30 ±0.69				
MHG 2	4.57± 2.11	8.79±0.30	10.02± 0.07	DBK 9	30.76± 0.46	34.16±2.88	71.85± 0.35				
MHG 3	5.57 ±0.98	11.34±0.37	10.66± 0.01	DBK 11	39.12 ±0.78	39.75±0.72	37.44 ±0.19				
MHG 4	3.52± 2.41	7.09±0.29	13.51±0.04	Mean	31.14	36.04	62.53				
Mean	5.03	9.56	11.49	Sum	93.43	108.12	187.59				
Sum	20.13	38.24	45.95	SD	7.79	3.21	21.97				
SD	1.27	1.99	1.53	%RSD	25.02	8.92	35.13				
%RSD	25.28	20.92	13.30								

5.3 The sum of PCB-101, 158 and 180 concentration at each sampling site after bioremediation

The site with the highest PCB concentration was found to be JSK112/5/4 with the value of 395.06 µg kg^{-1} followed by DBK177/4 with the value 389.14 µg kg^{-1} JGC106/3/14 with the value of 105.57 µg kg^{-1}, MBR46/2 with a value 88.99 µg kg^{-1} and MHG204 with the value 26.08 µg kg^{-1} (figure 5.1).

At site JSK112/5/4, PCB-158 concentration in the soil was found to be with the highest heterogeneity, followed by that of PCB-101 and least PCB-180. The highest PCB heterogeneity in the soil at site MBR46/2 was found to be PCB-158, followed by PCB-180 and least PCB-101. At site DBK177/4, PCB-180 in the soil was found to have the highest heterogeneity, followed by PCB-101 and least PCB-158. The highest PCB heterogeneity in the soil at site JGC106/3/14 was found to be that of PCB-180 followed by PCB-158 and least PCB-101. At site MHG204, PCB-158 concentration in the soil showed highest heterogeneity followed by PCB-180 and least PCB-101. However, based on the error bars displayed on figure 5.1, PCB concentrations were all heterogeneous in the soil samples. The reasons behind this could be due to soil displacement which happened during bioremediation process followed by leaching of the PCBs during rainfall. Figure 5.1 clearly shows that PCB-180 was most dominant in the soil samples at all the sites followed by PCB 158 and least PCB-101.

The PCB studies conducted in England reported PCB-153 as the dominant congener, followed by PCB-138 and least PCB-77. The studies in Northern Ireland reported PCB-52 as dominant followed by PCB-153 and least PCB-47 while in Scotland PCB-153 was dominant followed by PCB-138 and least PCB-118 (Environment Agency UK Soil and Herbage Pollutant Survey, 2007). This therefore indicates that it cannot be concluded that PCB-180 dominates other congeners in every case.

