BIOREMEDIATION OF POLYCHLORINATED BIPHENYLS (PCBs)-CONTAMINATED SOIL BY PHYTOREMEDIATION WITH *CHROMOLAENA ODORATA* (L) R. M. KING AND ROBINSON

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DECLARATION

I, Raymond Oriebe ANYASI sincerely and solemnly declare that the work: **BIOREMEDIATION OF POLYCHLORINATED BIPHENYLS (PCBs)-CONTAMINATED SOIL BY PHYTOREMEDIATION WITH *CHROMOLAENA ODORATA* (L) R.M. KING AND ROBINSON** is my work and that all sources quoted were indicated and acknowledged by means of complete reference.

Signed ....

For student

Signed ....

Supervisor

DEDICATION

This research dissertation is dedicated to God in three Persons: God the Father, Son and Holy Spirit. To my loving parents; Sir and Mrs. Cyril Chukwu Anyasi (aka Interior Minister), and to the loving memory of my late grandmother; Lolo Uzor Nwajah.

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ABSTRACT

The ability of *Chromolaena odorata* propagated by stem cuttings and grown for six weeks in the greenhouse to thrive in soil containing different concentrations of PCB congeners found in Aroclor and transformer oil, and to possibly remediate such soil was studied under greenhouse conditions. *Chromolaena odorata* plants were transplanted into soil containing 100, 200, and 500 ppm of Aroclor and transformer oil (T/O) in 1L pots. The experiments were watered daily at 70% moisture field capacity. Parameters such as mature leaves per plant, shoot length, leaf colour as well as the root length at harvest were measured. *C. odorata* growth was negatively affected by T/O in terms of shoot length and leaf numbers, but no growth inhibition was shown by Aroclor.

At the end of six weeks of growth, Plants size was increased by 1.4 and 0.46%, but decreased at -1.0% in T/O, while increases of 45.9, 39.4 and 40.0% were observed in Aroclor treatments. Mean total PCB recoveries were 6.40, 11.7, and 55.8µg in plants tissues at Aroclor treated samples resulting in a percentage reduction of PCB from the soil to 2.10, 1.50, and 1.10 at 100, 200, and 500mg/kg Aroclor treatments respectively. There was no PCB recovery from plants in transformer oil treatments as a result of its inhibition to growth. Root uptake was found to be the probable means of remediation of PCB-contaminated soil by *C. odorata*, this was perhaps aided by microbes. This study has provided evidence on the ability of *C. odorata* to remediate PCB contaminated soil. However, the use of *C. odorata* for phytoremediation of PCB contaminated soil under field condition is therefore advised.

KEYWORDS: Phytoremediation, bioremediation, PCB, bioaccumulation factor, organic compounds, *Chromolaena odorata,* Aroclor, Transformer oil

LIST OF ABBREVIATIONS

AFCEE   Air Force Center for Engineering and Environment

AGOC   Aramco Gulf Operations Company

Ah   Aryl hydrocarbon

ATSDR-TP   Agency for Toxic Substance Disease Registry-Technical Program

BAF   Bio-Accumulation Factor

BTEX   Benzene, Toluene, Ethyl benzene, and Xylene

CARA   Conservation of Agricultural Resource Act

C-atoms   Carbon atoms

CBA   Chloro-Benzoic Acid

CNMI   Commonwealth of the Northern Mariana Island

CO_2 Carbon dioxide

DCM   DiChloroMethane

DDT   Dichlorodiphenyl trichloroethane

EPA   Environmental protection agency

$FeSO_4$ Iron II sulphate

FSM   Federated state of Micronesia

$g\ cm^{-3}$ Gram per cubic centimeter

GC-MS	Gas chromatography mass spectroscopy
H_2O	Water
HRP	Horseradish peroxidase
IBA	Indole butyric acid
ICP	Inductively coupled plasma spectroscopy
I-TEF	International toxicity equivalent factor
IUPAC	International union of pure and applied chemistry
LD_{50}	Median lethal dose ('lethal dose, 50 %')
$LogK_{ow}$	Octanol water partition coefficient
Mg/kg	milligram per kilogram
Ml/kg	milliliter per kilogram
MLPP	Mature leaves per plant
MRS	Moisture replacement system
MS3-O2	*Janibacter*
Nacl	Sodium chloride
NATO	North atlantic treaty organisation
PAH	Polycyclic aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated debenzo-p-deoxin

PCDF	Polychlorinated debenzo furan
PCN	Polychlorinated naphthalene
PCP	Polychlorinated phenol
PCQ	Polychlorinated quaterphenyls
PGPR	Plant growth promoting rhizobacteria
PNG	Papua New Guinea
POP	Persistent organic pollutant
PPM	Parts per million
PVC	Polyvinyl chloride
RBBR	Ramazol brilliant blue R
RCRA	Reserve conservation and recovery act
RF	Remediation factor
S_1	Soil sample 1
S_2	Soil sample 2
SADC	South African Development Corporation
SE Asia	South East Asia
T/O	Transformer oil
TCE	Trichloroethylene
TLF	Translocation factor

TNT   Trinitrotoluene

TOC   Total organic carbon

TPH   Total petroleum hydrocarbon

TSCA   Toxic substance control act

UNEP   United Nation Environmental Program

USEPA   United states environmental protection agency

WHO   World health organization

$Wm^{-1}K^{-1}$ Watts per meter Kelvin

IPEN   International POP elimination network

LIST OF TABLES

LIST OF FIGURES

PICTURES

TABLE OF CONTENT

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER FIVE

CHAPTER ONE

Introduction

1.1 Overview

The past two decades have been marked by an increasing deterioration of the environment resulting from the incessant increase in industrial production of chemicals. The unprecedented growth in agriculture, chemical industries, oil production, transportation, military activities and mining have all contributed in the generation of intensive pollution to the environment (Graham and Ramsden, 2008). The concentrations of anthropogenic toxic substances in the environment has risen beyond set limits, although quantitation of such increase had been difficult to ascertain, annual estimation of the spread has been reported to be in billions of tons (USEPA, 1993a; Kvesitadze *et al*., 2004). In South Africa for example, industrialization has resulted to an increase in industrial waste of environmental concern. This sudden rise in waste generation could result in dysfunctional hydrology as well as acidification and salination of the soil and groundwater leading to nature cycling and environmental degradation. Environmental degradation causes loss in biodiversity and the ecosystem which eventually impacts on human health if proper measures are not employed to checkmate it (Alcock and Jones, 1993; Pilon-Smith, 2005). There are different types of contaminants found in the environment. The most dangerous among them are those that have high persistence in bioaccumulation as well as toxicity capabilities to man as they occur in the food chain. Persistent organic pollutants (POPs), as those contaminants are called and which include polychlorinated biphenyls (PCBs) accumulate in different niches of biosphere significantly affecting ecological balance (Graham and Ramsden, 2008).

The international character of environmental degradation determined by factors such as global migration of contaminants (migration between soil, air and water), consists in overall distribution of contaminants of different structure and level of toxicity (Idris *et al*., 2004). Plants which are regarded as a natural ecological tool occupying approximately 47% of the total land surface of the earth are capable of purifying the air, water and soil. This means that plants are potential universal detoxifiers (Kvesitadze *et*

al, 2004). It has been reported that plants in addition to accumulation of heavy metals, carry out intracellular degradation process which leads to decomposition of carbon skeleton of different contaminants (Kvesitadze *et al*, 2009). Different species of plant have potentials to assimilate toxic compounds at different rates, removing them from the environment thereby providing long term natural protection as well as monitoring the environment against contamination (Berrow and Burridge, 1991). Heavy metals as well as higher molecular weight POPs are usually very difficult to remediate at waste sites by the various methods employed in their removal. Such methods which include physical, chemical, biological as well as high temperature incineration are met with some limitations and disadvantages (Babish *et al*., 1981). There have been growing interests in phytoremediation as it seems to be the alternative and cheap way of traditional clean up technology. Although phytoremediation technologies are still in the research and developmental stages, their various applications have been successfully used (Schnoor *et al*, 1995; Robinson *et al*., 2003; Singh *et al*., 2009; Kvesitadze *et al*, 2009).

Plants usually take up considerable amount of water, solutes, and organic matter as part of their normal physiological process. This action can be exploited to improve degraded environments by establishing, removing, or breaking–down contaminants in the substrates (Cho *et al*., 2003; Cho and Seo, 2005; Robinson *et al*., 2003). In recent years, a number of articles have described the role of plants in remediating contaminated soils and ground waters. Zeive and Peterson (1984), Brown *et al*, (1987), Huang *et al*, (2004/2005) and Glass (1999) describe how plants promote by various processes the remediation of wide range of chemicals at toxic waste sites. These processes include:

1. Modifying the physical and chemical properties of the contaminated soil;

2. Releasing root exudates thereby increasing organic carbon;

3. Improving aeration by releasing oxygen directly to the root zone, as well as increasing the porosity of the upper soil zones;

4. Intercepting and retarding the movement of contaminants; effecting co-metabolic microbial and plant enzymatic transformation of recalcitrant chemicals; and

5. Decreasing vertical and lateral migration of pollutants to ground water by extracting available water and reversing the hydraulic gradient.

These result in phytoremediation, which is referred to as the use of plants to improve degraded environments. Studies have continued to provide evidences that PCBs and other semi-volatile organic compounds are capable of being remediated by the use of plants (Pier *et al*., 2002). PCB is a toxic substance, which could impact on the metabolism of plants (Wilken *et al*., 1995; Ye *at al*., 1992). Therefore a potential phytoremediation plant for PCB should be a plant that has the ability to thrive in a highly contaminated environment. It should be able to withstand or perhaps neutralize the toxicity of the contaminant. Such plants should also show premise in the absorption of an appreciable solutions containing the contaminant, equally possess high growth rate as well as have the ability to accumulate organic compounds in its shoot, to reduce the cost of harvest. Hence the needs for plants that can efficiently take up PCBs and concentrate them in thier aerial parts (Singh *et al*., 2009; Atagana, 2001a).

Chromolaena odorata (L.) King & Robinson (Asteraceae. Eupatorieae), known as Siam Weed, is a perennial shrub that forms dense tangled bushes and grows wild as a weed in different geographic locations. Due to its prolific, wind-dispersed seed production and a short term persistent seed bank, the plant spreads very easily in different geographical areas (Singh *et al*., 2009). *Chromolaena odorata* is a major weed in Africa, India, Sri Lanka, SE Asia, Australia and its native land (neotropics from eastern USA, Central America, most West Indian Islands to Paraguay), and is predicted to invade most countries between the tropics of cancer and Capricorn (Witkowsky and Wilson, 2001). It has been described as the most problematic non-native invasive plant species in Kwazulu-Natal, South Africa (Macdonald and Jarman, 1985). It tends to be prevalent on nutrient rich soil and is replaced by C4 grasses on nutrient poor fallows in NE India due to lower nitrogen (N) and phosphorus (P)-use-efficiencies. Thus *C. odorata* affects both the persistence of native species and the appearance of the virtual environment (Usher, 1988). In its native range, *C. odorata* is not a weed hence no control is required. In contrast, it is a serious weed in many of the countries where it has been introduced: Africa, South and Southeast Asia. This is because of the plants ability of growing in wild (McFadyen 1991; Vwioko and Fashemi, 2005).

Previous studies on biodegradation of PCBs by phytoremediation used plants which are food crops (Iwata and Gunther, 1976; Suzuki *et al*., 1977; Weber and Mrozek, 1979; Buckley, 1987; Sawney and Hankin, 1985; Ye *et al*., 1992; Zeeb *et al*., 2006; Mackova *et al*., 2007,2009). To the best of my knowledge, there has not been any literature on the remediation of PCB with a complex plant such as *Chromolaena odorata*. This particular plant belongs to the worst class of the invasive alien plants (IAPs) in South Africa, with little or no favorable economic importance. As a result, the Department of Agriculture, Forestry and Fishery (DAFF), spends a lot of money annually in their control even till late (Macdonald and Jarman, 1985; de Lange and van Wilgen, 2010). Furthermore, the Conservation of Agricultural Resources Act (CARA), 1983 (South African National Department of Agriculture Act No. 43 of 1983), put *Chromolaena odorata* in category one of the alien invaders. This means that it should be removed as soon as possible when dictated (Macdonald and Jarman, 1985). *Chromolaena odorata* plants have the ability to grow successfully in any type of soil; this is because it is a perfect competitor hence suppresses the growth of any other plant in its invaded environment. This means that the plant could posses the structural composition capable of enabling it to thrive in a PCB-contaminated environment, by so doing degrading the contaminant. The above characteristics however, underpin the use of *C. odorata* in this study. The aim of the study therefore is to investigate the potential of *Chromolaena odorata* in the remediation of PCB-contaminated soil under greenhouse conditions.

1.2 Problem Statement

Polychlorinated biphenyls (PCBs) are world-wide environmental contaminants, resistant to degradation, but subject to biological magnification, and have been reported in human, animal, bird tissues and in milk (Iwata *et al*., 1974). Literatures reported that PCB is not biodegradable, even with the bioremediation practices that have been employed (EPA, 1983; Bedard *et al*, 1987). Attempts were made in the past to remediate PCB-contaminated soils with incineration, land filling and soil washing (Semple *et al*., 2001). But the high cost and intense disturbance associated with these techniques created interest in the use of biological degradation systems (Mikaszewski, 2004; Li Xu *et al*., 2010). However, in the phytotoxicity

study of PCB, it was observed that the compound is toxic to plants. In this study, Weber and Mrozek (1979) reported that PCB applied to the soil significantly inhibited height and fresh weight of above groung parts of soybeans plants at high rate of application (1000 ppm of PCB), this was shown by the malformation (twisting and curling) of newly developing leaves as was observed in plants growing in high PCB-treated soil. Low rate of PCB (1-100 ppm) was also inhibitory as the measurements were significantly different from the untreated control (Weber and Mrozek, 1979).

The recalcitrance of PCB in the environment can be attributed to low bioavailability of the compound in soil which tends to limit its direct extraction, removal, degradation, or stabilization by plants (Joner *et al*., 2001; Johnson *et al*., 2004). It is a contradiction to the recent biological degradation of PCB by plants and its associated microbes in the rhizosphere soil as reported by Li Xu *et al*, (2010). According to Li Xu *et al*, (2010), an average of 36% decrease in PCB levels as compared to a 5.4% decrease in the unplanted soil was observed. The decrease was further enhanced when plants were inoculated with symbiotic Rhizobium. Moreover, there is mounting evidence that plants including carrot, alfalfa, switch grass and others (Webber *et al.,* 1994; Chekol *et al*., 2004; White *et al*., 2006; Aslund *et al*., 2007), may play an important role in removal of PCB from soils (Schnoor *et al*., 1995; Schnoor, 1999; Cunningham and Ow, 1996). Among these postulations and trials, no consideration has been made with regards to a weed such as *Chromolaena*; a stress resistant, deep-rooted perennial plant with high-yield and ability to thrive in any kind of soil. This is similar to alfalfa plants that share almost these characteristics and were reported to possess strong potential for use in the remediation of organic contaminants (Chekol and Vough, 2001). From the background above, some problems were identified and deduced from literature. They include:

1. Most result in phytoremediation studies using *C. odorata* were obtained with inorganic chemicals or metals. For example 'the potential of *Chromolaena odorata* for phytoremediation of [137]cesium from solution and low level nuclear waste' (Singh *et al*., 2009).

2. Phytoremediation on most PCBs gave a very little remediation factor and only affects low chlorobiphenyls. Higher chlorobiphenyls are not affected unless when amended by a rhizosphere microorganisms (Mehmannavaz *et al*., 2002; Chekol *et al*., 2004; In Iwata *et al*., 1974).

3. PCBs is said to be toxic to plants as was indicated by the altered biomass distribution exhibited as a result of changes in the aerial and below ground biomass ratio (Mrozek *et al*., 1983; Keil *et al*., 1972; Mahanty and Gresshoff, 1978)

4.The non translocatability of PCBs in plants as was demonstrated by Moza *et al*, (1979); Ye *et al*, (1992); Fismes *et al*, (2002).

5. The effect of bioconcentration which is of great concern because of its significant toxic health effect in the ecological system (Singh and Jain, 2003; Singh *et al*., 2009). Bioconcentration is a consequence of bioaccumulation resulting in continuous proliferation of PCBs in the environment (Nolan *et al*., 2003)

1.3 Rationale

The use of food crops in the phytoremediation of PCB as was the case in various past studies may have inpacted in the continued bioaccumulation of PCB (Suzuki *et al*., 1974; Weber and Mrozek, 1979; Mackova *et al*., 2007/2009). Bioaccumulation of PCB is the enabling factor in the continuous persistence of the compound in the environment (Tanabe, 1988). Bioaccumulation is the uptake and retention of pollutants from the environment by organisms via any mechanism or pathway (Connell and Miller, 1984: In Wang *et al*., 1997, Nolan *et al*., 2003). The process of bioaccumulation of PCB in plants is a complex blend of the physico-chemical nature of the substance and its interaction with the plant biota (Wang *et al*., 1997). Accumulation and ingestion of contaminated food opens the possibility of pollutant transfer from one tropic level to another and therefore possible biomagnifications of the pollutant (Nolan *et al*., 2003).

Typically, plants have extensive and fibrous roots which form an extended rhizosphere. Plant rhizospheres are ideal location for studies of competitive interaction of microorganisms. These are area of dynamic microenvironments in which microbial communities have access to elevated supply of carbon

and energy rich materials from the plants root to the bulk soil and sediments (Gregory, 2006). These communities supported by high levels of carbon resources, should be capable of both quantitative and qualitative changes in composition of organic compounds. Rhizospheres are stable physically avoiding the potential compounding effects on naturally occurring disturbances on microbial community composition and activities (Piceno and Lovell, 2000). According to the work of Walton *et al*, (1994), it was reported that when a chemical stress is present in the soil, plant may respond by increasing or changing exudation to the rhizosphere, which modifies rhizosphere micro flora composition or activity. As a result, the microbial community would therefore increase the transformation rate of the toxicant (Molobela, 2005). This means that phytoremediation of organic contaminant can occur either through phytostabilization, phytostimulation, or by phytotransformation of the contaminant by the plants; these will be explained in the later part of this work.

In Singh *et al*, (2009) however, *C. odorata* was found to translocate cesium much to the shoot than the root when planted in soil highly contaminated with cesium. If this could be true with PCBs, along with the fact that *C. odorata* could be toxic to grazing animals, it will present the weed as a potential plant for remediation of PCBs contaminated soil (Singh *et al*., 2009). Uptake of PCBs by terrestrial organism occurs by absorption of PCBs in the soil and water mass through the epidermis or by direct consumption of contaminated fog. A potential phytoremediation plant should have the ability to accumulate PCBs in the above ground part of the plant which may not be eaten by herbivores. Therefore, the PCB-accumulated shoot can be pruned off allowing the weed to develop another shoot and thus forming a continous process (Tanhan *et al*., 2011). This could serve as a model that could be used in breaking bioaccumulation of PCBs if successfully employed especially in field trial. In addition to these processes, *C. odorata* is known to posses other useful characteristics that present it as a better candidate plant for this study. It grows as a weed and colonizes wide geographical location. It has extremely fast growth (up to 20mm per day) and is toxic to livestock (Asumbiade and Fawale, 2009; Singh *et al*., 2009; Atagana, 2011a/b; Tanhan *et al*., 2011). *Chromolaena odorata* also has the capability to translocate contaminants

to its shoot. Therefore combining this factor with the high reproducibility could make phytoremediation a continual process since pruning the contaminated shoot will make room for a new shoot that keeps sopping the contaminant (Singh *et al.*, 2009).

Moreso, this study has contributed in:

1. broadening the science of phytoremediation

2. presenting *Chromolaena* as a phytoremediation tool

3. providing for the elimination of bioaccumulation of PCBs and

4. providing cheap means of clean up of PCB.

1.4 Aim of the study

The aim of this study was to investigate the prospect of remediating PCB-contaminated soil with *C. odorata* under greenhouse conditions.

1.5 Research objectives

The main objective of this study was to explore the capability of *Chromolaena odorata* in the removal of PCBs in contaminated soil. This was achieved through the following measures:

❖ The determination of the effects of PCB on the growth and biomass accumulation of the plant in PCB contaminated soil.

❖ Determination of the ability of *C. odorata* to translocate PCB from contaminated soil to plant body.

❖ The measurement of the concentrations of PCBs in contaminated soil after the experimental period

❖ The determination of the overall efficacy of *C. odorata* in remediating PCB-contaminated soil.

CHAPTER TWO

Review of literature

2.1 Introduction

Advances in science and technology have enabled man to exploit natural resources to a great extent, generating unprecedented disturbances in global elemental cycles (Susarla *et al*., 2002). The relatively recent introduction of man-made toxic chemicals, and the massive relocation of natural materials to different environmental compartments; soil, ground water, and atmosphere, has resulted in severe pressure on the self-cleansing capacity of recipient ecosystems. Various accumulated pollutants are of concern relative to both human and ecosystem exposure and potential impact. However, efforts have been intensified by many countries environmental angencies to control the release of contaminants (Schnoor *et al*., 1995; UNEP, 2005), and to accelerate the breakdown of existing contaminants by appropriate remediation techniques. For example, existing *ex-situ* methods for remediation of contaminated ground water, which included extraction and treatment by activated carbon adsorption, microbes or air stripping. Meanwhile, all of these technologies involve relatively high capital expenditure and man power as well as long term operating cost. Hence, there are effort towards developing more cost effective approach to treat large volumes of contaminated natural resources such as soil, ground water and wetlands (Anyasi and Atagana, 2011).

Phytoremediation is an emerging technology that utilizes plants and the associated rhizospheric microorganisms to remove, transform, or contain toxic chemicals located in soils, sediments, ground water, surface water, and even the atmosphere. Currently, phytoremediation is used for treating many classes of contaminants including petroleum hydrocarbons, chlorinated solvents, pesticides, explosives, heavy metals and radionuclides as well as landfill leachates (Susarla *et al*., 2002). Phytoremediation has been used for hundreds of years to treat human waste, reduce erosion, and protect water quality (Robinson *et al.*, 2003). Research focusing specifically on the phytoremediation of contaminated soils has only

grown significantly in the last 25 years (Barman *et al*., 2000; Sarma, 2011). In this study, *C. odorata* (Siam weed), was grown in polychlorinated biphenyls (PCB)-contaminated soil in order to study the effect of the plants on soil-PCB contamination.

Polychlorinated biphenyls (PCBs) are a family of compounds produced commercially by direct chlorination of biphenyls. The molecule of biphenyls is made up of two connected rings of six carbon atoms (Figure 2.1), it contains one or more chlorine atom attached to the biphenyl nucleus. (Annema et al., 1995).
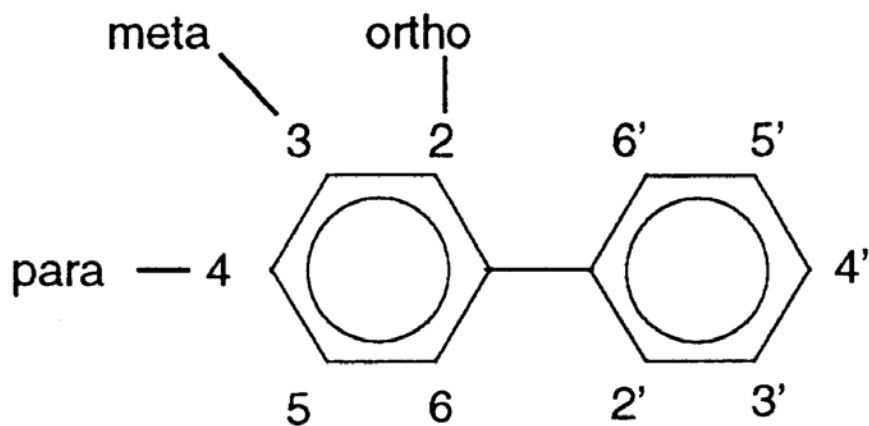
Figure 2.1: General structural formulae and numbering of the chlorine substitution position of the PCBs with the spatial terms (*orthor*, *meta*, and *para*) (Annema *et al*, 1995)

Carbon has a high selectivity for those PCBs which can assume a planar conformation; these PCBs contain no *ortho*-chlorines. As the degree of *ortho*-substitution increases (up to four chlorines in 0, 0' positions), the retention time of the compound decreases. Thus, a PCB with four *ortho*-chlorines would elute from a carbon column before other PCBs. This selectivity is useful for fractionation of PCBs. In the toxicity of PCBs, the degree of *ortho* substitution affects the toxicity (Erickson, 1997). Therefore, isolation and characterization of different fraction of the congeners from commercial mixtures has been of interest. Although the non-*ortho* PCBs are often described as "the coplanar congeners", all PCBs regardless of substitution pattern are twisted (McKinney and Singh, 1982). The energy barrier of rotation

increases as the number of chlorine atoms in *ortho* position increases. The electron diffraction technique has been used to estimate the dihedral angles of some PCBs. For instance, Almenningen *et al*, (1985) reported a non-*ortho* PCB-2 angle to be 44 ^{o} and Bastiansen (1950) reported 74°for the di-*ortho* PCB-4. The barrier of internal rotation for the tri- and tetra-*ortho* PCBs severely restricts their rotation (Kaiser 1974). Among the 209 PCBs, 19 are predicted to be atropisomers, i.e. they are conformationally stable and optically active under most environmental conditions (Kaiser, 1974). The atropisomers can be isolated by liquid chromatography with chiral stationary phases (Haglund, 1996). Furthermore, the biological potency, both *in vitro* and *in vivo*, has been shown to differ between enantiomers of the same atropisomeric PCB (Puttmann *et al*., 1989).

The PCB molecule consists of two phenyl molecules joined together with two or more hydrogen atoms replaced by chlorine atoms. PCB comprises of a group of 209 structurally different congeners with the empirical formula $C_{12}H_{10-n}CL_n$ (n=1-10; see figure 1) (Larsson *et al*., 2000). The congeners of PCBs are created by replacing the chlorine atoms at the various corners of the carbon ring (Gray *et al*., 2005). A good commercial form of PCB is Aroclor 1254, although other brand names exist. The first two digits designate the number of carbon atoms in the molecule while the last two stands for the weight percentage respectively in each type (Cogliano, 1998). The environmental occurrence of PCBs was first reported in 1966 by Jensen, who found extremely high levels of PCBs in a white-tailed sea eagle found dead in the Stockholm archipelago (Andersson, 2000). Today, PCBs can be found in all environmental compartments from the bottoms of the oceans to the aerial Polar Regions. They spread into the environment from dumps, landfills, combustion process, and from their use in various open and close systems. PCBs are lipophilic and are enriched in adipose tissues of predators, mainly through consumption of contaminated food. They have also been found to cause a multitude of toxic responses in wildlife and humans (Giesy and Kannan, 1998; Safe, 1994, van den Berg *et al*., 1998; Fitzgerald *et al*., 2001). The effects of toxicity of PCB were brought to public awareness by the Yusho incident in Japan 1968, where in a sudden epidemic in Western Japan, more than 1800 persons suffered from toxicity due to consumption of

contaminated rice oil (Jensen, 1966; Kuratsune *et al*., 1996). In Sweden and many other industrial countries therefore, the production and use of PCBs have been strictly restricted since the 1970s (Safe, 1985; Andersson, 2000). The United Nation Environmental Programme (UNEP) has established a list of 12 classes of persistent organic pollutants (POPs) , including the PCBs, along with substances such as the polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), dichlorodiphenyl trichloroethane (DDT), toxaphene, and dieldrin (UNEP,1998). These substances are listed for global priority action to eliminate discharges, emissions, and losses (Jansson *et al*., 1993).

PCBs are a group of synthetic oil-like chemicals of the organochlorine family (Erickson *et al*., 1989). It was first described in technical literature in 1881 and commercial production commenced in the late 1920s. PCBs were widely used in different ways for example as insulation in electrical equipment, particularly transformers, until their toxic nature was discovered leading to their ban in the early 1980s. All commercially produced PCBs are complex mixtures of many different congeners (PCB molecule containing a specific number of chlorine molecules at specific sites). PCB are synthetic organic compounds that exhibit high environmental persistence due to their high chemical stability, relatively low volatility, high dielectric constant, and elevated resistance to thermal decomposition (Leiva *et al*., 2010). Although there are no natural sources of PCBs, hence they persist in many environmental matrices such as water, sediments, biosolids, and soil as a result of its profuse bioaccumulation. They enter some of these matrices through the production process, use, disposal, spillage, leakage and fires of PCB containers. The chemical structure and the numbering of the C-atoms of PCB are as shown in Figure 2.2 below.
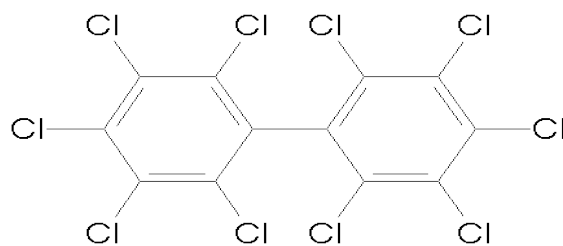
Figure 2.2: Structure and arrangement of chlorine atoms in PCB (Fiedler, 1998)

The dielectric properties of PCB gained their rapid and widespread industrial use as electrical insulators, lubricants, hydraulic fluids, diffusion pump oils, and plasticizers. However, PCBs are used frequently in day to day activities as flame retardants, polyvinyl chloride (PVC) toys, and molded containers, protectants in rubber, in weather proof coatings, stucco, waxes, varnishes, paints, inks, duplicating fluids, and pesticides formulation. PCB residues appeared first in 1949 and the level increased progressively through 1965 (Jensen 1966). And later, the residues were detected in a variety of birds, fish, and marine life in most part of the world, indicating pervasive contamination of environmental media and entry into the food chain (Risebrough *et al*, 1969 in Wang *et al*, 1997). Various studies were undertaken to determine the widespread and persistence of PCB in the environment and to evaluate the toxicological and physiological effects on the biota. Their harmful biological effects linked to mutagenesis and teratogenesis, resulted in the ban of their application and manufacturing in July 1979 in the United States of America under the Toxic Substance Control Act (TSCA). Consequently, production of PCBs in Europe, Canada and other areas ceased years ago as a result of its toxicity and persistence (Pross *et al*., 2000). The problems of environmental contamination resulting from PCBs have been widely publicized and well documented. Today, PCBs are known as the most ubiquitous and persistent contaminants on the planet (Mahler *et al*., 2005).

PCBs are synthetic compound and can be found everywhere therefore South Africa environment is not an exception in the adverse effect of its contamination. According to reports by Glenn Ashton in EKOGAIA.com (cited 23/11/2009), "South Africans are certainly not immune to the threat on pollution; Agricultural pesticides, industrial chemicals, antibiotics, and the growth of our local chemical industry

under the aegis of development have caused similar increases in chemical use and exposure. Many people assumed they were immune to these problems locally but after testing have been found to have alarmingly elevated levels of many of such products" (Glenn, 2010). According to the above quoted statement, PCBs belong to the group of chemicals known as the dirty dozens; most people have these chemicals in their body, several of which are indestructible. Problems of this nature will continue especially in the developing nations as can be found in Africa where the capacity to monitor the removal of these chemicals from their national territory or the body of their people is limited (Glenn, 2010). Recently the South African Department of Environmental Affairs and the South African Revenue Service moved to adopt a measure in examining the environmental and health costs of direct and indirect support to polluting industries and chemical clusters. They are considering allowing agricultural pesticides to be zero-tax rated. Glenn (2010) suggested self-education as well as simple livelihood as the best safeguard from exposure to these chemicals, but the question remains "what happens to other life outside human, what will be the fate of our agricultural and biological systems?" The best solution perhaps would be to harness measures that would control the presence of these chemicals in the environment or better still be removed completely.

