

**Bioremediation of Polychlorinated Biphenyls (PCBs) on
Contaminated Soils: A Case Study at Rietvlei Farm Borehole
No. 11, Limpopo Province, South Africa.**

by

SENGANI DAVID

(Student number 11521512)

**A thesis submitted in partial fulfillment of the requirements for the
degree of Masters in Environmental Management (MEnv.M) in the
Department of Ecology and Resource Management**

Supervisor: Prof. N. Potgieter

Co-Supervisor: Mr. M.P. Mojapelo

February 2015

Declaration

I Sengani David (student no. 11521512) hereby declare that the submission of this dissertation for the Master in Environmental Management degree at the University of Venda, I declare that the entirety of the work contained therein is my own, original work in design and execution that I am the owner of the copyright thereof (unless to the extent explicitly otherwise stated) and that I have not previously in its entirety or in part submitted previously for a degree at this or any other University, all reference material contained therein has been duly acknowledged



.....
Signature (Student)

14/02/2015
Date.....

Copyright © 2013 University of Venda
All rights reserve

Table of Contents

Contents	Page
Declaration.....	i
Table of Contents	ii
List of Figures.....	v
List of Tables	vi
Abstract.....	vii
Dedication	viii
Acknowledgements	ix
List of Abbreviations	x
CHAPTER ONE: GENERAL INTRODUCTION AND OBJECTIVES	1
1.1 General Background	1
1.2 Problem Statement	3
1.3 Motivation.....	4
1.4 Hypothesis.....	4
1.5 Research Objectives.....	4
1.5.1 General Objectives.....	4
1.5.2 Specific Objectives	4
CHAPTER TWO: LITERATURE REVIEW.....	6
2.1 Introduction.....	6
2.2 Origin of Polychlorinated Biphenyl (PCBs).....	7
2.3 The impact of PCBs on the Environment	7
2.3.1 The impact of PCBs on Air.....	8
2.3.2 The impact of PCBs on Soil.....	8
2.3.4 The impact of PCBs on human health	9
2.4 Polychlorinated Biphenyl (PCBs) in South Africa	10
2.5 Methods for PCBs remediation on the contaminated Soils	11
2.5.1 Physico-chemical methods of remediation of PCBs contaminated soil	12
2.5.2 Microbiology and biochemistry of PCB biodegradation	12
2.5.2.1 Role of bacteria in PCBs degradation.....	13
2.5.2.1.1 Aerobic bacteria in degradation of PCBs.....	13
2.5.2.1.2 Anaerobic Bacteria degradation of PCBs	15

2.5.2.2 Biochemical degradation of PCBs	15
2.6 Factors affecting PCB degrading Bacteria.....	17
2.6.1 Biological factors.....	17
2.6.2 Environmental factors.....	18
2.7 Methods of studying microbial biodiversity of soil.....	19
2.7.1 Biochemical-based techniques to study microbial population.....	19
2.7.2 Sole carbon Source utilization (SCSU) patterns.....	19
2.7.3 Plate Counts	20
2.7.4 Fatty Acid Methy Ester (FAME) Analysis	20
2.7.5 Molecular-Based Techniques.....	21
2.7.6 Guanine plus Cytosine (G + C) content.....	21
2.7.7 Nucleic acid reassociation and hybridization	21
2.7.8 PCR – Based techniques	22
2.7.9 Denaturing Gradient Gel Electrophoresis (DGGE).....	22
2.7.10 Restriction Fragment Length Polymorphism (RFLP).....	23
2.7.11 Terminal Restriction Fragment Length Polymorphism (T-RFLP)	23
2.7.12 Ribosomal Intergenic Spacer Analysis (RISA)/ Automated Ribosomal Intergenic Spacer Analysis (ARISA)	23
2.7.13 Highly Repeated Sequence Characterization or Microsatellite Region.....	24
2.8 Summary of Literature Review	24
CHAPTER THREE: MATERIALS AND METHODS	26
3.1 The Study Area	26
3.2 Soil samples collection	27
3.3 Assessment and Quality Control of PCB in the Laboratory	28
3.3.1 Infrared Spectroscopy (IR) Analysis of PCBs.....	28
3.3.2 Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis of PCBs	28
3.4. Isolation and testing of PCB degrading Bacteria.....	30
3.4.1. Culture Medium.....	30
3.4.2 Chemicals and reagents.....	30
3.4.3 Isolation of PCB degrading bacteria from contaminated soils	31
3.4.4 Identification of the isolated Bacteria Strains	32

3.5. The biodegradation ability of the isolated bacteria on PCBs in soil.....	32
3.5.1 Assessment of ability of isolated bacteria to degrade PCBs.....	33
3.5.2. Total plate count.	36
3.6 Statistical Analysis.....	36
CHAPTER FOUR: RESULTS AND DISCUSSION.....	37
4.1. Assessment and Quality Control of PCBs in the Laboratory	37
4.1.1 Infrared Spectroscopy (IR) analysis of PCBs	37
4.1.2 Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis of PCBs	38
4.2 Isolation and testing of PCB degrading bacteria.....	40
4.3 The Biodegradation ability of the isolated bacteria on PCBs in soil	41
4.3.1 Degradation ability of <i>Burkholderia cepacia</i>	41
4.3.2 Degradation ability of <i>Pasteurella pneumotropica</i>	43
4.3.3 Degradation ability of <i>Enterococcus faecalis</i>	45
4.4 Comparison of the effect of bacteria on different PCB concentrations	50
4.5 Correlation between PCB degradation and growth of bacteria isolates	52
CHAPTER FIVE: CONCLUSION AND RECOMENDATION	55
5.1 Conclusion	55
5.2 Recommendation	56
CHAPTER SIX: REFERENCES	58
Appendix A	68
Appendix B	69

List of Figures

Figure 1.1: The chemical formula for all PCBs: C ₁₂ H ₁₀ -xCl _x	1
Figure 2.1: Pathways of aerobic PCB degradation	16
Figure 3.1: Map of Rietvlei farm at borehole No. 11, Limpopo Province	26
Figure 3.2: Rietvlei farm Borehole No.	27
Figure 3.3: Perkin Elmer Clarus 500: Gas Chromatography	29
Figure 3.4: Demonstrate the 10 fold serial dilutions of soil samples.....	33
Figure 3.5: Anaerobic incubation outlay of Gram positive bacteria experiment.....	34
Figure 3.6: The schematic outlay of the laboratory experiment	35
Figure 4.1: IR test showing the detection of Transformer Oil compounds	38
Figure 4.2 Chromatogram peak areas for PCBs analysis	39
Figure 4.3 Response curve of the PCBs calibration process	40
Figure 4.4: The growth of Burkholderia cepacia on the different PCBs concentrations .	42
Figure 4.5: Degradation studies of PCBs by Burkholderia cepacia.....	43
Figure 4.6: Growth of Pasteurella pneumotropica at different PCB concs after 72 hours	44
Figure 4.7: Degradation studies of PCBs by Pasteurella pneumotropica	45
Figure 4.8: The growth of Enterococcus faecalis at different concentrations of PCBs....	46
Figure 4.9: Degradation studies of PCBs by Enterococcus faecalis	47
Figure 4.10: The growth of mixed culture at different PCBs Concentrations	48
Figure 4.11: Degradation studies of PCBs by mixed cultures	50
Figure 4.12: Comparison of effectiveness of the bacteria isolates on PCBs degradation	51
Figure 4.13: Bacteria growth in the different concentration of PCBs	54
Figure 4.14: Degradation studies of PCBs.....	54

List of Tables

Table 4.1 Number of bacteria isolates: growing in PCBs contaminated soil samples after 72 hours.....	41
Table: 4.2. The analysis of variance (Anova) of bacteria growth and PCBs degradation.	52
Table: 4.3. The analysis of variance means of bacteria growth.....	53
Table: 4.4. The analysis of variance means of PCBs degradation.....	53

Abstract

Polychlorinated biphenyls (PCBs) are worldwide environmental pollutants which contaminate the environment through careless disposal practices and accidental spills or leakages from electrical transformers. These organic compounds are lipophilic chemicals soluble in fats, slightly soluble in water and readily bioaccumulated in the fatty tissues of fish, birds, animals and humans. The main objective of this study was to isolate and identify PCB degrading bacteria from PCB contaminated soils and test them for their degradation ability of PCBs in natural habitat conditions. Three bacteria species which comprise of Gram negative and Gram positive microorganisms were isolated and identified through biochemical tests, catalase tests, oxidase tests and morphological study and included *Burkholderia cepacia*, *Pasteurella pneumotropica* and *Enterococcus faecalis*. The results indicated that, there was an overall decrease of PCB concentration level and the readings ranged between -1.51 and -1.79 respectively for all the microorganisms. *Enterococcus faecalis* remove as much as 32% of PCBs in the contaminated soil samples. Whereas *Pasteurella pneumotropica* could remove 24% of PCBs, *Burkholderia cepacia* 21% of PCBs and the mixed culture removed 23%. Data showed that the 3 bacterial strains could tolerate high concentration of PCBs. The results provided the evidence that naturally occurring bacteria in soil contaminated with PCBs have the potential to degrade PCBs. Statistical analysis showed that there was a significant positive correlation between bacteria growth and treatment with a coefficient of $(r) = 0.1459$ and p value < 0.001 .

Keywords: Bacteria, Bioaccumulation, Biodegradation, Bioremediation, Polychlorinated Biphenyls.

Dedication

I dedicate this work to:

My Lord and Saviour, Jesus Christ

God gives just amount of light I need for the exact moment I need it. I walk forward, one step at a time, fully trusting that the light God sheds is absolutely sufficient.

(Stormie Omartian 1999)

My Fiancé and dearly, Wendy Mosibudi Cherane. My Father and my late Mother Anna Sengani.

Acknowledgements

I would like to sincerely thank:

Professor Natasha Potgieter, my supervisor for her encouragement, support and valuable guidance in finishing this thesis.

Mr. M.P. Mojapelo my co-supervisor, for encouragement and valuable advice on the intervention studies.

Mr. J.P. Kabue, for his assistance and support with laboratory experiment and results analysis.

Mrs. Liesl Morey from Agricultural Research Council (ARC) Statistical and Biometry Unit Hatfield, Pretoria South Africa, for her guidance and analysis of my MSc data.

National Research Foundation (NRF) for funding this project.

University of Venda; Research Publication Committee (RPC) for supporting this project financially.

My family and friends for believing in me and encouraging me to reach for the top, and for always making me feel like a winner. Luambo, Shumani, Rudzani, Nndweleni, Ndandu, Khaukhanani, Murendeni, T.A Nonyana, Mr J.N Steyn, Roderick .M. Baxter, Prof. Van Heerden, for loving me and bringing happiness and love to my life.

List of Abbreviations

% - Percentage

°C – Degree Celcius

µg – microgram

µl – microliter

µm – micrometer

ATSDR- Agency for Toxic Substances and Disease Registry

BHI – Brain Heart Infusion Agar

CBA – Colombia Blood Agar

CFU – Colony Forming Unit (s)

cm – Centimeter

CO₂ – Carbon dioxide

DEA – Department of Environmental Affairs

DGGE- Denaturant Gradient Gel Electrophoresis

DNA- Deoxy Ribonucleic Acid

DPD – N,N-diethyl-phenylenediamine

FAME- Fatty Acid Methyl Ester

FID – Flame Ionization Detector

g – gram

G + C – Guanine + Cytocine

GC – Gas Chromatography

GC- Gas Chromatography

GPS – Global Positioning Satelite

h – hour

H₂O – Water

HPLC- High Performance Liquid Chromatography

IEC – International Electrochemical -Commission

IR – Infrared Spectroscopy
ISO – International Standardization Organization
km – kilometer
mg – milligram
ml – milliliter
NA – Nutrient Agar
NaCl – Sodium Chloride
NB – Nutrient Broth
ng – nanogram
O₂ - Oxygen
PCBs – Polychlorinated Biphenyls
PAHs – Polyaromatic Hydrocarbon
POPs – Persistence Organic Pollutants
PCR- Polymerase Chain Reaction
PLFA- phospholipids Fatty Acid
ppm-parts per million
RAPD- Random Amplified Polymorphism DNA
rpm – revolutions per minute
rRNA- ribosomalribonucleic Acid
RSA – Republic of South Africa
Temp – Temperature
T-RFLP- Terminal Restriction Fragment
Turb – Turbidity
UK – United Kingdom
UN – United Nations
USA – United State of America
UV- Ultra-Violet
VOC-Volatile Organic Compound

CHAPTER ONE: GENERAL INTRODUCTION AND OBJECTIVES

1.1 General Background

Characteristics of Polychlorinated biphenyls (Figure 1.1) are a class of organic compounds with 1 to 10 chlorine atoms attached to a biphenyl molecule composed of two benzene rings; each of these rings contain six carbon atoms (Borja et al., 2013; Brody et al., 2008; Jamshidi et al., 2007). These organic compounds are complex chemical mixtures that comprise of 209 congeners, some of which are known to cause a wide range of adverse effects on humans and animals (Jamshidi et al., 2007; Charles et al., 2007). They have half-lives ranging from months to years, have high chemical stability, low water solubility and has the ability to absorb in the soil and sediments, which results in their accumulation in aquatic ecosystems and bioaccumulate in fish, wildlife and humans through the food chain (Joutey et al., 2013; Jamshidi et al., 2007, Charles et al., 2007).

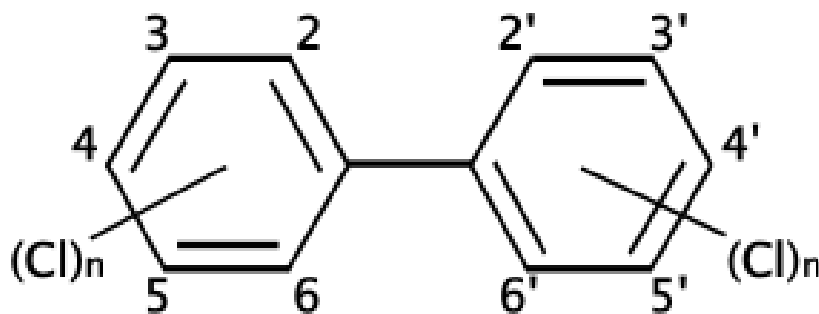


Figure 1.1: The chemical formula for all PCBs: $C_{12}H_{10-x}Cl_x$ (Brody et al., 2008).

They are viscous liquids in nature, odourless, tasteless, and clear to pale yellow in colour and are formed by electrophilic chlorination of biphenyl with chlorine gas (Furukawa and Fujihara, 2008). Their water solubility is low ranging from 0.0027 - 0.42 ng/l and low vapor pressures at room temperature. They have high solubility in most organic solvents, which include oil and fats (Furukawa and Fujihara, 2008; Charles et al., 2007). These

compounds have high dielectric constants and very high thermal conductivity. The flash point of PCBs lies within the range of 170 - 380°C and they are resistant to oxidation, reduction, addition, elimination and electrophilic substitution. The density varies from 1.182 to 1.566 kg/l. The other physical and chemical properties of PCBs vary widely across the class and as the degree of chlorination increases the water solubility decrease (Kafilzadeh et al., 2011; Jamshidi et al., 2007).

These organic compounds were manufactured worldwide in 1929 by Montesano Company and the production ended in 1977 in America. Manufacture and unauthorized use of PCBs was proscribed by United States Environmental Protection Agency (USEPA) in 1978 (Kafilzadeh et al., 2011; Gray, 2004). They were originally called “chlorinated biphenyls, and produced as complex mixtures with multiple isomers of different degrees of chlorination (Jamshidi et al., 2007). Due to the higher demand for electricity, the manufacturing of transformers and capacitors was increased. The PCB containing transformers were used because they were safer, fire resistant, cooling and protecting fluid for transformer and capacitor equipment (Joutey et al., 2013; Brody et al., 2008).

Generally PCBs can enter the environment during their manufacture, use and disposal (Brody et al., 2008). According to the Agency for Toxic Substances and Disease Registry (ATSDR), (2001) Polychlorinated biphenyls have been detected in soil, water, air, sediments, plants and animal tissues globally, due to accidental spills and leaks during their transportation and even in other products containing PCBs. People who are exposed to PCBs normally show symptoms such as chloracne, rashes, headache, tiredness, cough and unusual skin sores (Young-Cheol Chang et al., 2013; ATSDR, 2001).

In addition, studies from the United State of America in 2008 have shown that workers who were exposed to high levels of PCBs had potential liver damage. Pregnant women exposed to relatively high levels of PCBs had premature babies with low birth weights and miscarriages (Young-Cheol Chang et al., 2013; Adeyemi et al., 2009; Broding et al., 2008). Animals that ate PCBs contaminated food showed various health effects such as

anemia, liver damage and thyroid gland injuries (Adeyemi et al., 2009). In order to achieve the sustainable development of the society, therefore there is a need to develop a method to reduce the wide range of pollutants, chemicals and waste from the environment with low environmental impact. One of the best methods to clean up contaminated soil is to make use of certain bacteria which degrade PCBs by means of bioremediation processes (Broding et al., 2008; Fatima et al., 2005; ATSDR 2001). Bioremediation processes are simple to perform, applicable over large areas, cost-effective and generally lead to the complete destruction of the contaminant in question (Hatamian-Zarmi et al., 2009; Begonja et al., 2007; Fatima et al., 2005).

1.2 Problem Statement

Polychlorinated biphenyls are major human health and environmental concern worldwide. In addition the threat posed by PCBs-oil leaking electrical transformers to the environment has increased globally during the past thirty years (Joutey et al., 2013; Adeyemi et al., 2009). Health problems have been reported in a number of countries including Australia, Brazil, China, England, South Africa and the United States of America (Hatamian-Zarmi et al., 2009; Atagana, 2009). Polychlorinated biphenyls are chemically stable and they accumulate in the aquatic ecosystems through run-off. PCBs bioaccumulate in the fatty tissue of fish, animals and human being through the food chain and they are known to be carcinogenic. In South Africa, especially the Limpopo province, which is mostly rural areas, oil leaking transformers and capacitors end-up contaminating the soil, which in turn could have an adverse effect on human health. Data on PCBs from Eskom reveals the existence of oil leaking electrical transformers in the province, which is caused by accidental bursting and theft of copper from the electrical transformer (Eskom, 2009). There is high probability that all Eskom oil leaking electrical transformers are still containing PCBs which could contaminate the soil. Eskom is presently trying to phase out PCBs containing materials and dispose them in the specific designed hazardous waste landfill sites (Eskom, 2009). Phasing out PCBs supplies can seriously cause the disruption of electricity supply to South Africa. Replacing PCB containing equipment requires the switching off of power and leave society with no

power for weeks to months. Therefore there is a need to conduct this study in order to address the problem and get the best solution for cleaning up PCB contamination.

1.3 Motivation

There are different PCBs degradation techniques which are widely investigated, this include hypothermal, mechanical, Rhizoremediation, phytoremediation and photodegradation (Joutey et al., 2013). These techniques are expensive due to maintenance (Atagana, 2009). Bioremediation is an attractive approach for cleaning up PCB contaminated soil because it is cheap and readily available (Hatamian-Zarmi et al., 2009). This study aimed to isolate and identify bacteria with biodegrading abilities from the PCB contaminated soil and assess their potential to degrade different PCB concentration from the contaminated soil.

1.4 Hypothesis

- ❖ Soil at Rietvlei farm borehole no. 11 is contaminated with high concentrations of PCBs that leaks from Eskom transformers and capacitors.
- ❖ Natural occurring environmental bacteria may rapidly co-metabolize PCBs as their sole carbon source.

1.5 Research Objectives

1.5.1 General Objectives

- ❖ Bioremediation of Polychlorinated Biphenyls on contaminated soils; a case study at Rietvlei farm borehole no. 11, Limpopo Province, South Africa.

1.5.2 Specific Objectives

- ❖ To determine characteristics of PCBs in transformer oil.

- ❖ To isolate naturally occurring Gram-negative and Gram-positive bacteria from PCBs contaminated soil at Rietvlei farm borehole no. 11.
- ❖ To evaluate the biodegrading abilities of these isolated bacteria on soil contaminated with PCBs.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Joutey et al., (2013) reported the existence of bacteria strains that are able to degrade Polychlorinated Biphenyls under aerobic and anaerobic conditions (Joutey et al., 2013). Based on the literature review, most of PCBs degradation studies have been conducted in major cities in the United State of America (USA), United Kingdom (UK) and Europe, most of this studies were focused on PCBs contamination on sediments, soil and water (Joutey et al., 2013; Leigh et al., 2006). Microorganisms including bacteria, yeasts and fungi capable of degrading PCBs from the different environmental media have been investigated and reported (Nwinyi, 2010; Atagana, 2009; Mailin and Firdausi, 2006). In South Africa (SA) there are limited PCBs degradation studies reported and especially in rural based areas. This study will aim to be the first to report on PCBs degradation by bacterial species on contaminated soil in rural areas.