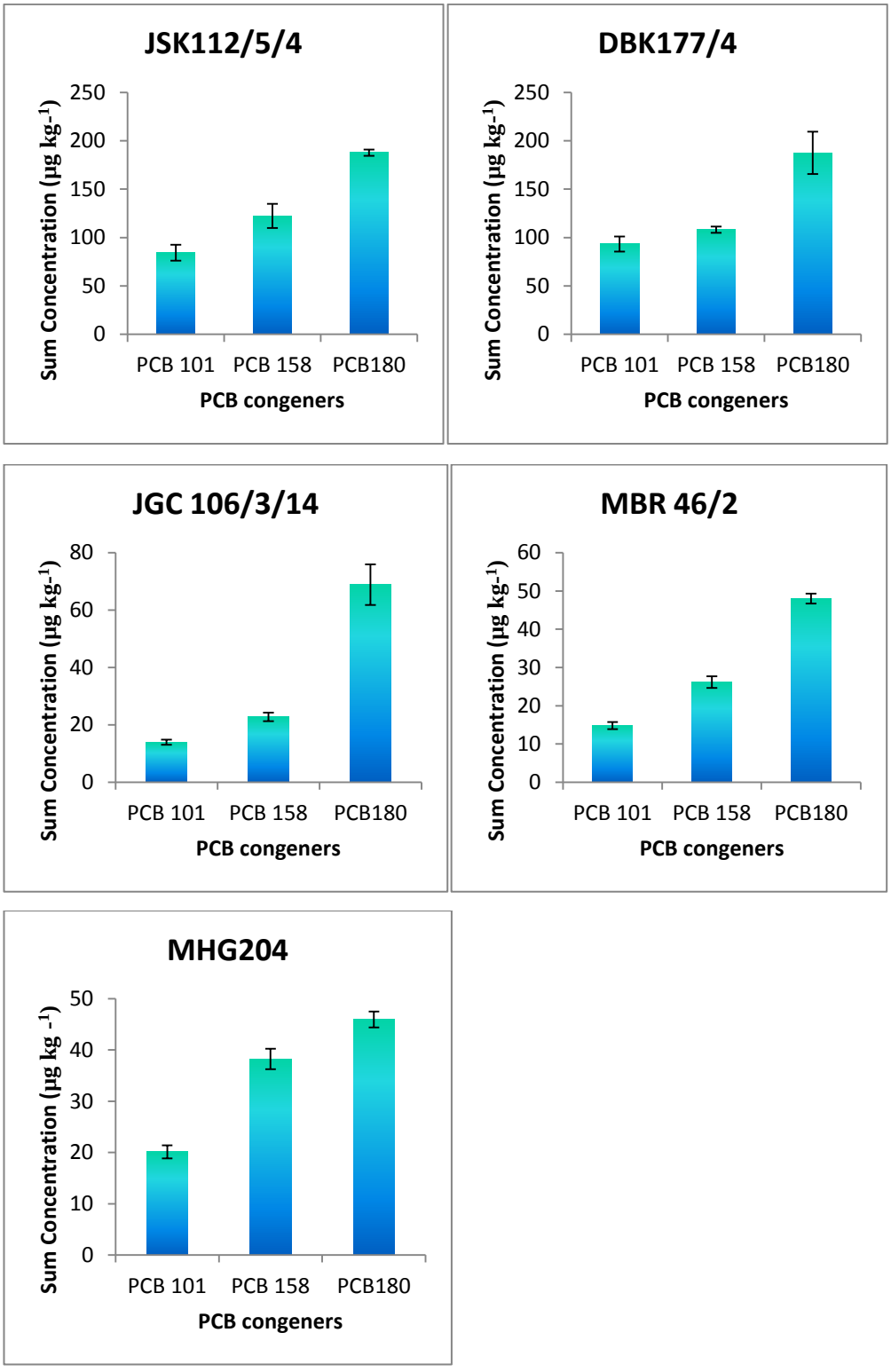

Figure 5.1: Sum PCB concentration (µg kg $^{-1}$) at each sampling site

5.4 Comparison of PCB concentration before and after bioremediation

PCB concentration at JSK112/5/4 before bioremediation was 90 µg kg^{-1} (figure 5.2), while the sum of PCB concentrations at this site after bioremediation is 395.06 µg kg^{-1} (figure 5.1 and table 5.1). The PCB concentration at this site after bioremediation is about 4 times higher than it was before bioremediation.

The PCB concentration at MBR46/2 was 13 µg kg^{-1} before bioremediation (figure 5.2), and the determined sum of PCBs concentration after bioremediation is 88.99 µg kg^{-1} (figure 5.1 and table 5.1). PCB concentration at this site is about 7 times more that it was before bioremediation.

The determined PCB sum concentrations after bioremediation at DBK177/4 is 389.14 µg kg^{-1} (figure 5.1 and table 5.1) while the PCB concentration before bioremediation was 130 µg kg^{-1} (figure 5.2). In comparison, concentration after bioremediation is 3 times higher than concentration before bioremediation.

The same trend was observed in all other sites where concentration of PCBs after bioremediation was found to be higher than the reported concentration before remediation. It is expected that the PCB concentration after bioremediation should be less than the PCB concentration before bioremediation. However the methods used during the PCB analysis of the soil samples before bioremediation is not known. The condition of the samples, for example, if the samples were wet or dried is also not known since the sample collection, extraction and analysis was done by a private lab and Eskom was just given the results without method procedures and data validation results. There is also no certainty as to which specific PCB congeners were used to represent the PCB concentrations at these sites during sample analysis by the private lab. It could be that one particular congener was used to represent the concentration of PCBs at various sites; therefore the comparison of concentration before bioremediation (figure.5.2) and after bioremediation (figure 5.1) cannot be concluded. The efficiency of bioremediation cannot be concluded either due to the reasons mentioned above.