Consequently, South Africa is also a member of UNEP's SADC-PCB inventory project. This project was formulated in order to prepare for the implementation of the Stockholm convention on Persistent Organic Pollutants (POPs), which entered into force in May, 2004 with the aim of protecting human health and the environment from the harmful impacts of POP's (UNEP, 2005). The function of the inventory project is to organize workshops on the identified needs which included inventory on PCB-containing equipments and contaminated media. Therefore, assessment of environmental health with regards to PCB as well as its ecological baseline was recommended (UNEP, 2005). Participants further emphasized the need to evaluate available PCB disposal options and existing capacities and to strengthen the legal framework with regard to PCBs. And in responding to these needs, it was concluded that the participants share the experience gained in the environmental sound management of PCBs among member countries of the

SADC region. For them to reach their goal, it was recommended they implement a network on PCB related issues and to undertake research on the impact of PCBs on human health and the environment (UNEP, 2005). This forms the need for a study of this nature in South Africa.

Two decades after the restriction imposed on the application and manufacturing of PCB, they are still found in the environment. However, monitoring programs within several countries revealed significant global environmental contamination from PCBs. Several investigations have also indicated the existence of significant quantities of PCBs as found in the soil, sludge, sediments, water, plants, fish, widelife, human blood, semen, milk, and biological tissues. (Safe *et al*., 1985; Bush *et al*., 1986; Jacobson *et al*., 1989). This is attributed to their environmental stability, global air transport in the form of vapour and particulate and improper disposal in landfills and dump sites. Thus, PCB continued to contaminate the food chain. Extensive study has been done both *in-vivo* and *in-vitro* on the toxicological effects of these compounds in different mammalian systems (Safe, 1994; Tilson *et al.,* 1990; Golub *et al*., 1991). PCBs fate in the biotic and abiotic systems has also been reviewed by Hooper *et al*, (1997).

2.2 Production and use of PCB

For decades PCBs were extensively used in a range of industrial applications. Amongst other uses already mentioned, they are also used as lubricants for turbines and pumps, in the formulation of cutting oils for metal treatments, and to a lesser extent, in applications such as plasticisers, surface coatings, adhesives, pesticides, carbonless copy paper, inks, dyes, and waxes. The commercial utility of PCBs is based largely on their chemical stability, low flammability, and their desirable physical properties as well as electrical insulating properties. According to Erickson, (1997), the increased concerns over the environmental impact of PCBs, the "open" uses which lead to direct disposal into the environmental compartment were voluntarily curtailed by Monsanto in 1970 when they invented the manufacturing of capacitors and transformers that used PCB-containing oil (Erickson, 1997).

The total production of PCBs was estimated at 1.5 million tons (de Voogt and Brinkman, 1989). The Monsanto Industrial Chemicals Co. (St. Louis Missouri, USA) was one of the largest producers and sold mixtures of PCBs under the trade name Aroclor until 1977. Other trade names existed, they included: Germany, where Bayer's produced as Clophen; Caffaro produced as Phenoclor in Italy; Japan as Pyralene by Kanegafuchi Chemical Company; Kanechlor in France by Prodelec; Fenchlor in Czechoslovakia by Chemko; and Delor in USSR by Sovo (Erickson, 1997). The production of PCB involves batch chlorination of biphenyl, and the congener pattern in the product is principally determined by the reaction time and the amount of chlorine. More than 140 congeners can be separated from the technical mixtures (Bossi *et al*., 1992). In addition, these mixtures also contain a number of contaminants in parts per million levels, such as polychlorinated dibenzodioxin/furans (PCDD/Fs), polychlorinated quaterphenyls (PCQs) and poly-chlorinated naphthalene (PCNs) (de Voogt and Brinkman, 1989).

The commercial PCB products, such as the Aroclor, typically consist of 50-70 congeners. Most of these mixtures are liquids at room temperature. The physico-chemical properties of the commercial mixtures depend on the congener composition, but generally they are resistant to acids and bases, resistant to oxidation and hydrolysis, thermally stable, excellent electrical insulators, sparingly soluble in water and have low flammability (Anyasi and Atagana, 2011). These characteristics made them very useful in diverse industrial applications, such as liquid components of transformers, capacitors, heat-exchangers, and vacuum pumps. PCB mixtures have also been used in open systems, such as plasticizers, drinking solvents, water-proofing agents, sealing liquids, fire retardants and pesticides (de Voogts and Brinkman, 1989).

2.3 Physico-chemical properties of PCBs

In 1980, Ballschmiter and Zell presented a numbering system for the 209 individual PCBs that follow the IUPAC rules. But three years later, minor amendments to this system were suggested by Andersson (2000). The molecular weights of the PCBs range from 188.7 to 498.7 based on the natural abundance of

carbon, hydrogen, and chlorine (de Voogt and Brinkman 1989). PCBs are soluble in organic solvents, oils and fats, but show an extremely low solubility in water, especially the more highly chlorinated biphenyls. Specific physico-chemical properties of individual PCBs may vary between measurements. These values are critical for modeling aspects such as the transport and fate, persistence, bioconcentration, and biological activity of the congeners. An important physico-chemical characteristic of the PCBs is their ability to rotate around the phenyl-phenyl bond. Arrangements of chlorine in PCBs have been shown to determine its toxicity, strength of adsorption to surfaces, and partition between various media. Although the non-*ortho* PCBs are often described as "the coplanar congeners", all PCBs regardless of substitution pattern are twisted (Mckinney and Singh 1982).

It has been reported that only 29 of PCB congeners are of environmental interest as a result of their toxicity (Willmann *et al*., 1997). Toxicological problems of PCB are associated with its co-planar congeners. The basic structure of PCB according to Fiedler (1998) is as shown in Figure 2.2 above. In the manufacture of PCB, a mixture of compounds with molecular weight ranging from 188-437.7, depending on the number of atoms attached to the biphenyl ring is produced. The congeners that are toxic carry between 5-10 chlorine atoms, mostly in the *para* and *meta* positions. Meanwhile, the congener's that substitute at the 3, 4-*ortho* positions are considered the most toxic. It is widely stated that *ortho* substitution increases toxicity (Erikson, 1997).

Properties of every PCB congeners depend entirely on the degree of its chlorination. These properties range from highly mobile colourless and oily liquids through the increasingly darker and more vicious liquids, to the yellow and black resins. The monos-, di-, tri-and tetra-chlorinated PCBs regarded as the lower ones are colourless, oily liquids while heavy ones are honey-like oils (Wiegel and Wu, 2000). The most highly chlorinated PCBs are waxy and greasy substances. PCBs have a low flash point which is from $140^{o}C$ to $200^{o}C$, but most of them have no flash points according to standard tests (Wiegel and Wu, 2000). Its vapour is invincible and has a very strong odour; this is one of the characteristic properties of the compound. Partition coefficient and water solubility of PCBs is low, but octanol partition is high as

well as its solubility in fats and oil. The solubility in water decreases with increase in the degree of chlorination. It ranges from 6 mg/l for monos, and about 0.007 mg/l for the octas but strangely, decachlorinated biphenyls although has a higher chlorine content, its solubility in water is twice that of octachlorinated biphenyls. This solubility is said to vary among congeners of same number of chlorine atoms (Borja *et al*., 2005).

Properties of PCB that lead to their being valuable for industrial applications include chemical inertness, high electrical resistivity and dielectric constancy, thermal stability, non-flammability and acute toxicity. Toxicity of PCB varies considerably among congeners. The coplanar PCBs is known as non-*ortho* PCBs because they are not substituted at the ring positions to the other ring, (i.e. PCBs 77, 126, 169 etc.). They tend to have dioxin like properties, and are generally among the most toxic congeners (UNEP, 1998). PCB effects on human health ranges from the skin conditions to acute liver damage as a result of man's exposure to the chemicals. Animals that eat PCB contaminated food even for a short period of time suffer from liver damage and may die (UNEP, 1998).

2.4 Environmental occurrences

From 1929 until 1977, 99% of all PCBs used by US industries were manufactured by Monsato chemical company at a production facility in Sauget, Illinois (Durfee, 1976; IARC, 1978b). During this period, about 571,000 metric tons ($1,250 \times 10^6$ pounds), were produced and or were used in the United States (Erickson, 1997; Hansen, 1999). In 1976, the US government banned the manufacturing, processing, distribution in commerce and use of PCB under Toxic Substance Control Act (TSCA), and The Reserve Conservation and Recovery Act (RCRA). Exemptions were granted to individual practitioners for use with optical microscopy and for research and development (EPA, 1998). However, production in other areas ceased years ago as a result of its recalcitrance and toxicity (Pross *et al*., 2000). Since PCBs were no longer manufactured or imported in large quantities, significant released of newly manufactured or imported materials to the environment were limited. However, predominance of PCB in the environment

continued from its bioaccumulation in the environment (e.g. soil to water, water to air, and sediments to water) (Eisenreich *et al*., 1999; Larsson and Okla, 1989). Thus, the majority of PCB in the air results from volatilization of PCBs from soil and water. Some PCBs were released to the atmosphere from uncontrolled landfills and from hazardous waste sites; incineration of PCB containing wastes; leakage from older electrical equipments in use and improper disposal of spills (Bremle and Larsson 1998).

PCBs are ubiquitous compounds and their levels generally increase from lower to higher tropic levels (Bright *et al*., 1995; Jensson *et al*., 1993; McFarland and Clarke, 1989; Willman *et al*., 1997). Pattern of the PCBs found in the biota does not resemble the composition of the commercial PCB products. PCBs released to the environment are partitioned between different media and transformed through a range of processes, such as photolysis, microbial activity, and metabolism. Among the 209 PCB congeners, McFarland and Clarke (1989) suggested 36 to be environmentally threatening due to their environmental prevalence, relative abundance in animal tissues, and potential toxicity. These 36 PCBs are listed in Table 2.1below. Total PCB level in muscle from herring caught along the Swedish coast ranged between 510 and 2400ng/g lipid (Bignert *et al*., 1998). These values can be compared with the Swedish national limit for PCB 153 in fish products of 100ng/g (Darnerud *et al*., 1996). For comparism, PCB 153 account for roughly 10-14% of total PCBs, and herring muscle consists of about 5-10% lipids (Atuma *et al*., 1996). Since the production and use of PCBs were restricted in most industrial countries, in the late 1970s, the levels in the environment have declined (de March *et al*., 1998; Sanders *et al*., 1995). However, the decrease in levels has been slower for the PCBs compared to the DDTs (Bignert *et al*., 1998). These authors concluded that most likely PCBs still enter the environment.

A retrospective study by Alc`ock and Jones, (1993) showed that the PCB levels in soil in the UK peaked during the late 1970s. The levels of PCBs have since then decreased to levels comparable with those found in the soil in the 1940s, i.e. 20-30 ng/g (dry weight). These authors also reported changes in the PCB patterns, towards greater proportions of highly chlorinated PCBs. In a sediment core from the northwestern Baltic Proper, the levels of PCBs peaked in the disk from 1978 (age range 1974-81) at

11ng/g (dry weight) and decreased in their subsequent disk to 2.6ng/g (Kjeller and Rappe, 1995). A decreasing trend of PCB levels has also been observed in archived herbage samples (Jones *et al*., 1992), peat and sediment cores (Sanders *et al*., 1992, 1995), and stored air filter samples (Jones *et al*., 1995). Air samples collected around the Baltic Sea indicated a median current concentration of total PCBs of 57pg/m^3 (Agrell *et al*., 1999), slightly higher PCB levels (89-370 pg/m^3) in the air were found at sites near the Great Lakes, atmospheric levels of PCBs are correlated with temperature. Thus, higher concentrations of the highly chlorinated PCBs are found during the summer (Haugen *et al.*, 1999).

Table 2.1: List of priority groups of important PCB congeners (Clarke *et al*., 1989).

Group 1. IUPAC no.		Group 2. IUPAC no.	Group 3. IUPAC no.	Group 4. IUPAC no.
A	B			
77	118	87	18	37
126	128	99	44	81
169	138	101	49	105
	156	153	52	114
	170	180	70	119
		183	74	123
		194	151	157
			177	158
			187	167
			201	168
				189

2.5 Sources of PCB

There are no known documented natural sources of PCB, yet they persist in the environment. They are found in air, water, soil and food (Borja *et al*., 2005). Majority of the PCBs in the environment finds its way during their manufacturing, usage as well as during disposal. This can be in the form of spillages and leakages during production, transportation and other exposure units. Other sources of PCB emission include treatments, storage, disposal facilities and landfills; hazardous waste sites; steel and iron reclamation facilities like auto scrap burning as well as in accidental release of PCB to the atmosphere (Borja *et al*., 2005). In water bodies, PCB concentrations are generally higher near human activity and

near shorelines. Therefore, the major source of PCB in surface water results from environmental cycling (i.e. from sediments, air and land) (Anyasi and Atagana, 2011). Sediments at the bottom of a water body can act as a reservoir from which PCBs can be released in small amounts to water. PCBs in fish can be hundreds of times higher than in water because they accumulate in the fish (USEPA, 1993a).

Another possible source of PCB exposure is the workplace. This can occur during repair and maintenance of PCB transformers, accidents, fires, spills, or disposal of PCB containing materials by breathing contaminated air and touching materials containing PCBs. Old appliances and electrical equipments are also believed to be the primary source of household contamination, since they may contain PCBs. Meanwhile PCB levels in indoor air are often much higher than outdoor air (Borja *et al*., 2005).

Commercial PCB mixtures were sold in many countries under a variety of trade names, but their PCB contents was very similar to an Aroclor series (Sawhney, 1986). This brings about author's interest in using Aroclor as source of PCB. Aroclor however, was mentioned earlier as the trade name of the United States of American version of PCB sample. Other common mixtures of PCBs are Aroclors 1221; 1232; 1242; 1248; 1254; 1260; and 1262, which contain 21; 32; 42; 48; 54; and 62% of chlorine by weight, respectively. Aroclors are designed by four –digit number, the first two digits: 12, represents the 12-carbon of biphenyl skeleton. The second two digits are represented as the weight percentage of chlorine in the mixture (Caims *and* Seigmund, 1986).

2.6 Health and environmental effects of PCB (Toxicity)

Polychlorinated biphenyls possess dioxin- like toxicity. Toxicity determination for any mixture needs to take into account international toxicity equivalents factor (I-TEF), e.g. 3,3',4,4'-tetrachlorobiphenyl has I-TEF of 0.0001, 3,3',4,4',5-pentachlorobiphenyls has 0.1. Recorded effects of its toxicity include dermal toxicity, immune-toxicity, reproductive effects and tera-toxicity, endocrine disruption and carcinogenicity (WHO, 1998). The first step in toxicity mechanism is mediated by the binding of PCB to the Aryl hydrocarbon (Ah) cellular receptor (Mukerjee, 1998, WHO, 1998, Sawhney and Hankin, 1985). Toxicity

of PCBs is said to range from low-moderate levels (Suominen *et al*., 1999). Treated samples of animal show an LD_{50} ranging from 0.5g/kg to 11.3g/kg of body weight. Most of the effects are as a result of repetitive or chronic exposure.

Absorption of PCBs by human and animals is through the skin, the lungs, and the gastrointestinal tract. Once inside the body, PCBs are transported through the blood stream to liver and to various muscles and adipose tissue where they accumulate (Safe *et al*., 1985/1990). Research has shown that effects on health depend on age, sex, and areas of the body where PCBs are concentrated. PCBs are carcinogenic in animals, this is because, according to the study of Borja *et al*, (2005), animals that ate food containing large amount of PCBs for short period of time had mild liver damage and some died. Occupational studies show some increase in cancer mortality in workers exposed to PCBs (Sawhney, 1986; Tsai *et al*., 2007). Furthermore, significant excess cancer mortality was found at all PCB sites combined and the gastrointestinal tract of workers exposed to PCBs contains 54 and 42 percent chlorine by weight. Likewise, Brown *et al*., (1987), found significant excess mortality from cancer of the liver, gall bladder, and biliary tract in capacitor manufacturing workers exposed to Aroclors 1254, 1242, and 1016. ATSDR-TP (1993) found significant excess malignant melanoma mortality in workers exposed to Aroclors 1241 and 1016. PCBs have also been implicated as a cause of mass mortality in seabirds. Environmental concerns over PCBs first surfaced in the late 1960s, some years after PCB was introduced. According to a study by a Swedish scientist, PCBs has anti-oestrogen properties that can inhibit calcium deposition during egg shell development, leading to insufficient strong shells and premature lost Larsson and Okla, 1989). It's Anti-oestrogen effects may also lead to adverse effects on male reproduction capabilities of birds and animal species (Borja *et al*., 2005).

PCBs can affect the productivity of phytoplanktons and the composition of phytoplankton communities. Phytoplankton is the primary source of all sea organisms and a major source of oxygen in the atmosphere. The transfer of PCBs up the food chain from phytoplankton to invertebrates, fish, and mammals can result in human exposure through consumption of PCB-containing food source (Tanabe, 1988).

2.7 Decontamination of PCB liquid

According to UNEP's Survey of Currently Available Non-Incineration Destruction Technologies (First Issue, August 2000), sodium treatment is the "most common technology to dechlorinate PCB molecules and yield oil which can be re-used". Here the basic chemical principle is the cleavage of the C – Cl bond to give sodium chloride (NaCl) and an organic molecule without chlorine. Residues from the treatment procedures above include sodium salts and various aromatic, non-halogenated hydrocarbons. The quantity of residues generated by the dechlorination procedure is in proportion to the PCB content of the treated liquid. Example, for oil with PCB content of 1000ppm, for instance, the total quantity of residues is usually less than 1% of the oil weight. This technology of dechlorination is available in South Africa but can only treat PCB contaminated fluids up to 2000ppm. Although the oil is re-refined to SANS 555 (2002), there are some concerns as to the effect of polyaromatic hydrocarbons (PAH). These structures are not fully understood and their long-term effect on the life span of electrical equipment has not yet been proven. Other option in South Africa for the treatment of PCB liquids above 2000ppm lies with the encapsulated method. The disadvantage being the "Polluter Pays Principle"(Gray *et al*., 2005), which implies that future pollution of the waste or components of the waste even after the waste has been disposed of, results in additional cost to the generator for any clean-up and rehabilitation resulting from the pollution.

2.8 Elimination of PCB from the environment

Environmental health effect of PCB does not just end with the discovery of the right technology, but its sustainability. They have usually been no single technology that is perfect and adequate in the destruction of any contaminated media to the level accepted by the community at risk (Chary and Yates, 2000). However, the media involved in PCB clean-ups are usually soil, water, sediment especially in rivers and lakes, sludges sometimes serve as medium also. Meanwhile, some technologies work perfectly in one medium than the other or require some level of pre-/post- treatment for them to be effective. Therefore, it

is imperative to keep this in mind when considering any appropriate method (UNEP, 1998). Some of the technologies that have been used for the elimination of PCBs include:- chemical (treatment with solvents); mechanical (soil escavation), thermal (incineration), and biological (use of microorganisms).

2.8.1 Thermal process

Thermal processes have always involved the transfer of pollutants from the soil to a gaseous phase. The pollutants are then released by vaporization and are burned at high temperature. Most thermal remediation is completed in three steps:

- Soil conditioning
- Thermal treatment and
- Exhaust gas purification (Van Deuren *et al*., 2002).

Soil conditioning is a process in which soil particles is broken into small grains and sieved in preparation for thermal treatment.

Thermal treatment heats the soil in order to transfer volatile pollutants (PCB) to a gaseous phase. Heating is done by using a sintering strand, fluid bed, or rotary kiln plants. The soil is usually heated to a temperature range of between $350\text{-}550^0\,C$. Combustion of the gases occurs over the top of the soil, but the volatile gases are not destroyed. The gases are then burned in an after-burner chamber at approximately $1200^0\,C$ and dioxins are destroyed (Koning, *et al*., 2000). Thermal remediation techniques can be used to remediate a lot of compounds including: total petroleum hydrocarbon (TPH); benzene, toluene, ethyl benzene, and xylene (BTEX); PCBs, polychlorinated phenols (PCPs), PCDD/Fs etc, this forms the basic advantage of the technique being that it can be used for almost every compound. Other forms of thermal remediation process also include: incineration, thermal desorption as well as plasma high temperature metal recovery. A major demerit of thermal process is that it is limited for use only in soil types with high permeability and low organic content and can only remove pollutants which can be stripped in the lower temperature range (Van Deuren *et al*., 2002). Also most thermal processes are high-tech procedure which

require huge outlay of fund for it to be executed hence making such an impossible venture in a situation whereby there is lean finance to carry out a remediation project.

The most widely accepted method for the destruction of PCBs is incineration (Waid, 1986: in Rodriguez and Lafuente, 2002). However, incineration is an expensive practice and often produces more toxic compounds as by-products (Erickson *et al*., 1989). About 1% of PCBs in a system was converted into polychlorinated debenzofurans/dioxins (PCDFs/Ds) during combustion of PCBs, therefore, incineration becomes inadequate for the treatment of PCBs especially when it is present at low concentration in aqueous media (Leung, 2004). Chemical remediation techniques currently under development emanated as a result of the demerits of incineration.

2.8.2 Chemical/physical process

This process is regarded as the pump and treats method; it involves pumping water into the surface in order to draw out the contaminants. Surfactants are sometimes added to the water to increase the solubility of the pollutants. The water is then treated with standard wastewater treatment techniques. This process, just like the thermal process is also limited by the permeability of the soil. Chemical/physical processes include:-

2.8.2.1 Oxidation

This is a common but highly active remediation technology for soil contaminated by toxic organic chemicals and cyanides. Oxidising agents used in this technology includes a wide range of substances, among which the most common are hydrogen peroxide, ozone and potassium permanganate. These chemicals are used to accelerate the destruction of the toxic organic compounds when injected into soil (Van Deuren, *et al*., 2002 in EPA, 2000).

C_2HCl_3 (TCE) + $3H_3O_2$ ---------> $3HCl + 2CO_2 + 2H_2O$
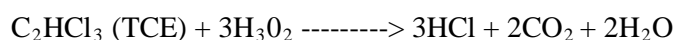
Ozone destruction of toxic contaminants takes place in the following manner-

$C_2HCl_3 + O_2 + H_2O$ ----------> $3HCl + 2CO_2$
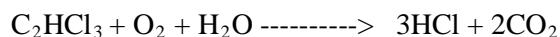
This method has been successfully used for *in situ* remediation at some source areas as well as for flume treatment. It is mostly used for benzene, ethylbenzene, toluene and xylene (BTEX) as well as for PAH, TCE, Phenols and alkenes.

2.8.2.2 Vapor extraction-

Here vacuum blowers are used to extract volatile pollutants from the soil through perforated pipes. The volatile pollutants are then treated at the site using activated carbon filters or compost filters. The effectiveness of this technique is dependent on soil characteristics such as moisture content, temperature, and permeability. A high percentage of fine soil or a high degree of saturation can also hinder the effectiveness of soil vapor extraction (Van Deuren *et al*., 2002). In vapor extraction, complete decontamination of the soil is rarely achieved. Other chemical methods include- substitution of chlorides, hydride reduction, hydrodechlorination, dechlorination using metals, photolysis, radiolysis, oxidation, electrolysis, supercritical degradation. Some of them are used commercially to treat mainly liquid PCBs, and PCB-contaminated soils, however, the low reactivity and/or selectivity of most reagents is manifested by the low applicability to dechlorination of especially multichlorinated aromatic compounds. Most vapor extraction processes require high heat, high pressure, radiation, stoichiometric reagents, vast amounts of catalyst and/or strongly-basic conditions, and usually many of them are frequently incomplete.

2.8.3 Biological transformation of PCBs

The ability of PCBs to be degraded or be transformed in the environment depends on the degree of chlorination of the biphenyl molecule as well as isomeric substitution pattern. At present, employing the biochemical abilities of microorganisms is the most popular strategy for the biological treatment of contaminated soils (Idris and Ahmed, 2003). Microorganism, more so than any other class of organisms, have a unique ability to interact both chemically and physically with a huge range of man-made and naturally occurring compounds. This usually leads to structural changes, or the complete degradation of

the target molecule (Borja *et al.*, 2005). The relatively recent development of bioremediation has added to existing cleanup strategies currently available for the restoration and rehabilitation of contaminated sites and can be conducted either in situ or ex situ. This biological strategy is dependent on the catabolic activities of the indigenous microflora, optimizing the conditions in situ for growth and biodegradation.

Organism may modify organic pollutants such as PCBs to the extent of reducing the negative effects of the contaminant to the barest minimum. Microorganisms lead this mode of biodegradation by producing enzymes, which modify the organic pollutants into simpler compounds (Dobbins, 1995; McEldowney *et al.*, 1993). Biodegradation is done in two ways: mineralization and co-metabolism. Mineralization is a process whereby the organic pollutant is used as a source of carbon and energy by the organism resulting in the reduction of the pollutant to its constituent elements. Co-metabolism on the other hand requires a second substance as its source of carbon and energy for the microorganisms but the target pollutant is transformed at same time. When the products of co-metabolism are ready for further degradation, they can be mineralized; otherwise incomplete degradation occurs (Aken *et al.*, 2010). This may then result in the formation and accumulation of metabolites that are more toxic than the present molecule requiring a consortium of microorganisms, which can utilize the new substance as source of nutrients (Dobbins, 1995). The effectiveness of biodegradation depends on many environmental factors. Rates vary depending on the conditions present in the environment. These factors include the structure of the compound, the presence of exotic substituent and their position in the molecule, solubility of the compound and concentration of the pollutant (Olusola and Ansalem, 2010). In the case of aromatic halogenated compounds, a high degree of halogenations requires high energy by the microorganisms to break the stable carbon-hydrogen bonds (Dobbins, 1995). Chlorine also acts as the substituent that alters the resonant properties of the aromatic substance as well as the electron density of specific sites. This may result in deactivation of the primary oxidation of the compound by microorganisms. There are also stereo-chemical effects on the affinity between enzymes and their substrate molecules on the positions occupied by substituent chlorines (Vasilyeve and Strijakova, 2007).

Water solubility of a compound has a vital role in its degradation. Compounds with high aqueous solubility are easily accessed by microorganisms than those with low solubility (Molebela, 2005). For the PCBs, highly chlorinated congeners are very insoluble in water. This could account for the resistance of highly chlorinated PCB congeners to biodegradation. Pollutant concentration is also a major factor affecting biodegradation. In general, a low pollutant concentration may be insufficient for the induction of degradative enzymes or to sustain growth of competent (remediation enabling) organisms. On the other hand, a very high concentration may render the compound toxic to the organisms (Silvestre and Sandossi, 1994). Under the low concentration range, degradation increases linearly with increase in concentration until such time that the rate essentially becomes constant regardless of further increase in pollutant concentration (Dobbins, 1995). Other environmental factors affecting degradation are temperature, pH, presence of toxic or inhibitory substance acceptors, and interactions among microorganisms. All these factors interplay and make the rates of biodegradation unpredictable. Biodegradation can be enhanced biologically leading to a modern day bioremediation.

2.8.3.1 Bioremediation.

Bioremediation is the use of living organisms to reduce or eliminate environmental hazards resulting from accumulations of toxic chemicals or other hazardous waste (Gibson and Sayler, 1992). Bacteria are generally used for bioremediation, but fungi, algae and plants could also be used. Bioremediation is not a new technology as evidenced by the compost piles which existed as far back as 6000BC, and the creation of the first biological sewage treatment plant in Sussex, UK. However, the word 'bioremediation' did not appear in peer-reviewed scientific literature until 1987 (Leung, 2004).

There are three classifications of bioremediation:

1. Biotransformation- the alteration of contaminant molecules into less or non-hazardous molecules

2. Biodegradation- the breakdown of organic substances in smaller organic or inorganic molecules

3. Mineralization- the complete biodegradation of organic materials into inorganic constituents such as CO_2 or H_2O (Leung, 2004).

These three classifications of bioremediation can occur either *in situ* (at the site of contamination) or *ex situ* (contaminant taken out of the site of contamination and treated elsewhere). There are advantages and disadvantages to both *in situ* and *ex situ* strategies. *Ex situ* strategies also known as 'pump and treat', removes the contaminants and places them in a contained environment. This allows for easier monitoring and maintaining of conditions and progress, thus making the actual bioremediation process faster (Aken *et al*., 2010). However, the removal of the contaminant from the contaminated site is time consuming, costly and potentially dangerous. By bringing the contaminant to the surface, the workers and the general public have increased exposure to the toxic material (Borja et al., 2005). There are several extraction strategies to facilitate ex situ bioremediation. The soil can actually be dug up and transported to a bioreactor. Soil washing is another method that can be used, where water is flushed through the contaminated region and then transferred to a bioreactor for treatment (Aken *et al*., 2010, Pinsker, 2011). Similarly, soil venting can be used, where air is flushed through the contaminated region and the air containing the contaminant is transferred to a bioreactor for treatment. The method of contaminant extraction depends on the nature of the contaminant in question (whether it is gas, liquid or solid phase, its chemical properties, and its toxicity) (Chaudhry *et al*., 2005).

In contrast, the *in situ* strategy does not require removal of the contaminant from the contaminated site. Instead either biostimulation or bioaugmentation is applied (Anyasi and Atagana, 2011). Biostimulation is the addition of nutrients, oxygen or other electron donors and acceptors to the coordinated site in order to increase the population or activity of naturally occurring microorganisms available for bioremediation. Bioaugmentation is the addition of microorganism that has ability to biotransform or biodegrade contaminants. The microorganisms added can be a completely new species or more members of a species that already exists at the site. One of the advantages of *in situ* bioremediation is that there is no need to extract the contaminant, so there is less exposure to workers, and it is also less costly (Silvestre and Sandossi, 1994). However, there are disadvantages associated to this strategy. The site of bioremediation is not contained, therefore, it is difficult to control conditions and monitor progress. One example of this is the attempt to biostimulate microbes at an oil spill site. Nutrients added to the site end up diffusing

through the water, and the result is a solute concentration the same as before when the supplementary nutrients were never added. Again, if the soil (or the media that contains contamination) is heterogenous, there will be uneven flow of liquid or gas containing the nutrients or microbes, so different areas will undergo different rates of remediation (Beebe, 2011). Despite the complications with bioremediation, it is still being used or studied for use in the remediation of crude oil spills, sewage effluents, chlorinated solvents, pesticides, agricultural chemicals, gasoline contaminants, and contaminants from wood processing, radioactive and toxic metals, this is shown in Figure 2.3 (Leung, 2004).
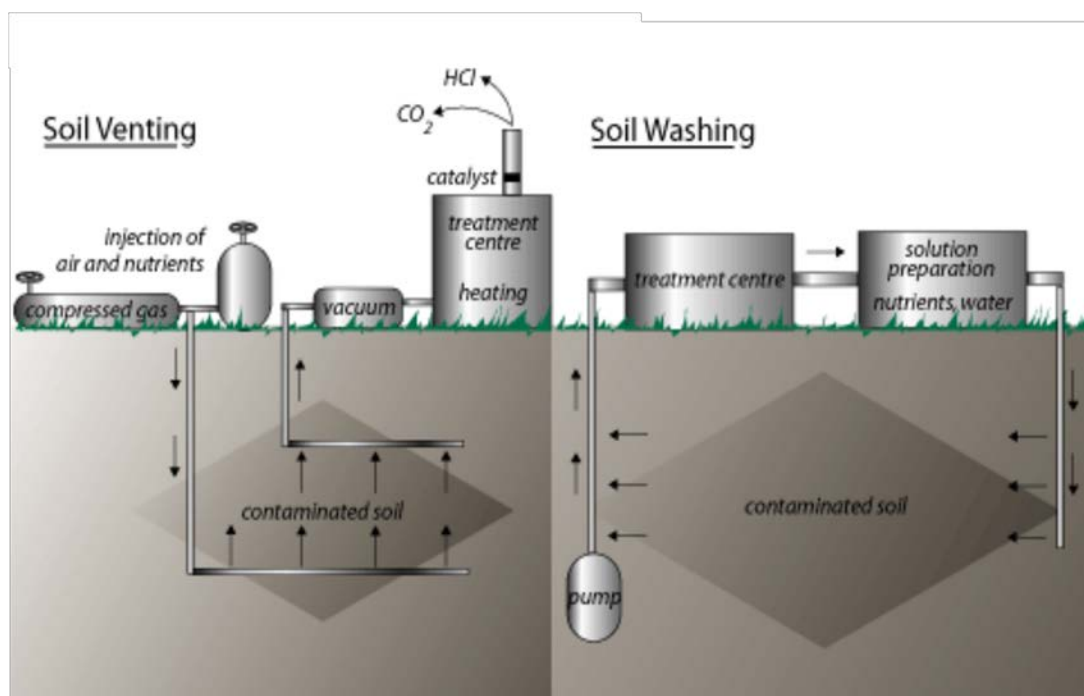
Figure 2.3: In-situ Bioremediation (Leung, 2004)

Bioremediation is often less expensive and disruption is minimal, it eliminates waste permanently, eliminates long term liability, and has greater public acceptance, with regulatory encouragement, it can also be coupled with other physical or chemical methods (Idris and Ahmed, 2003). Bioremediation has its limitations; some chemicals are not amenable to bioremediation, for instance, heavy metals, radionuclides and some chlorinated compounds. In some cases, microbial metabolism of contaminants may produce toxic metabolites. Bioremediation therefore is a scientifically intensive procedure, which must be tailored

to the site-specific conditions. This means that one has to do treatability studies on a small scale before the actual cleanup of the sites (Idris and Ahmed 2003). Some of the questions one has to answer before using bioremediation technique are: is the contaminant biodegradable? Is biodegradation occurring in the site naturally? Are environmental conditions appropriate for biodegradation? If the waste does not completely biodegrade, where will it go? These questions can be answered by doing site characterization and also by treatability studies (Idris *et al*., 2004).