Polychlorinated biphenyls (PCBs) and other related polycyclic aromatic hydrocarbons (PAHs), Benzoapyrene (BaP), Naphthalene and Phenanthrene, are ambiguous environmental contaminants (Kafilzadeh et al., 2011; Atagana 2009). Polychlorinated biphenyls commonly enter the environment through electrical transformer leakage and accidental spillage from the materials which contain PCBs. These PCBs then persist in the environment and end up contaminating ground water, surface water, soil, air and food (Jong-Su Seo et al., 2009; Demnerova et al., 2005).

However, a few studies have focused on the contribution of individual microorganisms in reducing the toxicity of PCB or PAH compounds in the environment. Microbial degradation of PCBs may result in the incomplete breakdown of the compound depending on the environmental conditions and the microbial population present (Kafilzadeh et al., 2011; Erdogan et al., 2011). Microorganism may be at a risk from the higher concentration and high molecular weight of PCBs in the soil. Previous studies have shown that PCBs can be totally degraded or particularly transformed either by a single bacterium or community of bacteria species (Kafilzadeh et al., 2011; Erdogan et

al., 2011). Different degradation techniques may be used for the remediation of PCBs contaminated media (Nwinyi, 2010). A suitable degradation technique however depends on many factors such as time of treatment, cost, and type of environmental media, site sensitivity, climatic factors, PCBs molecular weight, and PCBs concentration (Field and Sierra-Alvarez, 2008; Leigh et al., 2006). The techniques may be limited by the high cost of maintaining their conditions. Bioremediation is an ideal treatment option in this case (Leaes et al., 2006).

Jong-Su Seo et al., (2009) determined the high risk posed by consumption of sea food marine prey species contaminated with PCBs to humans and to Chinese white dolphin (*Sousa chinenses*) (Jong-Su Seo et al., 2009). Atagana, (2009) reported that toxicity of PCBs in sediments to marine *Rhepoxynius abronium* was enhanced by irradiation during 27-83 days of exposure (Atagana, 2009).

2.2 Origin of Polychlorinated Biphenyl (PCBs)

These organic compounds were firstly called "Askarel, chlorinated biphenyls," and produced as complex combinations of a multiple isomers in the different degrees of chlorination (Jong-Su Seo et al., 2009). Due to the higher demand for electricity supply, the manufacturing of transformers and capacitors was increased. They were used for cooling of the electrical transformer, because they are stable in the high temperature and have excellent electrical insulating properties (Jong-Su Seo et al., 2009; Brody et al., 2008).

2.3 The impact of PCBs on the Environment

Polychlorinated biphenyls spillage and leaking transformers have been shown to cause serious damage to natural ecosystem (Nwinyi 2010; ATSDR, 2001; Chaineau et al., 2000, Jensen and Sprensen, 1986). Nobbs and Chipman, (2003) observed the wide spread manifestation of PCBs in the environment, by demonstrating the concentration of PCBs in fish from the Swedish fresh water.

Polychlorinated biphenyls have a negative effect on the soil profile more especially on topsoil. Polychlorinated biphenyls suppress the growth of the plants, which leads to susceptibility of soil to natural hazards e.g. floods, as a results they contaminate the environment (Erdogan et al., 2011). Previous studies indicated that reduction of PCBs in soil and sediments can be achieved through biodegradation by microorganisms that can possess metabolic physiological and kinetic characteristics (Erdogan et al., 2011; Molobela 2005; ATSDR, 2001; Nobbs and Chipman 2003, Bolin et al., 2002).

2.3.1 The impact of PCBs on Air

Field et al., (2008) and Al-khalid et al., (2012) indicated that, atmosphere serves as the primary route of PCBs transportation globally, especially those congeners with 1 to 4 chlorine atoms. Polychlorinated biphenyls are volatile pollutant when it is heated, small amount of PCBs have been detected globally in the atmosphere, mostly in urban areas. Extremely high levels of PCBs in the atmosphere could cause skin rashes and cancers to humans being through breathing PCB contaminated air. Animals that inhale certain amount of PCBs in the air could develop liver damage, anemia and thyroid gland injuries (Al-khalid et al., 2012; Field et al., 2008; Woods et al., 1999).

2.3.2 The impact of PCBs on Soil

Polychlorinated biphenyls in the soil originate primarily from leaking electrical transformers. PCB binds very strongly to soil particles and resistant to physicochemical degradation (Al-khalid et al., 2012). Generally the absorption of PCBs onto soil particles depends on factors such as the degree of chlorination of the individual congeners, the soil type, the organic matter content, the soil pH and the soil moisture (Al-khalid et al., 2012; Environment Canada 1999).

Previous studies reported that the movement of PCBs in soil is directly proportional to the solubility of PCBs leaching solvent (Al-khalid et al., 2012; Field et al., 2008 and

Demnerova et al., 2005). In addition, PCBs are nonpolar and sparingly soluble in water. Therefore, the permeation of PCBs into the soil profile through the water flow is very limited. Research has shown that PCBs tend to be highly mobile when leached with organic solvents (Al-khalid et al., 2012; Field et al., 2008; Woods et al., 1999). The uptake of PCBs from soil by invertebrates occurs through direct dermal adsorption, soil contact, ingestion when grazing (Environment Canada, 1999). The uptake of PCBs in humans occurs through eating of termites' soil, fish and fruits and vegetables (Al-khalid et al., 2012).

2.2.3 The impact of PCBs on Water

Adeyemi et al., (2009) reported that, PCBs leakage contaminates the soil and as a result they cause health hazards in the water resources. Water resources especially in Nigeria's Lagos Lagoons are most affected by environmental stress imposed by PCBs as the major contaminant (Al-khalid et al., 2012; Adeyemi et al., 2009; Duffy et al., 2002). Polychlorinated biphenyls fail to degrade due to their high solubility in water, bioaccumulate throughout the food chain (Duffy et al., 2002). In Lagos, the detection of persistent organic pollutants (POPs) including PCBs in 40 fresh water fish samples have shown the ranged from 8.0 – 13.0 ng/g (Adeyemi et al., 2009; Duffy et al., 2002).

2.3.4 The impact of PCBs on human health

The impact of PCBs on human health is predominantly through the diet or food stuffs and especially in seafood's such as fish. In addition, red meat, poultry, eggs and dairy products also may be important dietary sources of PCBs (EPA, 1999). The use of old fluorescent lighting fittings and electrical devices and appliances such as television sets and refrigerators that dates 30 or more years ago, may leak small amount of PCBs into the air when they get hot during their usage or operation. In the work place during repair and maintenance of PCB transformers, accidents, fires or spills involving transformers, fluorescent lights and other old electrical devices and disposal of PCB materials may result in skin exposure (Joutey et al., 2013; Adeyemi et al., 2009; ATSDR 2001).

Individuals in the general population who may be exposed to higher than average levels of PCBs include recreational and subsistence fishers who routinely consume large amounts of locally caught fish, subsistence hunters who routinely consume the meat and organ tissue of marine mammals or people who live near hazardous waste sites contaminated with PCBs (EPA, 1999). Polychlorinated biphenyls have impacted the pregnancy women who are exposed to relatively high concentration of PCBs (Aluyor and Ori-Jesu, 2009). The most likely ways by which infants are exposed to PCBs are from the breastfeeding or trans-placental transfer. Previous studies have indicated that PCBs alter the estrogen levels in the body and contribute to reproduction problems (Adeyemi et al., 2009; Atagana 2009). Biological magnification of PCBs has imposed the detrimental health effect on the polar bears in both male and female, including whales were unable to reproduce the young's (Aluyor and Ori-Jesu 2009; Woods and Trobaugh 1999).

2.4 Polychlorinated Biphenyl (PCBs) in South Africa

In South Africa oil leaking electrical transformers and capacitors are still in use, especially in the Limpopo province. Eskom oil leaking electrical transformers might still contain PCBs and they are contaminating the soil. In this stage, Eskom is replacing PCBs-containing transformers and by 2025 they are expected to get rid of all the PCBs equipment (Eskom, 2009; Demnerova et al., 2005).

Eskom is participating in the National Implementation Plan (NIP) of the Department of Environmental Affairs (DEA) in terms of the Stockholm Convention for Persistent Organic Pollutants (POPs) Sectorial Focus Group 3: PCB Assessment (Eskom, 2009). Eskom is not the company which manufactures the PCBs but is found in dielectric fluid used in some electrical equipment such as transformers and capacitors, used for electrical insulation and thermal cooling. Over the past 12 years Eskom management practices relating to PCBs have included the following:

- ❖ Handling, storage, testing and labeling of PCB-contaminated equipment based on South African National Standard (SANS) 0290: (2008): Mineral Insulating Oils, Management and Handling of PCB. The standard covers the requirements, classification, labeling, handling, storage, transportation, decontamination and disposal of PCB-contaminated oil.
- ❖ Compilation of inventories of PCB-containing equipment.
- ❖ Development of phase-out plans that meet the requirements of the Stockholm Convention.
- ❖ Verification of the inventories regularly to ensure that the management of PCBs is in line with procedures and in line with the requirements of the SANS 0290: (2008) standard.

An overview of Eskom PCB inventory indicating the equipment that contains 50 ppm or more of PCBs. Over the last seven years Eskom has phased out over 950 tons of PCB-containing equipment through thermal destruction. Equipment items are thermally treated and the solid residue tested for the presence of PCBs after treatment. The residue is reclassified as non-hazardous and deposited in the land-fill (Eskom, 2009). Eskom (2009) reported that 17 086 PCBs containing equipment comprises of transformers and capacitors are currently in use with the PCBs concentration of ≥ 50 ppm. Eskom have shattered some of the transformers and capacitors and dispose them at registered landfill site. Removal of PCB containing transformers started in 2007 and they destroyed 10.0 tons of PCBs containing equipment, in 2008 they destroy 17.0 tons and in 2009 they destroyed 505.6 tons and deposited at registered landfill site (Eskom, 2009).

2.5 Methods for PCBs remediation on the contaminated Soils

Soil may require remediation when significant concentrations of one or more pollutants are detected. Several remediation technologies have been developed for the cleaning-up of these chemical elements in soil samples (Joutey et al., 2013). However traditional methods, such as excavation, thermal treatment and chemical soil washing are expensive and destructive (Joutey et al., 2013; Nwinyi 2010).

Remediation is generally the breaking down of complex and toxic materials into simple and common forms (Aluyor and Ori-Jesu, 2009). Any substance placed in the environment begins to undergo degradation processes due to the action of substrate utilizing microorganisms (Adeyemi et al., 2009). A large diversity of microorganisms such as bacteria and fungi are capable of degrading a wide range of PCB congeners (Adeyemi et al., 2009; Atagana 2009, Aluyor and Ori-Jesu, 2009).

2.5.1 Physico-chemical methods of remediation of PCBs contaminated soil

Physico-chemical remediation is the process where the physical and chemical properties of the contaminants at the contaminated media are destroyed, converted or separated (Hatamian-Zarmi et al., 2009). Physico-chemical treatments are cost-effective and can be completed in a short period of time in comparison with biological treatments. The technique is readily available. The technique includes processes such as liming and clay flocculation (Hatamian-Zarmi et al., 2009; Adriano et al., 2004).

Kafilzadeh et al., (2011) indicated that, lime is an important management tool in reducing toxicity in the soils. The effect of liming on Cd adsorption and uptake largely depends on the relative change in pH and Ca^{2+} concentration in soil solution. In addition of other alkaline materials such as coal fly ash has resulted in the decrease of the Cd content in soil (Young-Cheol Chang et al., 2013; Kafilzadeh et al., 2011).

2.5.2 Microbiology and biochemistry of PCB biodegradation

Basic information such as residual oil concentration, population of the PCBs – degrading microorganisms and the biodegradation potential, are the important factors to be considered for bioremediation of PCB polluted sites (Young-Cheol Chang et al., 2013). Biological degradation is an attractive alternative method for the treatment of PCB contaminated soils and sediments (Young-Cheol Chang et al., 2013; Aluyor and Ori-Jesu 2009). There is a wide diversity of microorganisms including bacteria and fungi which

are capable of degrading a wide range of PCBs congeners (Hatamian-Zarmi et al., 2009). Under aerobic conditions, lower chlorinated of PCB congeners can be degraded and serves as growth supporting substrates of the bacteria. Anaerobic bacteria are able to degrade higher chlorination of PCB congeners. The reductive dechlorination of PCBs provides the electron-donating substrates for the bacteria to grow (Aluyor and Ori-Jesu 2009; Field and Sierra-Alvarez 2008).

2.5.2.1 Role of bacteria in PCBs degradation

Bacteria that are mostly found in soils, acidic hot springs and radioactive wastes have the ability to biodegrade a variety of organic compounds including PCBs both through aerobic and anaerobic pathways (Nwinyi, 2010). Biodegradation is a natural way of reprocessing wastes, or breaking down of the organic matter into useful nutrients for the microbes in the soil (Joutey et al., 2013; Nwinyi, 2010). Biodegradation technique involves the decaying of the organic materials which is carried out by bacteria, yeast and fungi, and possibly other organisms. Bioremediation and biotransformation methods endeavor to harness an amazing naturally occurring microbial with the ability to degrade PCBs (Joutey et al., 2013; Nwinyi, 2010).

2.5.2.1.1 Aerobic bacteria in degradation of PCBs

Aerobic bacteria are capable to degrade PCBs and use it as their primary substrate for their growth and transform it into their energy source for survival (Field et al., 2007). The biphenyl metabolic enzymes encoded by the bph gene cluster are responsible for the attack of PCB cleavage (Bojar et al., 2013; Furukawa and Fujihara, 2008). Several studies have isolated PCB degrading bacteria which are as follows: gram negative *pseudomonas*, *pasteurella*, *spingomona*, *Alcaligenes*, *Achromobacter*, *Burkholderia*, *Comamonas*, *Sphingomonas*, *Ralstonia*, *Acinetobacter*; and gram-positive strains *Rhodococcus*, *Enterococcus*, *staphylococcus*, *Corynebacterium* and *Bacillus* (Borja et al., 2013; Joutey et al., 2013, Furukawa and Fujihara, 2008). An aerobic bacterium is known

to co-metabolize lower chlorinated PCBs congeners (Hatamian-Zarmi et al., 2009). The trend reflects that aerobic bacteria PCB biodegrading ability decreases with the increase of the chlorine (Hatamian-Zarmi et al., 2009; Field and Sierra-Alvarez, 2008).

The genus *Burkholderia* is a phylogenetically well-defined group of organisms which occupies very diverse ecological niches in the environment (Mailin and Firdausi, 2006). There are more than 30 *Burkholderia* species that are described as soil dwellers. Quite a number of studies have shown the ability of several *Burkholderia* strains on degradation of PCBs and other aromatics compounds (Furukawa and Fujihara et al., 2008; Mailin and Firdausi, 2006; Goris et al., 2004). This strain has the ability to co-metabolize many polychlorinated biphenyls congeners when grown into the biphenyls contaminated soil (Goris et al., 2004). They has extensive characteristics pathways for PCBs degradation at both genetically and in molecular level (Mailin and Firdausi, 2006; Goris et al., 2004).

The degradation of PCBs by the mixed microbial cultures was reported by Jun Xu et al., (2008). Al-khalid and EL-naas (2012) studied the enriched of mixed cultures which was obtained from PCBs contaminated river sediments and found to be more effective on PCBs degradation (Field and Sierra-Alvarez, 2008). Reports indicate that PCBs isomers which are more water soluble and low chlorination are degraded at faster rate than the less water soluble and high chlorination (Ashrafosadat et al., 2009; Mailin and Firdausi, 2006). Mixed cultures have shown a complete degradation than a pure bacteria strain (Mailin and Firdausi, 2006). This is due to the fact that, in nature all the bacterial populations occur in culture rather than solitary colonies (Jun Xu et al., 2008; Al-khalid and EL-naas 2012).

The PCB degrading bacteria degrade biphenyl and metabolize PCB with the same suite of enzymes employed in biphenyl catabolism (Ashrafosadat et al., 2009). Large varieties of aerobic bacteria are capable of using the PCBs as their sole carbon source and energy (Mailin and Firdausi, 2006). The PCBs degradation is accomplished through benzene ring cleavage mediated by intracellular enzymatic reaction. The use of pure cultures,

especially adapted to metabolize the contaminant can be foreseen attractive alternative (Mailin and Firdausi, 2006).

2.5.2.1.2 Anaerobic Bacteria degradation of PCBs

Anaerobic degrading bacteria attack more highly chlorinated PCB congeners through a process called reductive dechlorination with the removal of *meta* and *para* chlorines of PCB congeners (Mailin and Firdausi, 2006; Abramowicz 1995). The importance of the anaerobic PCB degrading bacteria is that they reduce the potential risk from the potential exposure of PCBs, and reduce the toxicity of PCBs, which will also reduce the carcinogenicity of PCBs (Borja et al., 2013; Abramowicz 1995).

Anaerobic reductive dechlorination of PCB was first observed by Field and Sierra-Alvarez (2008). Comparison of the congener patterns in anaerobic sediments, led the authors to conclude that, the higher PCB chlorination can degrade faster than lower chlorinated PCBs. After 16 weeks, 53% of the total chlorine in Aroclor 1242 applied at 700 ppm was removed. Field and Sierra-Alvarez, (2008) chlorine atoms are preferentially taken out from the meta- and para- positions on the biphenyl structure, thereby leaving lower chlorinated ortho substituted congeners. The activities above are schematically represented thus: $R - Cl + 2e^- + H^- \rightleftharpoons R - H + Cl^-$. Research groups' world-wide has succeeded in demonstrating the anaerobic dechlorination of PCB in sediment cultures from different environmental sources (Alvarez 2008; Field and Sierra-Alvarez, 2008).

2.5.2.2 Biochemical degradation of PCBs

Polychlorinated biphenyls can be degraded aerobically and anaerobically by microorganisms (Borja et al., 2013). Aerobic PCB degraders can be isolated as biphenyl (BP) - utilizing bacteria, which are widely distributed in the environment. PCB degradation by such microbes is through the co-metabolism by BP-metabolic enzymes (Furukawa and Fujihara, 2008). The major pathway of PCB degrading bacteria proceeds

via 2, 3-dioxygenation, in which molecular oxygen is introduced at the nonchlorinated 2, 3 sites (Kafilzadeh et al., 2011). The group of PCB degraders shows a narrow range of PCB specificities for degradation. A large number of *Pseudomonas* strains along with some strains of *Alcaligenes*, *Achromobacter*, *Acinetobacter*, and *Moraxella* have been isolated and characterized so far. Gram-positive strains such as *Arthrobacter* and *Rhodococcus* are also known to degrade PCBs through the same 2, 3-dioxygenation. On the other hand, *Alcaligenes eutrophus* and *Pseudomonas* sp. degrade PCBs primarily through 3, 4-dioxygenation (Borja et al., 2013; Kafilzadeh et al., 2011; Furukawa and Fujihara, 2008).

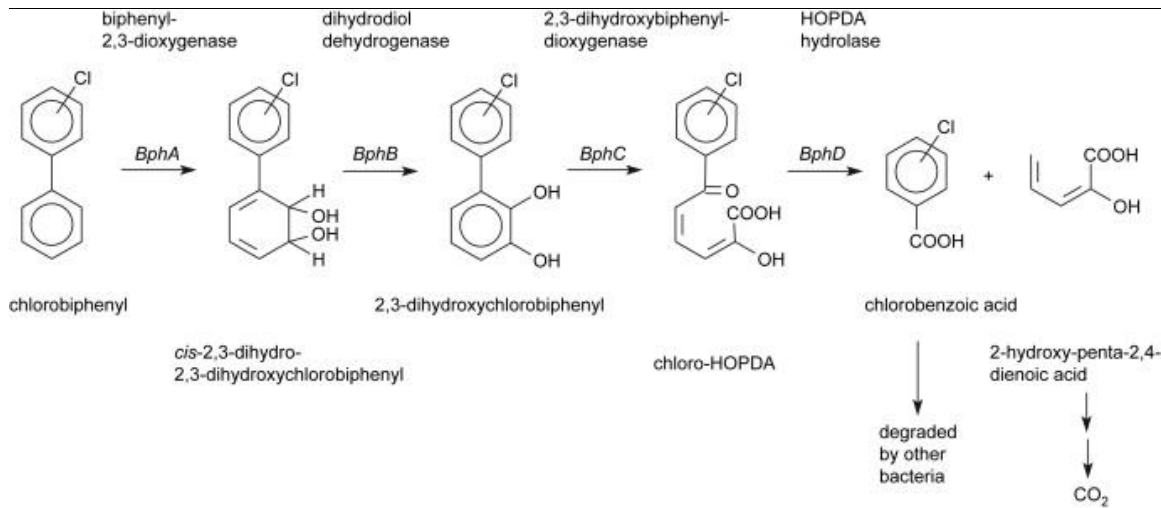


Figure 2.1: Demonstrate the pathways of aerobic PCB degradation bacteria (Strand, 2011).