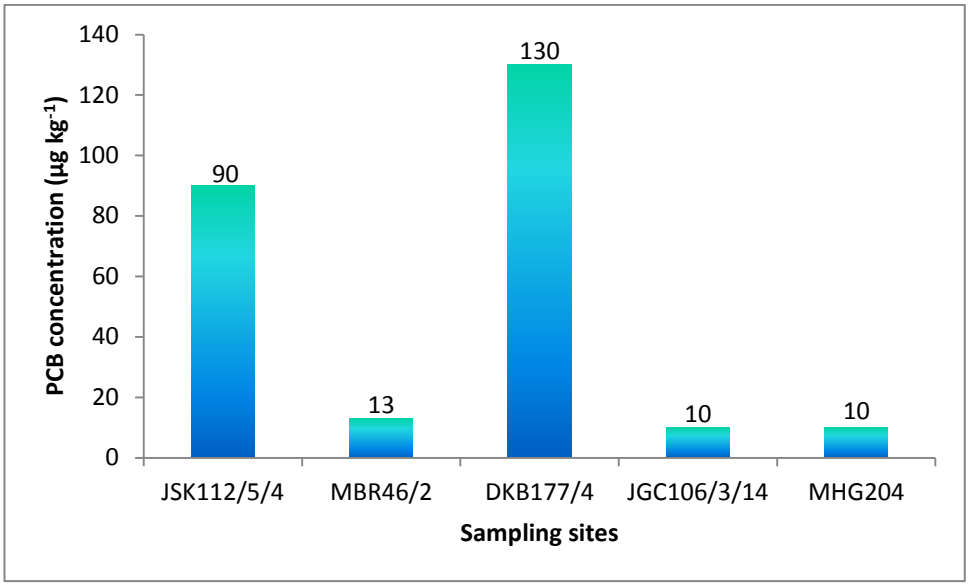

Figure 5.2: Sum PCB concentration at sampling sites before bioremediation

5.5 Comparison of PCB concentration with international standards

There are regulatory standards which are set out by various countries for the purpose of protecting the environment against PCB contamination. The broader aim is to make sure that PCB-containing equipments are phased out, decontaminated, labelled, registered and disposed safely.

There was a Stockholm convention held in Sweden on the 23 May 2001 whereby over 100 countries world-wide signed an agreement which is aimed at controlling and phasing out POPs, this include PCBs. This convention is mainly aimed at protecting human health and the environment. South Africa was one of the countries which signed the agreement (South African National Implementation Plan for Stockholm Convention on POPs, 2012).

For example, Environmental Protection Agency regulates the use, storage and disposal of PCBs as per the requirements of Toxic Control Substance Act of 1976 (TSCA) in the United States of America. South African PCB legal requirements are governed by National Environmental Management Act 108 of 1997 (NEMA). South African standard called SANS 290:2007 was developed to manage PCB oils

and PCB-contaminated equipments (National Implementation Plan for Stockholm Convention on POPs, 2012). The standard focuses on identifying materials with the PCB concentration of 51-500 mg kg^{-1} and ensures that the risk associated with such materials is properly mitigated. According to Stockholm convention requirements (Bouwman, 2004), TSCA and NEMA, any material with a PCB concentration of less than 50 mg kg^{-1} is referred to as non-PCB material and it is not regulated.

The PCB concentration in this study is compared with some of the international PCB standards to determine if the PCB levels at these sites are compliant with the standards. The PCB concentration in soil which is considered environmentally safe as per the PCB regulations of various states, namely United States, United Kingdom, Australia and South Africa is 50 mg kg^{-1}. The PCB levels at all sites before and after bioremediation is below 50 mg kg^{-1} therefore the sites are complying with the PCB standards of these states.

5.6 Comparison of PCB concentration with literature

Table 5.2 summarises PCB concentrations reported in various countries from literature in solid samples including soil samples. In comparison with PCB concentrations globally, the PCB concentration detected in soils of Polokwane, South Africa, are found to be considerably higher than those reported in Midway Atoll, Hawaii islands (2.6-148.8 ng g^{-1}), Russia (2-34 ng g^{-1}), Poland (80-680 ng g^{-1}), China (0-55 ng g^{-1}) and France (0.1-150 ng g^{-1}).

The PCB concentration detected in soil from Boston, USA ranges between 3.3 and 3.4 mg kg^{-1} (table 5.2); this concentration is significantly higher than concentrations determined in this study (26.08-395.06 µg kg^{-1}). The PCB concentration in Durban, South Africa (1-10 ng g^{-1}) is significantly lower than concentrations determined in this study. This is not surprising because here we are dealing with pollution hot spots of PCBs in the country. The reported concentrations in this study are significantly higher than those reported in some

African countries like Morocco (Less than 1 ng g^{-1}), Egypt (0.9-1210 ng g^{-1}) and Tunisia (0.89-6.63 ng g^{-1}).

Based on the results from this study and the comparison above, South Africa is more contaminated with PCBs than the rest of the countries in table 5.2 except for Boston, USA. However it cannot be concluded that South Africa, for example is more contaminated with PCBs than other countries because the concentrations were detected in samples collected from PCB-contaminated hotspots. Those with lower concentrations might have been collected further away from the hotspots.

Table 5.2: PCB concentrations in soils and sediments of various countries and regions world-wide

Country/Region	**Concentration range**	**Environmental media**	**References**
Midway Atoll, Hawaii islands	2.6 -148.8 ng g^{-1}	Soil samples	Ge et al., 2013
Moscow in Russia	2- 34 ng g^{-1}	Soil samples	Wilcke et al., 2006
Poland	80-680 ng g^{-1}	Soil samples	Falandysz et al., 2001
South East China	0-55 ng g^{-1}	Soil samples	Liao et al., 2012
East coast of Antarctic and France	0.1-150 ng g^{-1}	Soil samples	Motelay-Massei et al., 2004
USA, Boston	3.3 to 34 mg kg^{-1}	Soil samples	Herrick et al., 2007
Durban, South Africa	1-10 ng g^{-1}	Soil samples	Batterman et al., 2009
Alexander harbour, Egypt	0.9-1210 ng g^{-1}	Estuarine and sediments	Barakat et el., 2002
Northern Morocco	Less than 1 ng g^{-1}	Sediments	Piazza et al., 2009
Bizerte lagoon, Tunisia	0.89-6.63 ng g^{-1}	Sediments	Derouiche et al., 2004
Polokwane, South Africa	26.08-395.06 µg kg^{-1}	Soil samples from 5 sampling sites	Rampjapedi, 2016