As already stated above, bioremediation could be *ex situ* or *in situ* depending on whether the soil is taken out from its source or not. *Ex situ* remediation includes: land farming, biopiling, *ex situ* thermal, chemical/physical process. A major advantage of *ex situ* technique is that most of the decontaminated soil can be reused. *In situ* remediation on the hand includes: bioventing, biosparging, bioslurping and phytoremediation along with in *situ* physical, chemical and thermal process (Koning *et al*., 2000). *In situ* remediation is less costly due to lack of escavation and transportation costs but it is less controllable and less effective.

Various studies have documented long term accumulation of PCBs in soils and sediments as well as its continuous bioaccumulation in food chains (WHO, 1976). The detection of PCB in blood, adipose tissue, breast milk and other tissue samples from the population indicate widespread exposure to PCBs from the environmental sources. People who live near hazardous waste site where PCBs have been detected may be exposed primarily by consuming contaminated fish from adjacent water bodies and by breathing air that contains PCB (Fitzgerald *et al*. 2001). Bioremediation is a natural process that can be harnessed or optimized to enhance the rate at which microbes biodegrade organic chemicals released in the environment. Chemicals like PCBs, released into the environment tend to severe remediation as a result of its stability in the atmosphere (Mackova *et al*., 2007). Though much effort has been made on the bioremediation of the PCB, little attention is been given to uptake, translocation and perhaps transformation of PCBs in the terrestrial plants. Therefore it is imperative we understand the metabolism

of these pollutants through vegetation so that the fate and effects of PCBs in the environment can be assessed and remedied.

An available way to achieve effectively *in situ* remediation is to perform rhizosphere bioremediation using plants. Plants can accerelate bioremediation for organic contaminated soil by stimulating the growth and metabolism of soil microorganisms through the release of root exudates. Some root exudates may serve as carbon and nitrogen sources for growth and survival of microorganisms that are capable of degrading organic pollutants (Suominen *et al*., 1999). Plants growing in organic polluted soils themselves increase the microbial population density and diversity in the rhizosphere soils (Fletcher *et al*., 1995). Densities of rhizospheric microorganisms can be as much as two to four orders of magnitude greater than those in the surrounding bulk soils, which displays higher metabolic capacities for organic pollutants in rhizosphere than in bulk soils (Alkorta and Garbisu, 2001). In addition, plants root exudates may also provide co-substrates for microorganisms. The roots may change the porosity of the soil, which benefit the organic pollutants degradation by microbes. It is therefore notable that plant roots may reach different layers of the soil thereby distributing microbes without need for soil mixing. The concept of enhancing phytoremediation through the addition of specific microorganisms to degrade organic pollutants in the root zone is new, and has been practiced in experiments. *Meadow* bromigrass inoculated with *Pseudomonas* spp. strain 14 reduces TNT level of soil by 30% compared with controlled soil and 50% more plant biomass than noninoculated plants (Siciliano *et al*., 2001). As reported by Siciliano and Germida (1998b), the degradation rate of 2-chlorobenzoic acid (2-CBA) under the effect of plants-bacteria associations was higher than that of any single bacteria or plants, i.e. inoculation increased the phytoremediation efficiency for 2-CBA.

 Truly, limited information on PCB uptake by plants has been shown in literature until recently; and up till this moment, report is confined mostly to single chlorinated biphenyl congeners or a single PCB formulation (Iwata and Gunther, 1976; Moza *et al*., 1979; Aken *et al*., 2010; Anyasi and Atagana, 2011). Many isomers, especially highly chlorinated PCBs have not been studied with respect to their uptake and

metabolism in plants, these brings about contradictory reports. Some of the investigations show that there is little or no active transport, that band uptake of PCBs by plants is primarily through vapour sorption (Iwata and Gunther, 1976; Babish *et al*, 1981; Fries and Marrow, 1975; Buchley, 1987). Other studies pointed towards an active uptake of PCBs (Wallnofer *et al*., 1973; Moza *et al*, 1979; Suzuki *et al*., 1977; Sawhney and Haukin, 1985).

2.8.3.2 Phytoremediation

Phytoremediation is the use of vegetation for *in situ* treatment of contaminants from soil and water body. It is a promising technique that can be used to manage pollution (Singh *et al*., 2009). Phytoremediation is cost effective and eco-friendly strategy that can compliment or replace conventional approaches especially in the remediation of soil contaminated by PCBs. The principle mechanism of phytoremediation is either by stimulation of soil microbial activity and degradation of contaminants or through plant uptake of contaminants or by even their degradation products (Sung *et al*., 2001; Macek *et al*., 1999). Consequently, phytoremediation is potentially associated with plant contamination, therefore information about contaminant distribution and concentration in the plants is essential in predicting the effectiveness of a phytoremediation operation to remove and process these contaminated plants.

There are different methods of phytoremediation, they includes: phytoextraction also known as phytoaccumulation, rhizofilteration and phytostabilization. It also includes: phytodegradation, rhizodegradation and phytovolatilization (Singh and Jain, 2003). While the first three methods is involved in metal contamination hence is not considered in this review, however, the later three is involved in organic contamination and is explained thus. In phytodegradation, the plants internal and external metabolic processes break down the organic contaminants into a form that can be absorbed by the plant. Rhizodegradation on the other hand uses microorganisms to break down contaminants in the soil. This process is accelerated by the use of certain plants that encourage microbial activities. Thirdly, phytovolatilization is referred to as the process whereby plants take up contaminants that are water

soluble and consequently release the contaminants in the atmosphere as they release the water through transpiration (EPA, 1998).

Phytoremediation is a word that was coined from:

Phyto: to bring forth using plants and

Remediation: to correct a problem.

Hence, phytoremediation is an emerging technology using green plants to clean up contaminated environmental media. Phytoremediation technology having been increasingly recognized has been applied in both *in situ* (at the point of contamination such as sludge, groundwater, surface water, wastewater and then remedying it) and *ex situ* (outside the area of contamination) (AFCEE, 2011). Although phytoremediation technology is gradually being used for environmental mediation, it is however not a new technology as it dates back to the Roman civilization when eucalyptus tree was said to be used to de-water saturated soil (Carman and Crossman, 2001). Application of phytoremediation technology increased dramatically at the early nineties because of its low cost and versatility and also because of the public's positive support towards the technique. Two contrasting approaches to remediation were being pursued: pollutant-stabilization and containment, where soil conditions and vegetative cover were manipulated to reduce the environmental hazard; and decontamination, where plants and their associated microflora were used to eliminate the contaminant from the soil. It has been known that life cycle of a plant has profound effects on the chemical, physical, and biological process that occur in its immediate vicinity (Li-zhong and Gao, 2003). Therefore growing plants over a period of time with proper agronomic techniques, organic pollutants in soil will be removed from the contaminated matrix, or their chemical and physical nature will be altered within soils so that it will no longer presents a risk to man and the environment (Li-zhong and Gao, 2003). Phytoremediation costs a very meager amount per square meter of soil that is many orders of magnitude less than costs associated with physical or chemical remediation technologies (Cunningham and Ow 1996). Consequently, phytoremediation is becoming a promising and prospective technique for organic contaminated soil (Salt *et al*., 1995;

Watanabe, 1997). Phytoremediation is an emerging green technology which uses plant to remediate organic contaminated soil. However, studies on this technique since the last decade focused mostly on:

1. the mechanisms of phytoremediation;

2. factors affecting phytoremediation efficiency; and

3. phytoremediation models (Li zhong, 2003).

Efforts therefore should be directed towards uptake and possible translocation/transformation of hydrophobics such as PCBs.

2.8.3.2.1 Characteristics of phytoremediation-

Phytoremediation is an effective, natural, non-intrusive, and inexpensive remediation technique for organic contaminated soil. Just as it is stated in Figure 2.4 below, phytoremediation processes include phytodegradation, phytovolatilization, phytotransformation, phytostabilization, and rhizofilteration. These processes rely on the ability of plants to take up, and metabolize pollutants to less toxic substances 'for some plant species' (Krishnan and Safe , 2000). At the same time, plants growing at an organic contaminated site can stimulate microbial population density and diversity of the soil (Romantschuk *et al*., 2000), and improve the aesthetic value of the contaminated site, that is to say that the appearance of the site is improved by plants rhizosphere. Plants roots prevent erosion and change the porosity of the soil. As an *in situ* remediation technique, phytoremediation process reduces movement of pollutants towards groundwater, sustains the soil structure, enhances the soil organic matter content, and improves the soil texture. Therefore, soil so remediated will still be or even more suitable for agricultural purposes (Wiltse *et al*., 1998).
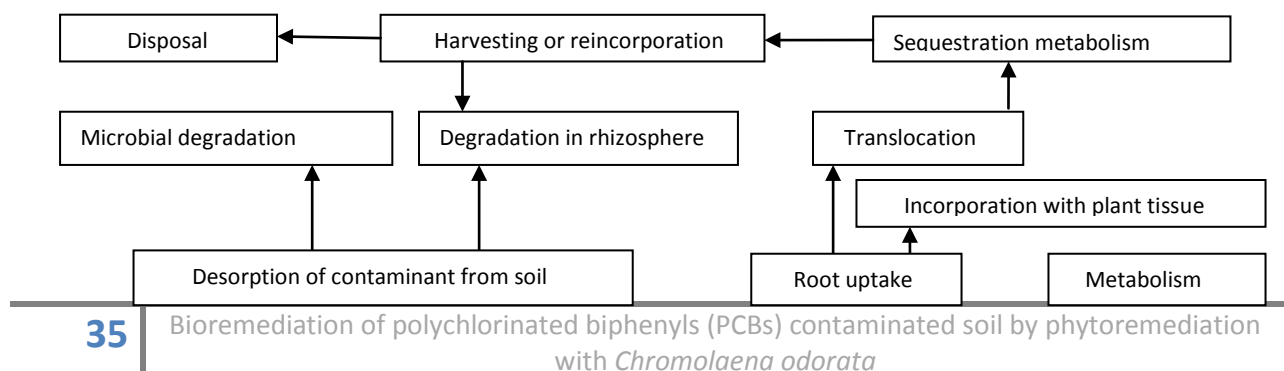
Figure 2.4: Phytoremediation process that removes organic pollutants from contaminated soil (Cunnigham and Ow, 1996)

As plant-based remediation technology, phytoremediation has its general limitations - the tolerance and uptake ability of different plants for organic pollutants differ widely. Pollutant concentrations and the presence of other toxins must be within the limits of plants tolerance. Phytoremediation is generally slower than physiochemical processes, and may be considered as a long-term remediation process (Cunningham and Ow, 1996). Moderate soil properties including soil water content, texture, organic matter content, temperature, and so on, are rigorously required to maintain plant's normal growth. Moreover, pollutants collected in leaves may be released again into the environment during litter fall. Hence, in the long run, phytoremediation still needs a deeper understanding of basic plants process (Li-zhong and Gao, 2003).

2.8.3.2.2 Mechanisms of phytoremediation for organic contaminated soils

Root uptake has always been the most sources through which plants accumulate the organics that contaminate the soil biosphere (Ryan *et al*., 1988). The process involved in this phenomenon will be explained thus:

2.8.3.2.2.1 Direct uptake of organic pollutants

Organic pollutant uptake by plants involves a complex process leading to a compound specific active and a passive process (Carman *et al*., 1998). In a passive process, the pollutants accompany the transpiration water through the plant. If the active process dominates, it is difficult to find a rigorous relationship between plant uptake and the pollutants physiochemical parameters, although some general guidelines are followed as listed below. However, if uptake of pollutants into the plants is a passive process, rigorous relationships exists (Ryan *et al*., 1988). Three main pathways through which organic pollutants enter a plant from soil has been established. These pathways include:

1. Root uptake and subsequent translocation by the transpiration stream;

2. Shoot uptake of organic pollutants from the air; and

3. Uptake and transport in oil cells which are found in oil containing plants like carrot and cress (Topp, 1986; Ryan *et al*., 1988; Eriksson, 1989; Zhang *et al*., 1999).

These pathways are a function of the physico-chemical properties of the pollutants, environmental conditions, and the plant species, these lead to variations in vegetative uptakes of organic pollutants (Schroll *et al*., 1994). However, lipophilic organic pollutants such as PAHs and PCBs, partition to the epidermis of the roots or to the soil particles and are not drawn into the inner root or xylem, since this part of translocation system is water based (Simonich and Hites, 1995; Kipopoulou *et al*., 1999). The main accumulation pathway for these somewhat pollutant is from the air to the leaf surface. Apparently, the partition of lipophilic organic pollutants from the outer leaf to the inner leaf is slow and these compounds are rarely transported by the phloem since it is water based too (Simonich and Hites, 1995; Boopathy, 2000). Recent studies have shown that the lower molecular weight PAHs dominate in both vegetable leaves and roots. Species and seasons are the main factors that significantly affect PAH concentration in inner vegetable tissues (Kipopoulou *et al*., 1999). Meanwhile, plants leaves have been found to contain PCBs absorbed from atmospheric vapor. Translocation of PCBs from soils to leaves through the root and vascular systems of plants contributed little to the PCBs found in foliage, even when plants grown in soil was inoculated with PCBs (Simonich and Hites, 1995; Meredith and Hites, 1987; Hermanson, 1990).

When plants are used to remediate organic contaminated soils, it is imperative to know the fates of organic parent pollutants and their metabolite (Alkorta and Garbisu, 2001). Therefore the fates of organic pollutants when entering into plant include:

1. Translocation to other plant tissue accompanying with the transpiration stream (Schroll *et al*., 1994);

2. Transformation to be less toxic chemicals by phytodegradation (Schnoor *et al*., 1995; Newman, 1997);

2. Incorporation with plant tissue to be nonavailable (Field and Thurman, 1996).

During chemical uptake and distribution within living plants, a lot of factors are involved in the processes, these factors are:

(a). the physical and chemical properties of the compound (e.g. water solubility, vapor pressure, molecular weight, and octanol-water partition coefficient, k_{ow});

(b). environmental characteristics (e.g. temperature, pH, organic matter, and soil moisture content);

(c). plant characteristics (e.g. type of root system, and type of enzymes). Some of the mechanisms used by plants to facilitate these remediation processes as stated earlier entail phytoextraction, phytopumping, phytostabilization, phytotransformation/degradation, phytovolatilization, and rhizodegradation (Susarla *et al*., 2002)

2.8.3.2.2.2 plant-derived degradative enzymes

About 20% of photosythates by plants, including plant enzyme, low molecular weight organic acid, and biosurfactants, are loaded into soil. Plants release a number of enzymes into soils and these enzymes may degrade organic pollutants. Plant-derived degradative enzymes in soils primarily include laccases, dehalogenases, nitroreductases, nitrilases, and peroxidases (Garrison *et al*., 2000). Several plants and their enzyme systems can degrade organic pollutants. For example, it has been proven of late that a positive correlation exists between peroxidase content and PCB disappearance during incubation of cultures in the presence of PCB. This was inferred when a study was carried out to evaluate the relationship between PCB transformation and oxidative enzymes taking part in PCB metabolism (Macek *et al*., 2002). Studies have shown that plants appear to contain certain sets of specific metabolic enzymes. Some of them can degrade organic pollutants within plant tissues, and some are released into the soils. However, the degree of enzyme released into soils remains poorly understood. Nonetheless, the measured half-life of these enzymes suggests that they actively degrade organic pollutants of soils for days following their release from plant tissues (Schnoor *et al*., 1995).

2.8.3.2.3 Factors affecting phytoremediation efficiency

There are several factors known to affect the effectiveness of any phytoremediation projects, they include:

1. Soil properties- Soils are not just the sinks of organic contaminants, but the sources of plant nutrients. Soil properties such as soil texture, water content, nutrient condition, temperature, organic matter content, and diversity of microorganisms clearly affect the phytoremediation process. The dieldrin concentration of some root crops growing in three well characterized contaminated soil (i.e. a sandy soil, a clay loam, and a muck soil, which differ widely in soil organic matter content from 1.4% to 66.5%) vary greatly. The dieldrin concentration are much lower for crops from the muck soil than from the sandy and the clay soils, whilst they are considerably higher in the muck soil than in the other two soils (Chiou *et al*., 1998). Wang *et al*. (1990/97) reported that the chlorobenzene concentrations of carrots generally increased with the increase of the corresponding soil chlorobenzene concentrations. More chlorobenzene residue was observed for sludge-amended soil than for direct spiked soil with the same amount of chlorobenzene added, which might be the results of competitive interaction between soil organic matter and organic pollutants (Wiltse, 1998; Migaszewski, 1999; Goodin and Weber, 1995), therefore soil properties affects the uptake of contaminants by plant.

2. Physiochemical properties of organic pollutants- Physical and chemical properties of organic pollutants affecting phytoremediation include water solubility, vapor pressure, molecular weight, octanol/water partition coefficients ($logK_{ow}$) (Zeive and Peterson, 1984). As stated by Simonich and Hites (1995), plants uptake of hydrophilic pollutants from soil through plant's root is a predominant pathway of plant accumulation. The uptake, translocation and distribution of these pollutants within plants depend on their lipophilicities. Whereas most lipophilic organic pollutants with ($logK_{ow}$ greater than or approximately 4), partition to the epidermis of the root or to the soil particles (particularly to the soil organic matter) and are drawn into the inner roots of xylem. The uptake of lipophilic organic pollutants through roots for most experimental species is not a significant pathway of accumulation. In general, lipophilic pollutants with half-life less than 10 days or Henry's law constants higher than 10^{-4} might not be suitable for

phytoremediation, whilst organic pollutants with $logK_{ow}$ greater than 5.0 would not be expected to be present in the above-ground plant tissue (Ryan *et al*., 1988).

3. Soil amendments- Phytoremediation efficiency, to a greater extent depends on the bioavailability of target organic pollutants; hence plenty of organic pollutants are hydrophobic. Many studies have focused on organic compounds' water solubility and availability enhancements under the effect of surfactants from bioremediation (Mulligan, 2001; Li, 1999; Kim, 2001). Some bacteria or plants produce biosurfactants that help them access hydrophobic pollutants as carbon and energy sources (Li, 1999). Other scholars have also confirmed this, therefore, the two main puzzling problems of bioremediation techniques for organic contaminated soils; long process time and residual pollutants, may be solved by the addition of surfactants.

4. Plant types- Plant uptake of lipophilic organic pollutants increases in one order with plant lipid content (Simonich and Hites, 1995; Meredith and Hites, 1987; Hermanson, 1990). Assuming that degradation of organic chemicals does not occur within the plants, and plant root uptake and translocation of organic chemicals from the soil is passive, plant uptake can be described as a series of consecutive partition reactions. These include partition between soil solids and water, soil water and plant roots, plant roots and transpiration stream, and transpiration stream and plant stems (Ryan *et al*, 1988). The hexachlorobenzene residue in some crops (barley, oats, maize, rape, lettuce, carrot, and radish) increases with the lipid content of this crop (Schroll *et al*., 1994). It has been shown that PCDD/F are high lipophilic compounds (log K_{om}>6), such compounds are primarily sorbed by plant roots or soil components and cannot be translocated within plants (Reischl, 1989). But past studies showed that clear bioconcentration of PCDD/F was observed for Zucchini. The PCDD/F concentration of Zucchini was about 2 magnitudes higher than that of pumpkin and cucumber, although they all belong to Cucurbita family (Hulster *et al*., 1994). PCDDs/Fs and PCBs all make up the persistent organic pollutants (POPs) available, this means they share the same characteristics. However, the mechanism of different plants as regards the uptake of

organics is still uncertain, and maybe it is correlated with the difference in root exudates (Hulster *et al*., 1994; Kerstin *et al*., 1995).

PCB is said to be a toxic substance (Winston and Gerstner, 1978), this means that it could impact on the metabolism of plants. Therefore a potential phytoremediation plant should have the ability to accumulate organic compounds in its part that is above ground level, hence the need for plants that can efficiently take up PCBs and concentrate it in its aerial parts.

2.9 Rhizo/phytodegradation of PCB

The rates of removal of pollutants in bioremediation are usually slower than those that can be achieved by the conventional methods. This is purely shown in remediation by plants in which its growth depends on some environmental factors. Therefore, the need arises for finding ways to enhance the entire scope and rate of bioremediation in order to present them as a competitive commercial technique (Chaudhry *et al*., 2005). PCBs are hydrophobic hence sorbs strongly to soil particles rendering its biotransformation property. The compounds are poorly taken up by plants tissues, but in the rhizospheres microbes play a dominant role in their remediation. They have been many reports of recent, showing significant increase in the reduction of PCBs in soil with different plants grown in it compared to unplanted soil (Chaudhry *et al*., 2005; Gerhardt *et al*., 2009). This section reviews the interactions of plants and microorganism in a rhizosphere looking at the effectiveness of remediation of PCB-contaminated soil with microorganism and plants explaining the differences between the two. It also throws more light in the combination of the two techniques using rhizodegradation technology of microorganisms and phytoremediation of plants.

2.9.1 Degradation of PCB by microorganisms

Recalcitrance of PCBs to biodegradation by microbes was as a result of its chemical stability (Furukawa and Fujihara, 2008). Just as higher chlorine constitution increases chemical stability and lowers water

solubility; it makes higher chlorinated congeners more resistant to remediation. Metabolism of PCBs is usually unfavourable energetically, thus requiring additional source of carbon to aid its co-metabolism. PCBs are regarded as persistant organic pollutants (POPs), however; its degradation by microbes has been well reported (Pieper and Seeger, 2008; Borja *et al.,* 2005; Field and Sierra-Alvarez, 2008; Vasilyeva and Strijekova, 2007). There are two known metabolic pathways of microbes in PCB;- aerobic and anaerobic, these depend on the degree of chlorination of the congener, the types of microbes involved as well as the redox conditions (Borja *et al.*, 2005: Aken *et al.,* 2010).

Rhizoremediation is based on the combination of microbial and plant growth process to enhance biomass accumulation, particularly plant roots in the soil, and thus, accelerating the remediation kinetics. In a natural environment, most of the demerits to remediation can be amended by the dynamic synergy existing between plants root and its associated microbes (Chaudhry *et al.*, 2005). This is because the effects of microorganisms around the root of a plant and the plants ability to withstand soil contamination could be more closely related than previously thought (Aken *et al.*, 2010). The actions of the microbes in and around the root seem to render the environment favourable for the co-metabolism of toxic chemicals abounds in the soil (Chaudhry *et al.*, 2005). Microbial transformation is not usually driven by energy need, but a quest for reduced energy. This is to enable secretion of root exudates that serve as energy source to microorganisms. Thus root exudates stimulates microbes and therefore aids degradation of phytotoxic compounds available as nutrients (Walton and Anderson, 1990; Shann *et al.,* 2001). The processes used include land farming, inoculation with contaminated degrading bacteria and growth of plants with plant growth promoting rhizobacteria (PGPR). The rhizo/phytodegradation was found to increase the overall rate of PAH remediation in creosote contaminated soil (Huang *et al.,* 2001, 2004). Therefore, combining two or more techniques in the remediation of persistent contaminants including PCB, can overcome many of the limitations that bemoan each particular method. For example, in phytoremediation, many plant species are quite sensitive to contaminants, including TPH (Huang *et al.,* 2004; Bock *et al.,* 2002). Therefore, either the plants do not grow or they grow slowly on contaminated soil. If growth is slow, the plants do not produce sufficient biomass to realize meaningful rates of

remediation. Sometimes, the number of microorganisms in contaminated soil is affected by inhibition thereby not having enough bacteria to aid degradation or even to support plants growth (Idris and Ahmed, 2003).

For effective remediation of variety of environmental contaminants, it is advantageous to use multiple techniques or processes to accelerate remediation kinetics and increase plant and microbial biomass (Huang *et al.,* 2001; Carrillo-Castaneda *et al*., 2001; Gerhardt *et al*., 2009). In the use of double or multi-process remediation, both plant growth promoting rhizobacteria (PGPR) and specific contaminant degrading bacteria was found to be vital for successful remediation (Huang *et al.,* 2001, 2004; Bhandary, 2007; Carrillo-Castaneda *et al*., 2001). For organic contaminants, use of bacteria as a pre-treatment that degrade organics in the soil can promote the remediation process (Shann *et al*., 2001; Walton *et al.,* 1994). Various bacteria can metabolize some readily available compounds-consuming bacteria that have been used on soils (Huang *et al*., 2001; Gerhardt *et al*., 2009). This will start the remediation process and can lower the toxicity of the compounds to plants when used prior to phytoremediation. Furthermore, there are bacteria called PGPR that increases the plant tolerance to organics and biomass accumulation (Gogoi *et al*., 2002). These growth promoting rhizobacteria work by preventing stress ethylene synthesis and providing auxins to the root, hence resulting to a greater biomass production (especially roots) and therefore faster remediation (Gioia *et al.,* 2006).

In a study by Huang *et al*, (2004), a series of laboratory experiments were carried out to determine the effectiveness of multi-process remediation for decontamination of creosote-spiked soil. The system consists of land farming, inoculation of degrading bacteria, and plant growth with PGPR. In a 4-month period, the multi-process remediation removed 50% more PAHs from the soil than any of the single process alone (Huang *et al.,* 2004). To further test the effectiveness of the system, remediation experiments with an environmentally aged soil from a contaminated site was used. The results showed that over an initial 4-month period, the average efficiency of removal of persistent TPHs by the system was twice that of land-farming alone, 50% more than bioremediation alone, and 45% more than

phytoremediation alone (Huang *et al*., 2004). Importantly, the system removed oil fractions 2, 3 and 4 with equal efficiency. About 90% of the total recalcitrant TPH was remediated from the soil after the second 4-months (Huang *et al*., 2005). Phytoremediation alone was able to remove only about 50% of TPHs in the same period. Therefore, rhizoremediation provides the key elements for successful remediation. With the use of plants specie which proliferates in the presence of high levels of contaminants, and strains of PGPR that increase plant tolerance to accelerate plant growth in heavily contaminated soil. The use of microorganism, both anaerobic and aerobic, is the only known process able to degrade PCBs appreciably in the soil systems or aquatic environments (Mackova *et al.,* 2007).

2.9.1.1 Anaerobic PCB-dechlorination

PCB congeners that contain four or more chlorine substituent undergo anaerobic reductive dechlorination (Aken *et al*., 2010). This is an energy yielding process in which PCBs serves as the electron acceptor for the oxidation of organic substrates. Anaerobic bacteria possess characteristics that are suited for high carbon-concentration pollutants because of the limitation in oxygen diffusion in a high concentration system (Borja *et al.,* 2005).   A predominant anaerobe environment is conducive for the reductive transformation resulting in the displacement of chlorine by hydrogen (Borja *et al.,* 2005). The dechlorinated compound is suitable for the oxidative attack of the aerobic bacteria. Aerobic bacteria grow faster than anaerobes and can sustain high degradation rate resulting in mineralization of the compound. Theoretically, the biological degradation of PCBs should give carbon dioxide, chlorine, and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting compound (Boyle *et al*., 1992).

Transformation of chlorinated organic compounds anaerobically, involves reductive dehalogenation where the halogenated organic compounds serve as the electron acceptor (Borja *et al*., 2005); the halogen substituent is replaced with hydrogen (Quensen III *et al*., 1990). Here chlorine atoms are preferentially taken out from the *meta-* and *para-* positions on the biphenyl structure, thereby leaving lower chlorinated *ortho-* substituted congeners (Olsen *et al.,* 2003). The activities above are schematically represented thus:

$$R - Cl + 2e^- + H^- == R - H + Cl^-$$

Electron acceptors are generally the factors limiting metabolism in anaerobic environment. Thus, any microorganism that could use PCBs as terminal electron acceptors would be a selective advantage (Brown *et al*., 1987).

Dechlorination in the absence of oxygen can attack a large array of chlorinated aliphatic and aromatic hydrocarbons. Several bacteria involved in this reaction have been isolated; they include *Desulfomanile tiedjel* (Mackova *et al*., 2010), *Disulfiro bacterium*, *Dehalobacter restricus*, *Dehalococcoides ethenogenes* and the facultative anaerobes *Enterobacter* strain MS1 and *Enterobacter agglomeraus*. Others are *Dehalospirillum multivoran* and *Desulforomanas chloroethenica*. Most of these bacteria reductively dechlorinate the chlorinated compounds in a co-metabolism reaction; others however utilize the chlorinated compounds as electron acceptors in their energy metabolism. Examples of phenomena that is common to the dehalogenators includes:

a. Aryl reductive dehalogenators function in a syntrophic communities and may be dependent on such a community.

b. This aryl reductive dehalogenation is catalysed by enzymes that are inducible.

c. There is exhibition of distinct substrate specificity by this enzyme.

d. Aryl dehalogenators obtain their metabolic energy from reductive dehalogenation. Hence micro-organisms with these distinctive dehalogenating enzymes each exhibit a unique pattern of congener activity (Borja *et al*., 2005).

Reductive dechlorination of PCBs occurs in soil and sediments under anaerobic condition and it is these microorganisms with the dehalogenating enzymes that are responsible. The route, extent and even the rate of these activities depend on the makeup of the active microbial community which tends to be influenced by the factors of the environment like the presence of carbon source, hydrogen or other electron donors, the presence or absence of electron acceptors other than PCBs, temperature and pH (Mackova *et al*.,

2010). For every anaerobically mediated dechlorination of PCB, the significant evidence was dependent on the observed modification of the substance in the sediments devoid of oxygen. When the distribution patterns of PCB in both the anaerobic sediments and commercial mixtures introduced to the river were compared , it showed that the sediments has a high proportion of the mono- and di- congeners and a reduction of the higher congeners (Borja *et al*., 2005). These inferences however were consistent with reductive dechlorination through *meta-* and *para-* chlorine removal. Confirmation of these findings were later done at the laboratory and the evidence was obtained that microbial numbers in the sediment could reductively dechlorinate most of the congeners of Aroclor 1242 at the *meta-* and *para-* positions, and proportions of mono- and di- chlorobiphenyls increased considerably (Quensen III *et al.,* 1990).