Previous studies reported that monochlorobiphenyls function as a sole source of carbon and energy of the PCB degrading bacteria (Ashrafosadat et al., 2009; Furukawa and Fujihara, 2008). On the other hand, congeners containing two or more chlorines are usually aerobically degraded via co-metabolism, requiring biphenyl as a growth substrate to induce the requisite enzymes (Ashrafosadat et al., 2009). Through the aerobic biodegradation of PCBs, bacteria degrade the more lightly chlorinated congeners. These organisms attack PCBs via the 2, 3 – deoxygenize pathways and convert the congeners to the corresponding Chlorobenzoic acids which can be further converted and the by-

products are carbon dioxide, water, and chloride (Figure 2.1); (Kafilzadeh et al., 2011; Furukawa and Fujihara, 2008).

2.6 Factors affecting PCB degrading Bacteria

Microorganisms have the ability to degrade different organic pollutants, through their metabolic processes (Joutey et al., 2013; Ines et al., 2007). However, their efficiency depends on many factors, including the chemical nature and the concentration of pollutants, their availability to microorganisms, and the physicochemical characteristics of the environment (Nwinyi, 2010). Factors that influence the rate of pollutants degradation include biological and environmental factors (Joutey et al., 2013; Nwinyi, 2010; Ines et al., 2007).

2.6.1 Biological factors

A biotic factor is the metabolic ability of microorganisms, which affect the microbial degradation of organic compounds (Joutey et al., 2013). These include direct inhibition of enzymatic activities and the proliferation processes of degrading microorganisms. The same factors can inhibit the process of microbial degradation of PCBs in the soil (Al-khalid and EL-naas, 2012). For example, competition between microorganisms for limited carbon sources, predation of microorganisms by protozoa and bacteriophages. The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of “catalyst” present (Hank et al., 2010). In this context, the amount of “catalyst” represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes produced by each cell (Erdogan et al., 2011). Furthermore, specific enzymes are required and the affinity of the organisms to metabolize the contaminant. In addition, availability of sufficient amounts of nutrients and oxygen is required in the process of co-metabolizing the contaminant without nutrient and oxygen this reduces the proper proportions for microbial growth (Hank et al., 2010). Other factors that influence the rate of biodegradation, is by controlling the rates

of enzyme catalyzed reactions such as temperature, pH and moisture (Erdogan et al., 2011; Hank et al., 2010; Leigh et al., 2006).

Joutey et al., (2013) and Hank et al., (2010) reported that biological enzymes involved in the degradation pathway have an optimum temperature. Indeed, the rate of biodegradation is decreased by roughly one-half for each 10°C decrease in temperature. Biodegradation process can take place under a wide-range of soil pH, however, a pH of 6.5 to 8.5 is generally optimal for biodegradation (Al-khalid and EL-naas, 2012; Hank et al., (2010).

2.6.2 Environmental factors

Soil type and soil organic matter content affect the potential for adsorption of an organic compound. Joutey et al., (2013) determine adsorption as an analogous process wherein a contaminant penetrates into the bulk mass of the soil matrix. Both adsorption and absorption reduce the availability of the contaminant to most microorganisms. Variations in porosity of the unsaturated and saturated zones of the aquifer matrix may influence the movement of fluids and contaminant migration in groundwater (Al-khalid and EL-naas, 2012). The ability of the matrix to transmit gases, such as oxygen, methane and carbon dioxide, is reduced in fine grained sediments and also when soils become more saturated with water. It affects the rate and the process of biodegradation to taking place (Hank et al., 2010). The oxidation-reduction potential of a soil provides a measurement of the electron density of the system. Biological energy is obtained from the oxidation of compounds in which electrons are transferred to various more oxidized compounds referred to as electron acceptors (Erdogan et al., 2011). A low electron density (Eh greater than 50 mV) indicates the oxidization of PCBs through aerobic conditions, whereas high electron density (Eh less than 50 mV) indicates reduction of PCBs through anaerobic conditions (Joutey et al., 2013; Al-khalid and EL-naas 2012; Erdogan et al., 2011).

2.7 Methods of studying microbial biodiversity of soil

Microbial biodiversity is the variety range of species and their distribution in the soil (Kirk et al., 2004; Bing-Ru et al., 2006). There is more than one method for studying microbial diversity in soil; they are classified into two groups' namely, biochemical-based techniques and molecular-based techniques (Kirk et al., 2004).

2.7.1 Biochemical-based techniques to study microbial population

Biochemical-based techniques have more advantages in terms of species counts. They are fast and simple to carry out, cost effective and can provide information with accuracy including characterization of the heterotrophic component of the population. Biochemical-based techniques also have the ability to culture a large number of bacterial and fungal species. The limitation of these techniques is when dislodging bacteria or spores from soil particles or biofilms in growth medium selections. It is difficult to maintain the growth conditions such as temperature, pH and light on the selected growth medium (Bing-Ru et al., 2006).

2.7.2 Sole carbon Source utilization (SCSU) patterns

Kirk et al., (2004) used the Sole Carbon Source Utilization (SCSU) patterns technique with commercially available 96-well microlitres plates to assess the potential functional diversity of the bacteria population through Sole Carbon Source Utilization (SCSU) patterns. The technique contains specific plates for identification of the Gram-negative and Gram-positive bacteria. Each plate contains 95 different carbon sources and one control without the substrate.

According to Bing-Ru et al., (2006), the Sole Carbon Source Utilization (SCSU) patterns technique is much simpler when it is used without automated measuring apparatus. SCSU-techniques give the accurate results about the importance of microbial

communities. The technique has become much more popular. Nonetheless, the analysis and interpretation of the information is often complicated. Limitations in the technique are encountered when soil contains too high or too low values of pH, temperature, light and growth conditions (Bing-Ru et al., 2006; Kirk et al., 2004).

2.7.3 Plate Counts

Plate count is a traditional technique which is used to select a direct viable count of specific bacteria species. According to Kirk et al., (2004), the plate count technique is accurate, cost effective and provides reliable information about the heterotrophic nature of the microorganisms in soil sample. The limitations of this technique are encountered when dislodging bacteria species from the soil samples. It is difficult to maintain the growth conditions such as temperature, pH and light on the selected growth medium. In addition, the plate count technique favors only those microorganisms which can grow fast (Kirk et al., 2004; Ranjard et al., 2000).

2.7.4 Fatty Acid Methy Ester (FAME) Analysis

Second studies reported that Fatty Acid Methy Ester (FAME) as the technique for studying microbial community composition and population change in the suitable soil samples due to the chemical contaminants (Bing-Ru et al., 2006; Kirk et al., 2004; Ranjard et al., 2000) . FAME analytical techniques provide reliable species composition based on the grouping of fatty acids. Signature fatty acids are used to differentiate the major taxonomic groups within a microbial community. Therefore, the change in the fatty acid profile will represent a change in the microbial population. FAME analysis is more convenient because fatty acids are extracted directly from the soil samples, methylated and analyzed by gas chromatography. Different soil samples can be compared through the use of multivariate analysis in order to check the FAME profile. This technique detects the change in the composition of the bacterial community. Although FAME analysis is the best technique for studying microbial diversity, some limitations are encountered when cellular fatty acid composition is influenced by factors

such as temperature, nutrition and the possibility of exists other organisms can confound the FAME profile. Fatty acids cannot represent specific species because individuals can incorporate numerous fatty acids and the same fatty acids can occur in more than one species (Bing-Ru et al., 2006; Kirk et al., 2004; Ranjard et al., 2000).

2.7.5 Molecular-Based Techniques

There are number of molecular-based approaches that have been developed to study the microbial diversity present in soil samples (Bing-Ru *et al.*, 2006). These approaches include DNA reassociation, DNA-DNA and mRNA, DNA hybridization and DNA cloning and sequencing. Which include PCR-based methods such as Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), Ribosomal Intergenic Spacer Analysis (RISA) and Automated Ribosomal Intergenic Spacer Analysis (ARISA) techniques (Kirk et al., 2004). Molecular based techniques are used to overcome some of the limitations from the culture-based techniques.

2.7.6 Guanine plus Cytosine (G + C) content

Difference in the Guanine plus Cytosine (G + C) content of DNA can be used to study the bacterial diversity of soil communities (Kirk et al., 2004). The principle of the test is based on the knowledge that microorganisms differ in their G + C content. This technique provides a course level of resolution with different taxonomy which can share G + C range. However, taxonomically related groups differ between 3% and 5%. G + C analysis is quantitative and does not require large quantities of DNA (Kirk et al., 2004).

2.7.7 Nucleic acid reassociation and hybridization

DNA reassociation measures the genetic complexity of the microbial community that is found in the soil. The total DNA is extracted from environmental samples, purified,

denatured and allowed to reanneal with the non-cultivability of the environmental species (Molobela 2005; Kirk et al., 2004).

2.7.8 PCR – Based techniques

PCR-based techniques are used for the identification of prokaryotes and predict the phylogenetic relationships of Gram-negative and Gram-positive bacteria. The advantage of PCR-based techniques is that they give more information on species composition than other techniques. They compare the common species present in soil samples with Nucleic acid reassociation and hybridization techniques (Bing-Ru et al., 2006; Kirk et al., 2004).

2.7.9 Denaturing Gradient Gel Electrophoresis (DGGE)

The Denaturing Gradient Gel Electrophoresis (DGGE) method is used to analyze the nucleic acid and to detect the mutation in DNA sequence extracted from the soil samples. It provides the first step for a more targeted and detailed analysis of composition and microbial diversity (Molobela 2005). Denaturing Gradient Gel Electrophoresis (DGGE) is reliable, reproducible, rapid and inexpensive. Multiple samples can be analyzed at the same time and they show changes of microbial population. The method has some limitation however, such as PCR biases and laborious sample handling. It is estimated that DGGE can only detect 1 – 2% of the microbial population that represent dominant species present in an environmental soil sample (Molobela 2005; Kirk et al., 2004).

2.7.10 Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) technique is also known as amplified ribosomal DNA restriction analysis (ARDRA). RFLP is a technique used for studying microbial diversity relying in DNA polymorphism. RFLP banding patterns can be used to measure bacterial community structure and detection of structural changes (Bing-Ru et al., 2006; Molobela, 2005).

2.7.11 Terminal Restriction Fragment Length Polymorphism (T-RFLP)

Terminal Restriction Fragment Length Polymorphism (T-RFLP) is a technique which addresses the limitations of the Restriction Fragment Length Polymorphism (RFLP) technique. T-RFLP and RFLP follows the same principles were one PCR primer is labelled with fluorescent dye. T-RFLP can analyze complex bacterial communities as well as provide information on the diversity of the visible taxonomic unit. It can also measure the species richness and species evenness. The procedures can be automated in order to allow the sampling and analysis of 5 soil samples. T-RFLP is limited on the choice of the universal primers, DNA extraction and PCR bias. Despite these limitations, T-RFLP can be used to study microbial diversity in the soil and is an excellent tool to compare the relationship between different soil samples. It can also be used to measure the spatial and temporal change in bacterial communities (Bing-Ru et al., 2006; Kirk et al., 2004).

2.7.12 Ribosomal Intergenic Spacer Analysis (RISA)/ Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Ribosomal Intergenic Spacer Analysis (RISA) or (ARISA) techniques provide the ribosomal-based fingerprinting of the microbial community. The intergenic spacer (IGS) in RISA region between the ribosomal subunits is the amplified by PCR. The region is useful for encoding tRNAs and differentiating bacterial strains and closely related species. Both techniques have the accuracy of producing highly reproductive bacterial community profiles. Limitations are only encountered when RISA requires larger

quantities of DNA, it is time consuming and the resolution tends to be low (Bing-Ru et al., 2006; Kirk et al., 2004, Ranjard et al., 2000).

2.7.13 Highly Repeated Sequence Characterization or Microsatellite Region

Highly repeated sequence characterization or microsatellite region technique contains the repetitive short DNA sequence which 1-10 based pair long. The technique is used to identify bacterial genomic fingerprint of chromosome structures (Ranjard et al., 2000). All chromosome structures identified are considered to be variable between bacteria strains. Microsatellite regions are used for identification of mycorrhizal fungi with highly repeated sequences. The similarity can be compared using the indices in order to investigate the difference between the interspecific and intraspecific level (Kirk et al., 2004). The highly repeated sequence characterization or microsatellite region technique encounters some limitations depending on the complexity of the bacterial community; microsatellite region needs to be known before primers are used. The technique is good for developing probes for detecting any change in the microbial community which is caused by environmental change (Bing-Ru et al., 2006; Kirk et al., 2004).

2.8 Summary of Literature Review

Polychlorinated biphenyls (PCBs) are major concern worldwide pollutants due to their low degradability and high toxicity on the environment. These compounds are known to cause a wide range of adverse effects in human and animal health (Charles et al., 2007). Several studies have shown different techniques to be used to indicate PCB degradation naturally in the environment, which include hypothermal, biodegradation, phytoremediation and photo-degradation (Joutey et al., 2013; Al-khalid and EL-naas, 2012; Erdogan et al., 2011).

These techniques however, were found to be limited by the high cost of maintenance and not easy to apply over a large area of contamination. Bioremediation using microorganisms was found to be an attractive method for restoring and/or rehabilitating sites which are contaminate by PCBs (Erdogan et al., 2011; Molobela 2005). The main

aim of this study was therefore to isolate, identify and evaluate the ability of PCB degrading bacteria to degrade PCBs on the contaminated soil. Little is known about bioremediation of PCBs in South Africa, especially in rural environments. To our knowledge this study will be the first in South Africa to use bioremediation of PCBs from contaminated soil in rural areas.

CHAPTER THREE: MATERIALS AND METHODS

3.1 The Study Area

The study area includes the borehole No. 11 at Rietvlei farm in the Makhado Municipality, Limpopo Province, South Africa. The GPS coordinates of the study area were: latitude of 23° 03' 56.31''S and longitude of 29° 52'57.98''E (Figure 3.1).

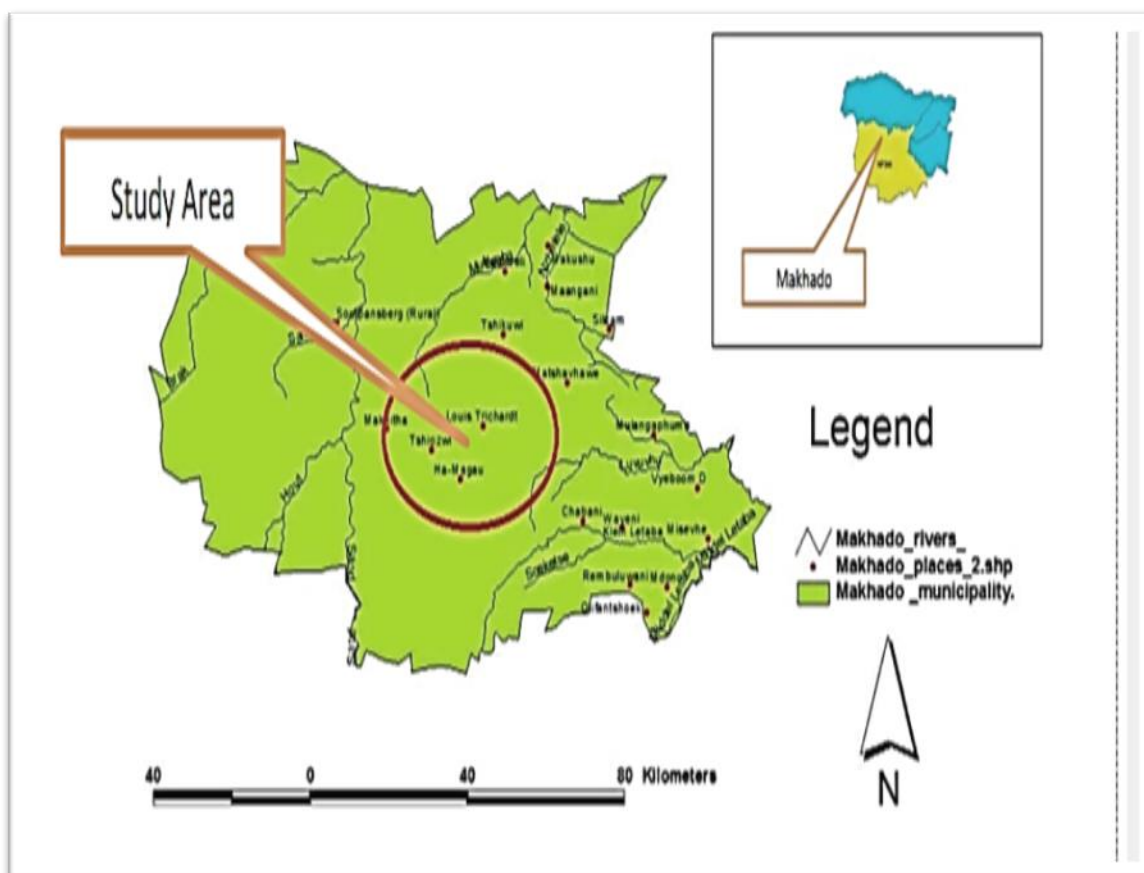


Figure 3.1: A map of Rietvlei farm at borehole No. 11, Limpopo Province, South Africa.

3.2 Soil samples collection

Soil samples were collected from Rietvlei farm borehole No. 11. The site was subdivided into two sections, section A (PCBs contaminated site) and B (uncontaminated site). Polychlorinated biphenyls contaminated soil samples were collected next to the utility poles where oil leaking transformers were located as indicated in Figure 3.2. Soil samples were collected using vertical soil sampling method which involves the use of the soil auger. The soil auger collects the soil vertically, which is called vertical sampling and works in a way similarly to a cork screw. The soil auger is inserted into the soil by applying a downward force while rotating it. It fills the soil auger with soil when goes deeper into the soil.

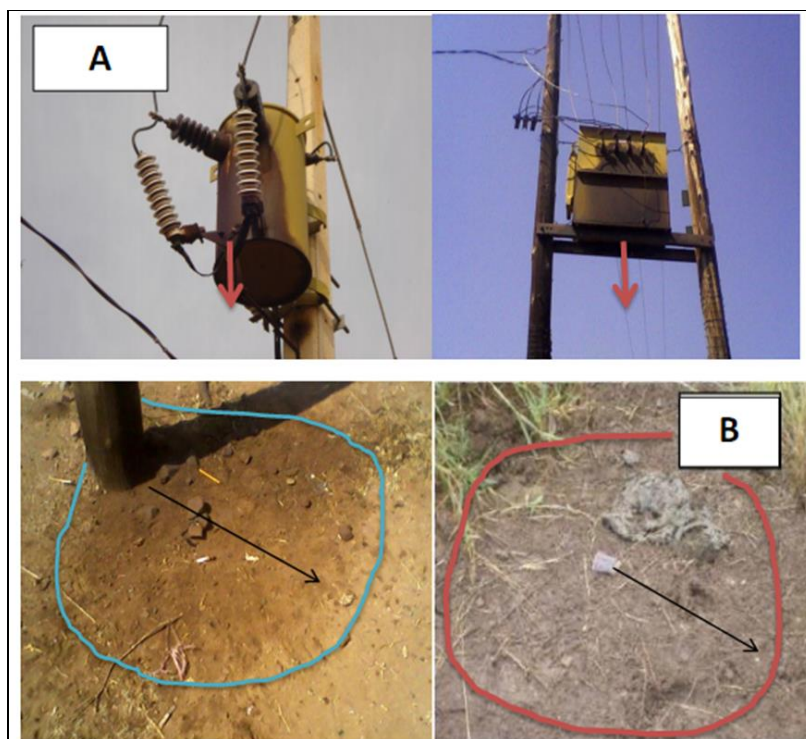


Figure 3.2: The study area map at Rietvlei farm Borehole No. 11. (A) Contaminated site from oil leaking electrical transformers; (B) uncontaminated site oil.

3.3 Assessment and Quality Control of PCB in the Laboratory

Two techniques namely Infrared Spectroscopy (IR) and Gas Chromatography-Flame Ionization Detector (GC-FID) were used to determine and characterize the chemical composition of the transformer oil (Zakharich et al., 2001). Clean oil used in the electrical transformer were obtained from Eskom and used as a reference source for testing oil and will be referred to as PCB no. 209. Transformer oil were also collected from the oil leaking transformer from Rietvlei farm borehole No. 11 and stored in the laboratory at ambient temperature were it ranged from 29°C – 37°C according to International Electrochemical Commission (IEC) procedures in order to be used for this assessment.

3.3.1 Infrared Spectroscopy (IR) Analysis of PCBs

A systematic procedure was used to identify the unknown composition of PCBs. The IR spectrum determines the wavenumber (cm^{-1}) of the absorbance of carbonyl groups and whether the structure is aromatic or aliphatic structure. Aromatic structure are structures with aromatic ring, typically bonded directly to the C=O. A transformer oil stock solution of 100 mg/l was prepared by placing 0.55 ml of transformer oil in a 10 ml volumetric flask and filling to the mark with heptane. This stock solution was further diluted by pipetting 2.5 ml into a 50 ml volumetric flask and filling to the mark with heptane. Calibration standards were prepared from the diluted stock solution. The absorbance measurements were performed using the PerkinElmer® Spectrum™ 400 IR spectrometer (PerkinElmer, Inc., Shelton, CT USA) in mid-IR mode equipped with ANB-315 filter IR analyzer (Martínez et al., 2005; Zakharich et al., 2001).