CHAPTER SIX-CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

PCB-180 was found to have the highest mean concentration at all sampling sites, followed by PCB-158 and PCB-101, this could also reflect similar trend in the original source. The sampling site with the highest total PCB concentration was found to be JSK112/5/4 followed by DBK177/4, JGC106/3/14, MBR46/2 and MHG204 and this could reflect the amount spilled from the transformers or differences due to variation in the bioremediation efficiency. The PCB concentration in the soil samples was found to be heterogeneously distributed and this could be attributed to soil disturbance during bioremediation followed leaching during rainfall.

The fact that there is no detailed analytical procedure used to extract and analyse PCB concentration before bioremediation by a private laboratory hired by Eskom, it is difficult to compare the obtained results with those before bioremediation
However, the results indicate that there are low levels of PCB which are still contaminating the environment after two to three years of in-situ bioremediation despite the fact that the concentration is less than 50 mg kg^{-1}, and it is within maximum allowable limit as per South African and other international PCB standards. In comparison with concentrations of PCB reported in other countries, PCB concentrations detected in this study are generally higher than those of other countries.

6.2 Recommendations

Further studies are required to determine PCB levels at all sites whereby all other potential PCBs are investigated instead of PCB-180, PCB-158 and PCB-101 studied in this project.

Further studies are also needed to check how far the PCBs are leaching into the surrounding environment. This is important because some poles mounted with

transformers are situated near residential areas, streams etc. This study can include depth profiling and spatial distribution around the sites as well as ground water and nearby streams and sediments

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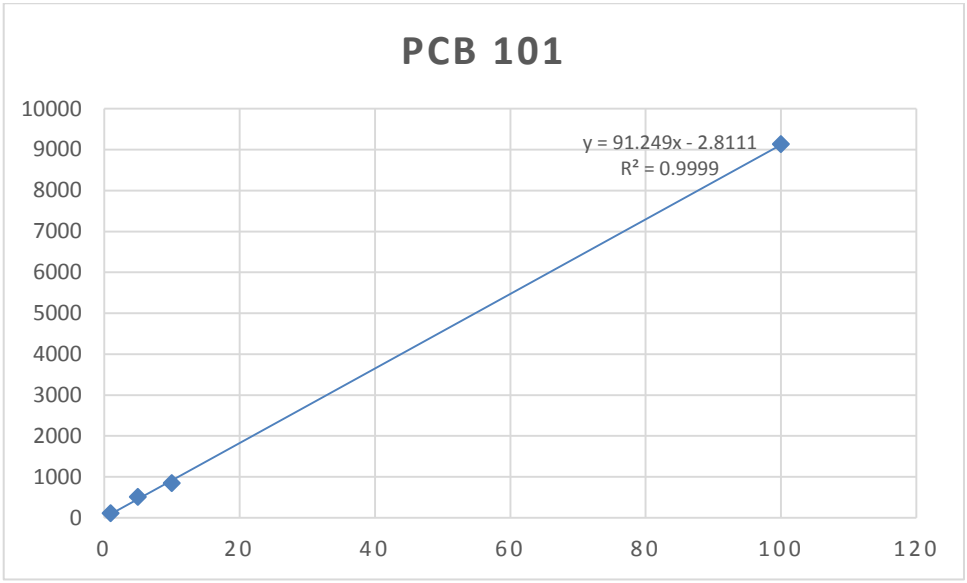
APPENDICES

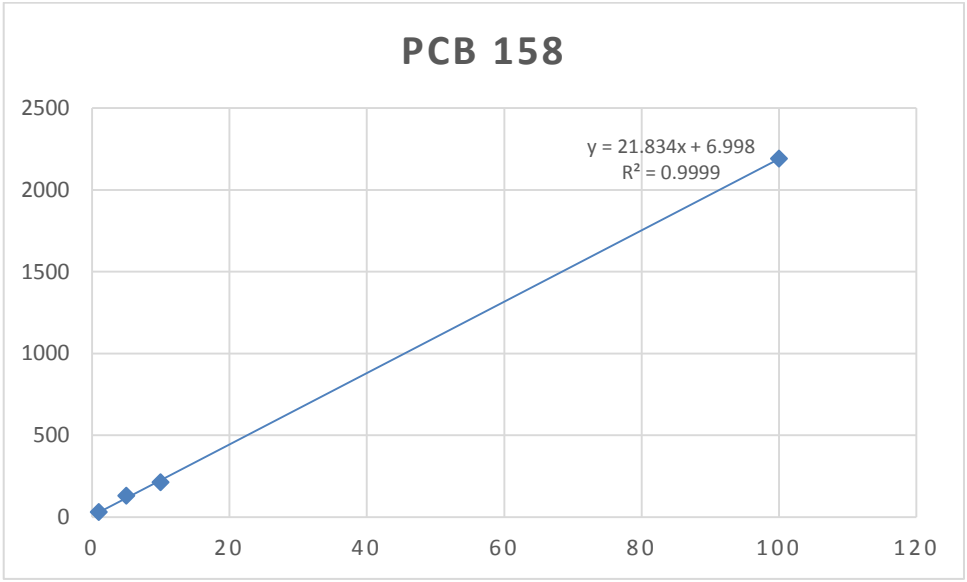
Appendix A: The structure, IUPAC identification number and RT at which the PCBs congeners eluted