Laboratory studies in the dechlorination of commercial mixtures of PCB showed that the rate and extent of dechlorination is inversely proportional to the degree of chlorination and dechlorination was said to be associated with synthropic communities attacking PCB at different positions with specificity for PCB dechlorination (Rezek *et al*., 2007). With microorganisms, the use of organic substrate as electron donors has also been shown to increase the rate of dechlorination of Aroclor 1242 (Newman and Reynolds, 2004). Even separate addition of glucose, acetone, methanol and acetate has almost the same pattern of dechlorination for each substrate, but the extent and rate of dechlorination were different. The rate of dechlorination was decreasing and greatest with methanol, glucose, acetone while acetate has least. As usual, dechlorination occurred primarily on the *meta-*and *para-* position of the highly chlorinated congeners resulting in the accumulation of less-chlorinated, primary o*rtho-*substituted products. The use of pyruvate and acetate as electron donors was also tested using microorganisms. Aroclors 1242, 1248, 1254, and 1260 were dechlorinated primarily at the meta- positions of the biphenyl molecule. Aroclor 1254 has the greatest dechlorination but with acetate, there was a kind of delay in its dechlorination (Newman and Reynolds, 2004). When Iron II sulphate ($FeSO_4$) was added to PCB-contaminated sediments, an almost complete *meta-* plus *para-* dechlorination of Aroclor 1242 was discovered (Borja *et al*., 2005). According to the study, while $FeSO_4$ was stimulating the growth of sulphate reducing organism responsible for PCB dechlorination, Fe^{2+} reduced the sulphide bioavailability and toxicity

through the formation of an insoluble FeS precipitate. The appreciable loss of *meta-* and *para-* chlorines catalysed anaerobic dechlorination leads to preferential reductions in the level of coplanar, dioxin-like congeners in the PCB mixtures (Abramowicz, 1995; Borja *et al*., 2005; Mackova *et al*., 2010). The decrease in risk is manifested in two ways:

1. Sparsely chlorinated congeners produced as a result of dechlorination can be degraded by indigenous bacteria (Borja *et al.,* 2005)

2. Dechlorination significantly reduces bioconcentration potential of the PCB mixtures through conversion to congeners that do not significantly bioaccumulate in the food chain (Magae *et al*., 2008).

PCB dechlorination is attributed to complex consortium of bacteria but little is known about the metabolic pathways, bases of the molecule and the enzymes involve in the process (Aken *et al*., 2010). The pollutants are widespread in contaminated sediments therefore are found to involve species related to *Dehalococcoids* (Abraham *et al*., 2002; Cho *et al*., 2002/2003; Bedard *et al*., 2006). It is of note however, that only very few bacterial species which are able to dechlorinate PCBs in pure culture were identified and the range of their activity is limited to just few congeners (ATSDR, 2000; Pieper and Seeger, 2008).

2.9.1.2 Aerobic biodegradation of PCB

Sparsely chlorinated PCB congeners which form as a result of dechlorination of the higher congeners are substrates for aerobic bacteria (Komancova *et al.,* 2003) Those PCB congeners undergo cometabolic aerobic oxidation which is mediated by an enzyme deoxygenases, bringing about a ring opening hence completing mineralization of the molecule (Kohler *et al*., 1989; Vasilyeva and Strijakova, 2007; Furukawa and Fijihara, 2008). A lot of bacterial strains are implicated in oxidative degradation of PCBs; among them are *Pseudomonas spp., Burkholderia spp., Comamonas spp., Rhodococcus spp.,* as well as *Bacillus spp*. (Cho and Seo 2005; Aken *et al*., 2010). Chlorine numbers per molecule and its placement are important factors in aerobic biodegradation (Furukawa *et al*., 2004). PCB congeners with three or less

chlorine atoms per molecule are easily degraded, but ones with more than three chlorine atoms are recalcitrant, therefore requires reductive dechlorination prior to oxidative mineralization (Aken *et al*., 2010). PCB-destruction in the presence of oxygen involves two gene clusters (Borja *et al*., 2005). The first one enables transformation of PCB congeners to chlorobenzoic acid and the second involves degradation of the chlorobenzoic acid. A common growth substrate for PCB –degradating bacteria is biphenyl or monochlorobiphenyls. During utilization of biphenyls, a yellow *meta*-ring cleavage product is formed as observed in most studied bacteria for example the *Pseudomonas spp. (*Boyle *et al*., 1992), and *Micrococcus* spp. (Benvinakatti and Ninncher, 1992). Through 1, 2-dioxygenative ring cleavage, benzoate results as a common by-product of biphenyl degradation. Some other bacterial species seem to produce benzoate through PCB metabolism, further breakdown differs among microbes but their by-products are less toxic compounds (Bianucci *et al*., 2004). Since PCBs persists more at increasing chlorination of the congeners, aerobic biodegradation involving ring cleavage is restricted to the lightly chlorinated congeners.

While biphenyls and monochlorobiphenyls can serve as growth substrates, the degradation of PCB congeners with more than one chlorine atom proceeds by a co-metabolic process in which biphenyl is used as carbon and energy source while oxidizing PCBs. Biphenyls therefore serve as an indicator of degrading enzymes. Earlier study reported that two species of *Achromobacter* are capable of growing on biphenyls and 4-chlorobiphenyl (Campanella-Bruno *et al*., 2002). The degradation of PCB by *Myocardial spp.*and *Pseudomonas spp.,* increased upon addition of biphenyls. This was reported to enhance co-metabolism of Aroclor 1242 in the presence of acetate using mixed cultures of *Alcalegenes odorans*, *A. denitrificans*, and an unidentified bacterium (Mackova *et al*., 2007). Increased mineralization of Aroclor 1242 by *Acineto bacteria spp*. strain P6 by addition of biphenyls and 4-chlorobiphenyl was also observed. Furthermore, these microorganisms co-metabolize Aroclor 1254 in the presence of biphenyl (Furukawa *et al*., 1978; Furukawa and Miyazaki, 1986).

In a recent study, a new bacterium, *Janibacter*, MS3-O2, was isolated from soil contaminated with PCB in form of Aroclor (Mackova *et al*., 2007). It was interesting to note that the degradation of Aroclor1242 was significantly higher in the liquid medium without biphenyl (70-100% after 7days). When biphenyl was added in the medium, degradation was only 84%. On soil medium, the soil native population was not able to degrade the PCB present in Aroclor 1242. Hence inoculation of the soil with MS3-O2 produced a decrease in some of the chromatographic peaks. Comparism of the result obtained in the soil and that of the liquid shows that the degradation was less efficient in the soil because of the effects of lower bioavailability of PCBs and its interactions with the surrounding soil microorganisms (Mackova *et al*., 2007).

Several studies on the microbial degradation of commercial PCBs show that certain patterns of chlorine substitution seriously hinder PCB degradation. For lightly chlorinated PCB congeners, a sequential enzymatic step involved in the degradation was however developed (Seeger *et al*., 1997). The complete degradation of PCB requires various microbial strains with specific congener preferences (Mackova *et al.,* 2007). In addition, the position and number of chlorine atom on the molecule can influence the rate of the first oxygenate attack. Mackova *et al*, (2007), proposed a mechanism for the oxidation of PCB by *A. Euterophus*, *P. Putida*, and a *Corynebacterium spp. Alcalegenes Odorans*, *A. Euterophus* and *P. Putida* bacteria strain degrade tetrachlorobiphenyl via 2,3- attack while C*orynebacteria spp.,* degrades the compound via 3,4- attack. In the study conducted to immobilize an SIRAN carrier, degradation of individual congeners with biphenyl as growth substrate showed a common metabolic pathway starting by oxidation at the 2, 3- position of the less chlorinated ring (Furukawa *et al*., 1978; Kumancova *et al*., 2003). The degradation for 2,4,4'-trichlorobiphenyl, a 2,3-deoxygenase attack of the less chlorinated ring was the primary reaction used by *Pseudomonas spp*., resulting in the formation of the yellow metabolite 3-chloro-2-hydroxy-6-oxo-6-(2,4-dichlorobiphenyl) hexa-2,4-dienoic acid; and a final product 2,4-dichlorobenzoic acid. The congener, 2, 2', 5, 5'-tetrachlorobiphenyl was degraded via 2, 3-dioxygenase attack, with the formation of 2, 5-dichlorobenzoic acid and trichlorobiphenyl. The identified metabolites indicated that *Pseudomonas spp.* 2 was capable of dehalogenating PCBs (Komancova *et al*., 2003). The

degradation of 2, 2', 5, 6'-tetrachlorobiphenyl confirms the ability of bacteria strain to dehalogenate PCBs (Borja *et al*., 2005). Degradation of this compound was via 2, 3-deoxygenase attack and the products formed correspond to (based on molecular weights) 4-(2, 5-dichlorophenyls)-oxobutanoic acid. Two other compounds, 2-chloro-3-(2, 5-dichlorophenyls)-2-acrylic acid and monochloroacetophenone, were also detected. These products are consistent with 3, 4-dioxygenase attack (Komancova *et al.,* 2003).

Furukawa *et al*. (1978) therefore summarised the relationship between chlorine substitution and the microbial breakdown of PCBs as follows:

1. The rate of degradation of PCBs is inversely proportional to the increase in chlorine substitution (Borja *et al*., 2005)

2. PCBs containing two chlorine in the *ortho-* position of a single ring (i.e.2, 3, 6-) and each ring (i.e. 2, 2') shows a striking resistance to degradation.

3. PCBs which have all of its chlorine on its single ring degrade much faster than those with same number on double rings (Cho and Seo, 2005; Bhandari, 2007).

4. PCBs having two chlorines at the 2,3- position of one ring such as 2,3,2',3'-, 2,3,2',5'-,2,4,5,2',3'-chlorobiphenyls are susceptible to microbial attack compared with other tetra-and penta-chlorobiphenyls, though this series of PCBs is metabolised through the alternative pathway.

5. Initial deoxygenation followed by ring cleavage of the biphenyl molecule occurs with a non-chlorinated or less chlorinated ring.

2.10 Anaerobic-aerobic transformation of PCBs

There have been a lot of studies on aerobic bacteria PCB-degradation (Seeger *et al*., 1997; Wiegel and Wu, 2000; Borja *et al*., 2006). From these studies, it was observed that only PCB congeners with four or less chlorine atoms were degraded. Highly chlorinated PCB congeners; those with five or more chlorine atoms, remain persistent to aerobic bacteria, though they had been few reports on the aerobic degradation of penta- and hexa- chlorobiphenyls (Borja *et al*., 2005). There were also various studies on the

transformation of PCBs using anaerobic bacteria eluted from PCB –contaminated sediments. Reports of preferential *meta-* and *para-*chlorine removal from highly chlorinated PCB congeners under an aerobic means producing lesser chlorinated congeners that can biodegrade aerobically abounds.The biotransformation pattern above however, is commonly found among halogenated aromatic compounds (Borja *et al*., 2005). Macek *et al*, (2002), reported a sequential anaerobic-aerobic treatment of PCBs in the soil microcosms, and the results of the batch soil-slurry microcosm showed dechlorination of several hexachlorobiphenyl to penta- and tetra-chlorobiphenyl by indigenous microorganisms. The availability of microorganism capable of degrading tri- and tetra-biphenyls was also shown in the aerobic microcosm experiment by Borja *et al,* (2005). According to the study, both aerobic and anaerobic metabolism modes transform PCBs. The difference in the pattern of degradation of PCB was as a result of preferential attack by different microorganisms (Quensen III *et al*., 1990). The degree of chlorination of the congeners is a major factor, which tends to influence degradation potentials of the compounds. Moreover, environmental factors such as temperature, pH, and the presence of other substrates affect the composition and growth of the microorganism. These factors should however be optimised to obtain high degradation efficiency (Anyasi and Atagana, 2011).

2.11 Rhizoremediation

PCBs are hydrophobic, hence possesses high affinity for soil particles. There are therefore taken up into the plants tissues sparingly (Campanella-Bruno *et al*., 2002; Chaudhry *et al*., 2005; Gerhardt *et al*., 2009; Wood *et al*., 2000). The mechanisms by which plants can stimulate microorganism activity in the soil to enhance the biodegradation of PCBs include:

(a) The release of organic compounds like suger, amino acids, and organic acids by plants root used as electron donor support for either aerobic or anaerobic metabolism of chlorinated compounds. In certain instances, microbial aerobic degradation consumes energy resulting in anaerobic processes which is usually favourable for PCB dehalogenation (Chaudhry *et al*., 2005).

(b) Extracellular enzymes that cause transformation of PCBs leading to further microbial metabolism are secreted by plants (Fletcher *et al*., 1995).

(c) Microbial degradation of PCBs are speed up by inducers which are secreted by plants, however, Hedge and Fletcher (1996), reported that *B. Xenovoranss* LB400 and its activity as a PCB degrader was induced by plants phenolic exudates.

(d) The effects of plants root increases the permeability of the soil and also oxygen diffusion in the rhizosphere. These induces microbial oxidative transformation by certain enzymes (Chaudhry *et al*., 2005)

(e) Growth factors are also known to be secreted by plants (Campanella-Bruno *et al*., 2002).

(f) Organic acids and molecules that act as surfactants come from the roots, they therefore help to mobilize PCBs making them more susceptible to plants tissues (Chaudry *et al.,* 2005).

Vicinity of plants root is the preferred environment for microorganisms. It has been reported that approximately 1.2×10^{11} cells/cm^3 of microbes live within a distance of less than 1mm to the roots, whereas only 1.3×10^{10} at a distance of 2cm (Paul *et al*., 2007). This means that about 5-10% of the root surfaces are covered with bacteria. Besides forming a habitat for microorganisms, plants roots also provide nutrients, e.g. sugars, in exchange for phosphates (fungi) or nitrogen (N$_2$-fixation). Mulberries (*morus rubra* L.) growing at PCB-polluted sites, excretes considerable amount of phenolic compounds which probably support the growth of PCB- degrading bacteria (Fletcher and Hedge, 1995), roots can also exude organic compounds which might mobilize indigenous soil pollutants e.g. saponines, proteins and enzymes. That roots and xylem exudates of zucchini (Cucurbitaceae) was also found to solubilise PCDD/F (Held and Door, 2000), probably by protein (Newman and Reynolds, 2004). However, plants hyper-accumulation of lipophilic compounds has not yet been established but with microorganisms, reasonable result is ensured (Hatamian-Zarmi *et al*., 2009).

The combined effort of microorganisms and plants on PCB was seen on report of the work of Dzantor *et al*, (2000), in which dissipation of Aroclor 1248 was enhanced using substrate amendment in the rhizosphere soil.

A lot of articles have elucidated the importance of root exudates on the activities of microbes in the soil and also on biodegradation of PCBs (Fletcher and Hedge, 1995). These exudates which are made up of water soluble and insoluble compounds in addition to the volatile components, enable the acquisition of minerals by plants thereby stimulating the growth of microbes in the rhizosphere (Chaudhry *et al*., 2005). Other factors affected by this synergistic effect of the root exudates include pH change, water flux, and oxygen availability in the rhizosphere. There was a report on the interaction on the difference in treatment in the degradation of Aroclor 1242 in soil (Mackova *et al*., 2007). In all the reports, degradation of higher molecular weight PCBs in the soil was a significant observation compared to non-vegetated control; hence the conclusion that plants enhances PCB degradation (Aken *et al.,* 2010). Plants perform this task through oxygen diffusion increase, infiltration amendment, and through enrichment of microbes. With the use of several plants in the phytoremediation of PCBs, there was less than 38% recovery as compared to 80% and above recoveries in non-planted controls (Chekol *et al*., 2004). It was also shown that increased soil enzymatic activities by plants were correlated with PCB-degradation level (Chekol *et al*., 2004). Other factors that enhance removal of PCBs from a contaminated soil include soil amendment (Smith *et al*., 2007). According to the author, organic amendment brings about oxygen consumption that is needed to achieve anaerobic dechlorination of PCBs (Aken *et al*., 2010). Molecular biology has also been used to develop a tool used to locate PCB-degraders in the roots of plants growing in a soil contaminated by PCB (Hogan *et al.,* 2004; Aken *et al*., 2010)

2.12 Uptake of PCBs by plants

Prediction of uptake of organic pollutants by plants depends on the octane rating of the pollutant (Schnoor, 1999). Based on this model, only moderately hydrophobic compounds ranging from 0.5-4.0 log

K_{ow} would be absorbed and consequently translocated within plant tissues. In addition, the effectiveness of uptake of PCBs by plants with its $logK_{ow}$ ranging from 4.5 (the monos) to 8.2 (the decas) will be expected to decrease synonymously with the degree of its chlorination. In the phytoextraction study done by Zeeb *et al*, (2006), there were variable concentration of Aroclor 1260 in root tissues, and lesser concentration in the shoot. According the study, those highly concentrated PCBs range from the tetras to the hexachlorobiphenyls. But the heptas and the nonas were also detected in minute quantities. This result however, counteracts the prediction based on octane rating of the pollutant and suggests the possibility of higher chlorinated PCBs taken up within plants tissues. In another development, Liu and Schnoor (2008), discovered that selected monos-tetrachloro PCBs were absorbed by plant roots, but only the lower chlorinated ones got translocated to the aerial parts of the plants. Aslund *et al*, (2008) also reported an increase in PCB concentration within stem and leaves of pumpkin plants following a short time of exposure in a field trial, but the concentration in the root remained unchanged. Therefore, the authors inferred that transfer of PCBs in plants primarily occurs through uptake and translocation, while other mechanisms have negligible effects (Aken *et al*., 2010).

PCB uptake into plants is through two general routes. One of the routes is through the root system and the other is through prior adsorption in the foliage and stems. It also involves subsequent movement through the epidermal layers into the apoplast or symplast (Mackova *et al.,* 2007). The former route is probably the most important way of uptake of applied PCBs, while the latter route probably predominates in the uptake of airborne PCBs by terrestrial plants and dissolved PCBs by aquatic plants and microorganisms. This means that uptake of PCBs from fallouts is unlikely to occur to any greater degree because the compound could adsorb to the outer surface of the plants and may not be truly present inside the plant. Plants cuticle contains many lipophylic compounds in which the PCB could effectively 'dissolve', limiting further internal migration (Gilbert and Crowley, 1997). In addition, unless PCB uptake by microbes can be differentiated into that which has adsorbed to the surface and that which has entered the protoplasm proper, uptake studies of this nature (using algae and bacteria) will become misinterpreted. Uptake of ^{14}C-labelled PCBs following application to leaves has been demonstrated, although in low

amounts (3.2-15.5%) of that applied; the greatest loss probably occurred through volatilization (Weber & Mrozek, 1979). According to Iwata and Gunter, (1976) as was reported by Strek and Weber, (1982a), PCB content of plant was dependent on the PCB concentration in the soil. This means that the amount of PCB absorbed by plant at any point in time depends on the initial concentration of PCB in the soil, plants species, and organic components of the soil as well as the ensuring temperature (Strek and Weber, 1982a). This however agrees with other studies on PCB (Smith *et al*., 2007; Mackova *et al*., 2007; Aken *et al*., 2010).

2.13 PCB metabolism by plants

Xenobiotics metabolism by plants is been described as a three way process in the green liver model as represented in Figure 2.3 below. It starts with the activation process consisting of oxidation of PCBs to hydroxylated products which are very soluble and reactive (Sandermann, 1994; Coleman *et al.*, 1997). The second process involves conjugation of activated compounds with plant molecules. Here, lesser toxic and more soluble compounds are formed. In the final process of sequestration, the conjugates are adsorbed into plant organelles (Sandermann, 1994; Coleman *et al*., 1997).
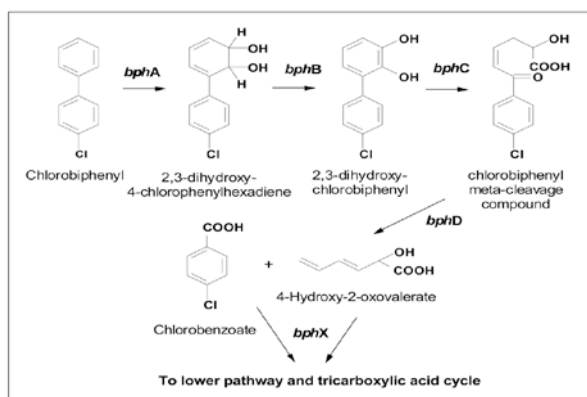
Figure 2.5: Aerobic bacteria of lower chlorinated PCB catalysed enzymes (bphADCDX) (Furukawa *et al*., 2004)
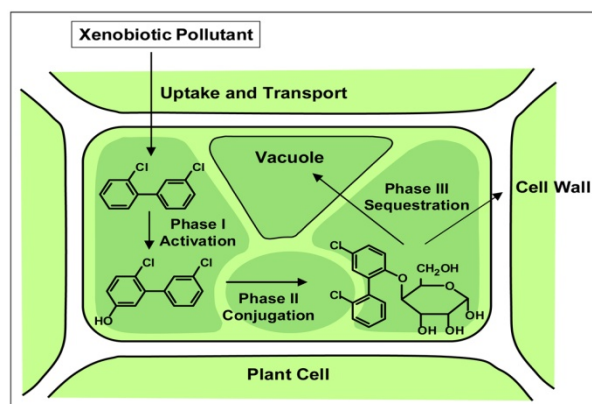
Figure 2.6: Three process of the green liver model of xenobiotic metabolism (Aken, 2008)

Studies on metabolism capabilities of PCB by plants has just recently begun (Aken *et al*., 2010), various studies have reported on the transformation of PCB-congeners in plants cells. Lee and Fletcher (1992) inferred that many individual congeners were metabolized by appreciable amount. Several mono- and dihydroxylated metabolites of PCB were detected in plant species in the study of Wilken *et al*, (1995), while Mackova *et al*, (2007), reported that transformation capability of PCBs differ according to strains. Plants ability to oxidize mono- and dichlorinated PCBs into mono- and dehydroxylated biphenyls were also reported by Kucerova *et al*, (2000). So many other studies with plant cell cultures have also highlighted on plants capability of PCB metabolism (Chroma *et al*., 2003; Harms *et al.,* 2003; Rezek *et al*., 2007). From these studies therefore, it was inferred that plants metabolism of PCBs could depends on the strain and the degree of chlorination of the compound.

Furthermore, metabolism of PCBs by plants is aided by several enzymes (Mackova *et al*., 2007; Aken, 2008). These enzymes include oxygenases, peroxidises, oxidases, and transferases. Cytochrome P-450 and peroxidises are also implicated in initial process of metabolism (Harms *et al*., 2003). Commercial horseradish peroxidase (HRP) was used to transform dichloro- and tetrachlorobiphenyls, and Remazol Brilliant Blue R (RBBR) oxidases with other enzymes were involved in *in vitro* cell culture of plants (Chroma *et al.*, 2003). Recently, Magae *et al*, (2008), reported dechlorination of biphenyl by extract of a reductase enzyme from *Medicago sativa* and *Zea mays.*

2.13.1 Effects of PCBs on plants

The inhibition of plants growth due to PCB effects has been well documented (Furukawa *et al.*, 2004). This report documented mainly for algae, denoted several deductions in algae cell numbers at a general low level (0.3-10ppm) of PCBs in aqueous solution. They had been scarce report on the growth inhibition of PCB to higher plants, although information on internal disorganization of front chloroplast cells of *Spirodela oligorrhiza* (Kurtz) Hegelm exposed to 5ppm of Aroclor 1242 was documented (Strek and Weber, 1982). Weber and Mrozek (1979) however reported malformations on newly developed soybean leaves on the plants growing in soil containing about 1000 ppm of Aroclor 1254. Reduction in plants height and fresh weight was noted for soybean, beets and pigweed *Amaranthus refloflexus L.* but only fresh weight reductions were reported for Fescue (Strek and Weber, 1982). At 1000 ppm rate of Aroclor 1254, soybean growth was inhibited by about 47%. However, the effect of PCB to water use by plants seem to be much pronounced than plants growth itself. This indirectly means that the effects on plant growth may be related to water use, following effects which may reflect on transpiration (Anyasi and Atagana, 2011).

In the work of Smith et al. (2007), which investigated the effects of PCB congeners found in Aroclor 1260 on plants growth. They used starch straw (as organic amendment) in soil to hasten the degradation. Significant differences between percentage losses of PCBs were found between treatments for some of the PCB congeners, but none of the expected degradation was detected (limits of quantification 0.1 mg/l in solution). A lot of differences between treatments were observed in the loss among penta-hepta chlorobiphenyls (Smith *et al.*, 2007). From the results, *C. aquatalis* with amendment had significant higher percentage loss than *C. Aquatalis* without amendment, *SD. Pectinata* with amendment, and *T. Dactyloides* with amendment; Mulberry (*M. Rubra*) with amendments was reported to have significantly higher percentage loss than did *S. Fluviatilis* with amendment. Aken *et al*, (2010), equally reported that highly chlorinated PCBs found in Aroclor 1260 requires reductive declorination as the first step in remediation, and this process require a treatment with low transpiration and high soil water content. The

study further stipulated that reductive dechlorination lead to accumulation of less chlorinated congeners that were possibly lost to aerobic microorganisms during the aerobic stage of the work (Anyasi and Atagana, 2011).

When water saturation is maintained in sediment, reductive dechlorination results with accumulation of cell chlorinated PCBs (Smith *et al.*, 2009). Therefore using plant species that remove water from the sediments and introduce oxygen into the rhizosphere through aerenchyma could greatly stimulate removal of lower-chlorinated PCBs from the environment but would have less impact on higher chlorinated congeners. Tang and Myers achieved a 40% reduction of PCB in dredged sediments (Borja *et al.*, 2005). The effect of plant in action on PCB in the soil according to various studies has been immense, but it is not devoid of demerits. Primarily, due to the fact that plants are autotrophs and not ideally suited for the metabolism and breakdown of organic compounds, therefore the use of plant-based technologies has a number of limitations. One of the major limitations with current phytoremediation is often slow time – scale for remediation to acceptable levels and also toxicity to the plants themselves. Mehmannavaz *et al*, (2002), reported that bioaugmentation of soil increases hardness of the soil in a significant way, and thus may have indirect effect on plants growth. The decrease in plant biomass, which however, causes poor PCB transformation in a PCB-contaminated soil, suggests that PCB and their bacteria products are phytotoxic to plants. This phytotoxicity is due to increased biotransformation, bioavailability or solubility (Mehmannavaz *et al*., 2002). However, the difference in plant growth and PCB depletion in bioaugmented and non-bioaugmented treatments may have been related to both the bacterial augmentation and the soil hardness. The study however, suggested additional studies to confirm these initial findings and to determine the effects of PCB and its product and of inoculums size on the growth of alfalfa in order to optimise phytoremediation of PCBs in the soil (Furukawa and Miyazaki, 1986).

2.14 Difference between PCB metabolism in bacteria and plants

Plants are implicated in the increase of both microbial numbers and activity in the soil, which usually results to a subsequent increase in the biodegradation of PCB (Limbert and Betts, 1996). Nevertheless, endogenous microbes capable of maintaining symbiosis with plant are however attracted to the rhizosphere by plants secretions. Although plants have shown capability of degradation of PCBs, it has rather been slowly achieved in field trials leading to accumulation and volatilization of compounds that are toxic (Aken *et al*., 2010; Anyasi and Atagana, 2011). Metabolism of PCB by plants is represented conceptually by a three way process of activation, conjugation and sequestration (Sandermann, 1994). Generally, the first stage of detoxification of PCBs called activation usually involves oxidation or hydroxylation reaction. It is a high reactive process producing soluble hydroxylated products (Aken *et al*., 2010). Following activation stage is the conjugation reaction involving endogenous hydrophilic molecules including glutathione, glucose or malonate that helps to increase the hydrophobicity of the parent compound (Rezek *et al*., 2007). The final stage of plant's PCB metabolism involves compartmentation of the inactive and conjugated water soluble compounds by exportation from cytosol into vacuole or apoplast of the plants cell (Coleman *et al*., 1997; Mackova *et al*., 2006b; In Rezek *et al.,* 2007). Microorganisms PCB metabolism on the other hand, requires a sequential anaerobic-aerobic processes (Borja *et al.,* 2005). Aerobic degradation is done through the biphenyl pathway and anaerobic is by dechlorination. The flow of the reaction here depends on the degree of chlorination of the PCB congener, the radox conditions, and the type of microbes involved (Mackova *et al.,* 2007). It can be easily deduced that while microbes depends on their sequential reactions which is usually activated by various enzymes to transform PCBs, plants involves direct uptake of PCBs, and subsequently transform the contaminant in a non phytotoxic form (Carman and Crossman, 2001; Mackova *et al*., 2007).

The main product of reaction (metabolites) of bacterial degradation pathway of PCBs as shown in Figure 2.5 above is chlorobenzoic acid while the transformation processes in plant leads to the formation of various hydroxylation products (Figure 2.6). A good example is shown in the use of plant cells in

oxidizing mono- and dichlorinated PCBs into mono- and dihydroxylated biphenyls (Kucerova *et al.*, 2000).

In transformation sequence of PCBs by microorganism, a lot of enzyme activitities is involved ranging from oxygenases, dehydrogenases, dioxygenases and also the conjugate enzymes; transferases. Cytochrome P450 and RBBR oxidase are all implicated in the process. So far little is known about the involvement of enzymes in plants PCB metabolism, but knowledge gained from the breaking down of other nucleophilic xenobiotics suggests that some enzymes may be involved (Chroma *et al.*, 2002b/3; Flocco *et al.*, 2004; Magee *et al.*, 2008).

In general, lower chlorinated congeners of PCBs are metabolized much frequently than the higher chlorinated ones. But the very high chlorinated ones are almost not involved in plant metabolism (Kucerova *et al.*, 2000). This indicates that amongst other factors, the number of chlorine atom, the position of chlorine substitution, and the molecular structure of the congener, all contributes in the metabolism of PCB in plants (Lee and Fletcher, 1992). In the biphenyls pathways of microorganisms, some bacterial cells degrade PCBs with different affinity, resulting in the type of products formed. Therefore microbial degradation of PCBs depends on the following; the strain of the microbes, chlorine substitution pattern on the reacting ring, radox condition, as well as the concentration of the contaminant (Bedard and Haberl, 1990; Kucerova *et al.*, 2000). Moreover, PCB congeners with lesser chlorines per molecule are easily degraded, and the ones with five and more chlorine atoms require anaerobic reductive dechlorination first before their metabolites are mineralized by aerobic microbes (Aken *et al.*, 2010). This means that even the high PCB congeners are likely to be degraded through the microbial process.

Therefore, complementing the shortcomings of each process by the combination of phytoremediation with microbial degradation mechanism will provide an improvement in the biological remediation of PCB (Aken *et al.*, 2010).

2.15 Challenges on the degradation of PCBs

Much work has been directed towards a better alternative technology for PCB destruction in the environment. Incineration although effective tends to be expensive and sometimes produces undesirable end products such as Polychlorinated dibenzo furans/dioxins (PCDF/Ds), which is a result of incomplete combustion of the parent material (Borja *et al*., 2005).

For the past 2 decades, many PCB remediation technologies have been proposed and some are already in use commercially. However, they have not been any of the methods that has gained wide acceptance like the conventional methods. This may be because of the following reasons:

1. None of the alternative technologies have been certified to be applicable to all PCB– contaminated media.

2. There is no certainty on the type of by-products which would be produced from some of the technologies.

3. The necessity of site specificity and treatability studies on most of the technologies.

4. The expensive nature of most of the alternative means has however prevented commercialization of these technologies (Cho *et al*., 2003).