3.3.2 Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis of PCBs

Following IR, further analysis of PCBs was carried out by Clarus 500 Perkin Elmer Gas Chromatography with the Flame Ionization Detector (GC-FID) equipped with manual injection system (Figure 3.3). Determination of the PCB content in the soil were performed as previously described (Smith et al., 2007; Pavia et al., 2006; Lundgren et al.,

2002). Transformer oil solution was prepared by pipetting 1000 μl in a volumetric flask 100 ml heptane (solvent). This was vigorously hand shaken and 5 μl extracts was injected into the Gas chromatograph using the Flame Ionization Detector (GC-FID) as indicated in Figure 3.3.

Transformer oil were extracted from the contaminated soil samples by taking 10 g of contaminated soil and soak in the 100 ml heptane and mixed with a spatula. Samples were stored in a dark place at room temperature for 24 hours. Extracts were then filtered using 0.20 μ filter paper inserted into a glassware funnel. The extracts were then injected to the GC-FID. The Eskom standard (PCB no. 209) was used as a reference at a concentration of 10 μml in heptane (Smith et al., 2007; Pavia et al., 2006; Martínez et al., 2005, Lundgren et al., 2002).

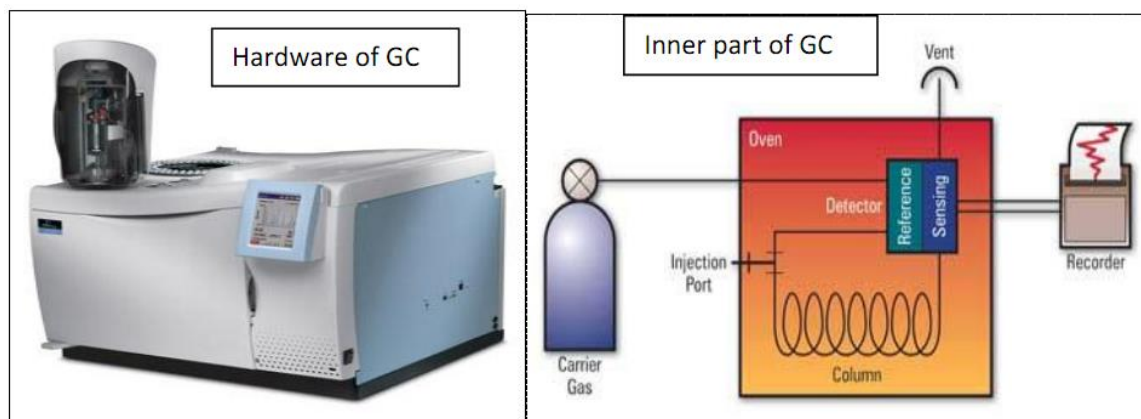


Figure 3.3 Perkin Elmer Clarus 500: Gas Chromatography (Lundgren et al., 2002)

The conditions were optimized and summarized as follows: high purity of Nitrogen gas was used as the carrier gas at a flow rate of 1 ml/minute. The Capillary column used were described as an OV17 consisting of 50% Phenyl Methyl composition with the diameter of Elite-Famewax, 30 m x 0.25 mm I.D. (0.25 mm thickness), Film 0.25 μm . The FID detector make-up gas was ultra-high purity hydrogen gas at a flow of 25 ml/minute. The column temperatures were initially held at 250°C held for 1 minute and ramped to 290°C, with 10°C /minute increase and held for 8 minutes. The total run time was 8 minutes and

determined the chromatographic peaks from the PCBs. The sample volume of 0.5 μL were picked up manually with a syringe and injected into a splitless injector. The temperature was maintained at 290°C and detector temperatures were also maintained at 290°C. The samples were processed by Totalchrom Workstation PAX Software. The solvent heptane, sample at the standard (PCB 209) were all analyzed by the GC-FID (Smith et al., 2007; Pavia et al., 2006; Martínez et al., 2005; Lundgren et al., 2002).

3.4. Isolation and testing of PCB degrading Bacteria

3.4.1. Culture Medium

Brain Heart Infusion Agar, Nutrient Agar, Nutrient Broth, Colombia Blood Agar, Streptococcus Selective Agar, Bile Esculin Agar (BEA) and Quarter Strength Ringer solution tablets were used and the preparation of the medium was performed according to manufacturer's instructions (Lehlabile Scientific; South Africa, Shimadzu Scientific; South Africa, Lasec Scientific; South Africa and Sigma-Aldrich Chemie CH-9471 Buchs; Switzerland).

3.4.2 Chemicals and reagents

Transformer oil, PCB no. 209 congener standard mixtures, Heptane and pentane was purchased from Merck, Fluka Analytical and Sigma-Aldrich (Chemie CH-9471 Buchs; Switzerland). Suitable identification kits and reagents used for identification of the isolates included: Analytical profile index (API) kit test purchased from BioMérieux® South Africa. The Gram staining kit test, Catalase test and Oxidase test kit were purchased from Lehlabile Scientific, South Africa. The preparations of all chemicals were performed according to manufacturer's instructions (BioMérieux® South Africa, Merck, Fluka Analytical, Sigma-Aldrich Chemie CH-9471 Buchs; Switzerland).

3.4.3 Isolation of PCB degrading bacteria from contaminated soils

Soil samples from Rietvlei farm borehole No. 11 were taken to the microbiology laboratory for further analysis. In the laboratory soil samples were air dried and passed through a 2 mm sieve (Sigma-Aldrich Chemie CH-9471 Buchs; Switzerland). A total of 10g of PCBs contaminated and uncontaminated soil samples were homogenized separately in 90 ml of sterile quarter-strength Ringer Solution in 250 ml Erlenmeyer flasks and allowed to shake in a shaker incubator at 200 rpm, and temperature was maintained at 37°C for 60 min. Samples were serially diluted from 10^{-1} up to 10^{-6} as indicated in Figure 3.4 (Fatima et al., 2005; Survery et al., 2004). A volume of 0.1 ml of soil sample was plated onto the Brain Heart Infusion (BHI) Agar plate and incubated at 37°C for 24 hours (Figure 3.4). After 24 hours of incubation, colonies were purified onto fresh nutrient agar for further identification (Prasanna et al., 2008; Survery et al., 2004; Ajaza et al., 2004).

The isolation of PCB degrading Gram positive bacteria was achieved by transferring 0.1 ml of soil samples onto the Streptococcus Selective Agar and Colombia Blood Agar (CBA), as indicated in Figure 3.4 and incubated in anaerobically conditions at 37°C for 48 hours using gas packs Figure 3.5. After incubation, purified colonies were picked and streaked out in Bile Esculin Agar (BEA) in tubes and incubated at 37°C for 24 hours. The presence of Gram positive bacteria were indicated by a black colour which confirms the positive result.

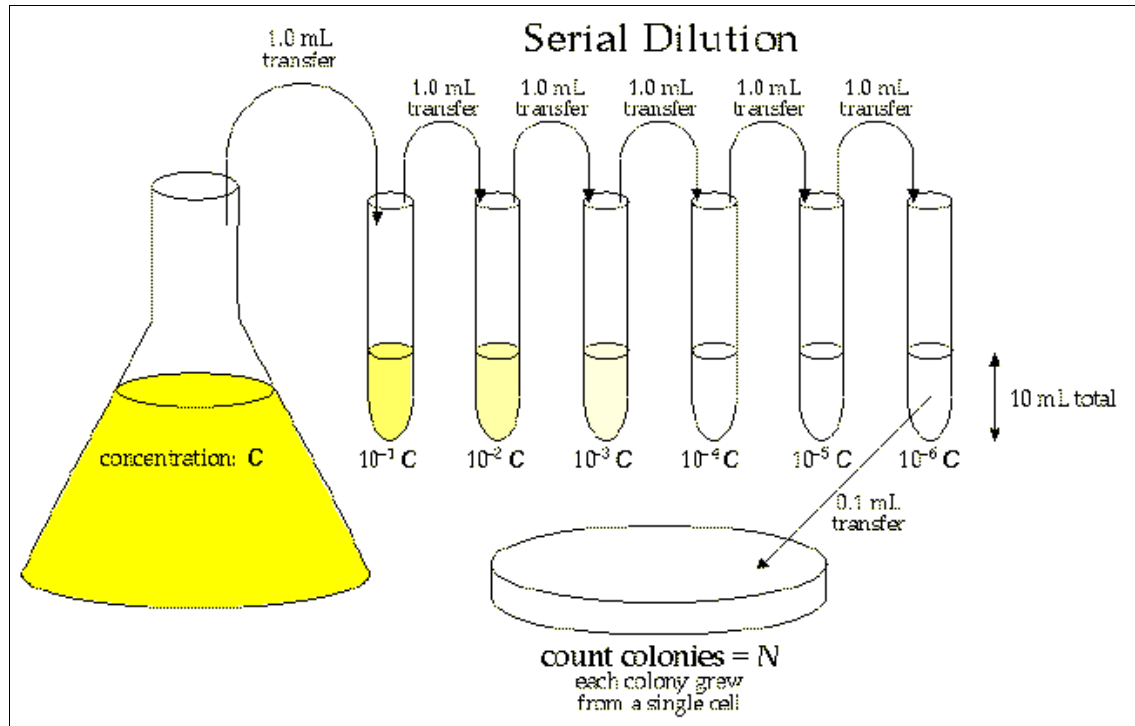


Figure 3.4: Demonstrate the 10 fold serial dilutions of soil samples.

3.4.4 Identification of the isolated Bacteria Strains

The three isolates obtained from the PCBs contaminated soil samples were identified based on morphological observation and biochemical characterization. Morphological characteristics were identified by Gram staining test kits. Biochemical characteristics were tested using the oxidase test; catalase reaction and API test kits.

3.5. The biodegradation ability of the isolated bacteria on PCBs in soil

Soil samples were prepared as follows; a total of 40 g of soil samples were collected within the University of Venda campus which contained no PCBs. The soil samples were initially tested for PCBs. Followed by sterilization of 40 g soil sample by autoclaving to avoid the presence of any microorganisms in the soil at 121°C for 15 min. A 30 g of soil sample was suspended in 250 ml pentane and stored in a dark place at room temperature for 24 hours covered with aluminum foil to avoid any evaporation. Extracts were filtered

using 0.20 μ filter paper inserted into a glass funnel. The filtrates were analyzed using Infrared Spectroscopy (IR) followed by Gas chromatography as described in sections 3.3.1 and 3.3.2.

The PCB concentrations were prepared as follows: a transformer oil stock solution of 100 mg/l was prepared by weighing 1 g of transformer oil in a 1 liter volumetric flask and filling to the mark with pentane. This stock solution was further diluted by pipetting 1 ml, 5 ml to 10 ml respectively into three separated 100 ml volumetric flasks and filling the flask to the 100 ml mark with pentane. Calibration standards were prepared from the diluted stock solution. All the volumetric flasks were clearly labeled as 100 mg/l, 50 mg/l and 10 mg/l PCBs concentration and stored in a dark place at room temperature (Figure 3.6).

3.5.1 Assessment of ability of isolated bacteria to degrade PCBs

All experiment was performed in triplicate for 3 days each (day 0, 1 and 2). The bacteria species capable of degrading PCBs were identified and included *Burkholderia cepacia*, *Pasteurella pneumotropica* and *Enterococcus faecalis*. A total of 40 g of soil sample were divided into 10 g samples, each were suspended in the 100 ml of sterile $\frac{1}{4}$ strength Ringer's solutions in 250 ml Erlenmeyer flasks. PCB concentration of 1 ml (10 mg/l), 5 ml (50 mg/l) and 10 ml (100 mg/l) were further pipetted and suspended in each of those 250 ml Erlenmeyer flasks. A 0.1 ml dense bacterial suspension was prepared according to McFarland 10^6 standard and 0.1 ml was suspended and mixed into 250 Erlenmeyer flask and this was clearly labelled as 10 mg/l, 50 mg/l, 100 mg/l and control. Erlenmeyer flasks were wrapped with aluminum foil and incubated in a shaker incubator at 200 rpm, and temperature was maintained at 37°C for 60 min. After 60 minutes of shaker incubation, the soil samples were incubated at 37°C for 24 hours in the incubator as indicated in Figure 3.6.

At day 1 after 24 hours of incubation, soil samples were transferred to a shaker incubator to mix at 200 rpm, and temperature maintained at 37°C for 60 min. This stock solution was further diluted by pipetting 1 ml of soil samples into the test tubes contained 5 ml of nutrient broth as indicated in Figure 3.6. A total of 0.1 ml was plated on to the Nutrient Agar petri-dishes and incubated at 37°C for 24 hours. After 24 hours bacteria colony forming units (CFU) were counted and expressed as bacteria number $\times 10^8$ CFU/ml, this were recorded as Day 1, these experiment were repeated three times respectively. Each day the mixture of bacteria and PCBs in 100 ml of Ringer solution (treatment) were tested for PCBs degradation. Quantifying of the PCBs concentration in the soil in contact with bacteria after 24 hours were performed as previously described (Smith et al., 2007; Pavia et al., 2006; Lundgren et al., 2002). The same procedure was followed for all 3 bacteria species. A mixed culture was also included and this culture all 3 species were added simultaneously to assess their collective ability to degrade the PCBs in the soil samples. The Gram positive bacterial plates were incubated anaerobically using gas packs (Figure 3.5).

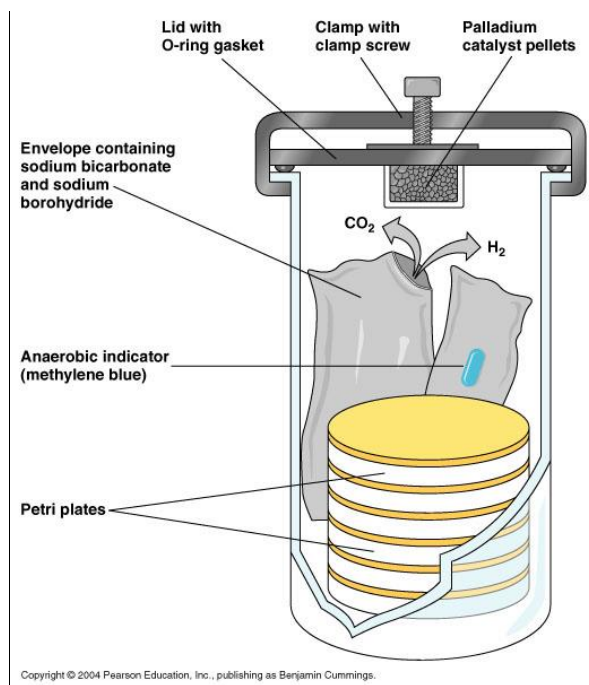
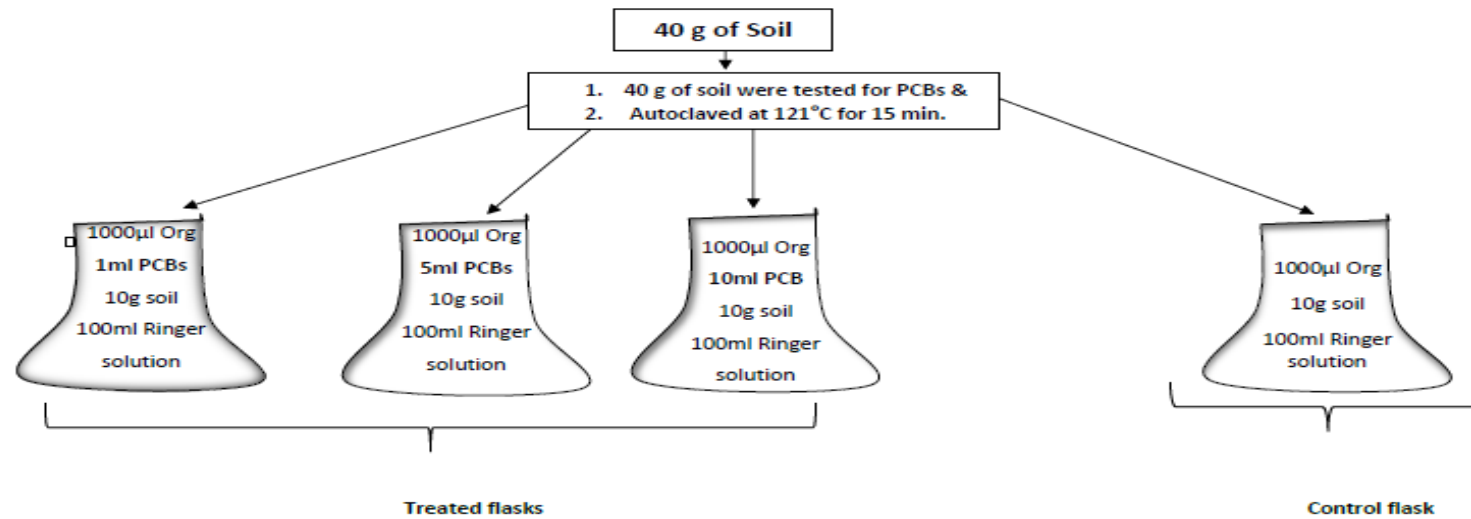


Figure 3.5: Anaerobic incubation outlay of Gram positive bacteria experiment.



- Flasks were closed with aluminium foil.
- Samples were mixed in a shaker incubator at 200 rpm; temperature maintained at 37°C for 60 min.
- After 60 minutes of shaker incubation, 10 folds of serially dilution were performed in 5 ml of nutrient broth.
- 0.1 ml was plated onto the fresh nutrient agar and incubated at 37°C for 24 hours in the incubator.

Figure 3.6: The schematic outlay of the laboratory experiment.

3.5.2. Total plate count.

Total plate count was performed as follows: the experiment was performed in triplicate from day 0, day 1 and day 2. Each day bacterial growth was monitored for the Colony Forming Units (CFU). The colonies were counted and expressed as bacteria number $\times 10^8$ CFU/ml and recorded (Leães et al., 2006; Molobela, 2011).

3.6 Statistical Analysis

Data was analysed using the statistical program GenStat (2012). The experiment was designed as a completely randomized design with three replicates. The treatment design was a split-plot with the four treatments the whole plots and the four concentrations randomly allocated within each whole plot. Difference between treatments, concentrations and interaction were tested for variance analysis. The data were acceptably normal with homogeneous treatment variances. Means of significant source effects were separated using Fisher's protected t-Least Significant Difference (LSD) at a 5% significance level (Snedecor & Cochran, 1980).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1. Assessment and Quality Control of PCBs in the Laboratory

The chemical composition of transformer oil leaking transformer at Rietvlei farm borehole No. 11 was assessed using the Infrared Spectroscopy (IR) and Gas Chromatography (GC) against a control oil sample (PCB 209).

4.1.1 Infrared Spectroscopy (IR) analysis of PCBs

Figure 4.1 shows the IR spectra from transformer oil sample obtain from the Eskom electrical transformer with high PCBs content. The component analysis of PCBs was initially done on the full spectral range ($3500 - 500 \text{ cm}^{-1}$) to characterize transformer oil spectra and detect outliers before establishing the bacterial isolates into the PCBs concentrations. The first two components of the PCBs were found to be Paraffinic, Aromatic, Naphthenic and Olefin. Figure 4.1 indicates that the important bands responsible for classification of the transformer oil sample are 2951, 2920, 2853, 1456 and 1376 cm^{-1} which are assigned to carbon-hydrogen (C-H) stretching vibrations. Figure 4.1 summarizes the results for the infrared spectra. (Smith et al., 2007; Pavia et al., 2006; Martínez et al., 2005, Lundgren et al., 2002).