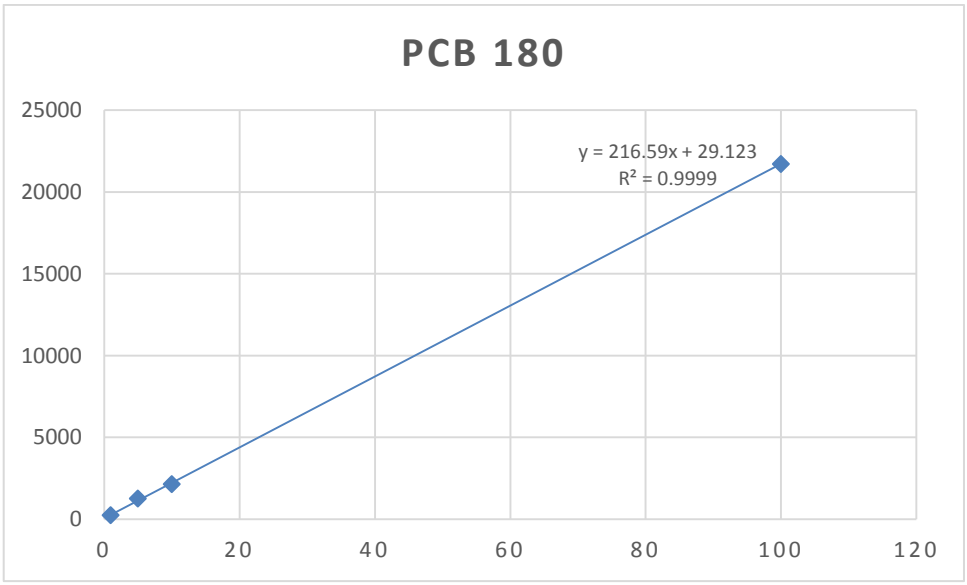
Substituted chlorine positions	IUPAC	RT (min)	Substituted chlorine positions	IUPAC	RT (min)	Substituted chlorine positions	IUPAC	RT (min)
4	3	8.10	2,2’,3’,4,5	97	22.23	2,2’,3,3’,4,4’,6	171	27.44
2,2’	4	9.85	2,2’,4,4’,5	99	21.40	2,2´,3,3’,4,5,5’	172	27.79
2,4’	7	11.28	2,2’,4,5,5’	101	21.13	2,2’,3,3’,4,5,6’	174	27.15
2,3,6	24	14.95	2,2’,4,5,6’	102	21.73	2,2’,3,3’,4,5’,6	175	26.35
2,4,4’	28	13.75	2,2’,4,5’,6	103	19.89	2,2’,3,3’,4,6,6’	176	25.69
2,4,6	30	13.04	2,3,3’,4,4’	105	25.40	2,2’,3,3’,4’,5,6	177	27.31
2,4’,6	32	14.95	2,3,3’,4’,6	110	22.96	2,2’,3,3’,5,5’,6	178	26.18
2,2’,5	39	12.96	2,3’,4,4’,5	118	24.08	2,2’,3,3’,5,6,6’	179	25.63
2,2’,4,4’	47	20.71	2’,3,3’,4,5	122	24.49	2,2’,3,4,4’,5,5’	180	27.97
2,2’,4,5’	49	17.66	2’,3,4,5,5’	124	23.69	2,2’,3,4,4’,5’,6	183	26.61