The above- mentioned factors have somewhat posed threats to researchers and government agencies by their effort in trying to come up with an alternative technology than incineration. There was suggestion for an extensive review of the extent of PCB problem of each country for an appropriate technology to suffice (Borja *et al*., 2005). Also the complexity of the microbial processes used to degrade these complex compounds was advised to be considered. All these and some other factors mentioned above gave a need for a more versatile and environmentally friendly method of PCB remediation, a method that can augment the singular actions of microorganisms or plants on PCB contaminated soil. Plant-microbial interaction in contaminated soil and the mutual relationship on the level of growth and support in degradation abilities of microbes were already studied and information regarding their cooperative mechanisms described (Hedge and Fletcher, 1996; Gilbert and Crowley, 1997; Leigh *et al*., 2006; Biancucci *et al*., 2004). There

is still lack of information on the possible combination of the metabolism of bacteria and plants because of the resultant metabolisation of intermediates and the metabolic products formed in primary degradation of PCBs by the two organisms (Mackova *et al*., 2007). The particular interest according to the study was directed towards the ability of plants in a PCB contaminated media, which was transforming PCBs initially, resorting to metabolising chlorobenzoates (bacterial PCB degradation products). Also to find out if rhizosphere-microbe degrading PCBs can transform plants primary metabolites (hydroxychlorobiphenyls) (Furukawa *et al*., 1978; in: Mackova *et al*., 2007). The fear above was diffused from the study of Kucerova *et al*, (1999, 2000 and 2001) and Bock *et al*., (2002), which reported the formation of different hydroxychlorobiphenyls in structure, as intermediates of plants PCB metabolisation. The study of Francova *et al*, (2004), also reported the transformation of commercially available hydroxychlorobiphenyls (found originally as metabolites of single PCB in plants), by bacterial enzymes *in vitro* during isolation from two PCB-degrading bacteria *Burkholderia xenovorans* LB400 and *Comamonas testeroni* B356 (Francova *et al*., 2004). In each step, the products of bacterial PCB pathway were detected after derivatization by GC-MS, and the results confirmed that both enzymes oxygenated hydroxychlorobiphenyls on the non-substituted ring producing three different metabolites of hydroxychlorobiphenyls. Mackova *et al*, (2007), revealed that bacterial enzymes of biphenyl operon, isolated from different bacterial PCB degraders LB400 and B356, can degrade mono-substituted hydroxyl- and hydroxychlorobiphenyls previously identified as products of transformation of plants PCB metabolism (Bock *et al*., 2002; In: Mackova *et al*., 2007). It also reported that certain plant species can degrade some chlorobenzoic acids entering the environment as a result of microbial PCB degradation and other means. This report however, created a further possibility of interactions between bacteria and plants in a PCB contaminated environment; it provided more information on the abilities of biological systems to degrade original xenobiotics as well as some of their intermediates and products (Mackova *et al*., 2007).

2.16 *Chromolaena Odorata*

Chlomolaena odorata (L) R.M. King & H. Robinson (Asteraceae) is an invasive bushy shrub of Neotropical origin; it is one of the world's worst tropical weeds (Holm *et al*., 1979). The plant is a member of the tribe Eupatoreae in the sunflower family Asteraceae. The weed goes by many common names including Siam weed, devil weed, bizattawbizat (Burma), tentrem khet (Cambodia), French weed (Laos), pokpok tjerman (Malaysia), communist weed (West Africa), triffid bush (South Africa), Christmas bush (Caribbean), hagonoy (Philippines), co hoy (Vietnam). In October 2000 '*Chromolaena*' was adopted as the standard common name by the International *Chromolaena* Working Group (ICWG) (Orapa, 2004).

2.16.1 Distribution

The native range of *C. odorata* is in the Americas, extending from Florida (USA) to the Northern Argentina. Away from its native range, *C. odorata* is an important weed in tropical and subtropical areas extending from west, central and southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, southern China, Taiwan, Indonesia, Timor, Papua New Guinea (PNG), and Guam, the Commonwealth of the Northern Mariana Islands (CNMI), Federated States of Micronesia (FSM), and Majuro in the Marshall Islands. The Majuro outbreak is being targeted for eradication. An outbreak found in northern Astralia during the mid 1990s is also being eradicated (McFadyen, 1991). *C. odorata* is supprisingly absent from Vanuatu, Solomon Islands, Fiji Islands, New Caledonia, all Polynesian countries and territories including Hawaii, and New Zealand.

2.16.2 Description, Biology and Ecology

Chromolaena odorata is a much-branched perennial shrub that forms dense impenetrable thickets which displace other vegetation and create fire hazards due to its flammability. It forms dense tangled bushes 1.5-3m height in open conditions (Orapa, 2004), and occasionally reaching 6-10 m by scrambling up other taller vegetation. The stems are circular, hairy or almost smooth and much branched. The leaves are

opposite, triangular shaped, young ones slightly reddish purple, have toothed margins, with three main veins and give off a pungent odor when crushed. The flowers are pale blue to white. Seeds are borne in the composite flower heads. The individual seed is about 5 mm long, with a pappus angled hooks to aid seed dispersal. *C. odorata* favours a wet dry seasonal climate, grows well in well-drained open areas and can tolerate all soil types and altitudes up to 1200 m above sea level (Zachariades *et al.*, 1999). It flowers once a year, May to August south of the Equator and October to April north of the Equator. It also produces massive amount of seeds: 93,000 to 1, 600, 000 viable seeds per plant (Blackmore, 1998). The seeds germinate during the rainy seasons. The lightweight parachutal structure of mature seeds can be windblown and spread over short distance. It can spread over long distances by attaching to clothing, vehicles, road works and farm machinery, seed contaminants, etc. seed longevity can be up to 4 years (Zachariades *et al.*, 1999, 2011). Under favourable conditions, single seeds can quickly give rise to infestation, which may spread further and become difficult to manage if unnoticed. Once established, *C. odorata* is difficult to eradicate because of the large number of seed, rootstocks that regrows, and also the difficulty of finding isolated plants, some of which can grow in inaccessible places like steep crevices (Blackmore, 1998; Orapa, 2004).

2.16.3 Significance

In its native range, *C. odorata* is not a weed so no control is required (McFadyen, 1991). In contrast, it is a serious weed in many of the countries where it has been introduced: Africa, South and Southeast Asia. It is increasingly becoming important in the western Pacific region. It has the potential to expand its range further into the small central and southern Pacific countries and territories if not prevented. *Chromolaena odorata* can grow rapidly and form infestations that can affect agriculture, pastures and biodiversity, as it interfares with the functions of natural ecosystems (Zachariades *et al.*, 1999). It can be very invasive, forming impenetrable thickets in open areas such as pastures and around villages and settlements, along roadsides, fallow areas, and allelopathy (releasing growth inhibitors). *Chromolaena odorata* leaves especially the young ones, are toxic due to high levels of nitrate. Thus grazing animals avoid it: if forced

to feed, animals can develop liver sclerosis and may even die. In seasonal dry areas, *C. odorata* can fuel hot bushfires after it dies back following flowering and seeding. This ultimately leads to death of other native flora and fauna. Homes and other property close to infestations can be at risk from such fires. *C. odorata* can also harbor pests such as locusts, rats, wild pigs and crop disease. In West Africa, the spread and increase of *C. odorata* resulted in the increase of the pest grasshoppers and their eggs from other natural predators and parasitoids (Boppre, 1991).

At present *C. odorata* forms two distinct centers of invasion in Africa: one is in Southern Africa, moving south and East and the other is in West Africa (McFadyen and Skarratt, 1996). It also invades most areas in the humid paleotropics and subtropics (India, South East Asia, Indinesia, Philippines, Papua New Guinea, parts of Oceania), and is predicted to spread further (McFadyen and Skarratt, 1996). Long distance dispersal in the bodywork of long distance vehicles has been reported in Australia (Vanderwoude *et al*., 2005). In all areas it impacts seriously on biodiversity and agriculture, due to its shrubby nature and its ability to re-sprout after cutting, this plant is difficult to control both chemically and mechanically. *C. odorata* thrives well during humid weather (wet-dry seasonal climate), therefore requires a neutral soil since it always tends towards alkalinity. Bright sunlight, higher soil moisture, relative humidity and low temperature also favors vigorous growth of the plant.

2.16.4 Management

The ideal strategy here is prevention. Control of *C. odorata* could either be through mechanical and cultural means, chemically or through biological means. Meanwhile it is generally recognized that biocontrol remains the most effective way to bringing *C. odorata* to manageable levels (Goodall and Erasmus, 1996; Zachariades *et al*., 2011).

2.16.5 *Chromolaena odorata* potentials in phyotoremediation

Chromolaena odorata is a perfect competitor; this means that it scavenges for available nutrients in the soil, as a result suppresses the growth of other plants even the weeds of its category. Areas invaded by the

plant are usually found to have twice the plant biomass of non-invaded areas. It is also known to contribute to increased litter (Nitrogen (N) and Potasium (K) inputs (Yonghachea *et al*., 2005). There are reports of association of *C. odorata* with presence of high soil fertility in Cameroon. Just as famers in Ghana consider both *C. odorata* and earthworm casts as indicating good soils and mechanistically link these by stating that the weed provides litter input, shade, and a moist environment which promotes earthworm activity (Adjei-Nsiah *et al*., 2004). This was complemented by the report that weed biomass dominated by *C. odorata* provided about 76% variation in earthworm casts aroung cropped fields (Norgrove *et al*., 2003). Farmers in Cote d'Ivoire reported that *C. odorata* helps to prevent the establishment of *Imperata cylindrica* (De Rouw, 1991). In South Africa, *C. odorata* is commonly known as triffid weed, *Chromolaena*, 'paraffienbos' (Afrikaans) and 'isandanezwa' (isiZulu) (Kluge, 1990; Goodall and Erasmus, 1996). It is regarded as the worst alien invasive plant species in the subtropical regions of South Africa (Zachariades *et al*., 1999; Zachariades and Goodall, 2002), and the plant poses the greatest threat of any invasive weed to the biodiversity of the KwaZulu-Natal province (where a good number of it can be found) in South Africa (Liggitt, 1983). In 1995, Working for Water (WfW) an agency from the Department of Water Affairs (DWAF) was created to work towards the control of the supposed effect of Invasive Alien Plants (IAP) which includes *C. odorata* on water supply. In about 16 years of its operation, with R4 billion of tax payers fund in expence trying to control IAP, *C. odorata* is still prominent and continue to expand (Moraise *et al*., 2009; van Wilgen *et al*., 2001, 2004, 2011). There is however a need to channel part of such a huge budget for other 'working for' projects for example Working for Energy (WfE) and Working for Health (WfH), as massive biomass generated by IAP could be harnessed towards energy generation and job creation (Preston, 2011). At same time IAP; *C. odorata* for example has been implicated in traditional medicine practices, in the removal of organics and inorganics from the environment among other things (Irobi, 1992; Singh *et al*., 2009; Atagana, 2011a/b, Tanhan *et al*., 2011).

It was stated in the previous chapter that plants should be considered for phytoremediation studies if it possess a number of growth characteristics listed out thus:

a. such plants should possess high germination rate

b. have ability to be propagated vegetatively

c. should be able to accommodate any soil condition and grow in it

d. the plant should be aggressively invasive

e. have the ability of surviving under stress conditions and at low nutrient availability

f. should be able to accumulate high biomass as well as the ability to dominate native vegetation in any new environment

g. ability to concentrated its absorbed compounds in the shoot

h. could illicit pungent odour to livestock and perhaps be a perennial plant (Singh *et al*., 2009; Atagana, 2011; Tanhan, 2011).

Having these factors in mind amongst others possessed by any phytoremediation plant which is equally possessed by *C. odorata*, it is evident therefore that these could be an added advantage. Weed control, nutrient availability and organic matter presence verifications in phytoremediation systems would be eliminated and cost of carrying them out channeled to other activities. These therefore present *Chromolaena odorata* as a potential phytoremediation plant for possible PCB remediation.

CHAPTER THREE

Material and methods

3.1 Soil

Soil samples were collected from a depth of up to 30 cm, one from the main campus of University of South Africa, Pretoria, refered to as (S_1) and the other from a construction site also at the University of South Africa, Pretoria also refered to as (S_2). The reason for the use of two soil samples was to compare the measured parameters within the two soil types. The soil samples were homogenized by mixing with hand to remove pebbles, stones and gravels and, air dried, kept in cellophane bags and stored at 4^0 C before use. Sub-samples of the soil (250 g) each were taken from the two soil types and used for soil characterization at the laboratory. Composite samples from the stored soil were separated as the cultured soil sample. Table 3.1 below present the characteristics of the soil used.

3.1.1 Characterization of soil samples used in the greenhouse experiment

Characterization of soil was done to provide an indication of mineral composition of the soil as well as checking for the presence of PCB in the soil. This will provide information on the conditions of the soil under which the plant will grow. Analysis of soil samples for total PCB was done at a private laboratory in Pretoria (Water Laboratories, Techno Park Meiring Naude Road, Pretoria). Furthermore analysis for metal was done using Inductively Coupled Plasma Spectrometry (ICP)- Perkin Elmer Optima 4300 (Uberlingen, Germany), equipped with Quartz touch, nickel sampler and skimmer cones, a peristaltic pump maintaining a 1ml min^{-1} sample uptake rate), a cross flow type pneumatic nebulizer and a double pass scott-type spray chamber. Other operating condition is summarized in Table 3.2.

Table 3.1: Properties of two soil samples used in the experiment.

Factor	Unit of measurement	Value		
		Soil 1	Soil 2	Horse manure
Clay	% wt	72.0	19.5	
Silt	% wt	18.5	15.0	
Sand	% wt	9.5	65.5	
Texture		Clay	Sandy loam	
pH		6.7	7.8	
Total organic carbon	% wt	7.0	0.5	52.7
Total Nitrogen (N)	% wt	0.03	0.02	81.0
Total Phosphorus (P)	ppm	9.0	4.2	50.0
Potassium (K)	ppm	15.5	2.9	
Calcium (Ca)	ppm	83.0	61.0	
Chromium (Cr)	ppm	79.0	5.8	
Lead (Pb)	ppm	46.0	-1.2	
Nickel (Ni)	ppm	10.0	0.4	
Copper (Cu)	ppm	35.0	0.5	
Zinc (Zn)	ppm	20.0	3.2	
Manganese (Mn)	ppm	76.0	10.0	
Iron (Fe)	ppm	58.5	77.7	

Cobalt (Co)	ppm	1.7	3.9
Magnesium (Mg)	ppm	1.2	8.8
Moisture content of soil	%	4.8	6.6
Thermal conductivity	$Wm^{-1}k^{-1}$	0.2	0.4
Soil density	$g\ cm^{-3}$	1.25	1.33
Total PCB	ppm	ND	ND

Values are means of three replicates. %wt=percentage weight, ppm=part per million, $Wm^{-2}k^{-1}$=watts per meter Kelvin, $g\ cm^{-3}$=gram per cubic centimeter, ND=not detected

Table 3.2: ICP-operating conditions

RF power (W)	1000
Plasma argon ($Lmin^{-1}$)	600
Plasma nitrogen ($Lmin^{-1}$)	400
Nebulizer flow ($Lmin^{-1}$)	0.9 1
Nebulizer	Cross-flow
Data acquisition	Peak hop transit
Resolution	Normal
Delay time (mins)	30
Number of replicates	3
Standards (ppm)	8,4,2,1

The method adopted was that formulated by the International Organization for Standardization (ISO 11466) known as Aqua regia involving leaching out of the metals from the soil with 3:1 ratio of HCl and HNO_3 and analyzing the metals with ICP.

3.2 Plants

Chromolaena odorata plants were collected from the Botanical gardens of the Department of Botany University of KwaZulu-Natal Pietermaritzburg and propagated by stem cuttings in the greenhouse at the University of South Africa. Soil samples were mixed with animal manure that was obtained from the

Department of Veterinary Science, University of Pretoria, Onderstepoort, at the ratio of 1:9 manure to soil. The carbon, nitrogen and phosphates values (CNP) of the animal manure were equally analyzed at Water Laboratories (see Table 3.1 above). The plant cuttings were planted into the prepared soil bed employing the method described by Clark *et al*. (1999). Plants rooting hormone "Indole Butyric Acid" IBA, supplied by Plantland Malanseuns in Roslyn was applied, this was to aid rooting of the cuttings. The plants in the soil bed were allowed to grow for three months and were then used for subsequent propagation and experimentation (see Picture 3.1 below). The bed was watered manually using watering can to maintain 70% moisture at field capacity.
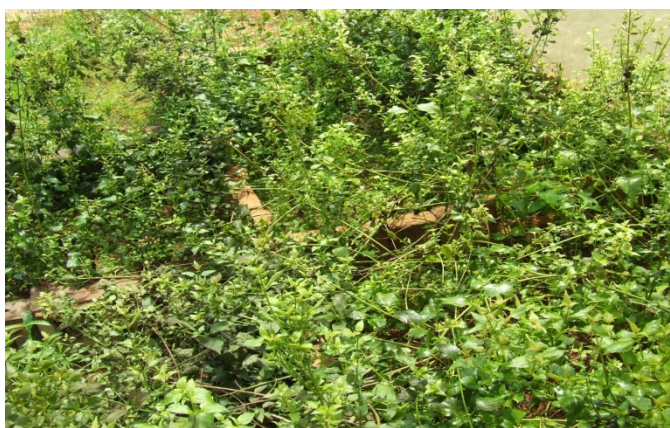
Picture 3.1: Cross section of *C. odorata* beds in the greenhouse

3.3 Chemicals and reagents

All chemical reagents used in this experiment were of analytical grade

Working standard solution was prepared from the surrogate standard using hexane fraction. Calculation of the required concentrations was based on the chemical formula:

$$C_1 V_1 = C_2 V_2$$

Where

C_1 = Concentration of stock solution

C_2 = Concentration to be made

V_1 = Volume to be determined

V_2 = Volume required

3.3.1 Chemicals used in Aqua regia digestion of soil samples

The chemicals employed in this experiment were:

- Nitric acid (98% pure) supplied by Merck chemicals

- Hydrochloric acid (98% pure) supplied by Saarchem (from Merck chemicals)

- Deionized water prepared from Milli-Q instrument (Millipore, Bedford USA) was used in making up the digested samples.

3.3.2 Chemicals used in Soxhlet extraction

The following chemicals were employed in soxhlet extraction:

- Hexane fraction supplied by Merck chemicals

- Acetone (99.5% pure) supplied by Merck chemicals

- Anhydrous sodium sulphate supplied by Merck

- Silica gel (70-230 mesh), supplied by Merck.

3.4 Green house experiment

There were four set of treatments in randomized block design adopted from Dzantor and Woolston, (2001), this design was amended to meet the scope of the experiment as described in 3.1.4.3 below.

3.4.1 Treatments

The following treatments were used in the experiment:

1. T/O amended soil taken as direct contamination (T/O $_D$): plant transplanted directly into contaminated soil;

2. T/O amended soil taken as Suzuki method (T/O $_S$): only the plants root protruded from the holes in the cellophane bag that was grown into the contaminated soil; sprout culture method (Suzuki *et al*., 1977); the implication of this amendment being that the contaminant can not access the entire plant but just the root.

3. Aroclor 1254 amended soil (T$_1$) and

4. Aroclor 1260 amended soil (T$_2$).

The Aroclor samples were made in composites of 100, 200 and 500 ppm concentration of the different contaminants, while the T/O amended samples co-contaminated with 100 ppm of Aroclor were made in composites of 100, 200 and 500 ppm soil. Each sample was duplicated according to the two soil samples. Equal set up as done in the treatments without T/O and or Aroclor amendments were made as controls viz:

3.4.2 Control samples

1. Amended soil planted without contamination to test the toxicity of the contaminants on plants (C1);

2. Amended soil contaminated without plants to test for other possible measures of dissipation of the contaminants (C2);

3. Amended soil contaminated, planted but covered with laboratory parafilm to study the effect of volatilization (C3);

4. Amended soil contaminated, planted but inhibited with 10 % sodium hypochlorite (local bleach) to study the effects of microbes on contaminant remediation (C4), (the soil microbial inhibitor was used as a source of water).

3.4.3 Experimental design

Ninety six (96) set of PVC cups of 2 L volume were used for the experiment each filled with 1 kg of soil. The cups were devided into two sets (48 each for the two soil samples), each section were further divided into two other sets (24 each for the two pollutants: Aroclor and T/O). The division was sub-divided into two more sets (12 each for the treatment and control section), which was sub-sub-divided into four sets (3 replicate each for the section according to Section 3.4.1: T/O $_D$, T/O $_S$, T_1 and T_2). Thus, one (1) *C. odorata* plant was tested in two (2) pollutants among two (2) soil types made into eight (8) divisions replicated into three(3). Thirty (30) sets of cellophane bags of 2 L volume were used for sprout culture method in T/O treatment. The cellophane bags were equally divided according to the design of the experiment: one plant each for two soil types at five sections replicated into three.

Five weeks old *C. odorata* plants were used in this study; it was planted into contaminated soil according to the treatment and was monitored for six weeks. In T/O direct treatments, plants were directly transplanted into the T/O $_D$ treated soil samples. In Suzuki (sprout culture) method however, plants were transplanted in a cultured soil which is contained in cellophane bags with holes at the bottom for protrusion of the roots. The bags containing the plants with protruded roots were placed on T/O $_S$ treated soil contained in the PVC cups. This adopted and amended Suzuki method was designed to avoid the toxic effects of T/O on plants which posed a problem during the preliminary stage of the study. With Aroclor treated soil samples (T_1 and T_2), C. *odorata* plants were introduced directly into the contaminated soil. The initial plant length, root langth and number of mature leaves per plant (MLPP) were noted: *C. odorata* grown for six weeks in Aroclor and Transformer oil treated soil was measured from the base of the root to the apex of the stem on the day of transplanting (before) and on the day of

harvest (final). The experiment was monitored for six weeks at prevailing environmental conditions (temperature, pressure and wind), watered to maintain moisture at 75% field capacity (Atagana, 2011) with manual watering can. Effort was made at ensuring that watering was done in such a way as to only wet the soil at any point in time avoiding much run off. The air inlets and outlet of the greenhouse were left open at intervals to allow for air flow and weeds were removed manually. Measurements were made at weekly intervals for the plants length (using string and tapes), MLPP (using manual counting), leaf colour (using eye observation) at different treatments and the root length which was only measured on the day of harvest using string and tapes. The choice of the parameters to be measured came from the fact that "plants when growing in a conducive environment show by an increase in size which will be complimented by development of new leaves as well as root elongation bringing about a great pull of nutrient from the soil in a mixture of water and other solvents". There was no application of inorganic manures to the soil mixes, but organic animal compost was used during the preparation of the soil at the ratio of 1:9 manure to soil. In order to avoid cross-contamination of the samples, each treatment was separated from each other though within the greenhouse. The control samples were also separated especially those that were not contaminated and those that were covered to avoid volatilization, untreated controls were kept at a distance from the contaminated samples.

3.5 Sampling

During the course of the experiment that lasted for six weeks, measurements and observation were taken at weekly interval in order to note the variations of growth parameters of the weed amongst different treatment in the entire experiment including the root length taken on the day of harvest. After six weeks of growth of the plant in the contaminated and control set up, the soil and plants were sampled. The plants were carefully removed from the PVC cups after loosening the soil around the cup using a kitchen knife; the roots were separated from the soil by shaking off the soil leaving only the rhizosphere soil. During this process, the entire plants were washed using running tape water, rinsed with distilled water and allowed to air dry, it was weighed afterwards to get the wet biomass. Root lengths which were initially

measured before contamination to get the initial measurement was also measure to get the final measurement. The plants were then separated into shoots and roots. Also separated were the shoot which was divided into leaves and stem, and the entire samples weighed using Mettler Toledo balance model PB1502 (MICROSEP, Switzerland) with maximum capacity of 1510 g. The soil samples were carefully collected also, homogenized and divided into sets together with the plants samples in preparation for subsequent extraction and analysis. Plants were harvested with a kitchen knife rinsed with acetone between uses to minimize cross contamination. Harvested and prepared plant samples were kept in Whirlpak[TM] bags (NASCO, South Africa) in the refrigerator until time for analysis. However any plant matter that was not collected for analysis was left in the green house in airtight containers for later use and appropriate disposal.

3.6 Determination of PCB concentration in soil and plant tissues after six weeks of treatment with PCB and T/O

All glass wares were washed with liquid detergent, rinsed with water and then soaked in Dichloromethane (DCM) over night. They were then rinsed with water, followed with distilled water and finally with acetone to remove any adhering organic substances (Perrin and Armarego, 1981; Winslow and Gestner, 1978). Soil and plant samples were thoroughly homogenized for analysis and sub-sampled for the determination of wet and dry weight ratio. The samples for biomass determination were dried at 50^0 C for 3 days, weighed at intervals until constant mass using Labcon industrial oven (Labcon, South Africa) with heating integration of $40\text{-}100^0$ C and were measured to obtain the dry mass. The dried plant samples were then ground using commercial blender, sieved at 2 mm and were stored prior to extraction while the soil samples were ground using a commercial mortar and was sieved at 2 mm. The extraction process adopted was 'Method 3540 Soxhlet Extraction' (Erickson, 1997) which was formally used by Chen *et al*, (2010). 5 g of 2 mm sieved dry soil as well as 5 g of 2 mm sieved plant samples were extracted using soxhlet apparatus for 4 hrs at 4-6 cycles per hour with 150 ml mixture of hexane-acetone (1:1, v/v), after which the extracted solution was concentrated to 2 ml in rotary evaporator (Buchi Rota vapor[TM] Japan model R-

200 with heating bath B-490 and heating intensity of 20-180^0 C). USEPA Method 3630B: Silica Gel Cleanup was used as this method has been shown to specifically address Aroclors (Erickson, 1997).The extract from soxhlet extraction was diluted with hexane to a volume of 10 ml and passed from a glass chromatographic column (i.d 20 mm and 400 mm height) parked with layers of silica gel and anhydrous sodium sulphate and then eluted with 100 ml of hexane. The eluent was finally concentrated with rotary evaporator for the second time to about 1ml and was analyzed using GC-MS.

3.7 Analysis and quantification of extracts from soil and plant samples for total PCBs

The method adopted here was the USEPA modified 8089/8081 method for the determination of total PCB. The analysis was conducted using Agilient 7890 GC equipped with 5975 Mass Spectometry and auto injector, an SupelcoWAX SPB^{TM}-1 (30 m x 0.25 mm x 0.25μm) column was used with N$_2$ as the carrier gas. Standard PCBs were graded Aroclor 1254 and 1260 in hexane in standard concentration of 1000 ppm (Sigma Aldriech Germany). This instrument works on the principle thus: a small amount of liquid extract injected into the instrument is volatilized at the hot injection chamber. The volatilized molecules are then swept by a stream of inert carrier gas by the help of a heated column that houses the stationary phase; a high boiling liquid. As the mixture moves along this column, its components bombard each other at different rates between the gas phase, dissolved in the high boiling liquid and is then separated into pure components. Just before the compounds leave the instrument; it passes through a detector which sends an electronic signal to the recorder which responds by peak formations. The peaks formed are therefore quantified by mass selective detector using the retention time of the relative compounds registered from a known standard. PCB congeners are identified by retention time matching to standards concentration. Prior to analysis of samples, recovery test was carried out using the standard aroclor samples to ascertain the linearity of the response. One micro litre of the sample extract was injected into the GC. Injector and detector chamber temperatures were 260^0 C and 300°C, respectively. The oven temperature was initially set at 180^0 C for 0.5 mins, ramped at 30^0 C per mins to 260^0 C, it was held for 18 mins then 15^0 C per mins to 270^0 C and held for 25 mins. PCB congeners were identified by

retention time matching to the surrogate standards which was prepared using the two commercial stock samples of PCB inform of Aroclor 1254 and 1260 prepared in concentrations of 1, 5, 10, 20, and 50 ppm in hexane. The value of the chromatogram was quantified using peak area integration.

3.8 Statistical analysis

Values from the analyses of samples were the mean values of three replicates. General linear model of analysis of variance (ANOVA) was used. Data were analyzed using ANOVA adopting the method described by Gomez and Gomez (1984), to describe the percentage of PCB removal from the soil and the mean values were compared using least significant difference calculated at $p \leq 0.05$ significant level. Superior Performance Software System (SPSS) (version 11.0 for windows) package was used.

<center>**CHAPTER FOUR**</center>

Results

4.1 Measurement of growth of *C. odorata* in Aroclor and T/O treated soil.

The results presented below were described according to the listed objectives and the methodologies employed.

4.1.1 Length of *C. odorata* in different concentrations of Aroclor and T/O treatments after six weeks of growth.

Lengths of plants used in every treatment were not uniform but the increase in length was deduced from the difference between the initial and the final length.

4.1.1.1 Length of *C. odorata* at different concentrations of Aroclor and T/O in S_1

Length of plants in S_1 at different treatment concentrations is presented below.

4.1.1.1.1 Length of *C. odorata* in S_1 at 100 ppm of Aroclor and T/O

The length of plant in S_1 at 100 ppm of Aroclor and T/O is presented in Figure 4.1.1 below.
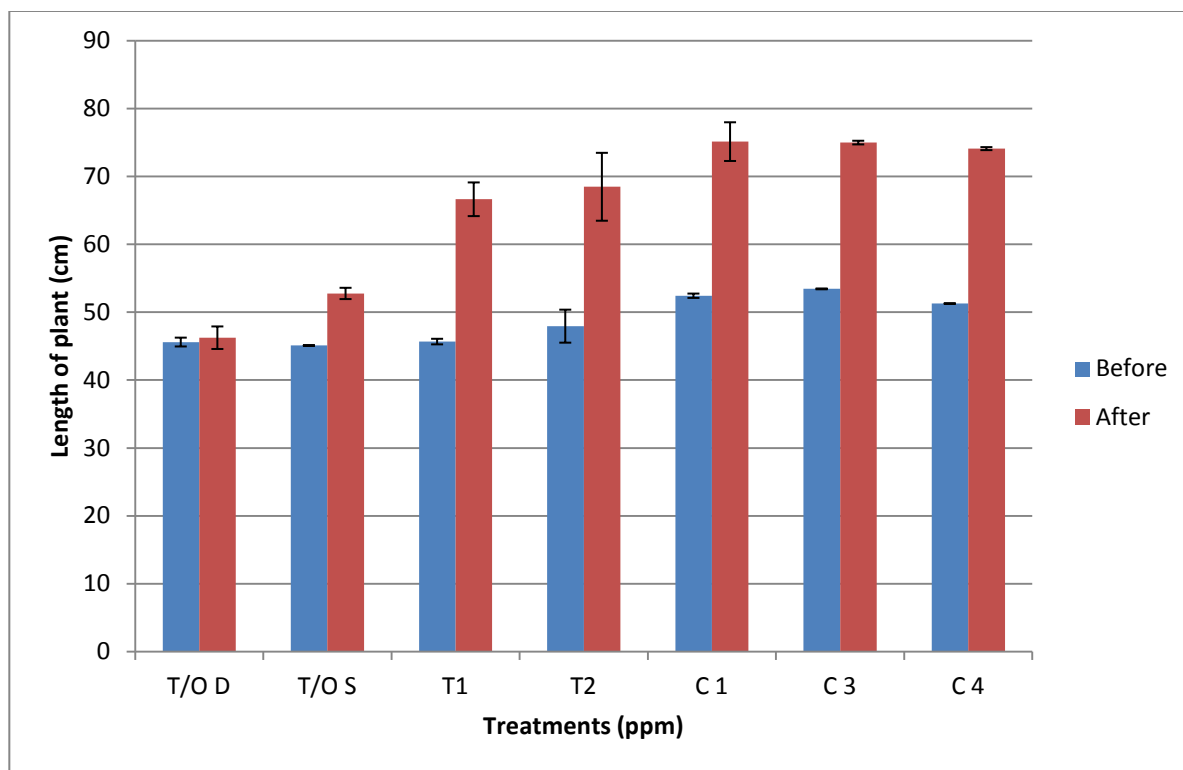
Figure 4.1.1: Length of plant at 100 ppm of Aroclor and T/O treatments in soil 1(Error bars indicate standard error of the mean), Before=Initial length, After=final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=control 1, C3=control 3, C4=control 4

From Figure 4.1.1 above, length of plant was highest at C1 (75.12 cm), this followed a tremendous increase from the initial plant's length hence achieving a growth length above 22 cm. C3 and C4 were equally of high values (74.98 and 74.09 cm) respectively and followed closely by T_1 and T_2 with lengths of 66.50 and 68.27cm respectively . Length of plant was however least at T/O $_D$ with value of about 46.24 cm which was not significant with that of T/O $_S$ (52.76 cm) (P = 0.14; LSD = 17.93). This evidently showed that Aroclor amended soil had almost equal growth parameters with untreated control. But T/O treatments were significantly different from Aroclor amended samples (P = 0.001; LSD = 9.14) as well as the untreated control (P = 0.0001; LSD = 4.88).

Bioremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

4.1.1.1.2 Length of *C. odorata* in S_1 at 200 ppm of Aroclor and T/O

The length of plant in soil sample 1 at 200 ppm of Aroclor and Transformer oil is presented in Figure 4.1.2 below.
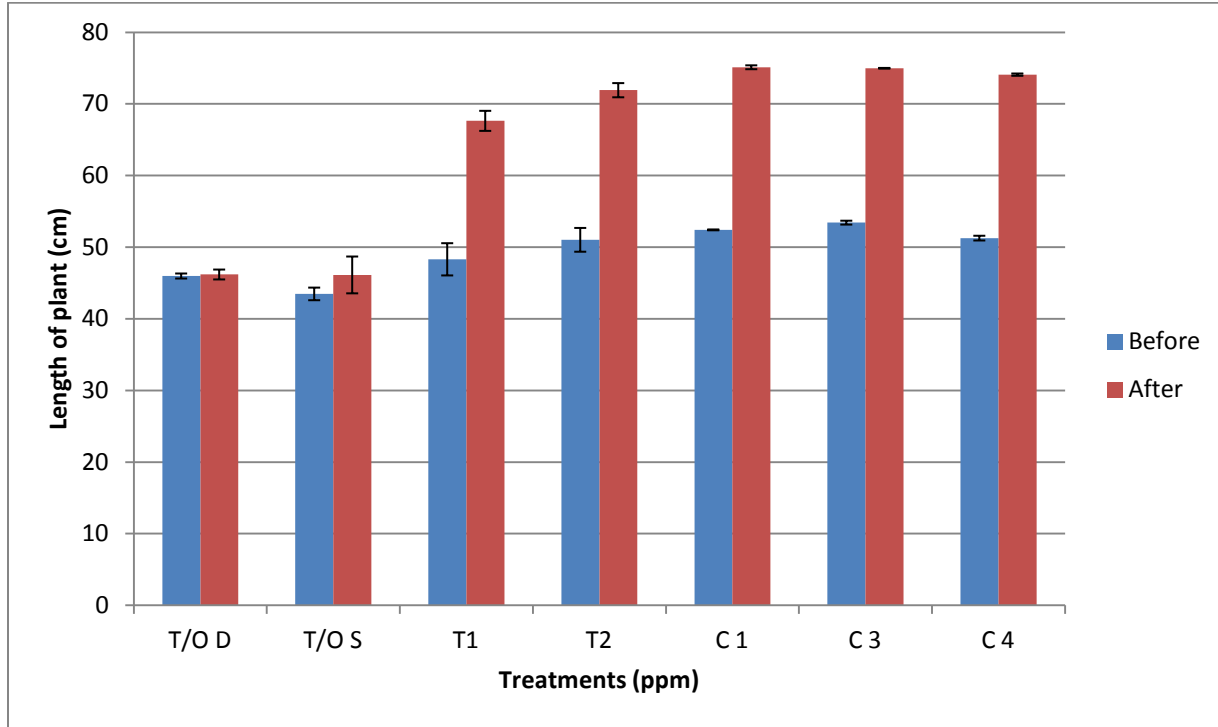
Figure 4.1.2: Length of plant at 200 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.1.2 above, plant length was highest in control 1 (73.60 cm), a reduced shift from the length at 100 ppm treatment, this resulted to about 42 % increase from the initial plants length of about 52.0 cm. It is followed by control 3 and 4 with increases of 73.09 and 72.49 cm respectively. Plant length was lowest at T/O $_S$ with 46.13 cm and 46.19 cm in T/O $_D$ respectively but growth was higher in T/O $_S$ as observed with its high percentage growth rate other than T/O $_D$. Therefore same growth trends as recorded in 100 ppm treatment were observed at 200 ppm.

4.1.1.1.3 Length of *C. odorata* in S_1 at 500 ppm of Aroclor of T/O

The length of plants in S_1 at 500 ppm of Aroclor and Transformer oil is presented in Figure 4.1.3 below.

Bioremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

Figure 4.1.3: Length of plant at 500 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.1.3 above, C1 made the highest increase in plant length with 72.88 cm followed by C3 and C4 with 71.98 and 71.38 cm respectively. The initial length of plant was about 52.0 cm hence had a percentage increase in plant length higher than what was observed in other treatments. The length of plant was also least in T/O $_D$ and T/O $_S$ with 42.40 and 45.12 cm respectively being their final length. According to the growth measured values, it could simply be explained that negative response was observed between Aroclor and T/O concentration and height gained in *C. odorata;* however such interaction was much pronounced in T/O treated samples which recorded a reduction of about 0.8% in length.

4.1.1.2 Length of *C. odorata* at different concentrations of Aroclor and T/O in S_2

The length of plant at S_2 between treatment concentrations of Aroclor and T/O is presented below.

4.1.1.2.1 Length of *C. odorata* in S_2 at 100 ppm of Aroclor and T/O

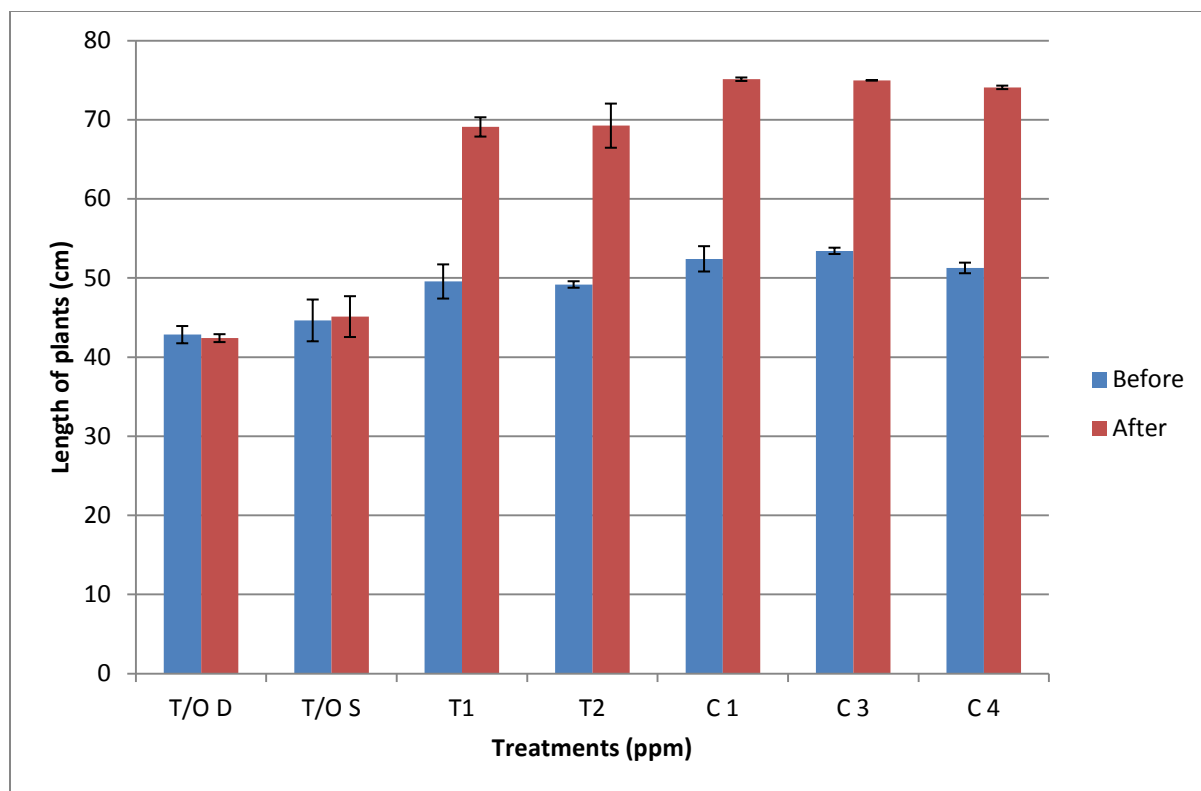The length of plants at 100 ppm of Aroclor and 100 T/O in S_2 is presented in Figure 4.2.1 below.
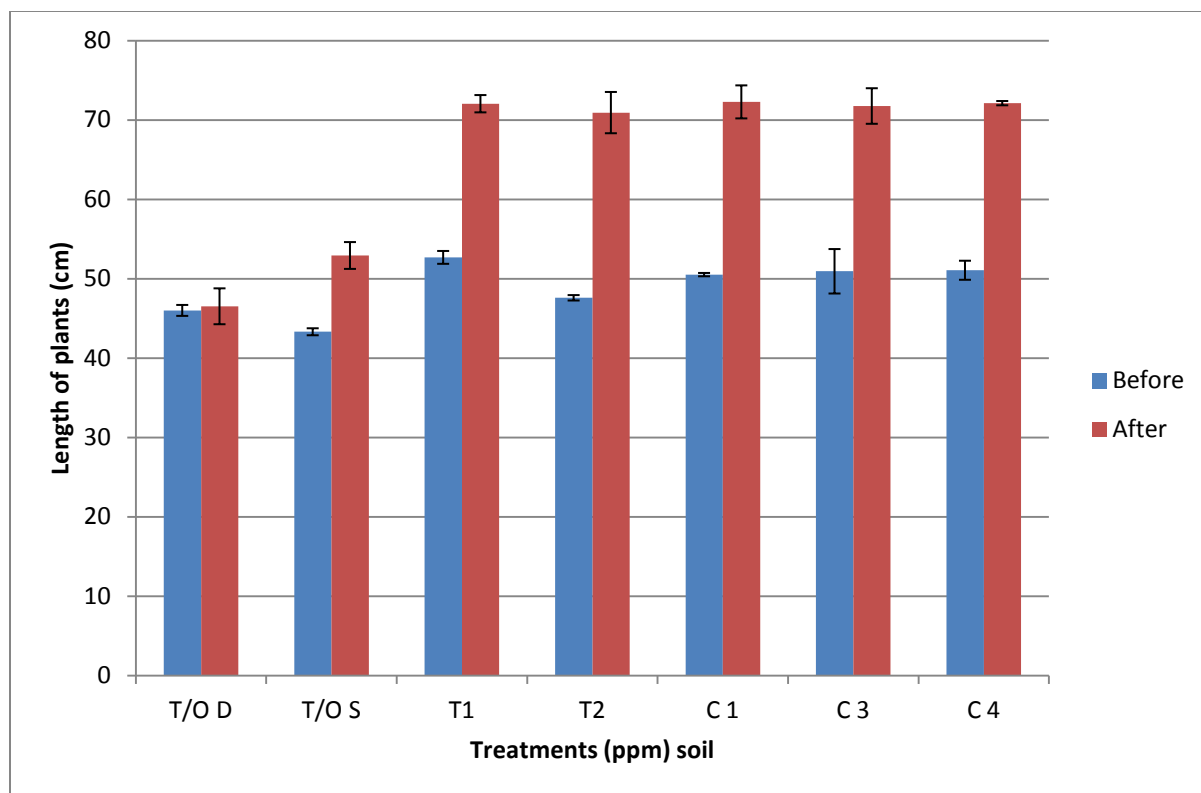
Figure 4.2.1: Length of plant at 100 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.2.1 above, the length of plants was highest in C1 with final plant length of 73.28 cm, followed by control 4 with value of 72.12 cm. Final Length of plant was low in T/O $_D$ treatment with value of 46.53 cm an increase from the initial of about 1 %. This is followed by T/O $_S$ with value 52.93 cm. Values of T_1, T_2, C1, C3 and C4 were not significant from each other (P = > 0.05). This then means that the same growth parameters measure at equal concentration of Aroclor and T/O in S_1 is observed in S_2.

4.1.1.2.2 Length of *C. odorata* in S_2 at 200 ppm of Aroclor and T/O

The length of plants at 200 ppm of Aroclor and T/O in S_2 is presented in Figure 4.2.2 below.

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Figure 4.2.2: Length of plant at 200 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.2.2 above, controls 1, 2 and 3 were highest in its final length in range (73.46, 73.20, and 72.46 cm) respectively and the range of the values were lower than that obtained in 100 ppm. The lowest values of final length were measured in T/O $_D$ and T/O $_S$ (43.80 and 52.70 cm) respectively. The values in T/O $_D$ and T/O $_S$ were not significant from each other ($P > 0.05$) but significantly different from the values of T_1 and T_2 ($P = 0.03$; LSD = 15.31). This followed thesame trend of increase in plant length as observed in S_1.

4.1.1.2.3 Length of *C. odorata* in S_2 at 500 ppm of Aroclor and T/O

The length of plants at 500 ppm of Aroclor and T/O in S_2 is presented in Figure 4.2.3 below.
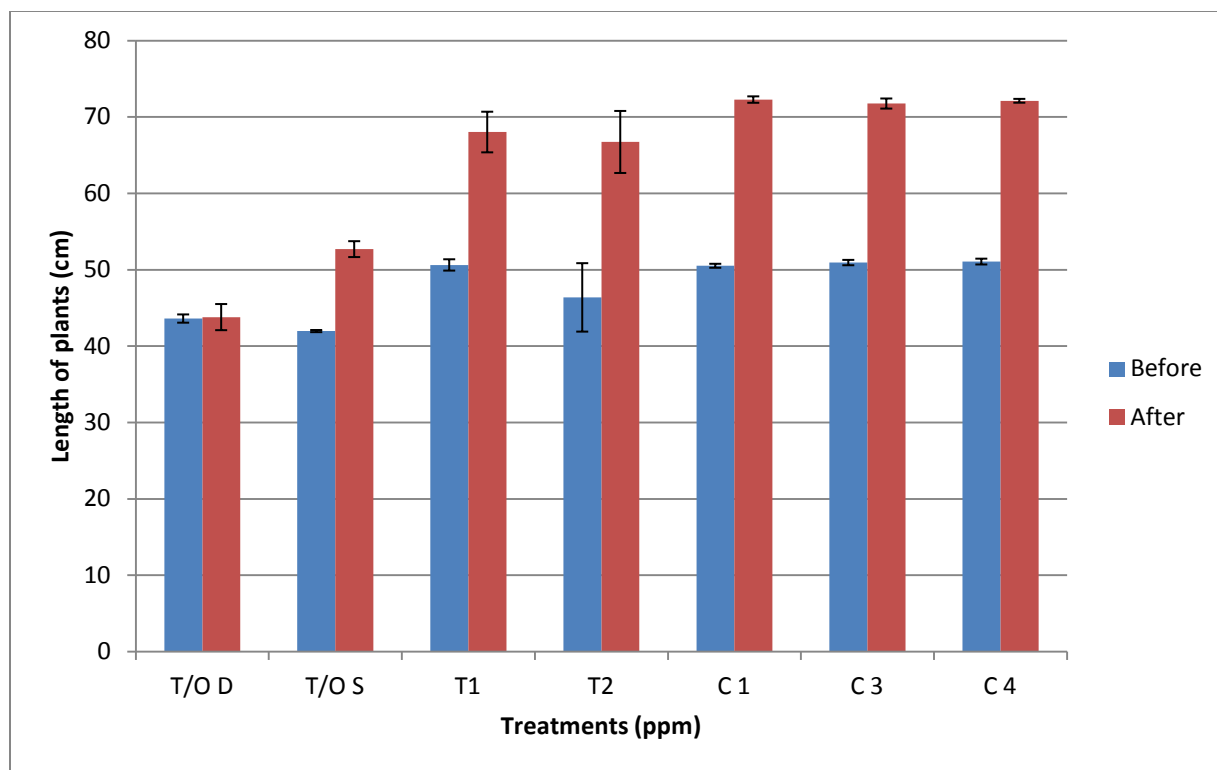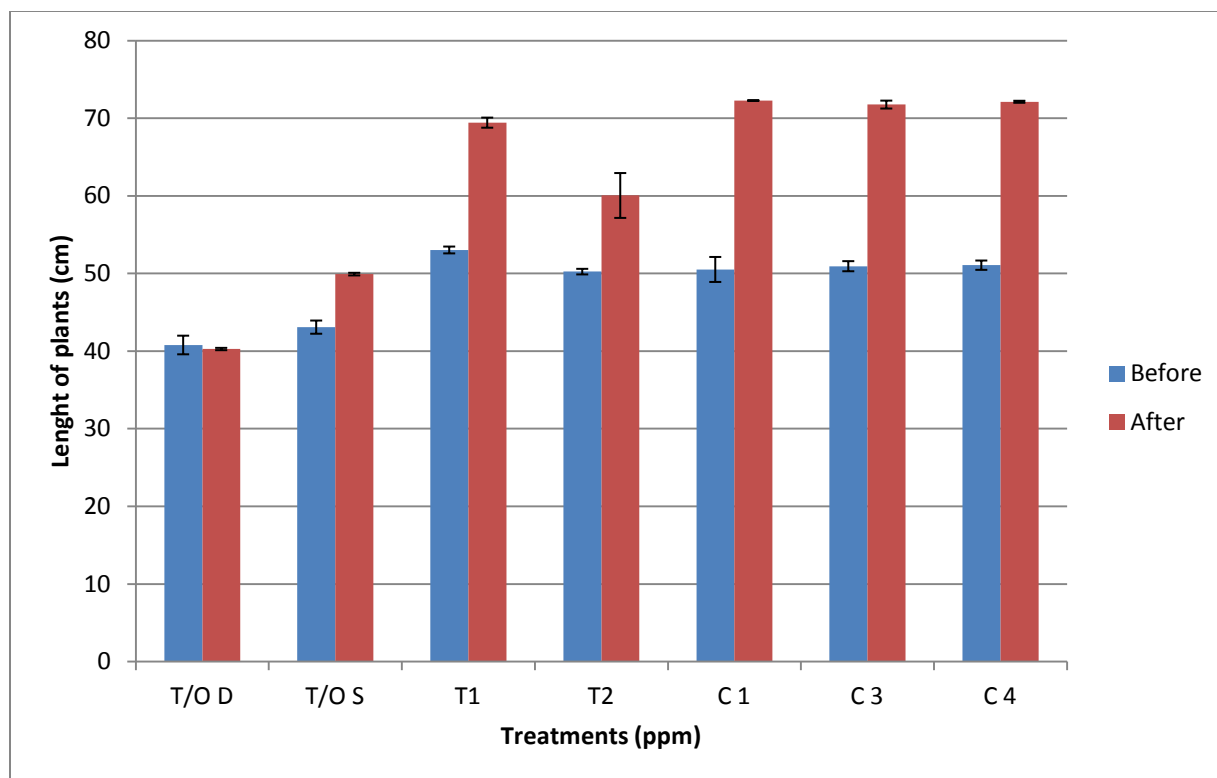
Figure 4.2.3: Length of plant at 500 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Iinal length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.2.3 above, C1, C2 and C3 maintains the highest values in final plant length among the 500 ppm treatments (65.83, 64.53, 62.65 cm) respectively. Lowest value in final plant length were recorded in T/O $_D$ and T/O $_S$ treatments (40.27 and 43.92 cm) respectively. However, in the T/O $_D$ treatment, percentage change in growth of the plant was less than zero an indication of reduction in growth, this is evidenced by the lower length of the final from the initial plant length. Equall growth parameters were recorded in the two soil samples as their values were not significant at p= 0.05. It is evident that the negative impact of the treatments to the plants was much obvious in transformer oil treated samples than in Aroclor treatments but the 500 ppm treatments impacted slowly.

4.1.2 Growth rate of *C. odorata* for six weeks at different concentrations of Aroclor and T/O treated soil

4.1.2.1 Percentage growth rate of *C. odorata* at different concentrations of Aroclor and T/O treated S_1

Measurement of growth which was calculated from the percentage increase in length of the plants in different treatments of S_1 at weekly intervals is presented in Table 4.1.1 below.

Table 4.1.1: Percentage growth rate of *C. odorata* at different concentrations of Aroclor and T/O in S_1.

Treatments (ppm)	Percentage growth in S_1		
	100 ppm	**200 ppm**	**500 ppm**
T/O $_D$	1.40^a	0.46^a	-1.03^a
T/O $_S$	17.01^b	6.09^b	1.08^a
T_1	45.89^c	40.01^c	39.41^b
T_2	42.82^c	40.94^c	40.81^b
C1	43.30^c	41.30^c	42.36^b
C3	40.33^c	40.53^c	40.55^b
C4	41.19^c	41.77^c	41.03^b

Values with the same alphabets in superscript in the same column were not significant at 5%. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.1.1, percentage growth rate was highest in T_1 at 100 ppm (45.89), which was even higher than (C1) though the difference was not significant (P = > 0.05). However, percentage growth was least in T/O $_D$ treatment (1.40), followed by T/O $_S$ (17.01) both of which are significant from each other (P = 0.044; LSD = 23.25) and also significantly different from the values of other treatments (P = 0.021; LSD = 13.03). Meanwhile, there was synonymous decrease in growth as the treatment concentration of both Aroclor and transformer oil was increased.

4.1.2.2 Percentage growth rate of *C. odorata* at different concentrations of Aroclor and T/O treatment in S_2

The percentage increase in length of plant in different treated soil contaminated samples in S_2 is presented in Table 4.1.2 below.

Table 4.1.2: Percentage growth rate of *C. odorata* at different concentrations of Aroclor and T/O in S_2.

Treatments (ppm)	Percentage growth in S_2		
	100ppm	200ppm	500pmm
T/O $_D$	1.13[a]	0.44[a]	-1.27[a]
T/O $_S$	22.18[b]	25.57[b]	1.93[a]
T_1	36.74[c]	34.37[c]	30.93[c]
T_2	49.01[d]	43.88[d]	19.53[b]
C1	43.07[d]	41.28[d]	42.93[d]
C3	40.87[d]	41.02[d]	39.81[c]
C4	40.38[d]	40.76[d]	41.41[d]

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=control 1, C3=control 3, C4=control 4

From Table 4.1.2 above, percentage growth was highest at T_2 at 100 ppm (49.01), followed by 200 ppm (43.88). These values were higher than what was obtained from the untreated control (C1) samples which were 43.07, 41.28, and 42.93% respectively between increases in treatments concentrations. However, the values were not significant at 0.05 levels. Percentage growth was least in T/O $_D$ at 500 ppm (-1.27), followed by T/O $_D$ at 200 ppm (0.44). The negative value of the percentage growth rate in T/O $_D$ at 500 ppm is an indication of reduction in plant length, a response to the adversity of T/O to *C. odorata* at high concentration. The same trend of decrease in plants growth as observed in S_1 as treatment concentration was increased also occurred in S_2. Therefore there is sence in arguing that both soil samples used in this greenhouse experiment were of equall constituents irrespective of the figures released by the

characterized results in Table 3.2. Hence either of the two plant sample could be used for the growth of *C. odorata.*

4.1.3 Plants biomass

Measured values of wet and dry weight at harvest were used to evaluate the percentage change in biomass among different treatment concentrations. This explains the effect of the treatments with Aroclor and Transformer oil on water retention ability of *C. odorata*. Uptake and retention of water is dependent amongst other factors on the physiologyof the plants especially at adverse environments. Therefore cumulative water use seems to be more sensitive than plant growth to PCB and indirectly reflects on transpiration.

4.1.3.1 Effect of different concentrations of Aroclor and T/O on water retention abilities of *C. odorata* in S_1.

Treatment concentrations of Aroclor and T/O effect on water retention abilities measured in percentage change from wet to dry biomass of *C. odorata* after six weeks of growth in the treated soil (S_1) are presented below.

4.1.3.1.1 Effect of 100 ppmof Aroclor and T/O treatments on water retention abilities of *C. odorata* in S_1

The results of the effect of 100 ppmof Aroclor and T/O treatment concentration on the change in plants biomass from wet to dry mass in S_1 is presented in Table 4.2.1.1 below.

Table 4.2.1.1: Percentage change in biomass in S_I of *C. odorata* at 100 ppm of Aroclor and T/O treatments.

S_1Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	8.95[a]	5.31[a]	3.64[a]	40.67[a]
T/O $_S$	18.66[a]	6.94[a]	11.72[a]	62.81[b]
T_1	91.22[c]	15.12[a]	76.10[c]	83.42[c]
T_2	79.56[c]	14.18[a]	65.38[c]	82.18[c]
C1	13.06[a]	1.33[a]	11.73[a]	89.82[c]
C3	25.35[b]	7.34[a]	18.01[a]	71.05[b]
C4	31.27[b]	7.07[a]	24.20[b]	77.39[b]

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.2.1.1 above, it is observed that a significant difference existed in the percentage change in plant biomass from wet to dry mass between T/O $_D$ and T/O $_S$ at 100 ppm contaminations (41 and 63%) respectively, but they was no such difference between T_1 and T_2 compared to the controls C1, C3 and C4 (83, 82, 90, 71 and 77%) respectively.

4.1.3.1.2 Effect of 200 ppm of Aroclor and T/O treatments on change in plants biomass in S_1.

The results of the effect of 200 ppm of Aroclor and transformer oil treatments on change in plants biomass in S_1 is presented in Table 4.2.1.2 below.

Table 4.2.1.2: Percentage change in biomass of *C. odorata* in S_I with 200 ppm of Aroclor and T/O treatments.

S_1Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	7.25[a]	4.75[a]	2.50[a]	34.48[a]
T/O $_S$	13.57[a]	5.45[a]	8.12[a]	59.84[b]
T_1	45.21[c]	8.59[a]	36.62[b]	81.00[c]
T_2	51.94[c]	10.11[a]	41.83[b]	80.54[c]
C1	92.07[d]	6.99[a]	85.08[c]	92.41[c]
C3	19.49[a]	6.60[a]	12.89[a]	66.14[b]
C4	23.41[b]	7.19[a]	16.22[a]	69.29[b]

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

In Table 4.2.1.2, percentage change in plants biomass maintained the same trend as was observed in 100 ppm treatment, but percentage changes in biomass was relatively lower in this case (200 ppm) than the former (100 ppm), except in C1 which was higher in 200 ppm than in 100 ppm treatment.

4.1.3.1.3 Effect of 500 ppm of Aroclor and T/O on the change in plants biomass in S_1.

Results of the effect of 500 ppm treatment concentration on the change in plants biomass in S_1 is as reported in Table 4.2.3 below.

Table 4.2.1.3: Percentage change in biomass in S_I with 500 ppm of Aroclor and T/O in soil.

S_1Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	5.69[a]	4.15[a]	1.54[a]	27.07[a]
T/O $_S$	6.64[a]	3.37[a]	3.27[a]	49.25[b]
T_1	65.94[b]	17.19[b]	48.75[c]	73.93[c]

T$_2$	62.25[b]	17.09[b]	45.16[c]	72.55[c]
C1	13.06[a]	1.76[a]	11.30[b]	86.52[d]
C3	19.49[a]	6.23[a]	13.26[b]	68.03[c]
C4	19.32[a]	6.27[a]	13.05[b]	67.55[c]

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.2.1.3 above, the entire treatment maintained relatively low percentage change in biomass compared to the values in 100 and 200 ppm of Aroclor and T/O except control 3 with a high percentage change in biomass (68), which was higher than the percentage change obtained in 200 ppm treatment (66%).

4.1.3.2 Effect of different concentrations of Aroclor and T/O on the change in plants biomass in S$_2$.

Treatment concentrations of Aroclor and T/O effect on percentage change from wet to dry biomass of entire plant of *C. odorata* after six weeks of growth in S$_2$ is presented below.

4.1.3.2.1 Effect of 100 ppm of Aroclor and T/O concentration on the change in plants biomass in S$_2$

The results of the effect of 100 ppm concentration of Aroclor and T/O on change in plants biomass in soil sample 2 is presented in Table 4.2.2.1 below.

Table 4.2.2.1: Percentage change in biomass in S$_2$ with 100 ppm of Aroclor and T/O treated soil.

S$_2$ Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	7.67[a]	4.79[a]	2.88[a]	37.55[a]
T/O $_S$	15.87[a]	6.33[a]	9.54[a]	60.11[b]
T$_1$	89.36[d]	16.75[a]	72.61[c]	81.26[c]

	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T$_2$	81.34^d	15.24^a	66.10^c	81.26^c
C1	12.23^a	2.08^a	10.15^a	82.99^c
C3	27.11^b	7.19^a	19.92^a	73.48^b
C4	54.28^c	12.06^a	42.22^c	77.78^b

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.2.2.1 above, direct co-contamination of the soil by PCB in transformer oil (T/O $_D$) affected the plants biomass in sandy loam soil as observed from their low mean percentage value (37.55), compared to the rest treatments. T/O $_D$ value is therefore significantly different (P = 0.02; LSD = 7.15) from other percentage changes in biomass.

4.1.3.2.2 Effect of 200 ppmof Aroclor and T/O treatment on the change in plants biomass in S$_2$.

The results of the effect of 200 ppm of Aroclor and T/O treatment on the change in plants biomass in S$_2$ are presented in Table 4.2.2.2 below.

Table 4.2.2.2: Percentage change in biomass in S$_2$ with 200 ppm of Aroclor and T/O treatments.

S$_2$ Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	7.95^a	5.31^a	2.64^a	33.21^a
T/O $_S$	15.47^a	7.06^a	8.41^a	54.36^b
T$_1$ 1254	49.57^c	11.13^a	38.44^b	77.55^c
T$_2$ 1260	60.52^d	14.41^a	46.11^c	76.19^c
C1	93.19^e	23.19^b	70.00^d	75.12^c
C3	21.22^b	5.75^a	15.47^a	72.90^c
C4	43.96^c	10.53^a	33.43^b	76.05^c

From Table 4.2.2.2 above, low percentage changes in plants biomass from wet to dry was evident from the increased concentration of the treatments. T/O $_D$ maintained its low percentage change in biomass (33.21), lower than its value in 100 ppm treatment. This value is significantly different (P = 0.021; LSD = 9.72) from the rest values for T/O $_S$, T_1 and T_2 with percentage change of 54.36, 77.55 and 76.19 respectively which is high compared to that of T/O $_D$. Control samples equally gave high percentage biomass change were also synonymous with the percentage values of T_1, T_2 and T/O $_S$.

4.1.3.2.3 Effect of 500 ppm of Aroclor and T/O treatment on the change in plants biomass in S_2.

The results of the effect of 500 ppm Aroclor and T/O contamination on the change in biomass of *C. odorata* grown in sandy loam soil is presented in Table 4.2.2.3 below.

Table 4.2.2.3: Percentage change in biomass of *C. odorata* in S_2 with 500 ppm of Aroclor and T/O treated soil.

S_2Treatments/Set-up (ppm)	Wet weight (g)	Dry weight (g)	Difference (g)	% change
T/O $_D$	7.59[a]	5.66[a]	1.93[a]	25.43[a]
T/O $_S$	4.23[a]	2.28[a]	1.95[a]	46.10[b]
T_1	79.97[c]	24.13[b]	55.84[b]	69.83[c]
T_2	62.25[c]	18.03[a]	44.22[b]	71.04[c]
C1	12.99[a]	3.35[a]	9.64[a]	74.21[c]
C3	21.04[b]	6.21[a]	14.83[a]	70.48[c]
C4	29.28[b]	10.01[a]	19.27[a]	65.81[c]

From Table 4.2.2.3 above, it is evident that increase in concentration of PCB as well as the contamination with oil in the soil has a negative effect in the ability of plant to retain water in its cells as seen with the reduced percentage biomass values compared to the 100 and 200 ppm soil treatment which have high percentage change in biomass of the plant. T/O $_D$ maintained a low value of 25.45% which is the least among the treatments, the highest value was found in C1 (74.48%) and is not significant (P > 0.05) with values for T_1, T_2, C3 and C4 respectively but   significantly different (P = 0.032; LSD = 10.13) from T/O values.

4.2 Mature leaves per plant (MLPP) grown in Aroclor and T/O treated soil during six weeks of phytoremediation study.

Fully expanded leaves per plant were manually counted at weekly intervals and the result is presented in the Figures and Tables below.

4.2.1 MLPP in S_1 at different treatments concentrations of Aroclor and T/O

The results of MLPP amongst different concentrations of Aroclor and T/O in S_1 are presented below.