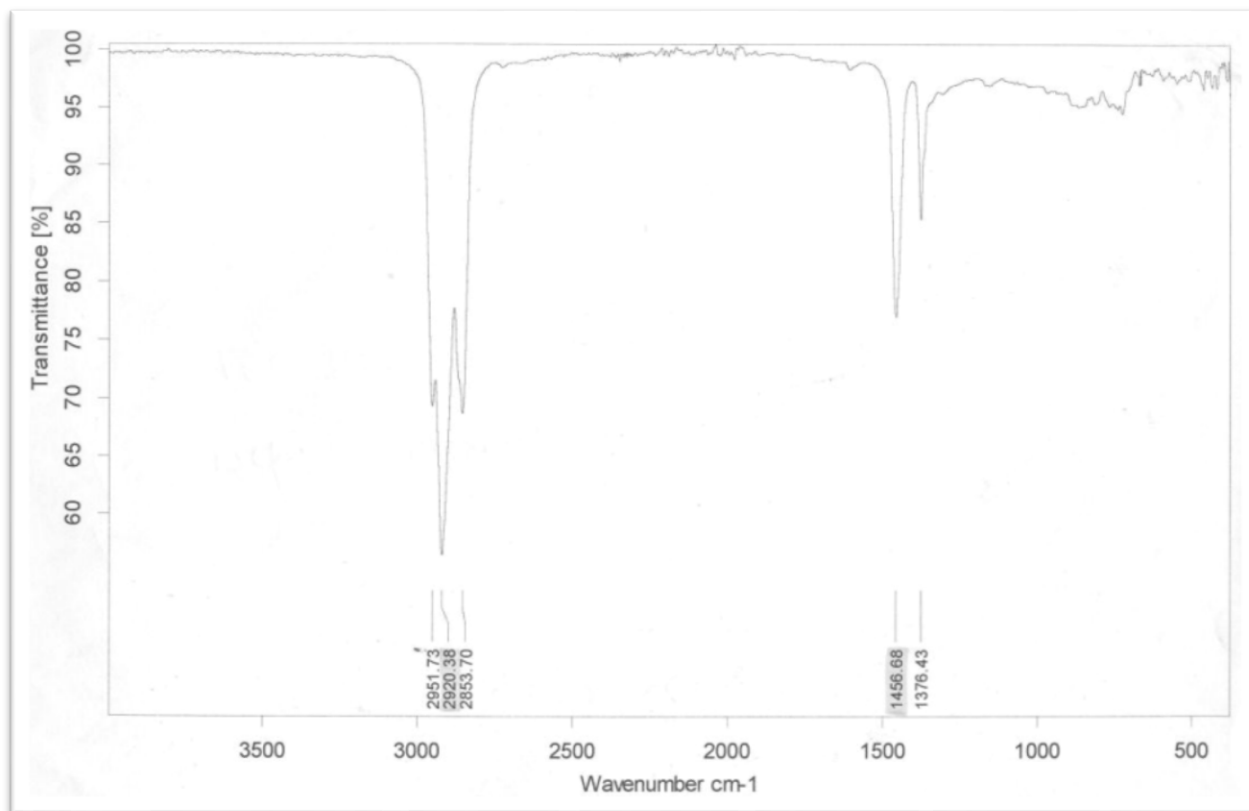


Figure 4.1: Infrared Spectroscopy (IR) test showing the detection of Transformer Oil compounds.

The typical IR Stretching Bond Absorptions of PCBs peaks were found at the range of 2951.73 to 1376.43 wavelength cm^{-1} resolution in transmission. One may conclude that due to the large and strong band of PCBs solution the area of 2951.73 cm^{-1} corresponding to carbon-hydrogen (C-H) stretching vibrations of PCBs.

4.1.2 Gas Chromatography-Flame Ionization Detector (GC-FID) Analysis of PCBs

Gas Chromatography-Flame Ionization Detector (GC-FID) was utilized as it is able to detect PCBs concentration on the contaminated soil samples and chromatogram was obtained. The peak areas of the chromatograms of the solutions of 10, 50, 100 mg/l were used to obtain the linearity. Each of them was analyzed in triplicate. Figure 4.2 shows the separation and identification of PCBs organic components by the analytical technique of gas chromatography. Chromatograms are co-eluted among themselves so only two peaks

of their elution were observed which the solvent (pentane) are and the PCBs sample. Generally, PCBs elute in GC in an increasing order of the number of chlorine atoms in the biphenyl molecule.

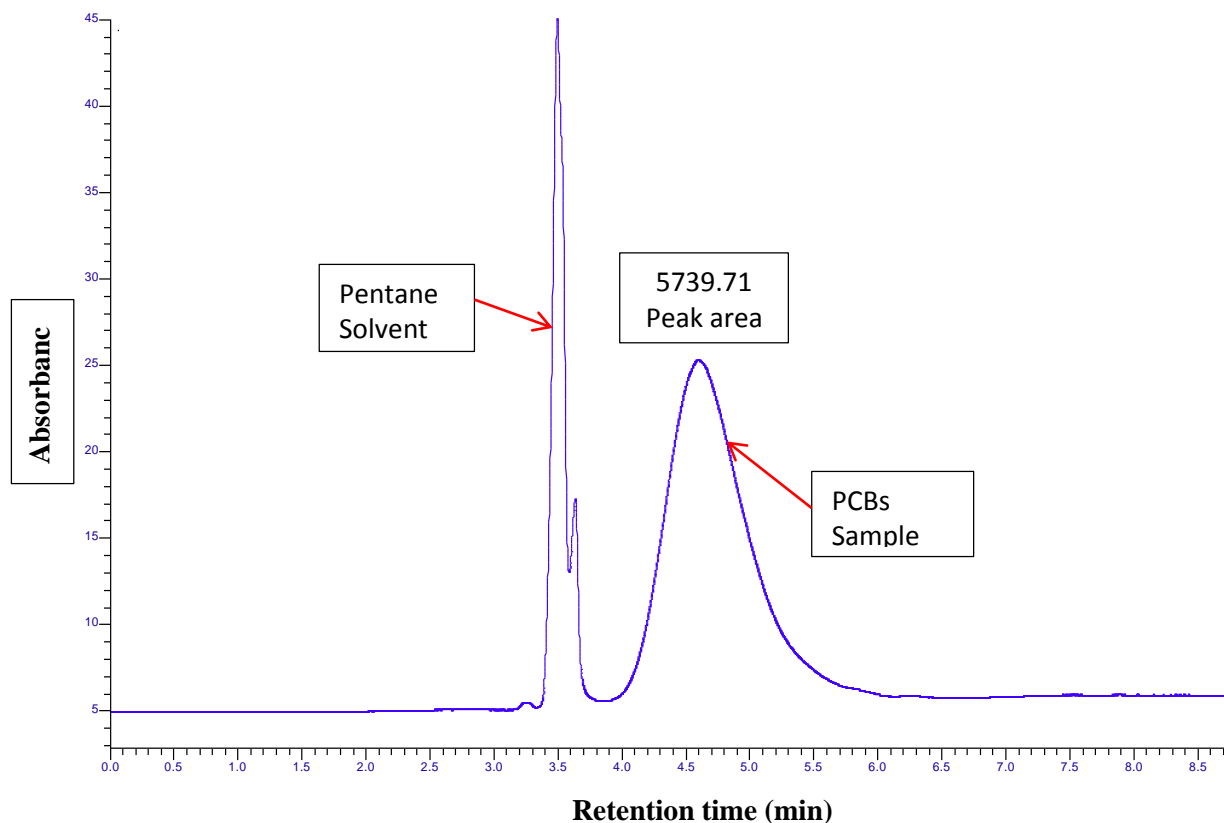


Figure 4.2 Chromatogram peak areas for PCBs analysis.

The areas of the chromatograms of the solutions of 100, 50, 10, 5 mg/l were used to obtain the linearity. Each of them was analyzed in triplicate. In such process, the number of concentrations of calibration was representative of the matrix to analyze. Figure 4.3 shows the response curve of the calibration, which corresponds to a straight line with the equation $y = 261223x$ and a correlation coefficient of 0.9967, which indicates that the samples with PCBs can be quantified in a wide range of concentrations within the response curve.

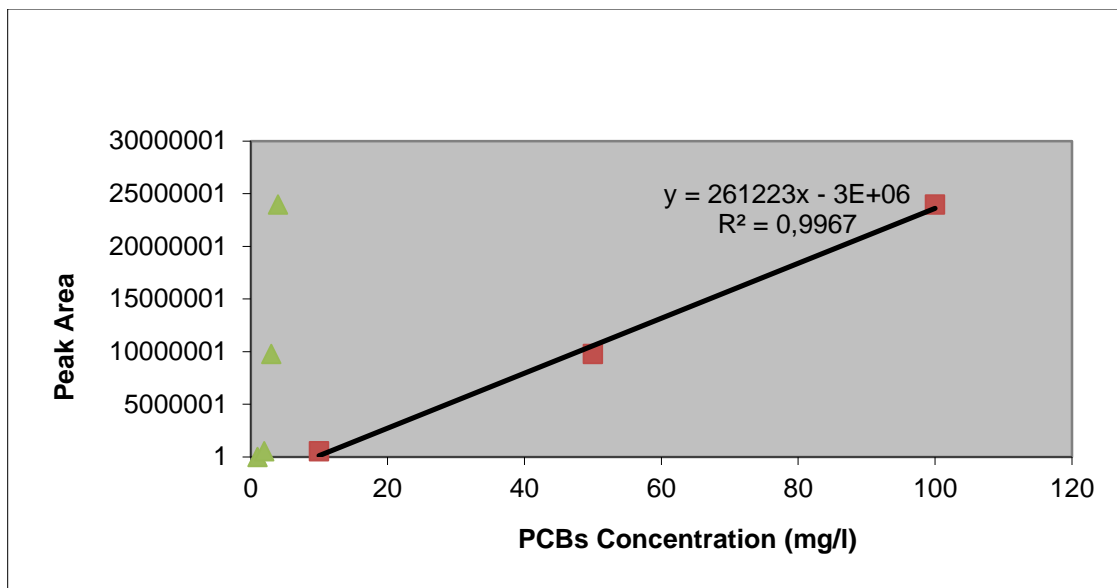


Figure 4.3 Response curve of the PCBs calibration process

4.2 Isolation and testing of PCB degrading bacteria

Bacteria species isolated from PCB contaminated soil was explored for their degrading ability of PCBs. The prominent bacteria isolates from the PCBs contaminated soils were identified using morphological and biochemical characteristics as indicated in chapter 3. Three species were identified and used in this study. These included *Burkholderia cepacia*, *Pasteurella pneumotropica* and *Enterococcus faecalis*. The potential of these 3 species utilize PCBs as carbon source of energy was investigated for a three day period (day 0, day1 and day 2) and the experiment was repeated three times. The procedure described in section 3.4 and Figure 3.3 was followed. Each day bacterial growth was monitored for the Colony Forming Units (CFU) which colonies were expressed as bacteria number X 10⁸ CFU/ml. The results are shown in Table 4.1 (Leães et al., 2006; Molobela, 2005).

Table 4.1 Number of bacteria isolates: growing in PCBs contaminated soil samples after 72 hours.

Bacteria strains	Treated soil with PCBs	Control soil (No PCBs)
<i>Burkholderia cepacia</i>	0.43 x 10 ⁸	0.360 x 10 ⁸
<i>Pasteurella pneumotropica</i>	0.020 x 10 ⁸	0.039 x 10 ⁸
<i>Enterococcus faecalis</i>	0.002 x 10 ⁸	0.001 x 10 ⁸
Mixed culture	0.038 x 10 ⁸	0.169 x 10 ⁸

CFU = Colony forming units: Data expressed as Bacteria number X 10⁸ CFU/ml

Burkholderia cepacia showed highest growth at 0.43 X 10⁸ cfu/ml on the contaminated soil, on the uncontaminated soil showed the growth of 0.36 X 10⁸ cfu/ml. In contrast, *Enterococcus faecalis* showed the lowest growth 0.0051 X 10⁸ cfu/ml on the uncontaminated soil, on the contaminated soil showed the growth of 0.00153 X 10⁸ cfu/ml. In contrast with *Pasteurella pneumotropica* and *Enterococcus faecalis*, *Burkholderia cepacia* was the bacteria which showed the highest growth rate on the contaminated and uncontaminated soil samples.

4.3 The Biodegradation ability of the isolated bacteria on PCBs in soil

The bacterial growths were assessed as previously described by Nwinyi and Obinna, (2010). Precisely, the pure colonies of isolated bacteria were inoculated into 3 different PCB concentrations namely 10 mg/l, 50 mg/l and 100 mg/l and plated onto Nutrient Agar. Bacterial growth was monitored in order to count the colony forming units (CFU) as all data were expressed as bacteria number X 10⁸ CFU/ml.

4.3.1 Degradation ability of *Burkholderia cepacia*

Figure 4.4 shows the ability of *Burkholderia cepacia* to grow on different concentrations of PCBs. According to Al-Khalid and El-Naas, (2012) *Burkholderia cepacia* is able to break down PCBs and convert it into carbon and use it as the sole source of energy. PCBs act as the growth supporting substrates of the microorganism.

Burkholderia cepacia showed highest growth at 2.7×10^8 CFU/ml in 10 mg/l of PCBs after 72 hours. The lowest growth was showed at 0.25×10^8 CFU/ml in 50 mg/l of PCBs. At higher concentration of 100 mg/l PCBs, *B. cepacia* showed lowest growth at 0.35×10^8 CFU/ml and the possible explanation is when the chlorine level increases as the by-product of PCBs degradation, the bacteria dies and decreases with time (Al-Khalid and El-Naas, 2012; Ines et al., 2007). Generally, this substrate overlaps means that other PCB concentrations may act as co-substrates, influencing the growth of the bacteria (Al-Khalid and El-Naas, 2012; Field and Sierra-Alvarez, 2008).

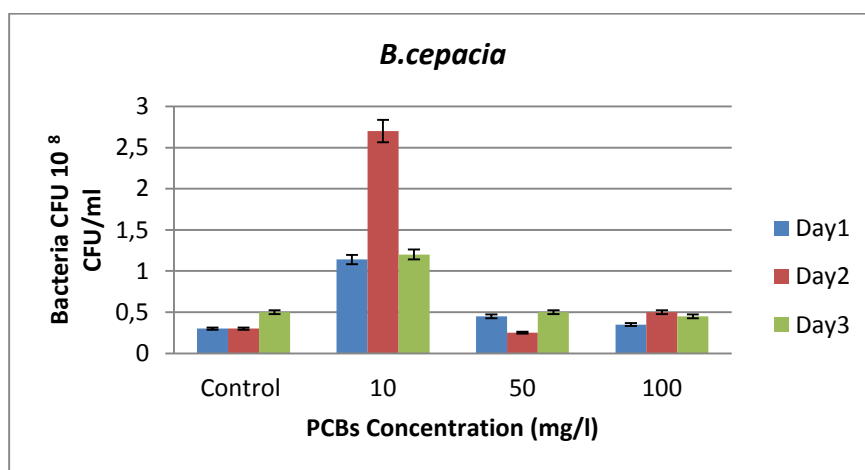


Figure 4.4: The growth of *Burkholderia cepacia* on the different PCBs concentrations.

According to Goris et al., (2004) *Burkholderia cepacia* has characteristic pathways for PCBs degradation at both genetically and in molecular level. A review by Mailin and Firdausi, (2006) on PCB degradation by *Burkholderia cepacia* has shown the model system for bacterial breakdown of PCBs (Mailin and Firdausi, 2006; Goris et al., 2004).

Figure 4.5 displays PCB degradation by *Burkholderia cepacia*. *Burkholderia cepacia* showed the potential to degrade PCBs at -1.5 in the 100 mg/l PCBs and -1.4 in the 50 mg/l PCBs. The lowest degradation rate was observed at -0.6 in the 10 mg/l PCBs. These finding is similar to Al-Khalid and El-Naas (2012) *Burkholderia cepacia* is more effective in PCBs degradation due to their faster growth and quickly transform PCBs into carbon as their sole energy source (Al-Khalid and El-Naas, 2012).

Burkholderia cepacia showed the ability to degrade PCBs congeners, the chlorine level when it increases, it affect PCBs metabolizing processes by the bacteria (Ines et al., 2007). The trend decreases with the increase of the chlorine level as the degradation by-product and cause death to the strain (Field and Sierra-Alvarez, 2008; Ines et al., 2007).

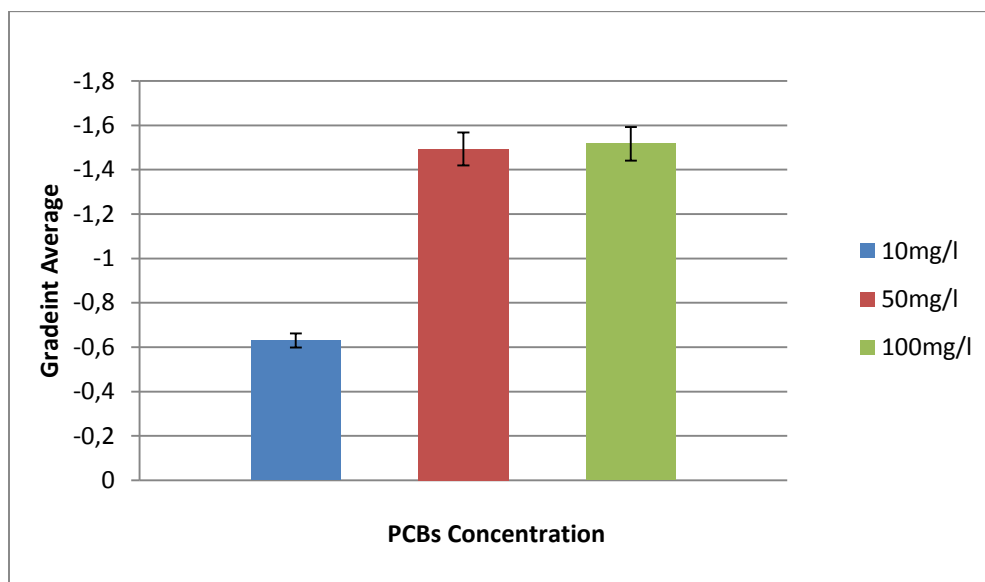


Figure 4.5: Degradation studies of PCBs by *Burkholderia cepacia*.

4.3.2 Degradation ability of *Pasteurella pneumotropica*

Figure 4.6 displays the growth of *Pasteurella pneumotropica* in different PCBs concentrations. *Pasteurella pneumotropica* showed the highest growth at 0.12×10^8 CFU/ml of 10 mg/l PCBs and 0.08×10^8 CFU/ml on the 50 mg/l PCBs. The lowest growth was observed on the control at 0.0001×10^8 CFU/ml and also on the higher concentration of 100 mg/l PCBs at 0.01×10^8 CFU/ml. One probable explanation could be, after 72 hours bacteria were not at the exponential phase of bacterial growth or the high levels of chlorine as the by-product of PCBs degradation which inhibit the bacterial growth and/or a lesser amount of nutrients to support the growth of bacteria. Studies have reported that, large varieties of aerobic bacteria are capable of using the PCBs as their sole carbon source and energy (Nwinyi and Obinna, 2010; Mailin and Firdausi,

2006). *Pasteurella pneumotropica* can tolerate high concentration such as 50 mg/l and 100 mg/l of PCBs (Hank et al., 2010; Nwinyi and Obinna, 2010; Mailin and Firdausi, 2006).

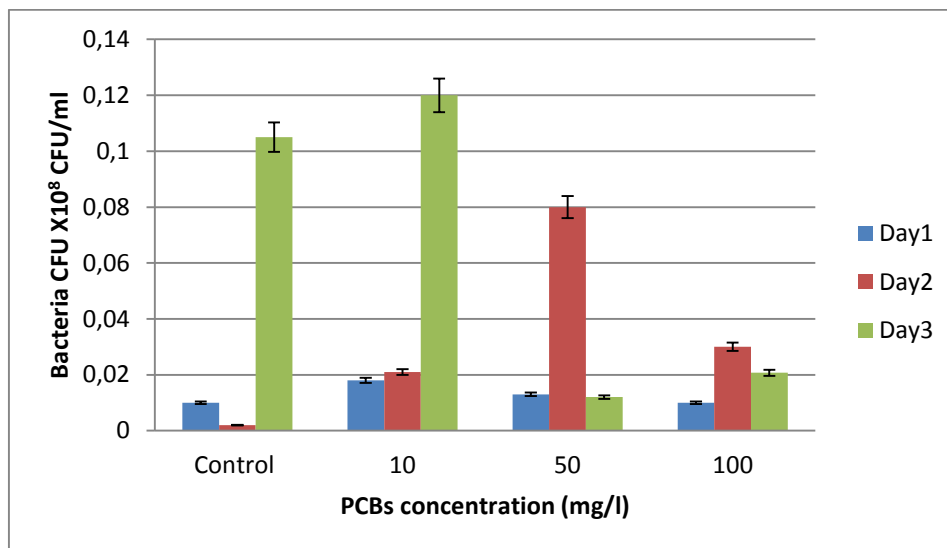


Figure 4.6: The growth of *Pasteurella pneumotropica* at different PCB concentrations after 72 hours.

Burkholderia cepacia and *Pasteurella pneumotropica* showed the same degradation average for PCBs as all PCBs degrading bacteria require corresponding set of enzymes vigorous to biodegrade and metabolize biphenyls (Ashrafosadat et al., 2009; Mailin and Firdausi, 2006). *Pasteurella pneumotropica* showed lower growth after 72 hours corresponding to the degradation of PCBs (Figure 4.7). *Pasteurella pneumotropica* showed higher level of PCB degradation at -1.5 in the 100 mg/l and at -1.4 50 mg/l PCBs. The gradient average confirms the ability of *Pasteurella pneumotropica* to degrade PCBs (Figure 4.7). The strain is able of using the PCBs as sole carbon source (Mailin and Firdausi, 2006). *Pasteurella pneumotropica* attacks PCBs via 2, 3-dioxygenase pathways, and converting PCBs congeners into chlorobenzoic acids. Chlorobenzoic acid can be further co-metabolized and resulting in the production of carbon dioxide, water, chlorine and biomass (Hank et al., 2010; Ashrafosadat et al., 2009; Mailin and Firdausi, 2006).