2,2’,5,5’	52	16.68	3,3’,4,4’,5	126	26.18	2,2’,3,4,5,5’,6	185	26.92
2,2’, 5,6’	53	15.48	2,2’,3,3’,4,4’	128	26.75	2,2’,3,4’,5,5’,6	187	26.45
2,2’,6,6’	54	16.87	2,2’,3,3’,4,6	131	24.54	2,3,3’,4,4’,5,5’	189	29.51
2,3’,4,5	67	18.66	2,2’,3,3’,4,6’	132	25.40	2,3,3’,4,4’,5,6	190	28.76
2,3’,4,5’	68	17.82	2,2’,3,3’,5,6’	135	23.69	2,3,3’,4,4’,5’,6	191	28.15
2,3’,4,6	69	18.27	2,2’,3,3’,6,6’	136	22.79	2,3,3’,4’,5,5’,6	193	28.01
2,3’,4’,5	70	19.47	2,2’,3,4’,5,5’	146	24.74	2,2’,3,3’,4,4’,5,5’	194	30.38
2,3’,4’,6	71	16.00	2,2’,3,4’,5,6	147	23.88	2,2’,3,3’,4,4’,5,6	195	29.88
2,3’,5,5’	72	19.68	2,2’,3,4’,5’,6	149	24.01	2,2’,3,3’,4,5,5’,6	198	28.88
2,4,4’,5	74	20.84	2,2’,4,4’,6,6’	150	27.44	2,2’,3,3’,4,5,6,6’	199	28.28
3,3’,4,4’	77	20.22	2,2’,3,5,5’,6	151	23.42	2,2’,3,3’,4,5’,6,6’	200	28.28
2,2’,3,3’,4	82	23.50	2,2’,4,4’,5,5’	153	24.96	2,2’,3,3’,4,5,5’,6’	201	28.99
2,2’,3,3’,5	83	21.96	2,3,3’,4,4’,5	156	25.90	2,2’,3,4,4’,5,5’,6	203	29.13
2,2’,3,4,4’	85	22.68	2,3,3’,4,4’,5’	157	27.64	2,3,3’,4,4’,5,5’,6	205	30.50
2,2’,3,4,5’	87	22.49	2,3,3’,4,4’,6	158	25.97	2,2’,3,3’,4,4’,5,5’,6	206	31.26

Appendix B: Linear calibration curves with a good regression line (r > 0.999).





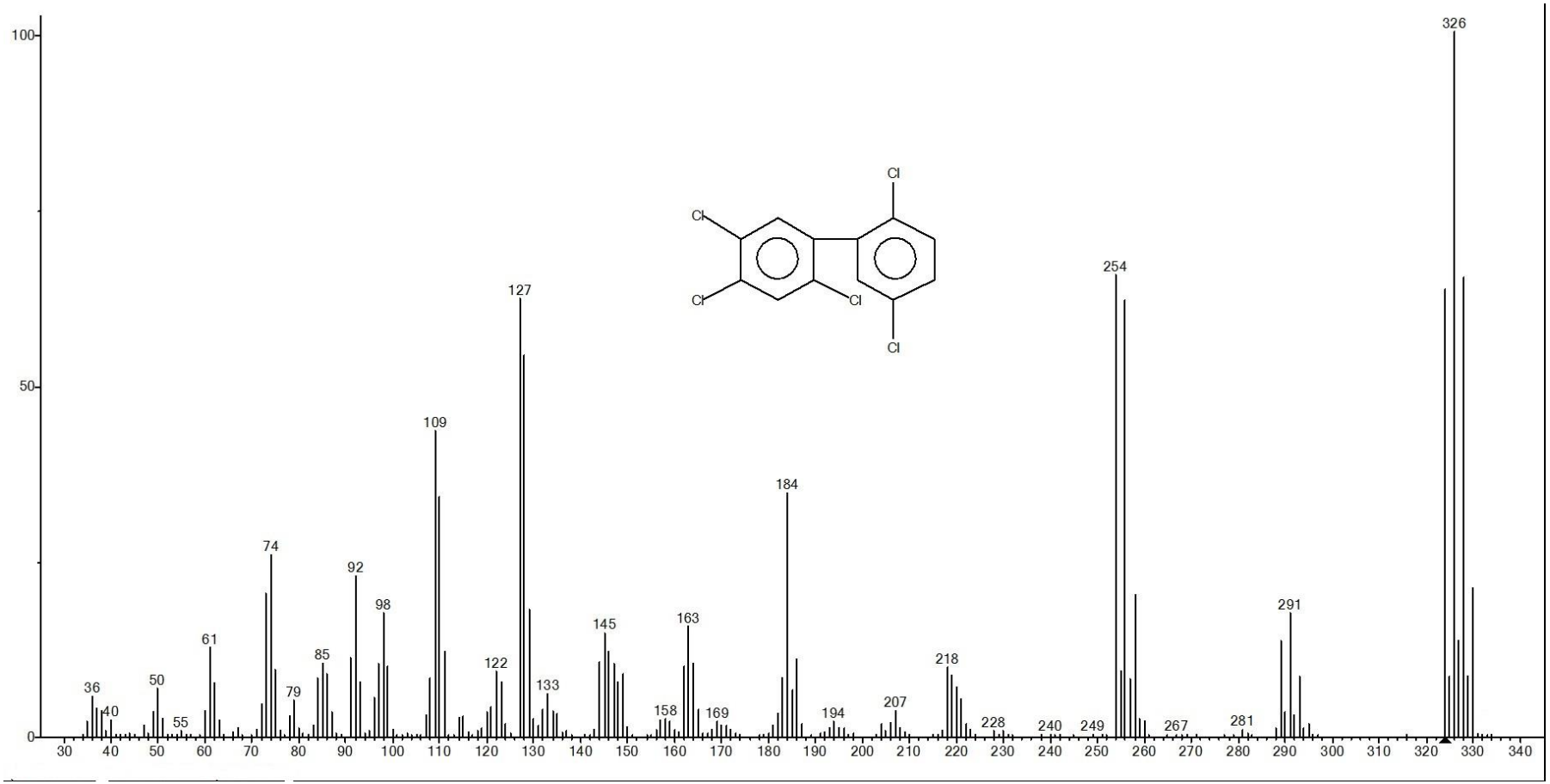

PCB 180
25000
20000
15000
10000
5000
0
0
20
40
60
80
100
120
y = 216.59x + 29.123
R² = 0.9999