4.2.1.1 MLPP at 100 ppm of Aroclor and T/O in S_1

The number of MLPP at 100 ppm of Aroclor and T/O treatments in S_1 is presented in Figure 4.3.1.1 below.
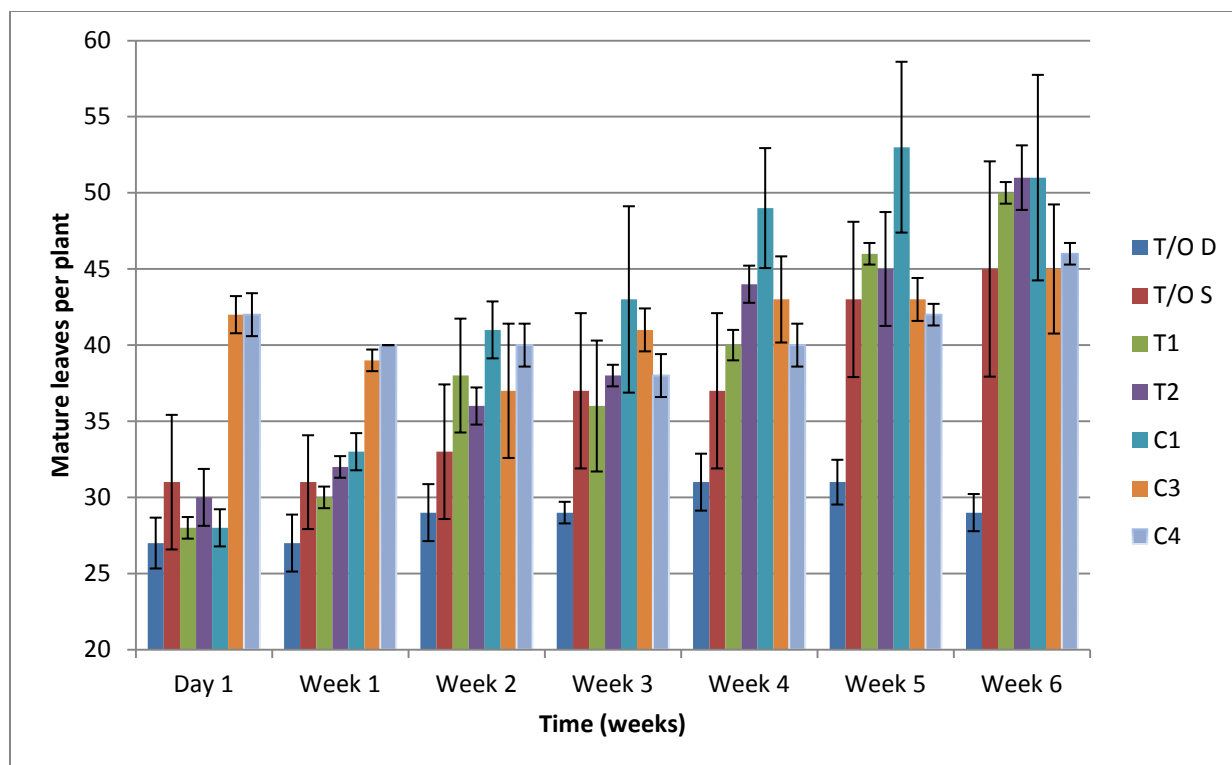
Figure 4.3.1.1: Mature leaves per plant at 100 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.1.1 above, MLPP maintained the same value from the initial on the first week; rose to 31 leaves on the fourth week and reduced to 29 leaves on the sixth week. In T/O $_S$, there was significant increase in MLPP from the second week to the sixth week where the value was 45 leaves. The same significant increase in MLPP was onserved in T_1 and T_2 with higher recorded values in MLPP of 50 and 51 leaves respectively on the sixth week. Equal trend of increase in leaf numbers was also observed in untreated control as well as in other controls. The highest least numbers was observed in C1on the fifth week with 53 leaves, but dropped to 61 on the sixth. MLPP percentage values in T_I and T_2 were 78.54 and 70.0 respectively; these values were not significant (P > 0.05) from the values obtained from the untreated control (82.14%).

4.2.1.2 MLPP at 200 ppm of Aroclor and T/O in S_1

The number of MLPP at 200 of Aroclor and T/O treatments in S_1 is presented in Figure 4.3.1.2 below.

Figure 4.3.1.2: Mature leaves per plant at 200 ppm of Aroclor and 200 T/O treatments in S_1 (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.1.2 above, evidence of the adverse effect of co-contamination of T/O to *C. odorata* is seen by the absence of T/O $_D$ bar at sixth week. T/O $_S$ increased from 29 leaves on day 1 to 35 leaves at sixth week. T_1 and T_2 maintained a tremedous increase and such value were not significant (P > 0.05) from the values obtained from the untreated controls.

4.2.1.3 MLPP at 500 ppm of Aroclor and T/O in S_1

The number of MLPP at 500 ppm of Aroclor and 500 ppm of T/O treatments in S_1 is presented in Figure 4.3.1.3 below.
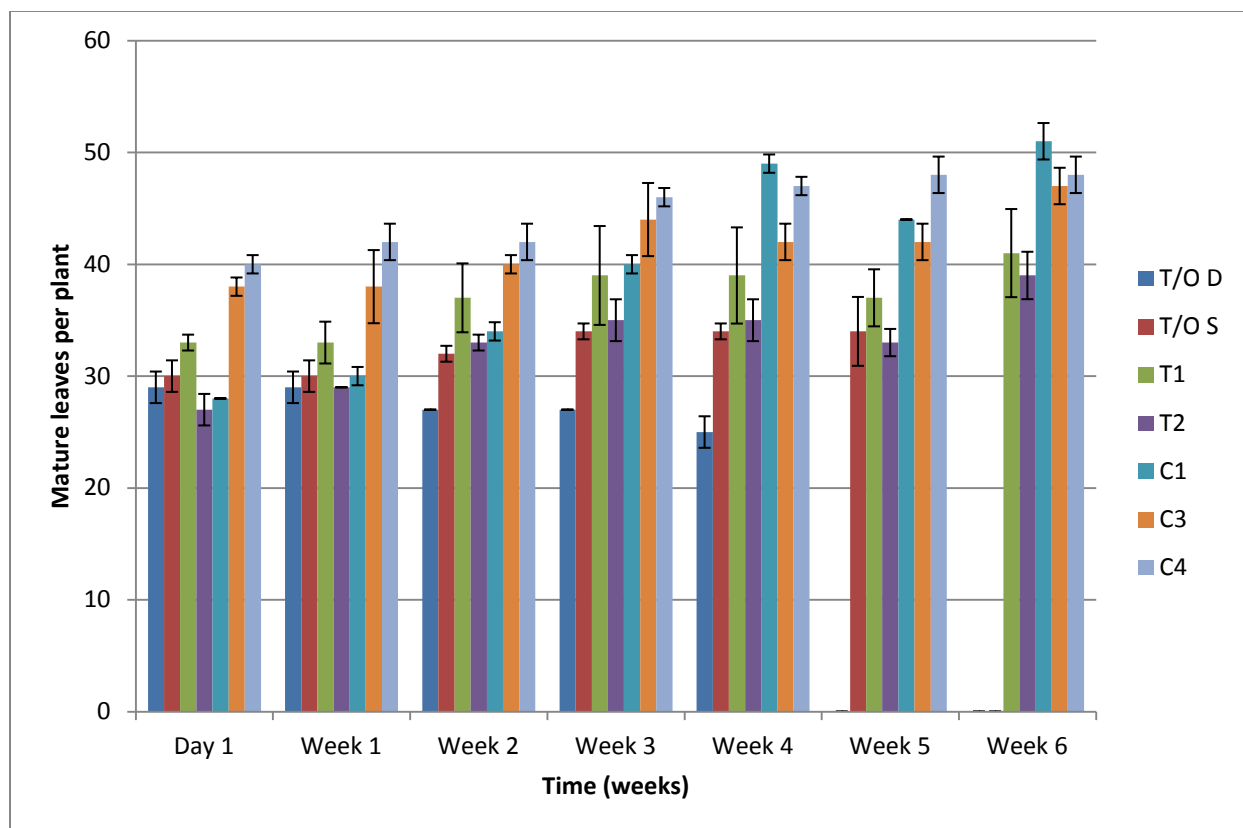
Figure 4.3.1.3: Mature leaves per plant at 500 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), T/O_D=Direct transformer oil, T/O_S=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.1.3 above, MLPP in both T/O_D and T/O_S were zero at fifth week, T/O_D was also absent after five weeks as shown by their absent on the sixth week. The MLPP in T/O started shrinking from the second week, dried at fifth week and were zero between the fifth and sixth week. T/O_S on the other hand shrank and got to zero on the sixth week. The drying of the leaves was an indication of the adverse effect of high co-contamination of the oil which was lethal at high concentration of T/O (500 ppm). MLPP in the Aroclor amended treatments increased considerably, though they seem to drop on fifth week but increased afterwards (41 and 39 leaves) for T_1 and T_2 respectively.

4.2.2 MLPP in S_2 at different treatments concentrations of Aroclor and T/O

The results of MLPP amongst different treatments of Aroclor and T/O in soil sample 2 are presented below.

4.2.2.1 MLPP at 100 ppm of Aroclor and T/O in S_2 treatments

The number of MLPP at 100 ppm of Aroclor and T/O in S_2 treatments is presented in Figure 4.3.2.1 below.
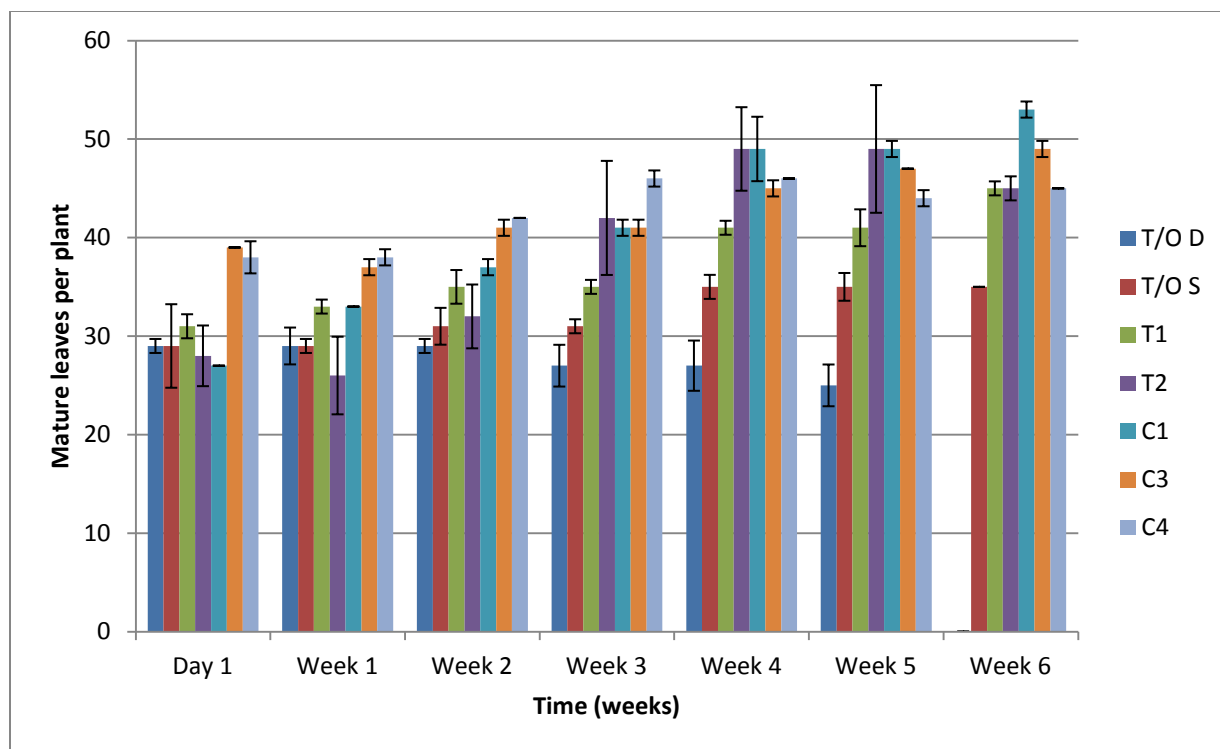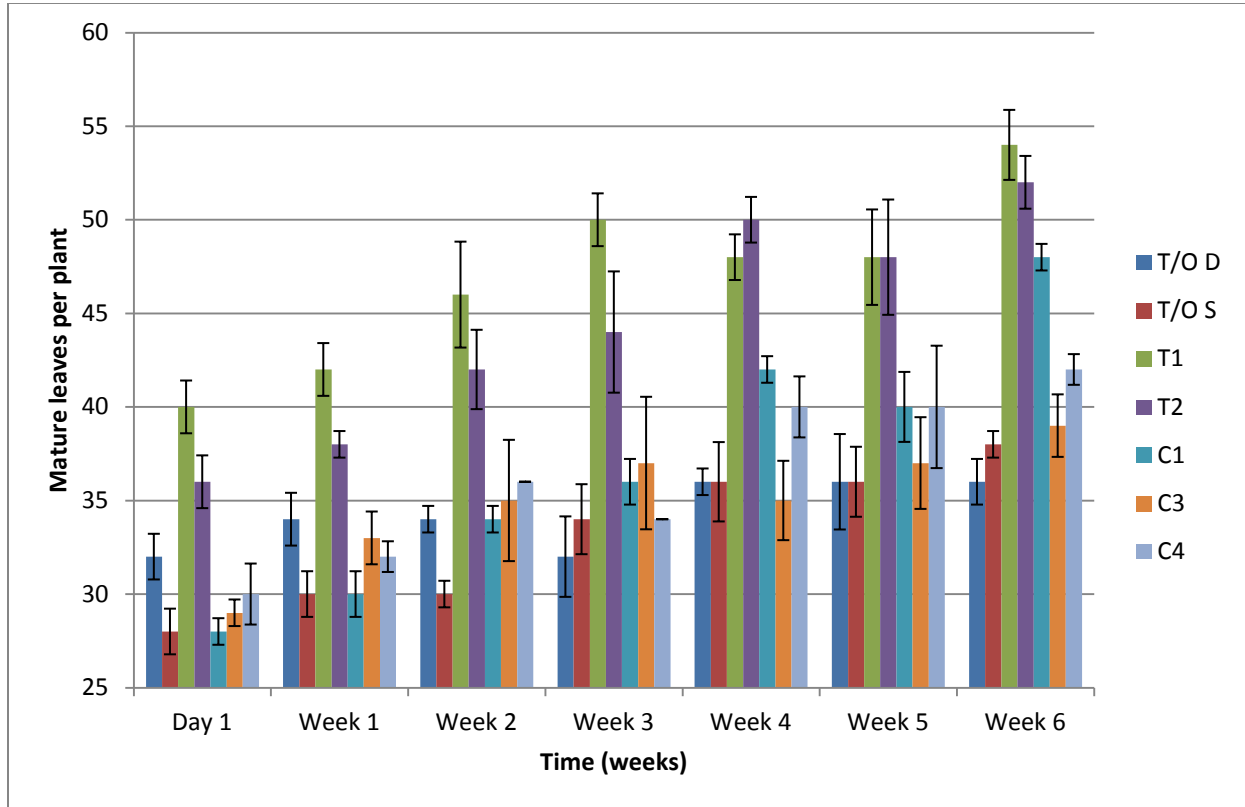

Figure 4.3.2.1: Mature leaves per plant at 100 ppm of Aroclor and T/O in S_2 treatments (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.2.1 above, MLPP of the entire treatments all increased considerably from day one to the sixth week. Increase in MLPP throughout the six weeks of growth in the PCB contaminated and T/O co-contamination were all synonymous with each other. At day one, MLPP was least but increased considerably from the first week all through the sixth week.

4.2.2.2 MLPP at 200 ppm of Aroclor and T/O in S_2 treatments

The number of MLPP at 200 ppm of Aroclor and T/O in S_2 treatments is presented in Figure 4.3.2.2 below.
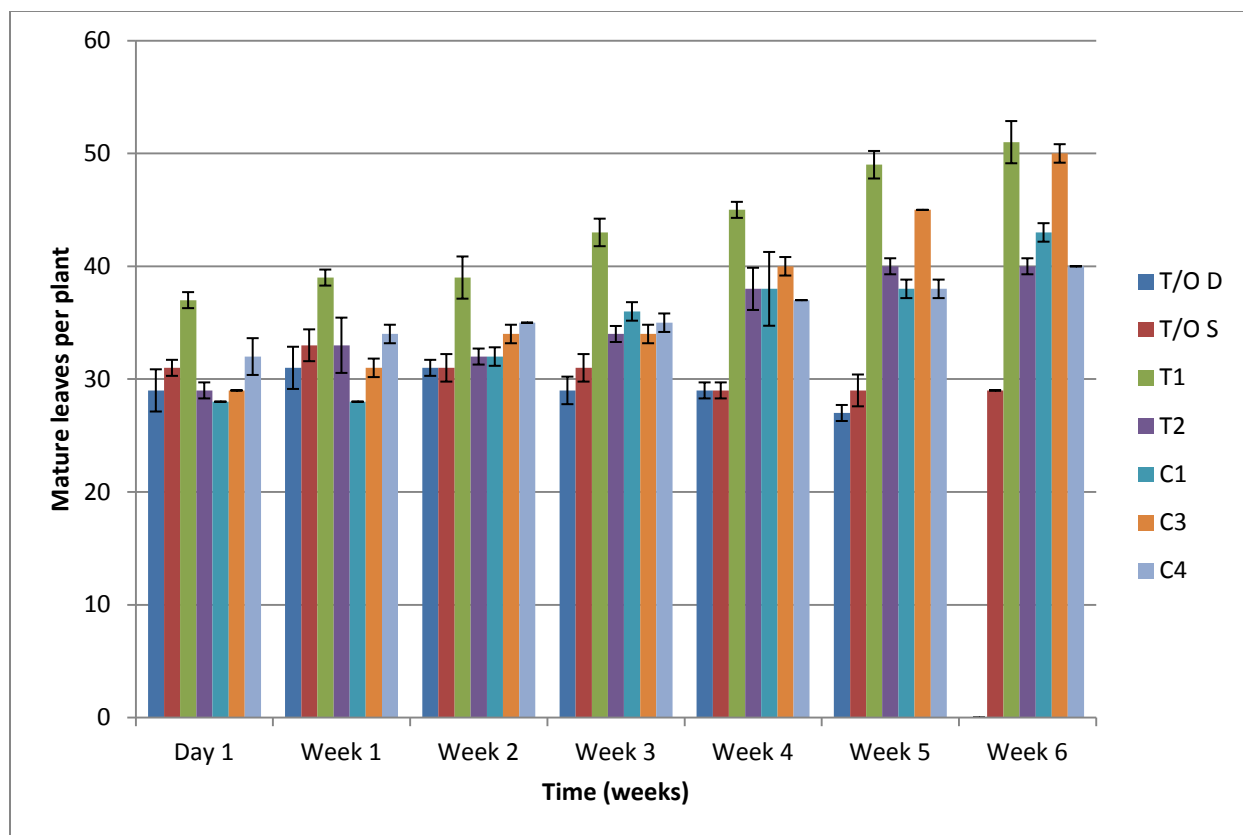
Figure 4.3.2.2: Mature leaves per plant at 200mg/kg of Aroclor and 200ml/kg of T/O in S2 treatments (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.2.2 above, MLPP in T/O $_D$ decreases and got dried up on the sixth week. This is shown by the disappearance of the T/O $_D$ bar on the sixth week. MLPP on T/O $_S$ on the other hand was decreased from 31 leaves on day one to 29 leaves on the sixth week. Aroclor amended samples all increased synonymously with control.

4.2.2.3 MLPP at 500 ppm of Aroclor and T/O in S$_2$ treatments

The number of MLPP at 500 ppm of Aroclor and T/O in S$_2$ treatments is presented in Figure 4.3.2.3 below.
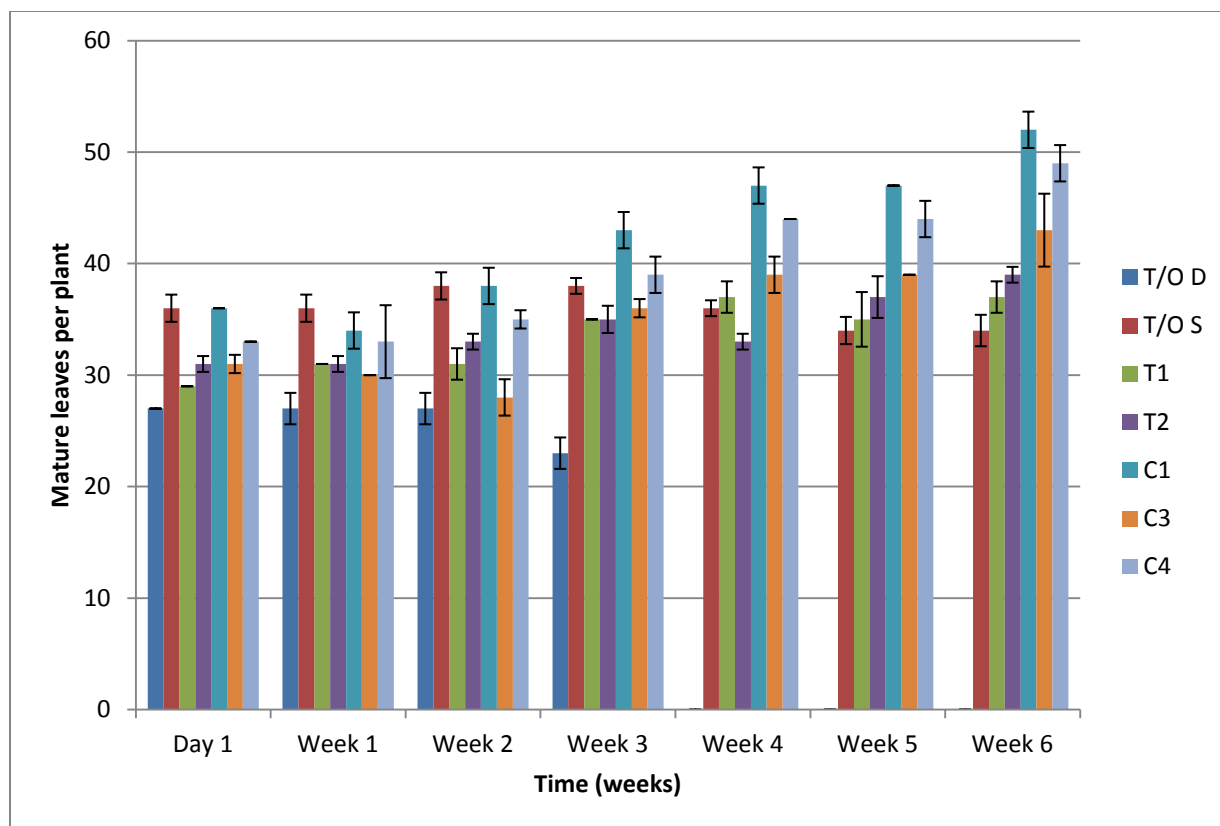
Figure 4.3.2.3: Mature leaves per plant at 500 ppm of Aroclor and T/O in S_2 treatments (Error bars indicate standard error of the mean), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.2.3 above, MLPP in T/O $_D$ decreased as well and dried up on the fourth week in the contaminated soil. The T/O $_S$ sample was reduced to 34 leaves from 36 on the sixth week of growth in the contaminated soil. T_1 and T_2 samples all increased throughout the duration, an indication that the plants survived the contamination. The highest leaf number was observed in the untreated control with 52 leaves on the sixth week.

4.2.3 Percentage change in MLPP of *C. odorata* at different concentrations of Aroclor and T/O treated soil after six weeks of growth.

The difference between the initial and final change in MLPP was evaluated and were used to calculate their subsequent percentage change.

4.2.3.1 Percentage change in MLPP of *C. odorata* in S_1 at different concentrations of Aroclor and T/O treated soil after six weeks of growth.

Percentage change in MLPP of *C. odorata* in S_1 at different concentrations of Aroclor and T/O treated soil after six weeks of growth is presented in Table 4.2.3.1 below.

Table 4.2.3.1: Percentage change in mature MLPP in different concentrations of Aroclor and T/O treated S_1.

Treatments (ppm)	Percentage increase in MLPP in S_1		
	100 (ppm) soil	200 (ppm) soil	500 (ppm) soil
T/O $_D$	7.41[a]	0.00[a]	0.00[a]
T/O $_S$	50.00[c]	20.69[b]	0.00[a]
T$_1$	78.57[d]	45.16[c]	24.24[b]
T$_2$	70.00[d]	60.71[d]	44.44[c]
C1	82.14[d]	96.30[e]	82.14[d]
C3	7.14[a]	25.64[b]	23.68[b]
C4	9.52[a]	18.42[b]	20.00[b]

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.2.3.1 above, percentage change in MLPP was highest in T$_2$ at 100 ppm (70.00), followed by T$_1$ (78.57). When the soil was treated with 200 and 500 ppm contamination, percentage change in MLPP at T/O $_D$ all reduced to zero, while they were zero only at 500 ppm treated soil in T/O $_S$. The percentage change in both T$_1$ and T$_2$ at different treatment concentrations was high compared to what was obtained in T/O samples and they were not significant (P > 0.05) with those in untreated controls. The zero percentage recorded was an indication of the adversity of the treatments to the growth of *C. odorata*.

4.2.3.2 Percentage change in MLPP of *C. odorata* in S_2 at different concentrations of Aroclor and T/O treatment after six weeks of growth.

Percentage change in MLPP of *C. odorata* in S_2 at different concentrations of Aroclor and T/O treatment after six weeks of growth is presented in Table 4.2.3.2 below.

Table 4.2.3.2: Percentage change in MLPP at different concentrations of Aroclor and T/O treated S_2.

Treatments (ppm)	Percentage increase in MLPP in S_2		
	100 (ppm) soil	200 (ppm) soil	500 (ppm) soil
T/O $_D$	12.50^a	0.00^a	0.00^a
T/O $_S$	35.71^b	-6.45^a	-5.56^a
T$_1$	35.00^b	37.84^b	27.59^b
T$_2$	44.44^b	27.93^b	28.81^b
C1	71.43^c	53.57^c	44.44^c
C3	34.48^b	72.41^d	38.71^c
C4	40.00^b	25.00^b	48.48^c

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=control 1, C3=control 3, C4=control 4

From Table 4.2.3.2 above, percentage change in MLPP of *C. odorata* was highest in T$_2$ at 100 ppm (44.44). MLPP reduced below zero at 200 and 500 ppm in T/O $_D$ were greater than zero at T/O $_S$ (-6.45 and -5.56) in 200 and 500 ppm respectively, but the values in T$_1$ and T$_2$ treatments were high and were not significant (P > 0.05) with the values of the untreated controls. Lethality of high concentration of transformer oil to the growth of plant*s* is an indication of the negative values in the 200 and 500 ppm of the oil on MLPP of *C. odorata*. *Chromolaena odorata* in T/O $_D$ treatment only increased slightly throughout the six weeks of growth at 100 ppm treated soil, signifying a percentage increase by 7.41. The plants in the 200 and 500 ppm treated soil were all dried by the sixth week of growth in T/O treatments, with percentage decrease to less than zero (negative increament). Plants MLPP in 200 ppm treatement

got completely dried on the sixth week while the 500 ppm sample got dried on the fifth week of growth. In T/O $_S$ treatments, MLPP of *C. odorata* increased appreciably from 31 on the day of transplant into the treated soil to 45 on the day of harvest in the 100 ppm treatment, maintaining a percentage increase of 50%. The 200 ppm increased to 35 from 29 leaves with a percentage increase of 20, while the 500 ppm was completely dried on the sixth week of growth after contamination. Percentage change in MLPP in T/O $_S$ 500 ppm was less than zero the same way the 200 and the 500 ppm samples of T/O direct samples respectively were. In the Aroclor amended samples, MLPP increased by 78% in the 100 ppm treaments in T$_1$ and 70% in T$_2$ samples which is an indication of luxiriant growth among the plants growing in that contaminated soil. The 200 ppm treatment allowed an average percentage growth of 45 and 60 respectively among the T$_1$ and T$_2$ set –up. Although the growth rate in this concentration was sperse, it was however better than the T/O contaminated samples in same concentration. On the other hand, the 500 ppm contamination saw the plants growth rate reduced to 24 and 44% respectively within the T$_1$ and T$_2$ treatments. It was easily deduced therefore that *C. odorata* supported the ranges of contamination of PCBs in form of Aroclor administered to the soil hence could survive high concentration of PCBs in the soil. Control 1 maintained a growth percetage increase of 82, which is quite high but was not significant (P > 0.05) with the 100 and 200 ppm contaminated treatments of T$_1$ and T$_2$. Control 3 of the Aroclor ammended samples also had a percentage increase in MLPP of 33% which was also not significant with the 500 ppm contaminated samples of T$_1$ and T$_2$ (P > 0.05). Meanwhile, percentage growth rate within T/O $_D$ treated samples were not significant (at same P value), those within T/O $_S$ samples were all significant among the treatments (P < 0.05). However, the values between T/O $_D$ and T/O $_S$ were significant (P < 0.05), and those between T$_1$ and T$_2$ were slightly significant at (P = 0.043; LSD = 7.1). The mature leaves per plant values is found in Table (4.3a-b) as well as in (4.4a-f). Both S$_1$ and S$_2$ used in the greenhouse maintained a somewhat similar trends in the numbers of their individual mature leaves but, they have their perculiarities. At 100 ppm direct T/O contamination, S$_1$ has a lower percentage value 7.41, while S$_2$ has a percentage of 12.50 both of which are not significant (P > 0.05). The two soil samples both maintained the same trend in values among the 200 and 500 ppm respectively. In T/O $_S$

treatments, S_1 has greater percentage value in the entire treatment concentration than S_2, the same trend was maintained at T_1 and T_2 treantment all through the treated control samples

4.3 Root length of *C. odorata* grown in different concentrations of Aroclor and T/O for six weeks.

Root length of the plants in the entire greenhouse study was measured first on the day of planting (initial measurement), and secondly on the day of harvest (final measurement). Percentage change in root length was however calculated from the initial measurement.

4.3.1 Root length in S_1 at different concentrations of Aroclor and T/O treatment

The length of roots in different concentrations of Aroclor and T/O in S_1 are presented in the Figures and Tables below.

4.3.1.1 Root length at 100 ppm of Aroclor and T/O treatment in S_1

The results of the measurement of root length at 100 ppm of Aroclor and Transformer oil in S_1 treatment is presented in Figure 4.4.1.1 below.
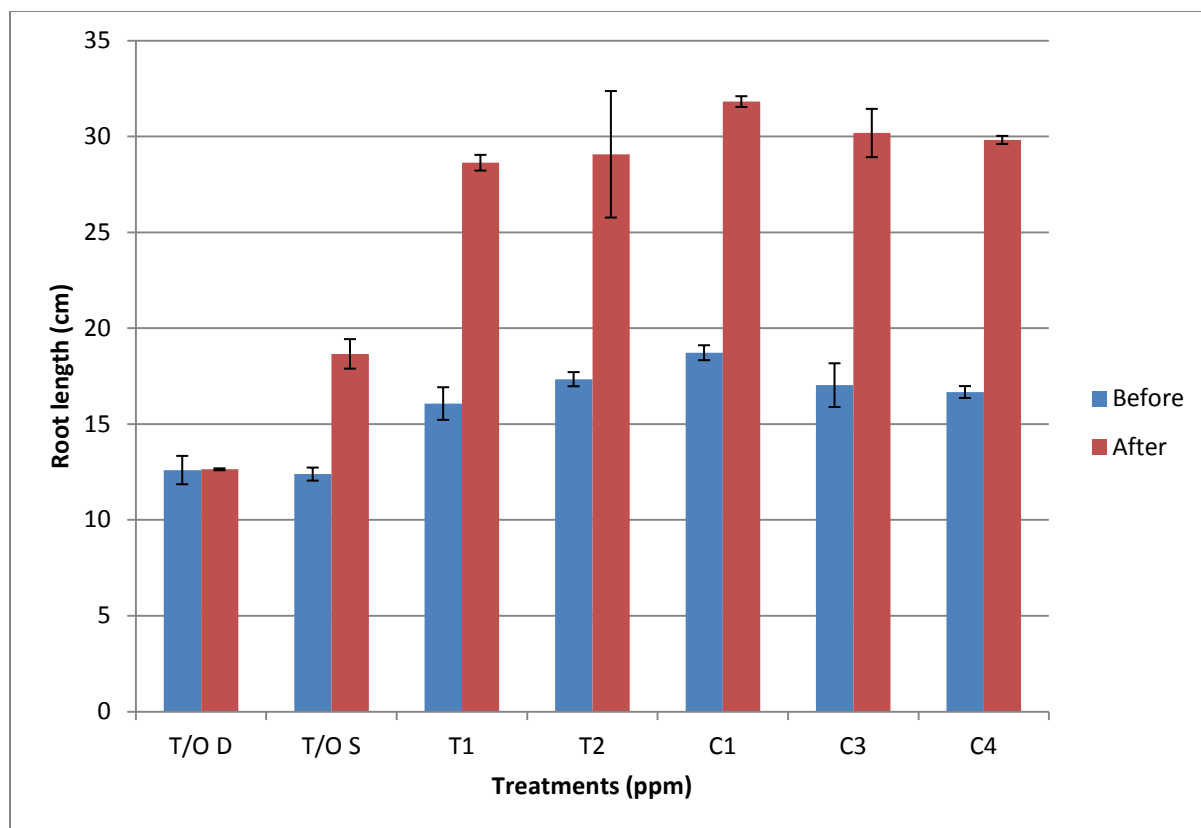
Figure 4.4.1.1: Root length at 100 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.4.1.1 above, root length was highest at C4, C3, and C1 with an increase in root length of 13.55, 13.15, and 13.10 cm respectively, the values were equivalent with each other. Increase in root length was however lowest in T/O $_D$ (0.04 cm), which is significantly different (P = 0.001; LSD = 5.61) from the value in T/O $_S$ (6.27cm). Increase in length among T_1 and T_2 were equally high (11.58 and 11.73cm) respectively, and were not significantly different (P > 0.05) from the values of the untreated control.