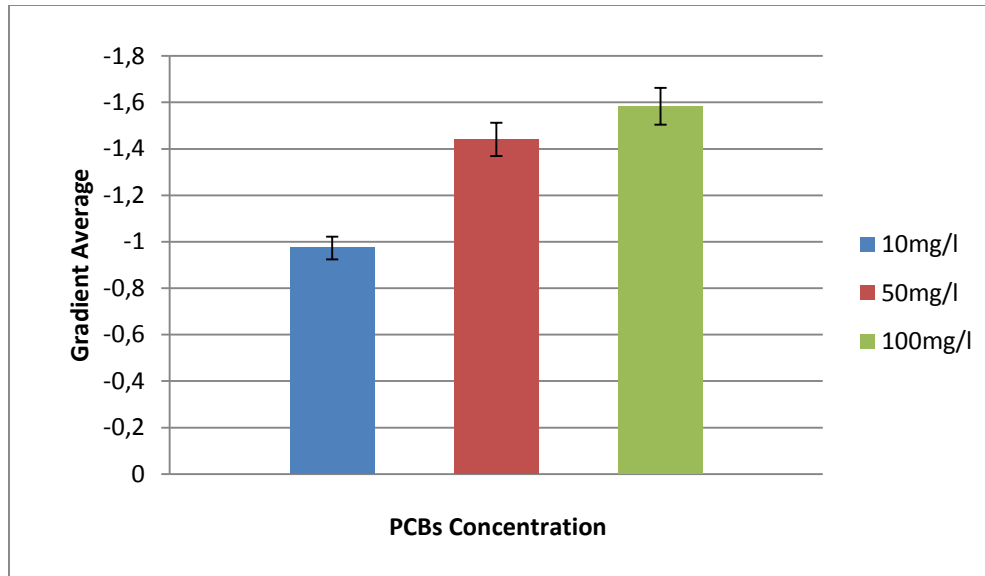


Figure 4.7: Degradation studies of PCBs by *Pasteurella pneumotropica*.

4.3.3 Degradation ability of *Enterococcus faecalis*

Figure 4.8 shows the growth of *Enterococcus faecalis* on the PCB contaminated soil. *Enterococcus faecalis* showed the highest growth after 72 hours of incubation. *Enterococcus faecalis* is able to degrade more highly chlorinated PCB congeners. In contrast of three concentrations of PCBs, *Enterococcus faecalis* showed the effectiveness growth on all concentrations and on the control. The *Enterococcus faecalis* showed maximum growth of 0.006×10^8 CFU/ml on the control and 0.0035×10^8 CFU/ml on the 100 mg/l PCBs. The results showed that *E. faecalis* can tolerate high concentration of PCBs and that this strain increased PCB degradation. Possible explanation could be that, PCBs components provide carbon as a growth substrate which enables *E. faecalis* to grow at high concentration of PCBs (Hank et al., 2010; Nwinyi and Obinna, 2010).

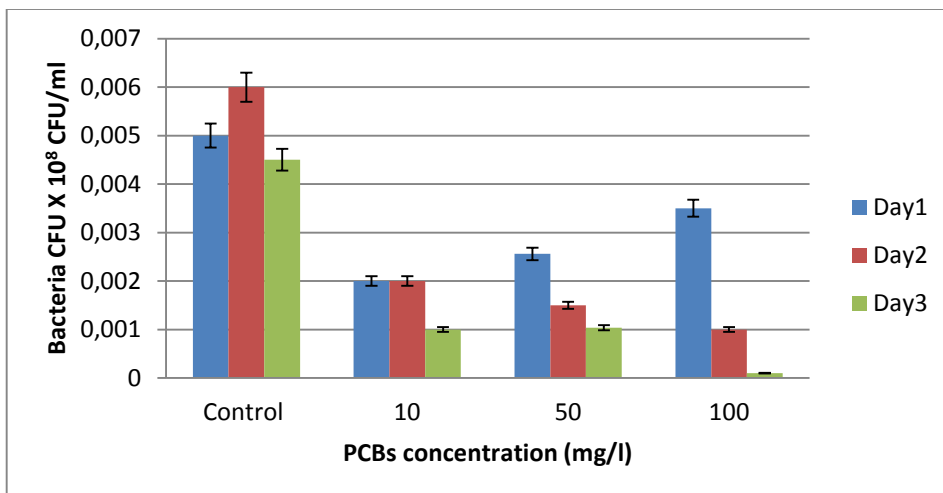


Figure 4.8: The growth of *Enterococcus faecalis* at different concentrations of PCBs.

In contrast with *B. cepacia* and *P. pneumotropica*, *Enterococcus faecalis* is an anaerobic Gram positive bacterium. The species is able to adapt pollutants with high carbon concentration due to their diffusional limitation of oxygen (Urbaniak, 2013). *Enterococcus faecalis* showed the highest degradation rate at -1.7 in 100 mg/l PCBs (Figure 4.9). Also the higher growth level was observed after 72 hours with a corresponding degradation at -0.6 in the 10 mg/l, -1.5 in the 50 mg/l PCBs respectively (Figure 4.9). Carbon dioxide is required for *Enterococcus faecalis*, it serves as growth supporting substrates. These strain attacks more highly chlorinated PCB congeners through reduction of dechlorination. In general, this organism preferentially removes the meta and para chlorines, resulting in depletion of highly chlorinated PCB congeners (Urbaniak, 2013; Field et al., 2008).

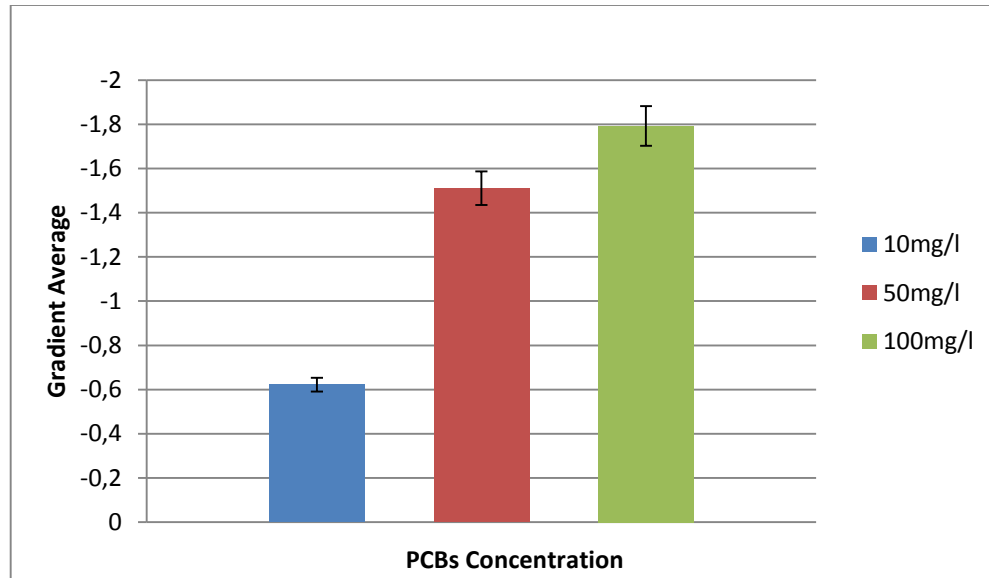


Figure 4.9: Degradation studies of PCBs by *Enterococcus faecalis*.

4.3.4 Degradation ability of mixed culture

The mixed culture showed higher growth rate at 0.4×10^8 CFU/ml in the 10 mg/l PCBs in contrast with the control mixed culture showed 0.3×10^8 CFU/ml after 72 hours (Figure 4.10). In the high concentration of PCBs lower growth was observed at 0.00111×10^8 CFU/ml which is the lowest growth rate. Mixed culture showed the lowest growth rate in contrast with other 3 pure strains in the different concentration of PCBs. There are factors that can inhibit the growth of the mixed culture in the different concentration of PCBs in the soil. These included direct inhibition of enzymatic activities and the proliferation processes of degrading PCBs. For example, competition between mixed cultures for limited carbon sources, predation of microorganisms by protozoa and bacteriophages (Al-khalid and EL-naas, 2012). The degree of contaminant degradation is often dependent on the concentration of the contaminant and the amount of “catalyst” present (Hank et al., 2010). In this context, the amount of “catalyst” represents the number of organisms able to metabolize the contaminant as well as the amount of enzymes produced by each cell (Erdogan et al., 2011). Bacteria require specific enzymes to metabolize PCBs. The availability of sufficient amounts of nutrients and oxygen is also necessary in the co-metabolizing process of the PCBs (Hank et al., 2010). Without

enough nutrients and oxygen the bacteria is then inhibited to grow and die. Other factors influence the rate of biodegradation in mixed culture is by controlling the rates of enzyme catalyzed reactions such as temperature, pH and moisture (Hank et al., 2010; Leigh et al., 2006). Joutey et al., (2013) and Hank et al., (2010) reported that biological enzymes involved in the degradation pathway have an optimum temperature. Meaning that, not all bacteria have the same metabolic turnover at the same temperature. The rate of biodegradation is likely decreased by roughly one-half for each 10°C decrease in temperature. Biodegradation process can take place under a wide-range of soil pH, however, a pH of 6.5 to 8.5 is generally optimal for biodegradation of PCBs (Al-khalid and EL-naas, 2012; Ines et al., 2007). The trend reveals that, the ability of mixed bacterial culture growing in the different PCB concentrations decreases with the increase of the chlorine level (Figure 4.10) (Joutey et al., 2013; Hank et al., 2010; Field and Sierra-Alvarez, 2008).

Although many bacteria are able to degrade high concentrations of PCBs, a single bacterium does not possess the enzymatic ability to degrade all or even most of PCBs congeners in a contaminated soil (Joutey et al., 2013). According to Joutey et al., (2013) and Prasanna et al., (2008) mixed culture have the most powerful ability to biodegrade PCBs, they contain genetically diversity which enable them to degrade the most complex mixture of PCBs congeners present in the contaminated soil (Joutey et al., 2013; Prasanna et al., 2008). Mixed cultures are more efficient in degrading the contaminants (Prasanna et al., 2008).

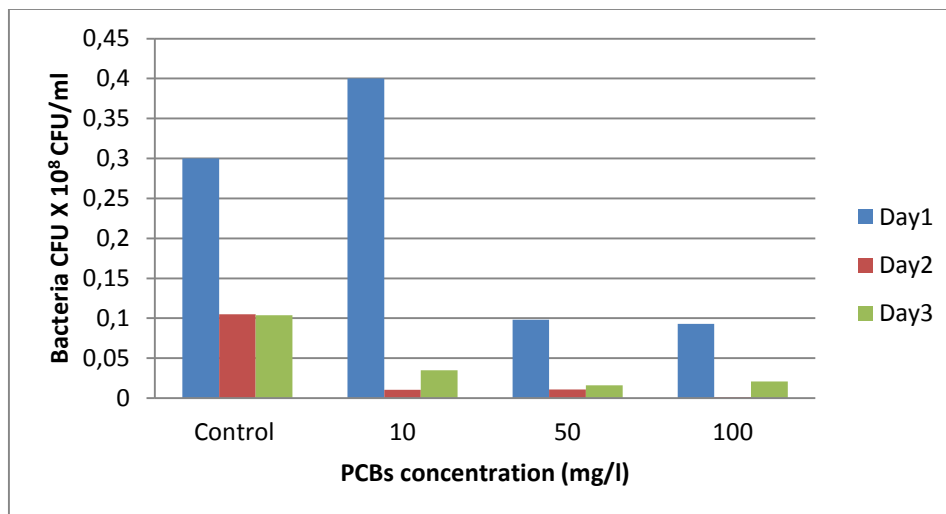


Figure 4.10: The growth of mixed culture at different PCBs Concentrations.

Mailin and Firdausi (2006) reported that mixed culture are more efficient in degrading high concentration of PCBs than one pure bacteria isolate. In nature bacterial populations occur in mixed culture rather than introverted colonies (Mailin and Firdausi, 2006). Mixed culture exhibited the higher degradation at -1.5 in 100mg/l of PCBs (Figure 4.11). The lower PCBs degradation by mixed bacterial culture was observed at -0.9 in the 10 mg/l of PCBs and -1.2 in 50 mg/l PCBs. It has been observed that mixed culture perform minimal in all PCBs concentration (Figure 4.11). This is due to a whole range of factors which include competition within microorganisms, insufficient supply with essential substrates, unfavorable external conditions (aeration, moisture, pH, temperature), and low bioavailability of the pollutant, biodegradation in natural conditions can be lesser (Urbaniak 2013; Mailin and Firdausi, 2006).

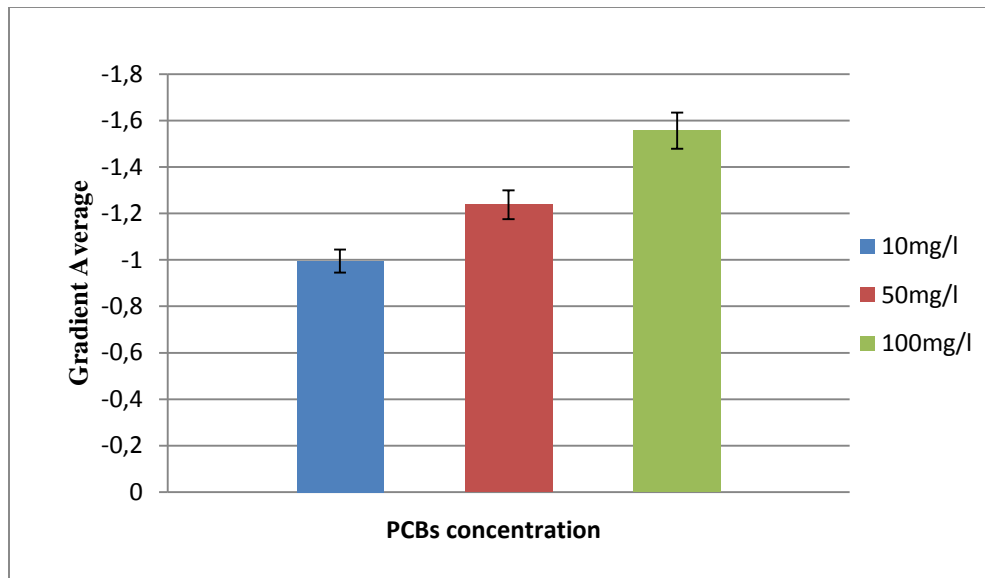


Figure 4.11: Degradation studies of PCBs by mixed cultures.

4.4 Comparison of the effect of bacteria on different PCB concentrations

Enterococcus faecalis showed the highest PCB degradation at 32% compared to *Burkholderia cepacia* and *Pasteurella pneumotropica*. *Enterococcus faecalis* is one among the 3 isolates which have shown the highest effectiveness growth corresponding to PCBs degradation (Figure 4.12). This bacterium was evaluated before for its efficiency to degrade different PCB congeners from the commercial mixture of Aroclor 1242 and reported as one of the bacteria with a potential to utilize the PCBs as its sole source of carbon and energy (Hank et al., 2010; Ashrafosadat et al., 2009; Hatamian-Zarmi et al., 2009, Mailin and Firdausi, 2006). This is in agreement with our study which showed that *E. faecalis* plays an important role in PCB degradation since it could remove about 32% of PCBs in contaminated soil. In contrast, *Pasteurella pneumotropica* showed the maximum degradation of 24%. Studies showed that *Pasteurella pneumotropica* can tolerate high concentrations of PCBs such as 100 mg/l (Figure 4.12). PCBs provide the carbon source of energy that enables the bacteria to grow and co-metabolize PCBs (Ashrafosadat et al., 2009; Hatamian-Zarmi et al., 2009; Mailin and Firdausi, 2006). In contrast with *Burkholderia cepacia* lower growth was shown in the 100 mg/l PCBs after

72 hours with the corresponding PCBs degradation at 21% (Figure 4.12). The increase of chlorine level as a by-product of PCBs degradation inhibits the bacterial growth and progressively the bacteria will be unable to metabolize the PCB congeners (Ines et al., 2007). The trend shows that *Burkholderia cepacia*'s ability to degrade PCBs decreases with an increase of the chlorine level (Field and Sierra-Alvarez, 2008; Ines et al., 2007). Mixed bacterial cultures showed lower growth corresponding to PCBs degradation at 23% in the high concentration of 100 mg/l PCBs respectively (Figure 4.12) (Field and Sierra-Alvarez, 2008; Ines et al., 2007).

According to Joutey et al., (2013) and Prasanna et al., (2008), mixed cultures are more efficient in PCBs degradation than pure strains. In nature all the bacterial population occurs in culture rather than in solitary colonies. In this study, quite a number of bacterial species were observed but only major bacteria were isolated to degrade PCBs. *E. faecalis* and *P. pneumotropica* they have shown the PCBs degradation ranging from 32% and 24% in the high concentration of 100 mg/l PCBs (Figure 4.12). Mixed culture can be inhibited by limited of carbon sources, antagonistic interaction and predation by protozoa and bacteriophages. Furthermore, specific enzymes are required for the organisms to be able to metabolize PCBs (Joutey et al., 2013).

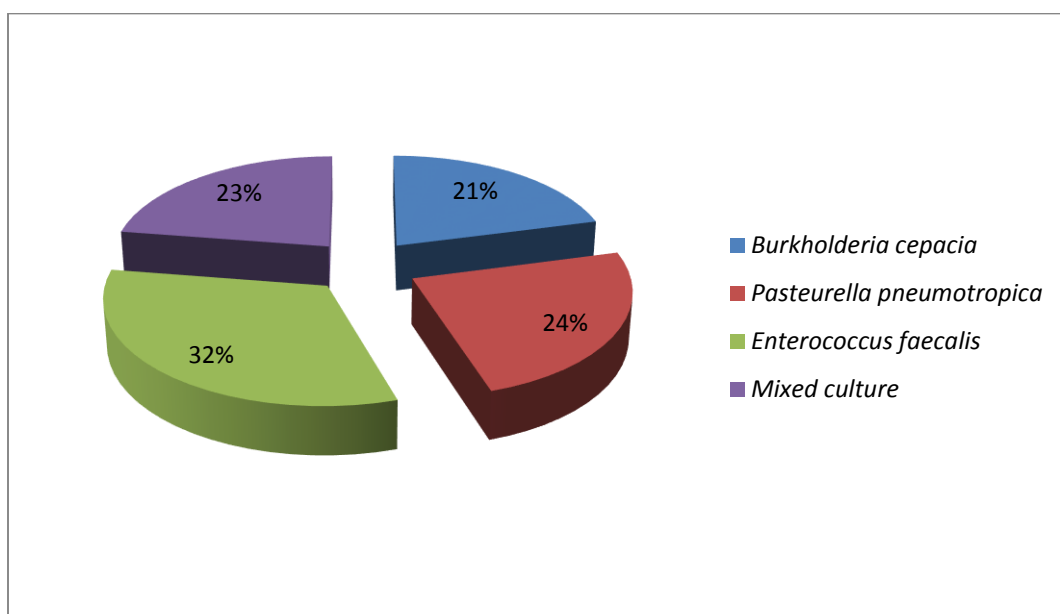


Figure 4.12: The comparison of the effectiveness of the bacteria isolates on PCBs degradation.

4.5 Correlation between PCB degradation and growth of bacteria isolates

Table 4.2 shows the results of statistical analysis of variance (Anova) comparing the p values between the bacteria growth and PCBs degradation. The statistical analysis showed a significant correlation between bacteria growth and treatment expression ($R^2=0.1459$ with a p value of <0.001) at a 5% significance level. The correlation between PCBs degradation and treatment was not statistically significant at ($R^2=0.9998$, $p<0.056$) (Snedecor & Cochran, 1980).

Table: 4.2. The analysis of variance (Anova) of bacteria growth and PCBs degradation

Source	Bacteria Growth			PCBs Degradation		
	Degree of freedom	Mean square	Probability	Degree of freedom	Mean square	Probability
Treatment	3	0.978	<0.001	3	0.02	0.961
Error (a)	8	0.014		8	0.26	
PCB Concentration	3	0.164	<0.014	2	2.13	<0.001
Treatment + Concentration	9	0.13	$<0.001^*$	6	0.098	$<0.056^*$
Error (b)	24	0.0058		16	0.037	
Total	47			35		

correlation						
-------------	--	--	--	--	--	--

*P<0.001 significance, *P<0.056 Not significance

Table: 4.3. The analysis of variance means of bacteria growth

Treatment	Bacteria Growth				Mean
10mg/l PCBs	1.247 a	0.053 cd	0.002 d*	0.149 cd	0.037
50mg/l PCBs	0.400 b*	0.035 cd	0.002 d*	0.042 cd	0.003
100mg/l PCBs	0.433 b*	0.020 cd	0.002 d*	0.038 cd	0.100
Control	0.367 b*	0.039 d*	0.005 d*	0.170 c	0.612

Least Significant Difference (LSD) $p=0.05 = 0.1504$

Table 4.3 shows the means between bacteria growth in the different PCBs concentration. The statistical analysis showed that, the means with the same letter or letters they do not differ significantly at a 5% significance level (Snedecor & Cochran, 1980).