Appendix C: PCB congeners composed in A1254 and A1260

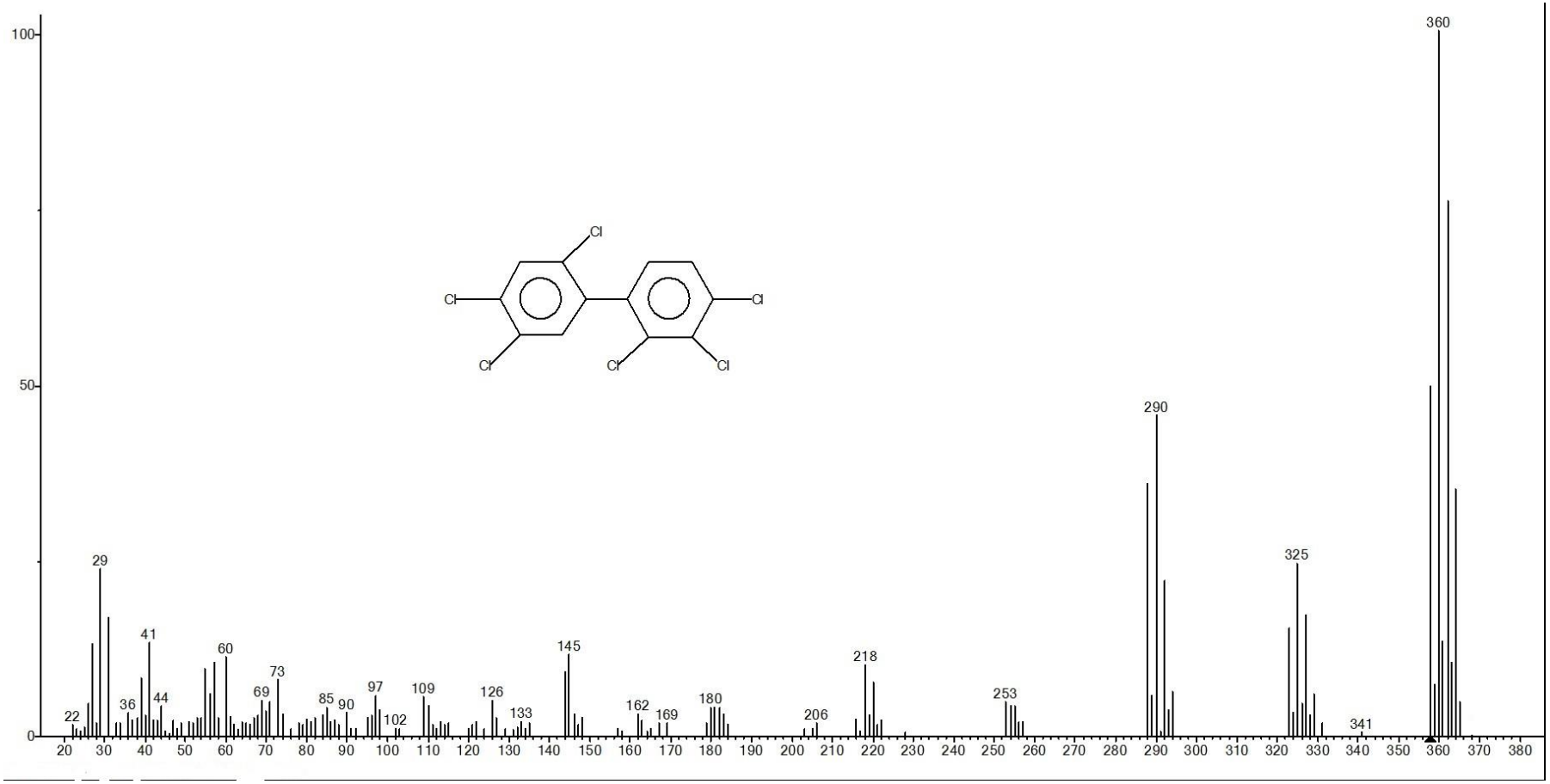
PCB congeners	Compounds	PCB congeners	Compounds
4	2,2'-Dichlorobiphenyl	83	2,2',3,3',4-Pentachlorobiphenyl
7	2,4-Dichlorobiphenyl	85	2,2',3,4,4'-Pentachlorobiphenyl
24	2,3,6-Trichlorobiphenyl	87	2,2',3,4,5'-Pentachlorobiphenyl
28	2,4,4'-Trichlorobiphenyl	92	2,2',3,5,5'-Pentachlorobiphenyl
30	2,4,6-Trichlorobiphenyl	95	2,2',3,5',6-Pentachlorobiphenyl
32	2,4',6-Trichlorobiphenyl	97	2,2',3,4',5'-Pentachlorobiphenyl
39	3,4',5-Trichlorobiphenyl	99	2,2',4,4',5-Pentachlorobiphenyl
47	2,2',4,4'-Tetrachlorobiphenyl	101	2,2',4,5,5'-Pentachlorobiphenyl
49	2,2',4,5'-Tetrachlorobiphenyl	102	2,2',4,5,6'-Pentachlorobiphenyl
52	2,2',5,5'-Tetrachlorobiphenyl	103	2,2',4,5',6-Pentachlorobiphenyl
53	2,2',5,6'-Tetrachlorobiphenyl	105	2,3,3',4,4'-Pentachlorobiphenyl
54	2,2',6,6'-Tetrachlorobiphenyl	110	2,3,3',4',6-Pentachlorobiphenyl
67	2,3',4,5-Tetrachlorobiphenyl	118	2,3',4,4',5-Pentachlorobiphenyl
68	2,3',4,5'-Tetrachlorobiphenyl	122	2,3,3',4',5'-Pentachlorobiphenyl
69	2,3',4,6-Tetrachlorobiphenyl	124	2,3',4',5,5'-Pentachlorobiphenyl
70	2,3',4',5-Tetrachlorobiphenyl	126	3,3',4,4',5-Pentachlorobiphenyl
71	2,3',4',6-Tetrachlorobiphenyl	128	2,2',3,3',4,4'-Hexachlorobiphenyl
72	2,3',5,5'-Tetrachlorobiphenyl	131	2,2',3,3',4,6-Hexachlorobiphenyl
74	2,4,4',5-Tetrachlorobiphenyl	132	2,2',3,3',4,6'-Hexachlorobiphenyl
77	3,3',4,4'-Tetrachlorobiphenyl	135	2,2',3,3',5,6'-Hexachlorobiphenyl
82	2,2',3,3',4-Pentachlorobiphenyl	136	2,2',3,3',6,6'-Hexachlorobiphenyl
PCB congeners	**Compounds**	**PCB congeners**	**Compounds**
146	2,2',3,4',5,5'-Hexachlorobiphenyl	177	2,2',3,3',4,5',6'-Heptachlorobiphenyl
147	2,2',3,4',5,6-Hexachlorobiphenyl	178	2,2',3,3',5,5',6-Heptachlorobiphenyl
149	2,2',3,4',5',6-Hexachlorobiphenyl	183	2,2',3,4,4',5',6-Heptachlorobiphenyl