Table: 4.4. The analysis of variance means of PCBs degradation

Treatment	PCBs Degradation				Mean
10mg/l PCBs	-0.630 a*	-0.973 ab	-0.621 a*	-0.995 abc	-0.805
50mg/l PCBs	-1.494 bcd	-1.441 cd	-1.511 bcd	-1.238 bcd	-1.421
100mg/l PCBs	-1.517 bcd	-1.583 cd	-1.793 d	-1.557 bd	-1.613
Control	0	0	0	0	0

Least Significant Difference (LSD) $p=0.05 = 0.5943$

Table 4.4 explains the means of the PCBs degradation. Statistical analysis showed that means with the same letter or letters they do not differ significantly at a 5% significance level (Snedecor & Cochran, 1980).

Figure 4.13 indicates the relationship between bacteria growth and the treatments in different PCB concentrations 10 mg/l, 50 mg/l, 100 mg/l and the control. The data was normal with homogeneous treatment variances. A significant positive relationship was found between bacteria growth and treatment with coefficient of correlation ($r = 0.1459$) and p value <0.001.

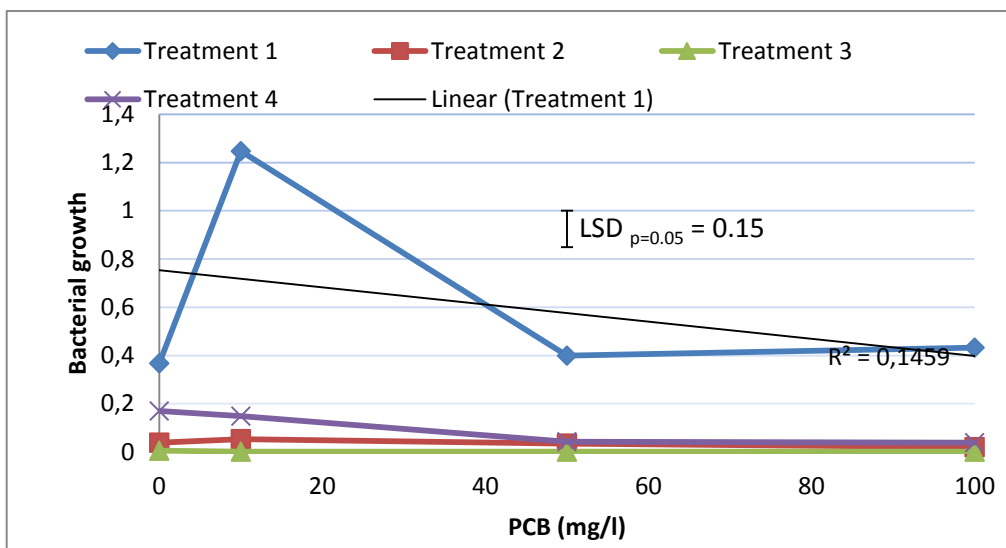


Figure 4.13: Bacteria growth in the different concentration of PCBs.

Figure 4.14 is showing the interaction for PCB degradation by bacterial isolates in four different treatments which include 10 mg/l, 50 mg/l, 100 mg/l and the control. The correlation between PCBs degradation and treatment was not significant with coefficient of correlation ($r = -0.9998$ and $p \text{ value} < 0.056$).

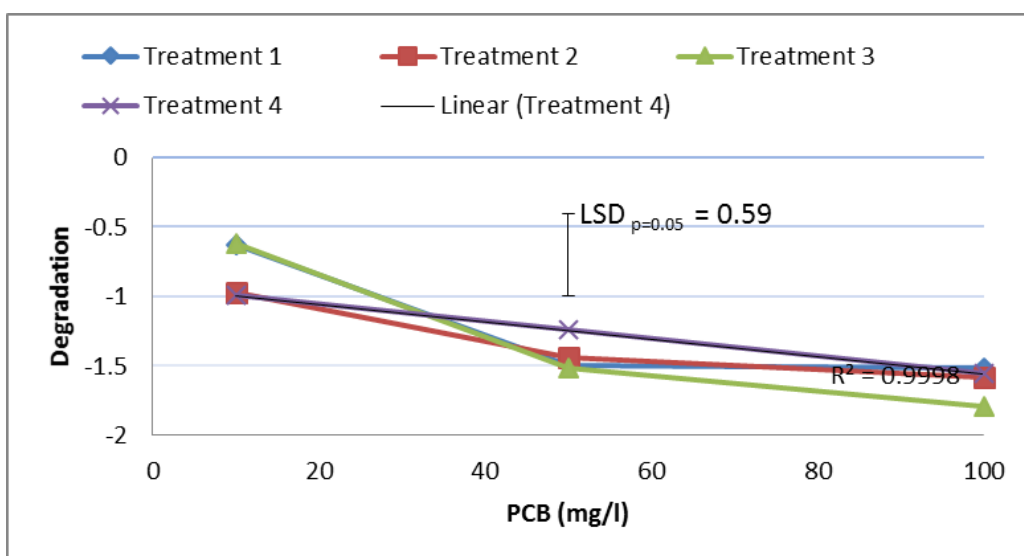


Figure 4.14: Degradation studies of PCBs.

CHAPTER FIVE: CONCLUSION AND RECOMENDATION

5.1 Conclusion

In this study the use of bacteria in the microbial degradation of PCBs was assessed, Gram positive and Gram negative bacteria isolated from the PCBs contaminated soils at Rietvlei farm borehole No. 11 were identified using morphological and biochemical characteristics. These included *Burkholderia cepacia*, *Pasteurella pneumotropica* and *Enterococcus faecalis*. The bacterial isolates potential to utilize PCBs as carbon source of energy was investigated for a three day period (day 0, day1 and day 2) the treatments and control were replicated three times. From the results obtained it was found that there was a decrease in the PCBs concentrations ranging from 21% to 24% (-1.5 to -1.7 (slopes)) respectively. *Enterococcus faecalis* and *Pasteurella pneumotropica* were able to utilize the PCBs in all concentrations. *Burkholderia cepacia* did so at lower concentrations and performed minimally at higher concentrations.

The same bacterial isolates were also used as a mixed culture together and investigated for PCB degrading ability on the contaminated soil. It has been observed that mixed culture perform minimal in all PCBs concentration. This could have been due to wide range of factors such as competition between microorganisms, insufficient supply of essential substrates, unfavorable external conditions (aeration, moisture, pH, temperature), and low bioavailability of the pollutant, biodegradation in natural conditions can be lesser. In nature all the bacterial populations occur in mixed cultures rather than solitary colonies. The results from this study provided evidence that microorganisms occurring in soil contaminated with PCBs have the potential to degrade Polychlorinated biphenyls.

5.2 Recommendation

These bacterial species are soil prevalent, and they can therefore be used for PCB degradation on contaminated soils with appropriate nutrients adjustment. The 3 organisms in this study can be cultivated in large quantities in the laboratories and applied on the contaminated soil. Their enzymatic systems responsible for the PCBs degradation i.e., 2, 3-dioxygenase biphenyl can be extracted from these organisms and produced for the use in biodegradation of PCBs contaminated soils. The results obtained in this study indicated that biodegradation of PCBs might be a useful means in future for degradation of these PCB compounds on the environment.

However, the bioremediation process alone is moderately effective, despite the fact that some by-products were nearly destroying the organisms. Furthermore, studies have reported that a chlorine atom remained in the organic parts of degraded PCB compounds which results in the increasing toxicity of the complicated mixture obtained after the process. The effectiveness of the PCB degradation process is strongly dependent on the structure of PCBs. Therefore, the rate of PCB degradation is strongly dependent on the number of chlorine atoms present in a molecule and probably on their location in the aromatic ring. This study recommends that old PCB-oil containing equipment should be phased out from the use in South Africa, proper environmental friendly disposal methods must be adopted.

Further research should be carried out in order to determine the gene responsible for conferring the degradative properties on these isolates. The gene can be extracted and modified to function better or transferred to other indigenous ubiquitous bacterial species; these will give them the potential to detoxify the PCB on the contaminated soils. For this reason, it is also necessary to support the activities of the indigenous microorganisms in polluted soils and to enhance their degradative abilities by bioaugmentation or biostimulation. Genetic engineering can be used to improve the biodegradation capabilities of those bacteria isolates. Whether or not such approaches are ultimately

successful in bioremediation of PCB and they can clean-up contaminated environment and enjoy a more sustainable future.

There is also a lack of information regarding the prevalence of PCB degrading bacteria in the soil mostly in rural communities. There is therefore a need for research on bacteria ecology and investigation of the factors affecting mixed bacterial cultures which might assist in determining better conditions for growing the mixed bacterial culture on the different concentrations of PCBs.

CHAPTER SIX: REFERENCES

Abramowicz DA (1995) **Aerobic and Anerobic Polychlorinated Biphenyl (PCB) Biodegradation in the Environment** *Journal of Environmental Health Perspective* 29: (2). 231-240

Ashrafosadat H.Z, Seyed A.S, E. Vasheghani-Farahani, S. Hosseinkhani, A. Emamzadeh (2009). **Extensive Biodegradation of Highly Chlorinated Biphenyl and Aroclor 1242 by Pseudomonas Aeruginosa TMU56 Isolated From Contaminated Soils.** *International Journal of Biodeterioration & Biodegradation* 63: 788–794

Adeyemi D, Ukpo G, Anyakora C and Uyimadu J (2009) **Polychlorinated Biphenyls in fish samples from Lagos Lagoon, Nigeria.** *African Journal of Biotechnology* 8: (12). 281-285

Adriano DC, Wenzel WW, Vangronsveld J and Bolan NS (2004) **Role of assisted natural remediation in environmental cleanup.** *Journal of Environmental Quality* 122: (3) 121-142

Agency for Toxic Substances and Disease Registry (ATSDR) (2001) **Toxicological Profile for Polychlorinated Biphenyls (PCBs);** *Article of Public health service* 25-69

Ajaz M, Noor N, Rasool SA, and Khan SA (2004) **Phenol resistant bacteria from soil: identification–characterization and genetical studies.** *Journal of Microbiology* 36: (2) 415-424

Al-khalid T and El-Naas M.H (2012) **Aerobic Biodegradation of Phenols: A Comprehensive Review;** *Journal of Environmental Science and Technology* 42: 1631-1690

Aluyor EO, and Ori – Jesu M (2009) **Biodegradation of minerals oils – A review.** *African Journal of Biotechnology* 8: (6) 915-920

Atagana H.I, (2009) **Biodegradation of PAHs by fungi in contaminated –soil containing Cadmium and Nickel Ions;** *African Journal of Biotechnology* 8: (21) 5780-5789

Avelizapa N.G.R, Vazouez R.R, Cruz J.M, Garcia F.E, Garcia A.M.D, Rios-Leal E, and Villagomez G.F (1999) **Isolation and Charaterization of Bacteria Degrading Polychlorinated Biphenyls from Transformed Oil;** *Journal of Folia Microbiology* 44: (3) 317 – 321

Begonja AK, Dubravka H, Fingler S, Petrić HČI and Kolić NU (2007) **PCB-degrading potential of aerobic bacteria enriched from marine sediments.** *Journal of Marine and Environmental Research* 8: (2) 341-350

Bernhard T and Petron S (2001) **Analysis of PCB Congeners vs. Arcolors in Ecological Risk Assessment:** automated categorization of Web resources. [Online]. Available at: <http://www.epa.gov/toxteam/pcbindex.html> [accessed on 10 March 2010]

Bing-Ru L, Guo_Mei J, Jian C and Gang W (2006) **A Review of Methods for Studying Microbial Diversity in Soils.** *Journal of Pedosphere* 16: (1)18-24

Bolin JT, Dai S, Vaillancourt FH, Maaroufi H, Drouin NM, Neau DB, Snieckus V and Eltis LD (2002) **Identification and analysis of a bottleneck in PCB biodegradation.** *Journal of Biological Sciences* 9: (12) 934-939

Broding HC, Schettgen T, Hillert A, Angerer J, Goen T and Drexler H (2008) **Subjective Complaints in Persons under chronic low-dose exposure to lower polychlorinated biphenyls (PCBs)**. *Journal of Applied Environmental Microbiology* 74: (7) 2089-9498.

Brody JG, Seryak LM, and Rudel RA (2008) "**PCB-containing wood floor finish is a likely source of elevated PCBs in resident's blood, household air and dust: a case study of exposure**". *Journal of Environmental Health* 14: (5) 411-415

Canadian Environmental Quality Guidelines (1999). **The protection of Environmental and human health. Polychlorinated biphenyls (TOTAL)**. *Canadian council of Ministers of the Environment* updated 2001. 03-08

Chaineau CH, Morel JL and Oudot J (2000). **Biodegradation of fuel oil hydrocarbons in the rhizosphere of maize**; *Journal of Environmental Quality*, 8: (29). 569-578

Chander M and Arora DS (2007). **Evaluation of some white-rot fungi for their potential to decolourise industrial dyes**; *Journal of Biotechnology*, 9: (72). 192-198

Chang yc, Takada K, Choi D, Toyama T, Sawada K and Kikuchi S, (2013). **Isolation of Biphenyls and Polychlorinated Biphenyl-Degrading Bacteria and their Degradation Pathways**; *Journal of Applied Biochemistry Biotechnology*, 170: 381-398

Charles FI, Gordon HG, Jelaso AM and Spitsbergen JM (2007). **Effects of polychlorinated biphenyls (PCBs) on expression of neurotrophic factors in C6 Glial cell in culture**; *Journal of Neurotoxicology*, 28: 111-120

Clark R.R, Chain E.S.K, and Griffin R.A, (1979). **Degradation of Polychlorinated Biphenyls by Mixed Microbial cultures**; *Journal of applied and Environmental Microbiology*; 37: (4). 680-685

Demnerova K, Mackova M, Spevakova V, Beranova V, Kochankova, Lovecka P, Ryslava E and Macek T, (2005). **Two approaches to biological decontamination of ground water and soil polluted by Aromatic-Characterization of Microbial populations**; *Journal of International Microbiology*, 8: 205-211

Duffy JE, Carlson E, Li Y, Prophete C and Zelikoff JT (2002). **Impacts of Polychlorinated Biphenyls (PCBs) on the immune function of fish: Age as a variable in determining adverse outcome**. *Journal of Marine Environmental Research*, 2 (54): 559-563

Erdogan E.E, Sahin F, and Karaca A, (2011). **Determination of petroleum-degrading bacteria Isolated from crude oil-contaminated soil in Turkey**; *African Journal of Biotechnology*, 35: 168 – 531

Eskom (2009). **Information sheet: Impact on the Environment: automated categorization of Web resources**. [Online]. Available [:http://www.eskom.co.za/annreport09/ar/htm](http://www.eskom.co.za/annreport09/ar/htm)[accessed on 05 September 2009]

Fatima MB, Camargo FAO, Okeke BC and Frankenberger WT (2005). **Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation**; *Journal of Bioresource Technology*, 9: (96). 222-229

Field J.A, and Sierra-Alvarez, (2008). **Microbial transformation and Degradation of Polychlorinated Biphenyls**; *Journal of Environmental pollution*, 155: 1-12

Field JA and Alvarez RS (2008). **Microbial degradation of chlorinated dioxins**. *Journal of Chemosphere*, 71: (8). 1005-1018

Gordon H, Garley AM, Jelaso C and Spitsbergen JM (2007). **Effects of polychlorinated biphenyls (PCBs) on expression of neurotrophic factors in C6 Glial cell in culture, Kalamazoo**, *Journal of NeuroToxicology*, 28: (6). 1264-1271

Goris J, De Vos P, Caballero-Mellado J, Park J, Falsen E, Quensen III E.J, Tiedje J.M and Vandamme P (2004). **Classification of the Biphenyl- And Polychlorinated Biphenyl-Degrading Strain LB400T and Relatives as Burkholderia Xenovorans Sp. Nov.** *International Journal of Systematic and Evolutionary Microbiology* 54: 1677–1681

Graciela ML, Aguiar RJ, Fernandez MS, Rodriguez RVH, and Varaldo P (2002). **Degradation by white-rot fungi of high concentrations of polychlorinated biphenyls (PCBs) extracted from a contaminated soil**; *Journal of Advance in Environmental Research*, 11: (6). 559-568

Gray IAR (2004). **Guide for Polychlorinated Biphenyl (Pcb) Management Of Insulating Oil In South Africa ABB Transformatoren**; Enviromental Services, Dortmund, Germany. P 101-109

Hank D, Saidani N. Namane A, and Hellal A, (2010). **Bacth phenol biodegradation study and application of factorial experimental design**; *Journal of Engineering Science and Technology*, 3: (1). 123 – 127

Hatamian-Zarmi A, Shojaosadati S.A, Farahani E.V, Hosseinkhani S and Emamzadeh A, (2009). **Extensive biodegradation of highly chlorinated biphenyl and Aroclor 1242 by Pseudomonas aeruginosa TMU56 isolated from the contaminated soils**; *Journal of international Biodeterioration and Biodegradation*, 63: 788-794

Ines P, Hrsak D, Flinger S, Voncina E, Cetkovic H, Kolar A.B, and Kolic N.U, (2007). **Enrichment and Characterization of PCB-Degrading Bacteria as Potential Seed cultures for Bioremediation of contaminated soil**; *Journal of Food Technology Biotechnology*, 45: (1). 11-20

Jamshidi A, Hunter S, Hazrat S and Harrad S (2007). **Concentration and chiral signatures of polychlorinated biphenyls (PCBs) in outdoor and indoor Air and soil in a Major U.K. Conurbation**; *Journal of Environmental Science Technology*, 41: (2). 153-2158

Jensen VA, and Sprengen LH (1986). **Microbial communities in soil**. Elsevier Science Publishing Co. Inc. *Elsevier Applied Science Publishers, London and New York Microbial Communities in Soil*. Pp. 35-48.

Joutey N.T, Bahafid W, Sayel and Ghachtouli N.E, (2013). **Biodegradation: Involved Microorganisma and Genetically Engineered Microorganisms**; *Journal of Biodegradation –Life of Sciences*, 10: 527 – 561

Kafilzadeh F, Sahragard P, Jamli H, and Tehery Y, (2011). **Isolation and Identification of Hydrocarbons degrading bacteria in soil around Shiraz Refinery**; *African Journal of Microbiology Research*, 4: (19). 3084 -3089

Kirk LJ, Beaudette LA, Hart M, Moutoglis P, Klironomos JN, Lee H and Trevors JT (2004). **Methods of studying soil microbial diversity**. *Journal of Microbiological Methods*, 10: (58). 169-188

Leães FL, Daniel AP, Mello GB, Battisti V, Bogusz S Jr, Emanuelli T, Fries LLM, and Costabeber L (2006). **Degradation of Polychlorinated Biphenyls (PCBs) by *Staphylococcus xylosus* in liquid media and meat mixture**; *Journal of Food and Chemical Toxicology*, 44: 847-854

Leigh M.B, Prouzova P, Mackova M, Macek T, Nagle D.P and Fletcher J.S, (2006). **Polychlorinated Biphenyl (PCB) Degrading Bacteria Associated with Trees in a PCB-Contaminated Site**; *Journal of Applied and Environmental Microbiology*, 27: (4). 2331-2342

Lundgren K, Van Bavel B, and Tysklind M (2002). **Development of a high-performance liquid chromatography carbon column based method for the fractionation of dioxin-like polychlorinated biphenyls**; *Journal of Chromatography Analytical*, 2: (962).79-93

Mackova M, Vrchotova B, Francova K, Sylvestre M, Tomaniova M, Loveck P, Demnerova K and Macek T (2007). **Biotransformation of Polychlorinated biphenyls (PCBs) by plants and bacteria – consequences of plant – microbe interaction**; *European Journal of Soil Biology*, 10: (43). 233-241

Mailin M and Firdausi R, (2006). **High Performance phenol Degrading Microorganisms isolated from Wastewater and Oil-contaminated Soil**; *Journal of Microbiology*, 2: (2). 32-36

Martínez HAR, Rodríguez GC, Castillo DH (2005). **Determination of PCBs in Transformer Oil using Gas Chromatography with Mass Spectroscopy and Aroclors (A1254:A1260)**; *Journal of Chemistry*, 49: (3). 263-270

Mesdaghinia AR, Nasser S, Arbabi M and Rezaie S (2005). **Isolation of polycyclic aromatic hydrocarbon-degrading bacteria associated with the petroleum contaminated soils in Iran**. Department of Environmental Engineering, Center for Environmental Research (CER) Tehran University of Medical Sciences, Tehran, Iran. P 211-216

Molobela IP (2005). **Development of rhizoremediation as a treatment technology in the removal of polycyclic aromatic hydrocarbons (PAHs) from the environment**, **MSc thesis**. University of Pretoria, Department of Microbiology and plant pathology, Faculty of Natural and Agricultural Science, South Africa. P 35-70

Nobbs D and Chipman G (2003). **Contaminated site investigation and remediation of chlorinated aromatic compounds**; *Journal of Separation and Purification Technology*, 8: (31). 37- 40

Nwinyi O.C, (2010). **Degradation of Askarel (PCB Blend) by Indigenous Aerobic Bacteria Isolates from Dumpsite in Ore, Ondo State of Nigeria**; *Journal of Basic and Applied Sciences*, 4: (8). 3938-3948

Pavia DL, Gary ML, George SK and Randall GE (2006). **Introduction to Organic Laboratory Techniques (4th Edition)**. Thomson Brooks/Cole. Saunders collage. Philadelphia. P 797-817

PerkinElmer Clarus 500 (2010). **Gas Chromatography: automated categorization of Web resources**. [Online]. Available: <http://www.perkinelmer.com/annreport10/ar> [accessed on 03 June 2010]

Pietrogrande MC, Benvenuti A, Previato S and Dondi F (2000). **HPLC Analysis of PCBs on Porous Graphitic Carbon: Retention Behavior and Gradient Elution**. *Journal of Chromatographia*, 52: (7/8). 425-480

Prasanna N, Saravanan N, Geetha P, Shanmugaprakash M and Rajasekaran P, (2008). **Biodegradation of Phenol and Toluene by Bacillus sp., Pseudomonas sp., and Staphylococcus sp., isolated from Pharmaceutical industrial Effluent**; *Article of Advanced Biotechnology*, 20

Ranjard L, Brothier E and Nazaret S (2000). **Sequencing bands of ribosomal intergenic spacer analysis fingerprints for characterization and microscale distribution of soil bacterium populations responding to mercury spiking**; *Journal of Applied Environmental Microbiology*, 8: (66). 5334-5339

Seo J.S, Keum Y.S, and LI Q.X, (2009). **Bacteria Degradation of Aromatic Compounds**; *International Journal of Environmental Resources & Public Health*, 6: 278-309

Smith KE, Schwab AP and Banks MK (2007). **Phytoremediation of Polychlorinated Biphenyl (PCBs) – contaminated sediments: A greenhouse feasibility study**; *Journal of Environmental Quality*, 7: (36). 239-244

Snedecor, GW & Cochran, WG. 1980. **Statistical methods (7th Ed.)**. Iowa State University Press.

Survery S, Ahmad S, Subhan SA, Ajaz M, and Rasool SA (2004). **Hydrocarbon degradation bacteria from Pakistani soil: Isolation, identification, Screening and genetical studies studies**; *Journal of Biological Sciences*, 7: (9). 1518-15522

Togers B.T, Anderson J. C, Palmer C.A, and Henderson W.G, (1973). **Septicaemia due to *Pasteurella pneumotropica***; *Journal of Clinical Pathways*, 26: 396-398.

U.S. Environmental Protection Agency (EPA), IRIS (Integrated Risk Information System) for Arochlor 1016. (1999). **National Center for Environmental Assessment Office of Research and Development. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, (3rd Ed)**. Washington, Cincinnati OH. USA. P 23-30

VSN International (2012). **GenStat for Windows 15th Edition**. VSN International, Hemel Hempstead, UK. Web page: GenStat.co.uk

Woods SL and Trobaugh DJ (1999). **Polychlorinated Biphenyl Reductive Dechlorination by Vitamin B12s: Thermodynamics and Regiospecificity**. *Journal of Environmental Science Technology*, 9: (33). 857-863

Yanagida F, Chen Y, Onda T and Shinohara T (2005). **Durancing L28-1A, a new Bacteriocin from Enterococcus Durans; L28- Isolated from soil**; *Journal of Applied Microbiology*, 97: (6). 1287-91

Zakharich MP, Zaitsev II, Komar, Nikonovich FN, Ryzhkov MP, and Skornyakov IV (2001). **Analysis of Transformer Oil using IR Analyzers: Journal of Applied Spectroscopy**; *Journal of Applied Spectroscopy*, 68: (1). 61-65

Appendix A

1.

$$C_1V_1 = C_2V_2.$$

$$C_1 = 1000\text{mg/l} - V_1?$$

$$C_2 10\text{mg/l} - V_2 100\text{ml}$$

$$100\text{mg/l} \times V_1 = 10\text{mg/l} \times 100\text{ml}$$

$$V_1 = \frac{10\text{mg/l} \times 100\text{ml}}$$

$$1000\text{mg/l}$$

$$= 1\text{ml}$$

2.

$$C_1V_1 = C_2V_2.$$

$$C_1 = 1000\text{mg/l} - V_1?$$

$$C_2 50\text{mg/l} - V_2 100\text{ml}$$

$$100\text{mg/l} \times V_1 = 50\text{mg/l} \times 100\text{ml}$$

$$V_1 = \frac{50\text{mg/l} \times 100\text{ml}}$$

$$1000\text{mg/l}$$

$$= 5\text{ml}$$

3.

$$C_1V_1 = C_2V_2.$$

$$C_1 = 1000\text{mg/l} - V_1?$$

$$C_2 50\text{mg/l} - V_2 100\text{ml}$$

$$100\text{mg/l} \times V_1 = 100\text{mg/l} \times 100\text{ml}$$

$$V_1 = \frac{100\text{mg/l} \times 100\text{ml}}$$

$$1000\text{mg/l}$$

$$= 10\text{ml}$$

Appendix B

B.cepacia

Data is expressed by 10^8 x bacteria colony per ml

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	10mg/l	250 x 10 ⁶ x 10/ml =25 x 10 ⁸ CFU /ml	960 x 10 ⁶ x 10/ml = 96 x 10 ⁸ CFU /ml	990 x 10 ⁶ x 10/ml =99 x 10 ⁸ CFU /ml	73.3 x 10⁸
Day 1		120 x 10 ⁵ x 10/ml =1.2 x 10 ⁸ CFU /ml	400 x 10 ⁴ x 10/ml =0.4 x 10 ⁸ CFU /ml	144 x 10 ⁵ x 10/ml =1.44 x 10 ⁸ CFU /ml	1.01 x 10⁸
Day 2		114 x 10 ⁵ x 10/ml =1.14 x 10 ⁸ CFU /ml	270 x 10 ⁵ x 10/ml =2.7 x 10 ⁸ CFU /ml	120 x 10 ⁵ x 10/ml =1.2 x 10 ⁸ CFU /ml	1.6 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	50mg/l	250 x 10 ⁶ x 10/ml =25 x 10 ⁸ CFU /ml	960 x 10 ⁶ x 10/ml = 96 x 10 ⁸ CFU /ml	990 x 10 ⁶ x 10/ml =99 x 10 ⁸ CFU /ml	73.3 x 10⁸
Day 1		100 x 10 ⁵ x 10/ml =1 x 10 ⁸ /ml	500 x 10 ⁴ x 10/ml = 0.5 x 10 ⁸ /ml	105 x 10 ⁵ x 10/ml =1.05 x 10 ⁸ /ml	0.85 x 10⁸
Day 2		450 x 10 ⁴ x 10/ml =0.45 x 10 ⁸ /ml	250 x 10 ⁴ x 10/ml =0.25 x 10 ⁸ /ml	500 x 10 ⁴ x 10/ml =0.5 x 10 ⁸ /ml	0.4 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean

Day 0	100mg/l	$250 \times 10^6 \times 10/\text{ml}$ = 25×10^8 CFU/ml	$960 \times 10^6 \times 10/\text{ml}$ = 96×10^8 CFU/ml	$990 \times 10^6 \times 10/\text{ml}$ = 99×10^8 CFU/ml	73.3×10^8
Day 1		$800 \times 10^4 \times 10/\text{ml}$ = $0.8 \times 10^8/\text{ml}$	$700 \times 10^4 \times 10/\text{ml}$ = $0.7 \times 10^8/\text{ml}$	$900 \times 10^4 \times 10/\text{ml}$ = $0.9 \times 10^8/\text{ml}$	0.8×10^8
Day 2		$350 \times 10^4 \times 10/\text{ml}$ = $0.35 \times 10^8/\text{ml}$	$500 \times 10^4 \times 10/\text{ml}$ = $0.50 \times 10^8/\text{ml}$	$450 \times 10^4 \times 10/\text{ml}$ = $0.45 \times 10^8/\text{ml}$	0.43×10^8

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	Control	$250 \times 10^6 \times 10/\text{ml}$ = 25×10^8 CFU/ml	$960 \times 10^6 \times 10/\text{ml}$ = 96×10^8 CFU/ml	$990 \times 10^6 \times 10/\text{ml}$ = 99×10^8 CFU/ml	73.3×10^8
Day 1		$490 \times 10^4 \times 10/\text{ml}$ = $0.49 \times 10^8/\text{ml}$	$360 \times 10^4 \times 10/\text{ml}$ = $0.36 \times 10^8/\text{ml}$	$160 \times 10^5 \times 10/\text{ml}$ = $0.6 \times 10^8/\text{ml}$	0.81×10^8
Day 2		$300 \times 10^4 \times 10/\text{ml}$ = $0.3 \times 10^8/\text{ml}$	$300 \times 10^4 \times 10/\text{ml}$ = $0.3 \times 10^8/\text{ml}$	$500 \times 10^4 \times 10/\text{ml}$ = $0.5 \times 10^8/\text{ml}$	0.36×10^6

P. Pasteurella

Data is expressed by $10^6 \times$ bacteria colony per ml

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	10mg/l	$1000 \times 10^6 \times 10/\text{ml}$ = 100×10^8 CFU/ml	$1200 \times 10^6 \times 10/\text{ml}$ = 120×10^8 CFU/ml	$6700 \times 10^6 \times 10/\text{ml}$ = 670×10^8 CFU/ml	296.7×10^8
Day 1		$200 \times 10^3 \times 10/\text{ml}$ = 0.02×10^8	$500 \times 10^2 \times 10/\text{ml}$ = 0.005×10^8	$147 \times 10^4 \times 10/\text{ml}$ = 0.147×10^8	0.057×10^8

		CFU /ml	10 ⁸ CFU /ml	10 ⁸ CFU /ml	
Day 2		180 x 10 ³ x 10/ml =0.018 x 10 ⁸ CFU /ml	210 x 10 ³ x 10/ml =0.021 x 10 ⁸ CFU /ml	120 x 10 ⁴ x 10/ml =0.12 x 10 ⁸ CFU /ml	0.053 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	50mg/l	1000 x 10 ⁶ x 10/ml =100 x 10 ⁸ CFU /ml	1200 x 10 ⁶ x 10/ml = 120 x 10 ⁸ CFU /ml	6700 x 10 ⁶ x 10/ml =670 x 10 ⁸ CFU /ml	296.7 x 10⁸
Day 1		160 x 10 ³ x 10/ml =0.016 x 10 ⁸ /ml	600 x 10 ² x 10/ml =0.006 x 10 ⁸ /ml	105 x 10 ⁴ x 10/ml =0.105 x 10 ⁸ /ml	0.042 x 10⁸
Day 2		130 x 10 ³ x 10/ml =0.013 x 10 ⁸ CFU /ml	800 x 10 ⁴ x 10/ml =0.08 x 10 ⁸ CFU /ml	120 x 10 ³ x 10/ml =0.012 x 10 ⁸ CFU /ml	0.035 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	100mg/l	1000 x 10 ⁶ x 10/ml =100 x 10 ⁸ CFU /ml	1200 x 10 ⁶ x 10/ml = 120 x 10 ⁸ CFU /ml	6700 x 10 ⁶ x 10/ml =670 x 10 ⁸ CFU /ml	296.7 x 10⁸
Day 1		120 x 10 ³ x 10/ml =0.012 x 10 ⁸ CFU/ml	150 x 10 ² x 10/ml =0.0015 x 10 ⁸ CFU /ml	100 x 10 ⁴ x 10/ml =0.1 x 10 ⁸ CFU /ml	0.038 x 10⁸
Day 2		100 x 10 ³ x 10/ml =0.01 x 10 ⁸ CFU /ml	300 x 10 ³ x 10/ml =0.03 x 10 ⁸ CFU /ml	207 x 10 ³ x 10/ml =0.0207 x 10 ⁸ CFU /ml	0.0202 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	control	1000 x 10 ⁶ x 10/ml =100 x 10 ⁸ CFU /ml	1200 x 10 ⁶ x 10/ml = 120 x 10 ⁸ CFU /ml	6700 x 10 ⁶ x 10/ml =670 x 10 ⁸ CFU /ml	296.7 x 10⁸
Day 1		170 x 10 ³ x 10/ml =0.017 x 10 ⁸ /ml	180 x 10 ² x 10/ml =0.0018 x 10 ⁸ CFU /ml	168 x 10 ⁴ x 10/ml =0.168 x 10 ⁸ CFU /ml	
Day 2		100 x 10 ³ x 10/ml =0.01 x 10 ⁸ CFU /ml	200 x 10 ² x 10/ml =0.002 x 10 ⁸ CFU /ml	105 x 10 ⁴ x 10/ml =0.105 x 10 ⁸ CFU /ml	

Enterococcus faecalis

Data is expressed by 10⁶ x bacteria colony per ml

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	10mg/l	9.4 x 10 ⁶ x 10/ml =0.94 x 10 ⁸ CFU /ml	920 x 10 ⁶ x 10/ml =92 x 10 ⁸ CFU /ml	960 x 10 ⁶ x 10/ml =96 x 10 ⁸ CFU /ml	62.98 x 10⁸
Day 1		250 x 10 ⁴ x 10/ml =0.25 x 10 ⁸ CFU /ml	450 x 10 ³ x 10/ml =0.045 x 10 ⁸ CFU /ml	500 x 10 ⁴ x 10/ml =0.5 x 10 ⁸ CFU /ml	
Day 2		200 x 10 ² x 10/ml = 0.002 x 10 ⁸ CFU /ml	200 x 10 ² x 10/ml = 0.002 x 10 ⁸ CFU /ml	100 x 10 ² x 10/ml =0.001 x 10 ⁸ CFU /ml	

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
-----	--------------------	---------------------	---------------------	---------------------	------

Day 0	50mg/l	$9.4 \times 10^6 \times 10/\text{ml}$ = 0.94×10^8 CFU /ml	$920 \times 10^6 \times 10/\text{ml}$ = 92×10^8 CFU /ml	$960 \times 10^6 \times 10/\text{ml}$ = 96×10^8 CFU /ml	62.98×10^8
Day 1		$120 \times 10^4 \times 10/\text{ml}$ = $0.12 \times 10^8/\text{ml}$	$300 \times 10^3 \times 10/\text{ml}$ = $0.03 \times 10^8/\text{ml}$	$150 \times 10^4 \times 10/\text{ml}$ = $0.15 \times 10^8/\text{ml}$	0.1×10^8
Day 2		$256 \times 10^2 \times 10/\text{ml}$ = 0.00256×10^8 CFU /ml	$150 \times 10^2 \times 10/\text{ml}$ = 0.0015×10^8 CFU /ml	$104 \times 10^2 \times 10/\text{ml}$ = 0.00104×10^8 CFU /ml	0.0017×10^8

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	100mg/l	$9.4 \times 10^6 \times 10/\text{ml}$ = 0.94×10^8 CFU /ml	$920 \times 10^6 \times 10/\text{ml}$ = 92×10^8 CFU /ml	$960 \times 10^6 \times 10/\text{ml}$ = 96×10^8 CFU /ml	62.98×10^8
Day 1		$110 \times 10^4 \times 10/\text{ml}$ = 0.11×10^8 CFU/ml	$250 \times 10^3 \times 10/\text{ml}$ = 0.025×10^8 CFU /ml	$250 \times 10^4 \times 10/\text{ml}$ = 0.25×10^8 CFU /ml	0.128×10^8
Day 2		$350 \times 10^2 \times 10/\text{ml}$ = 0.0035×10^8 CFU /ml	$100 \times 10^2 \times 10/\text{ml}$ = 0.001×10^8 CFU /ml	$100 \times 10^1 \times 10/\text{ml}$ = 0.0001×10^8 CFU /ml	0.00153×10^8

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	control	$9.4 \times 10^6 \times 10/\text{ml}$ = 0.94×10^8 CFU /ml	$920 \times 10^6 \times 10/\text{ml}$ = 92×10^8 CFU /ml	$960 \times 10^6 \times 10/\text{ml}$ = 96×10^8 CFU /ml	62.98×10^8
Day 1		$150 \times 10^4 \times 10/\text{ml}$	$100 \times 10^4 \times 10/\text{ml}$	$250 \times 10^4 \times 10/\text{ml}$	0.166×10^8

		=0.15 x 10 ⁸ /ml	=0.1x 10 ⁸ CFU /ml	=0.25 x 10 ⁸ CFU /ml	
Day 2		500 x 10 ² x 10/ml = 0.005 x 10 ⁸ CFU /ml	600 x 10 ² x 10/ml =0.006 x 10 ⁸ CFU /ml	450x 10 ² x 10/ml =0.0045 x 10 ⁸ CFU /ml	0.0051 x 10⁸

Mixed culture

Data is expressed by 10⁶ x bacteria colony per ml

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	10mg/l	100 x 10 ⁷ x 10/ml =100 x 10 ⁸ CFU /ml	890 x 10 ⁶ x 10/ml =89 x 10 ⁸ CFU /ml	880 x 10 ⁶ x 10/ml =88 x 10 ⁸ CFU /ml	92.3 x 10⁸
Day 1		100 x 10 ⁵ x 10/ml =1 x 10 ⁸ CFU /ml	250 x 10 ⁴ x 10/ml =0.25 x 10 ⁸ CFU /ml	700 x 10 ³ x 10/ml =0.07 x 10 ⁸ CFU /ml	0.44 x 10⁸
Day 2		400 x 10 ⁴ x 10/ml = 0.4 x 10 ⁸ CFU /ml	105 x 10 ³ x 10/ml = 0.0105 x 10 ⁸ CFU /ml	350 x 10 ³ x 10/ml =0.035 x 10 ⁸ CFU /ml	0.1485 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	50mg/l	100 x 10 ⁷ x 10/ml =100 x 10 ⁸ CFU /ml	890 x 10 ⁶ x 10/ml =89 x 10 ⁸ CFU /ml	880 x 10 ⁶ x 10/ml =88 x 10 ⁸ CFU /ml	92.3 x 10⁸
Day 1		161 x 10 ⁵ x 10/ml =1.61 x 10 ⁸ /ml	100 x 10 ⁴ x 10/ml = 0.1 x 10 ⁸ /ml	250 x 10 ⁴ x 10/ml =0.25 x 10 ⁸ /ml	0.653 x 10⁸
Day 2		980 x 10 ³ x 10/ml	109 x 10 ³ x 10/ml	160 x 10 ³ x 10/ml	0.0416 x 10⁸

		=0.098 x 10 ⁸ CFU /ml	= 0.0109 x 10 ⁸ CFU /ml	=0.0160 x 10 ⁸ CFU/ml	
--	--	--	--	-------------------------------------	--

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	100mg/l	100 x 10 ⁷ x 10/ml =100 x 10 ⁸ CFU /ml	890 x 10 ⁶ x 10/ml =89 x 10 ⁸ CFU /ml	880 x 10 ⁶ x 10/ml =88 x 10 ⁸ CFU /ml	92.3 x 10⁸
Day 1		200 x 10 ⁵ x 10/ml =2 x 10 ⁸ CFU/ml	103x 10 ⁴ x 10/ml = 0.103 x 10 ⁸ CFU /ml	100 x 10 ⁴ x 10/ml =0.1 x 10 ⁸ CFU /ml	0.734 x 10⁸
Day 2		930 x 10 ³ x 10/ml =0.093 x 10 ⁸ CFU /ml	111 x 10 ² x 10/ml =0.00111 x 10 ⁸ CFU /ml	208 x 10 ³ x 10/ml =0.0208 x 10 ⁸ CFU /ml	0.0383 x 10⁸

Day	PCBs concentration	1 st run	2 nd run	3 rd run	Mean
Day 0	control	100 x 10 ⁷ x 10/ml =100 x 10 ⁸ CFU /ml	890 x 10 ⁶ x 10/ml =89 x 10 ⁸ CFU /ml	880 x 10 ⁶ x 10/ml =88 x 10 ⁸ CFU /ml	92.3 x 10⁸
Day 1		250 x 10 ⁵ x 10/ml =2.5 x 10 ⁸ /ml	200 x 10 ⁴ x 10/ml =0.2 x 10 ⁸ CFU /ml	107 x 10 ⁴ x 10/ml =0.107 x 10 ⁸ CFU /ml	0.935 x 10⁸
Day 2		300 x 10 ⁴ x 10/ml = 0.3 x 10 ⁸ CFU /ml	105 x 10 ⁴ x 10/ml =0.105 x 10 ⁸ CFU /ml	104 x 10 ⁴ x 10/ml =0.104 x 10 ⁸ CFU /ml	0.169 x 10⁸