150	2,2',3,4',6,6'-Hexachlorobiphenyl	185	2,2',3,4,5,5',6-Heptachlorobiphenyl
151	2,2',3,5,5',6-Hexachlorobiphenyl	187	2,2',3,4',5,5',6-Heptachlorobiphenyl
153	2,2',4,4',5,5'-Hexachlorobiphenyl	189	2,3,3',4,4',5,5'-Heptachlorobiphenyl
156	2,3,3',4,4',5-Hexachlorobiphenyl	190	2,3,3',4,4',5,6-Heptachlorobiphenyl
157	2,3,3',4,4',5'-Hexachlorobiphenyl	191	2,3,3',4,4',5',6-Heptachlorobiphenyl
158	2,3,3',4,4',6-Hexachlorobiphenyl	193	2,3,3',4',5,5',6-Heptachlorobiphenyl
159	2,3,3',4,5,5'-Hexachlorobiphenyl	194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl
167	2,3',4,4',5,5'-Hexachlorobiphenyl	195	2,2',3,3',4,4',5,6-Octachlorobiphenyl
171	2,2',3,3',4,4',6-Heptachlorobiphenyl	198	2,2',3,3',4,5,5',6-Octachlorobiphenyl
172	2,2',3,3',4,5,5'-Heptachlorobiphenyl	199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl
174	2,2',3,3',4,5,6'-Heptachlorobiphenyl	200	2,2',3,3',4,5,6,6'-Octachlorobiphenyl
175	2,2',3,3',4,5',6-Heptachlorobiphenyl	201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl
176	2,2',3,3',4,6,6'-Heptachlorobiphenyl	203	2,2',3,4,4',5,5',6-Octachlorobiphenyl
PCB congeners	**Compounds**		
205	2,3,3',4,4',5,5',6-Octachlorobiphenyl		
206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl		
207	2,2',3,3',4,4',5,6,6'-Nonachlorobiphenyl		

Appendix D-Mass spectrum for PCB-101



Appendix E- Mass Spectrum for PCB-158



Appendix F-Mass Spectrum for PCB-180