4.3.1.2 Root length at 200 ppm of Aroclor and T/O treatment in S_1

The results of the measurement of root length at 200 ppm of Aroclor and T/O treatment in S_1 are presented in Figure 4.4.1.2 below.

Figure 4.4.1.2: Root length at 200 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.4.1.2 above, increase in root length was highest in C1 with 13.29 cm increase, but lowest in T/O $_D$ with value of 0.01 cm. The value of T/O $_S$ was low (2.05 cm), but significantly different from that of T/O $_D$ (P = 0.027; LSD = 9.29). The same trend in growth observed at the above ground tissue of the plant was recorded at the below ground tissues (root), therefore inhibition of stem tissue growth was equally observed in the root tissues.

4.3.1.3 Root length at 500 ppm of Aroclor and T/O treatment in S_1

The results of the measurement of root length at 500 ppm of Aroclor and 500 ppm of T/O treatment in S_1 is presented in Figure 4.4.1.3 below.

Figure 4.4.1.3: Root length at 500 ppm of Aroclor and T/O treatments in S_1 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From figure 4.4.1.3 above, there was decrease in root length till it was less than zero (< 0) among the entire concentration of T/O $_D$ and the higher concentrations of T/O $_S$ (-0.04 and -0.02 cm) respectively. This is an indication that the plants could not survive the duration of the experiment. Highest increase in root length was obtained in C4 (11.49cm), followed by C1 (11.23cm). These values were not significantly different from that of Aroclor treated samples (P > 0.05).

4.3.2 Root length in S_2 at different concentrations of Aroclor and T/O treatment.

The length of roots in different concentrations of Aroclor and T/O in S_2 are presented in figures and tables below.

4.3.2.1 Root length at 100 ppm of Aroclor and T/O treatment in S_2

The results of root length at 100 ppm of Aroclor and T/O treatments in S_1 are presented in Figure 4.4.2.1 below.
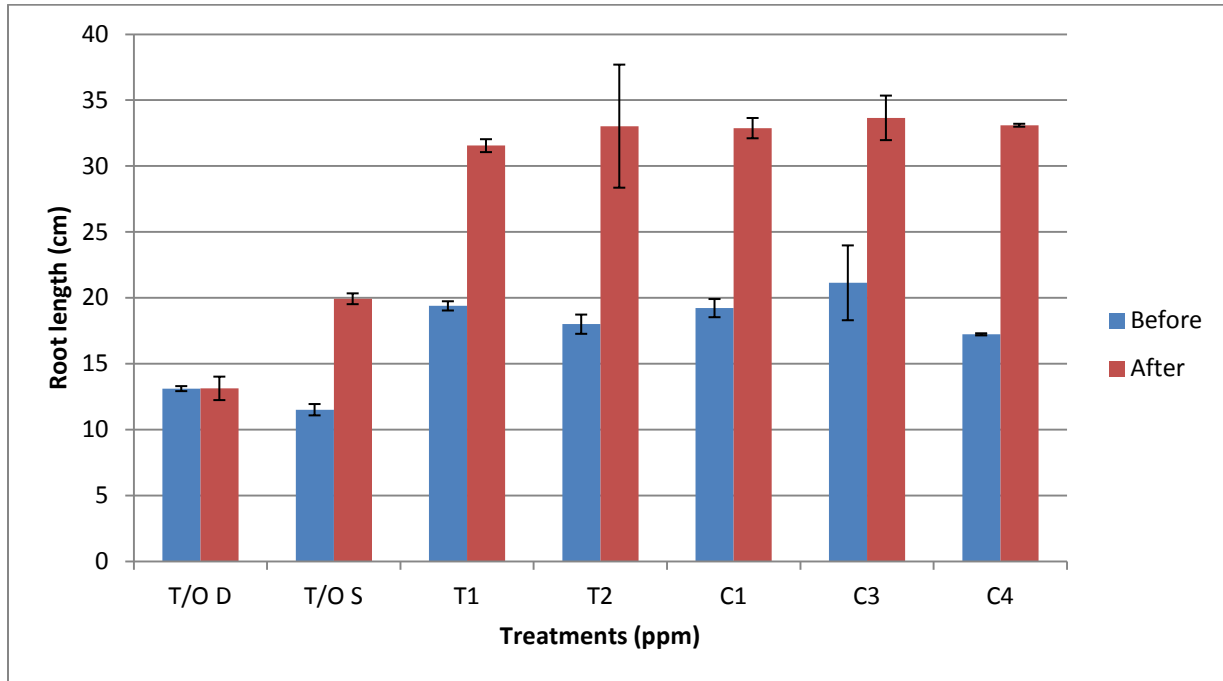
Figure 4.4.2.1: Root length at 100 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.4.2.1 above, the highest value of the increase in root length was obtained in T_2 (15.03 cm), this value was higher than the value obtained in untreatred control (C1). Increase in root length at T_1 was 12.16 cm, -0.01cm in T/O $_D$ and 2.03 cm in T/O $_S$. Increases in root length among T_1, T_1, C1,C3 and C4 were not significantly different (P > 0.05) from each other (12.16, 15.03, 13.66, 12.52 and 15.75cm) respectively. The value of T/O $_D$, signifies that there was no increase in length of root in that treatments, this is an evidence of unconducive environment which rather caused retardation in root length perhaps as a result of the shrinking effect.

Bioremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

4.3.2.2 Root length at 200 ppmof Aroclor and T/O treatment in S_2

The results of root length at 200 ppm of Aroclor and or 200 ppm of T/O treatments in S_2 are presented in Figure 4.4.2.2 below.
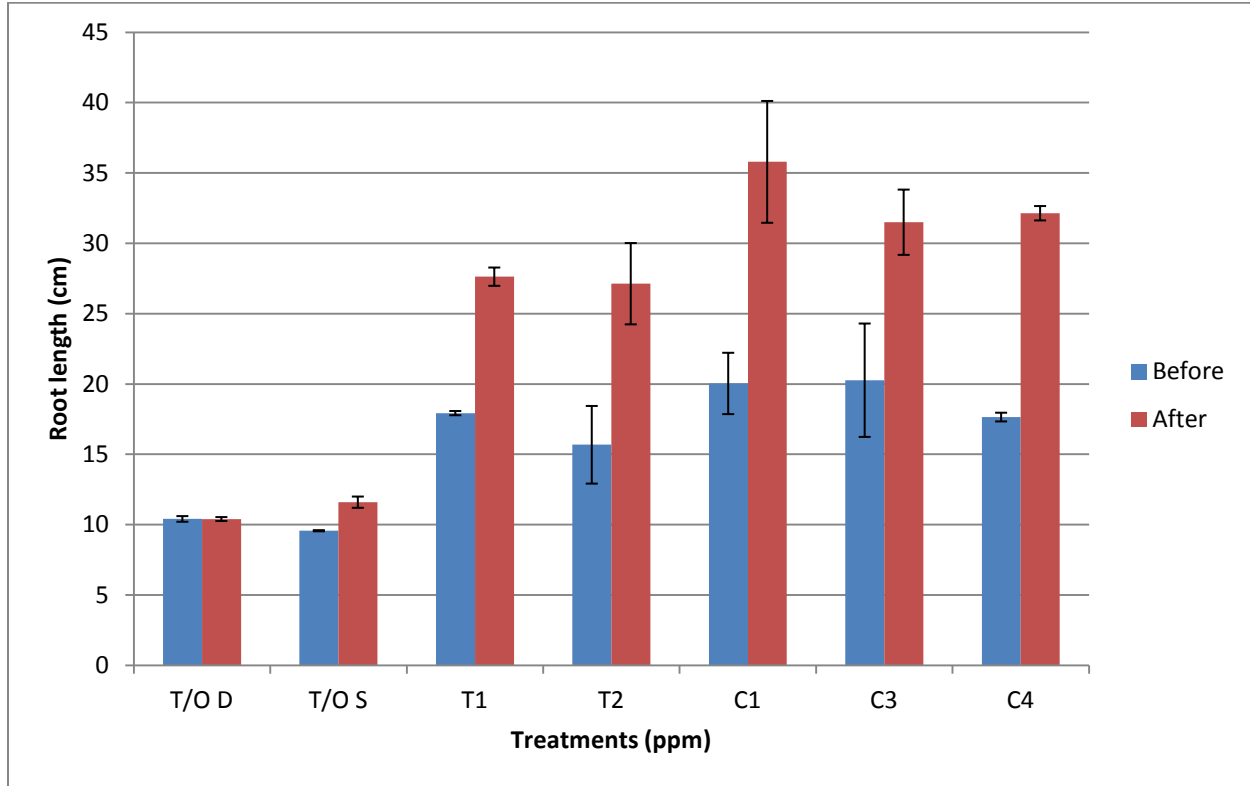
Figure 4.4.2.2: Root length at 200 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.4.2.2 above, increase in root length observed in T_1 was 11.45 cm, this value was lower than the untreated control 1 (15.75 cm). T/O $_D$ values was less than zero (-0.01 cm), and 2.03 cm for T/O $_S$. Values of the increase in C3 and C4 were 11.23 and 14.45 cm respectively. The values of the entire treatments were lower than the values obtained at 100 ppm concentration.

4.3.2.3 Root length at 500 ppm of Aroclor and T/O treatment in S_2

The results of root length at 500 ppm of Aroclor and or 500 ppm of T/O treatments in S_2 are presented in Figure 4.4.2.3 below.
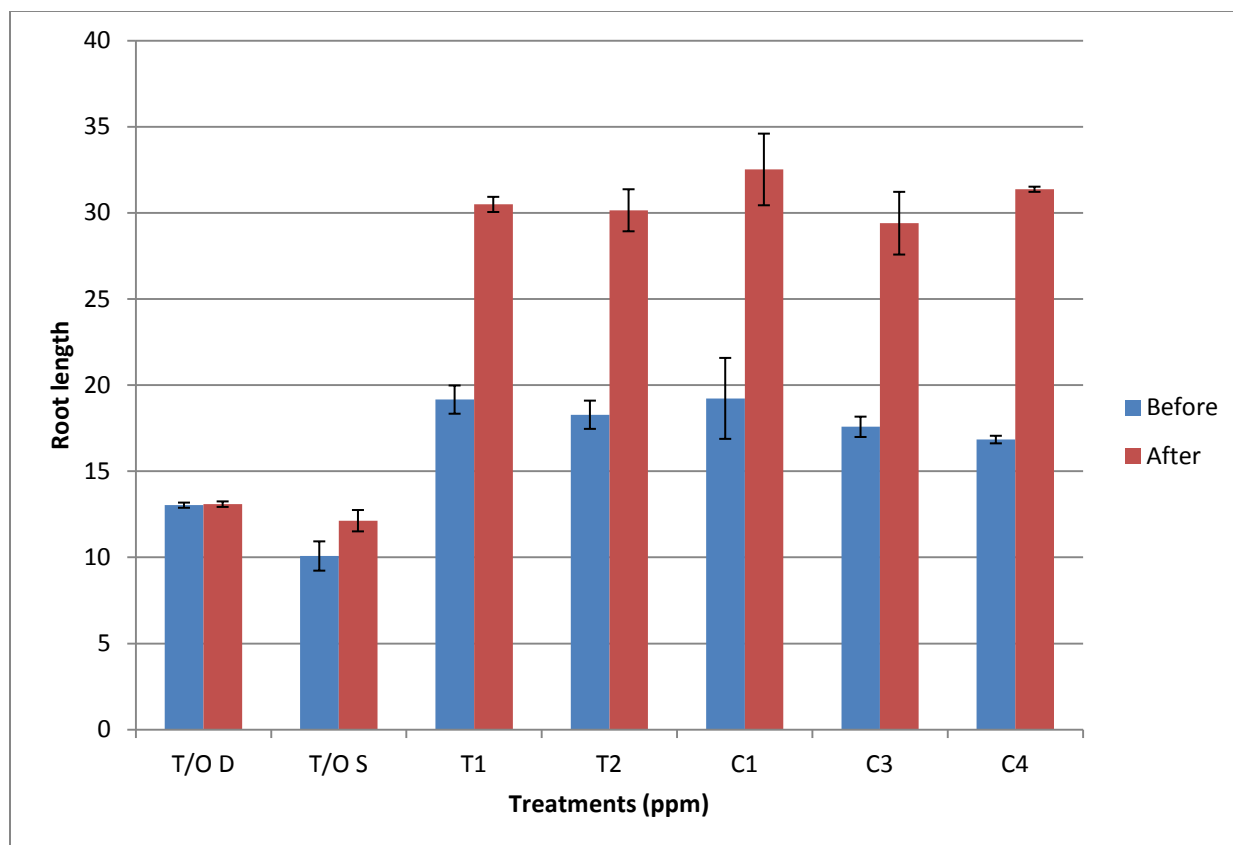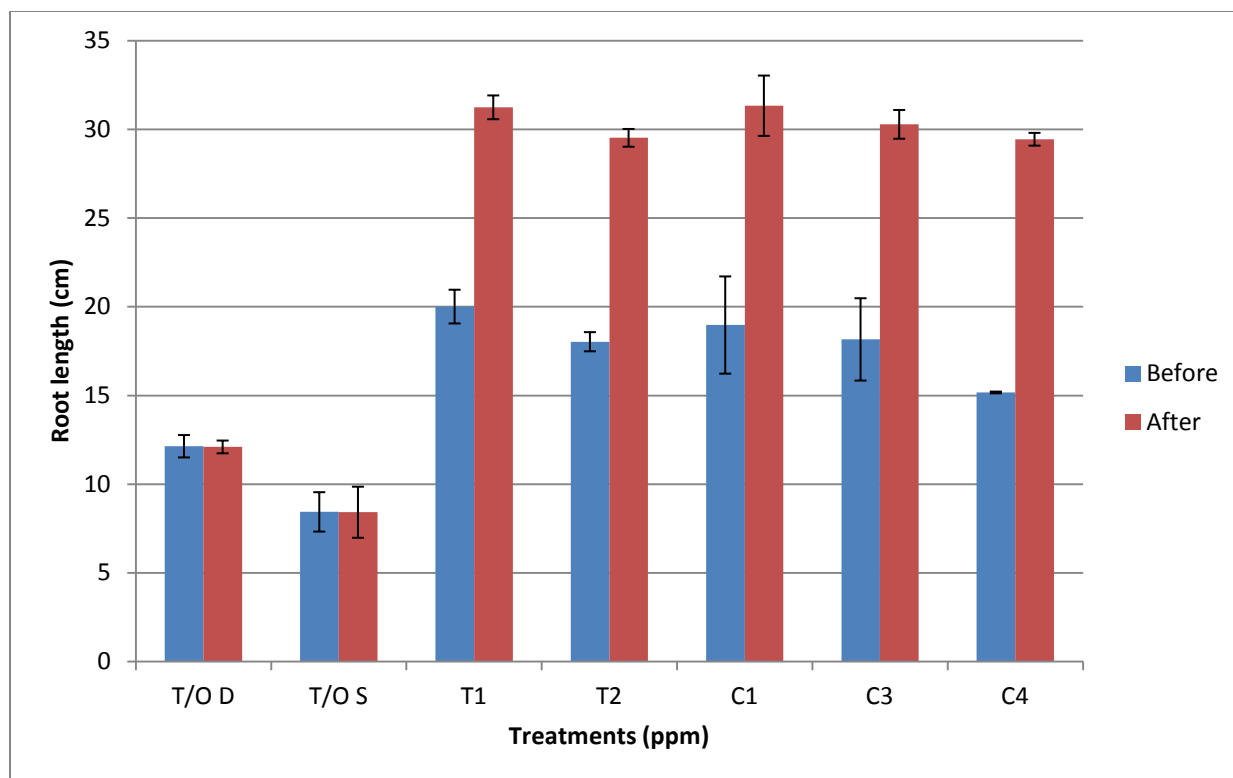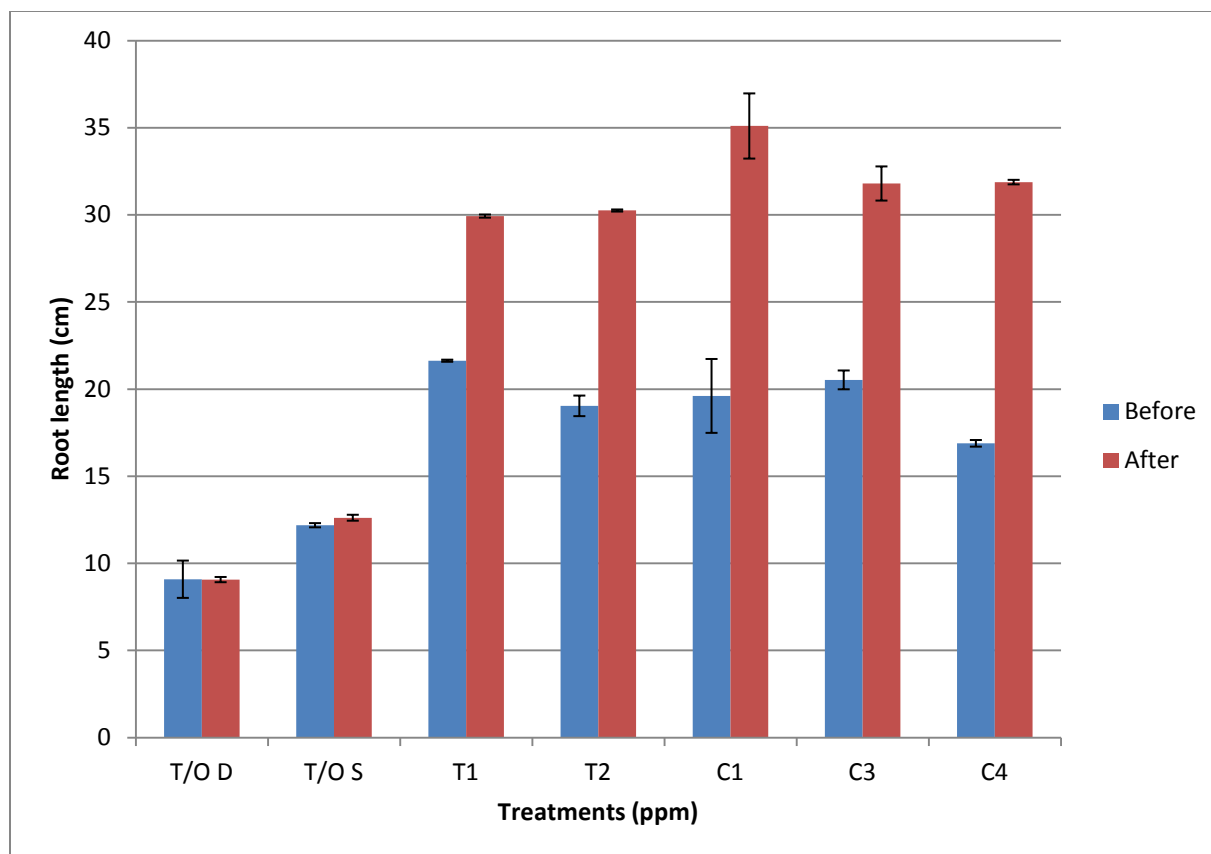
Figure 4.4.2.3: Root length at 500 ppm of Aroclor and T/O treatments in S_2 (Error bars indicate standard error of the mean), Before=Initial length, After=Final length, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

In Figure 4.4.2.3 above, it is evident that there was slight reduction in root length at the end of six weeks of growth in the contaminated soil. In T/O $_D$, root length was reduced by 0.02 cm, but increased by 0.43 cm in T/O $_S$. Root length also increased by 8.30 cm in T_1, 11.21cm in T_2 compared to 15.49 cm increase in untreated control. C3 and C4 were 11.27 and 14.99 cm respectively.

4.3.3 Percentage change in root length at the end of six weeks of growth of *C. odorata* in Aroclor and T/O treatments.

Changes in root length within the treatments deduced from the initial and final length of root and translated into percentages is presented in the Tables below.

4.3.3.1 Percentage change in root length at different concentrations of Aroclor and T/O treatments in S_1

The percentage change in root length at different concentrations of Aroclor and T/O treatments in S_1 is presented in Table 4.3.1 below.

Table **4.3.1**: Percentage change in root length at different concentrations of Aroclor and T/O treatments in S_1.

Treatments (ppm)	Percentage change in root length in S_1 treatments		
	100 ppm	**200 ppm**	**500 ppm**
T/O $_D$	0.30^a	0.08^a	-0.33^a
T/O $_S$	50.60^b	20.34^b	-0.24^a
T_1	78.28^d	59.13^c	56.12^b
T_2	67.65^c	64.93^c	63.73^b
C1	69.98^c	69.13^c	65.18^b
C3	77.22^d	67.25^c	66.61^c
C4	78.88^d	86.28^d	94.06^d

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Table 4.3.1 above, percentage change in root length at 100 ppm was highest in C4 (78.88), followed by T_1 (78.28). Percentage change in root length was least in T/O $_D$ (0.30), and T/O $_S$ (50.60). Values of T/O $_D$ and T/O $_S$ were significantly different from each other (P = 0.039; LSD = 13.72) as well as the control samples (P = 0.022; LSD = 9.11). At 200 ppm, highest value was recorded in C4 (86.28) and lowest value in T/O $_D$ (0.08). T/O $_D$ and T/O $_S$ were less than zero (-0.33 and -0.24) respectively, indicating a reduction in root length between planting and harvest at high concentration of 500 ppm an indication of actual reduction in root length compared to the value on the first day of contamination. The values of T_1, T_2, C1, C3 and C4 were equivalent with the percentage increases in 100 and 200 ppm treatments.

4.3.3.2 Percentage change in root length at different concentrations of Aroclor and T/O treatments in S_2

The percentage change in root length at different concentrations of Aroclor and T/O treatments in S_2 is presented in Table 4.3.2 below.

Table 4.3.2: Percentage change in root length at different concentrations of Aroclor and T/O treatments in S_2.

Treatments (ppm)	Percentage change in root length in S_2 samples		
	100 ppm	**200 ppm**	**500 ppm**
T/O D	0.15^a	-0.10^a	-0.22^a
T/O S	73.15^c	21.21^b	3.53^a
T1	62.71^b	54.10^c	38.37^b
T2	83.50^c	73.02^d	58.88^c
C1	71.07^c	78.57^d	79.00^d
C3	59.22^b	55.39^c	54.91^c
C4	92.11^c	81.87^d	88.75^d

Values with the same alphabets in superscript in the same column were not significant at 5% level. T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From Figure 4.3.2 above, the highest percentage change at 100 ppm, was at C4 (92.11), T_2 (83.50) and lowest at T/O $_D$ (0.15). At 200 ppm, it followed the same sequence as seen in 100 ppm, but the values were lower. In 500 ppm treatments, the highest value was lower than that of 200 ppm, but the lowest value was lower than that in 200 ppm and 100 ppm respectively. High percentage change in root length means that there was a significant increase ($P > 0.05$) in root length while low percentage change is an indication of poor growth. Relatively, the values of the percentage increase in S_2 were equivalent at the Aroclor treated samples as well as in treated controls.

4.4 Results of GC-MS analysis of PCB recovery from plants and soil samples after six weeks of growth of *C. odorata* in different concentration of Aroclor and T/O treated soil

PCB recovery from sampled plant tissues as well as the residual soil extracts analyzed and quantified using GC-MS is reported and explained below.

4.4.1 PCB recovery

Result of the GC-MS analysis and quantification of PCB recovery is presented in Table 4.4 below. The result encompasses initial soil PCB concentration (total PCB) and the final concentration after planted with *C. odorata* for six weeks.

Table 4.4: PCB recovery results: final soil PCB concentration, total PCB concentration, percentage PCB absorbed, percentage change in PCB, and PCB concentration factor

Treatments	Initial soil PCB conc. (ppm)	Final soil PCB conc. (ppm)	Total PCB in plants tissue (µg)	% PCB absorbed	% change in PCB	PCB concentration factor (PCB-RF)
T_1	100	97.9	6.40	6.4	2.1	0.022
	200	197.0	11.70	5.9	1.5	0.032
	500	494.5	55.80	11.2	1.1	0.065
	C 1	BD	--	--	--	--
	C 2-200	196.2	NP	NP	1.9	NP
	C 3-500	495	28.20	5.6	1.0	0.038
	C 4-500	495.5	3.10	0.62	0.9	0.006
T_2	100	98.2	33.90	33.9	1.8	0.38
	200	197.2	25.00	17.5	1.4	0.012
	500	494	31.70	6.34	4.2	0.083
	C 1	BD	--	--	--	--
	C 2-200	196.6	NP	NP	1.7	NP
	C 3-500	496.5	64.60	12.92	0.7	0.096
	C 4-500	500	58.00	11.60	0.01	0.095
T/O $_D$	100	BC	--	--	--	--
	200	BC	--	--	--	--
	500	99.6	--	--	0.4	--
	C1	NP	--	--	0	--
	C2-100	100	NP	NP	0	NP
	C3-100	99.5	--	--	0.5	--
	C4-100	99.7	--	--	0.3	--

Bioremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

T/O$_S$	100	BD	--	--	--	--
	100	99.7	--	--	0.32	--
	100	99.1	--	--	0.87	--
	C 1	--	--	--	--	--
	C 2-100	99.1	NP	NP	0.90	NP
	C 3-100	99.1	--	--	0.88	--
	C 4-100	98.9	--	---	1.08	--

Conc.=Concentration, BD=Below detection, NP=Not planted, RF=Remediation factor, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4

From GC-MS results of PCB recovery in Table 4.4 above, mean total PCB concentration in plant tissues of T_1 were 6.4, 11.7 and 55.8 µg for 100, 200 and 500 ppm treatments respectively. The controls C3 and C4 had 28.20 and 3.10 µg in the plants tissue, while C1 and C2 were zero. Final soil PCB concentrations were measured to be 97.3, 197.0, and 494.5 ppm for 100, 200, and 500 ppm treatment concentrations respectively. At same time, 196.2, 495 and 495.5 ppm were the residual concentrations for C2, C3, and C4 respectively. In T_2, total PCB concentrations in the tissues were higher in form: 33.90, 35.0, and 31.7 µg for the respective contaminant concentrations of 100, 200, and 500 ppm. C2 and C4 were 64.60 and 58.0 µg respectively. Final PCB concentrations in the soil of T_2 treatment were 98.2, 197.2, 494, 196.6, 494.5 and 500 ppm for 100, 200, 500 ppm initial concentrations, C2, C3, and C4 respectively. In T/O $_S$, final soil PCB concentration at 100 ppm was surprisingly analyzed beyond detection. But the rest treatments were 99.7, 99.1, 99.1, 99.1 and 98.9 ppm for 200, 500 ppm, C2, C3 and C4 treatments respectively.

PCB concentration factors (PCB-CF) were higher in T_2 (0.38, 0.012 and 0.083), compared to T_1 (0.022, 0.032, and 0.065), but alternated at 200 ppm treatment where PCB-CF of T_1 became higher than that of T_2.

4.4.2 Effect of initial concentration of PCB to phytoremediation ability of *C. odorata*.

The relationship between initial concentration of PCB and ability of *C. odorata* to remediate PCB treated soil is presented in Figure 4.5 and 5.6 below.
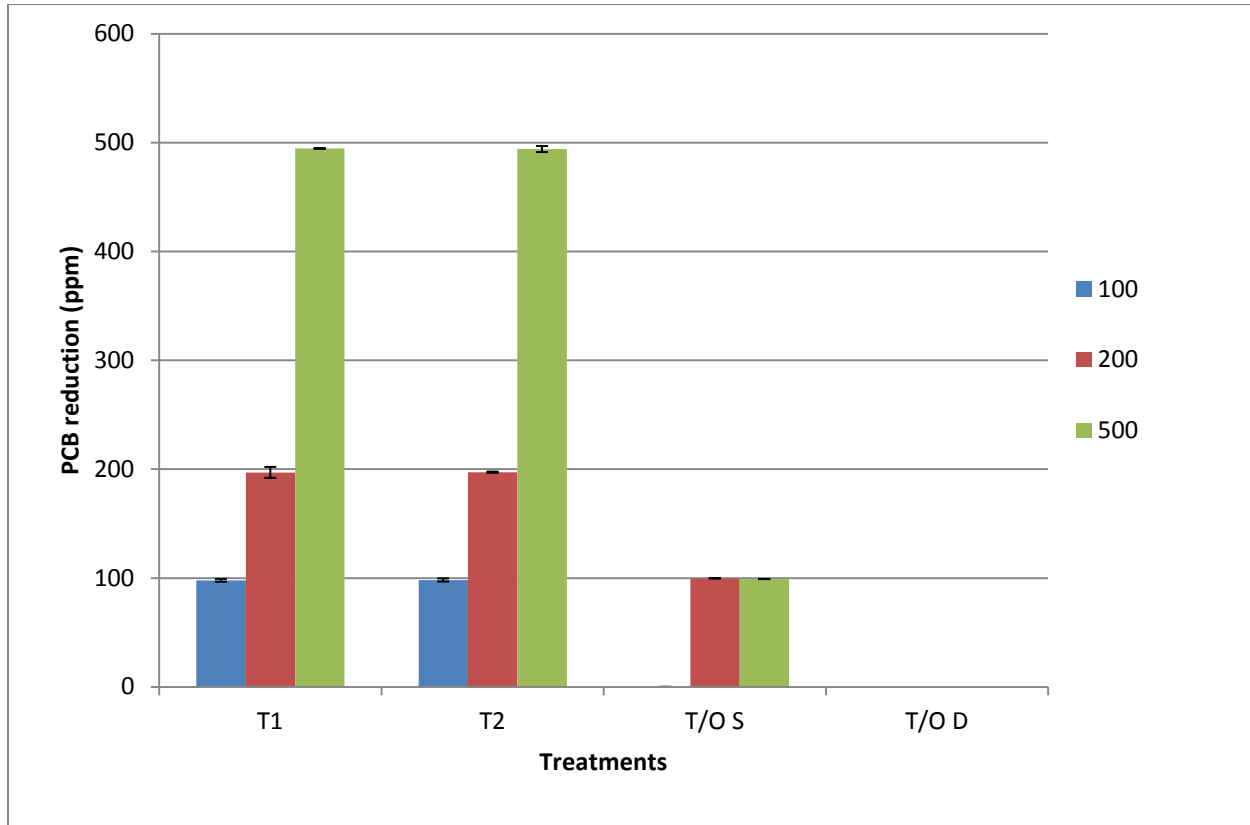
Figure 4.5: Relationships between initial PCB concentration and reduction of PCB *by C. odorata.* (Values are means of three replicates), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4
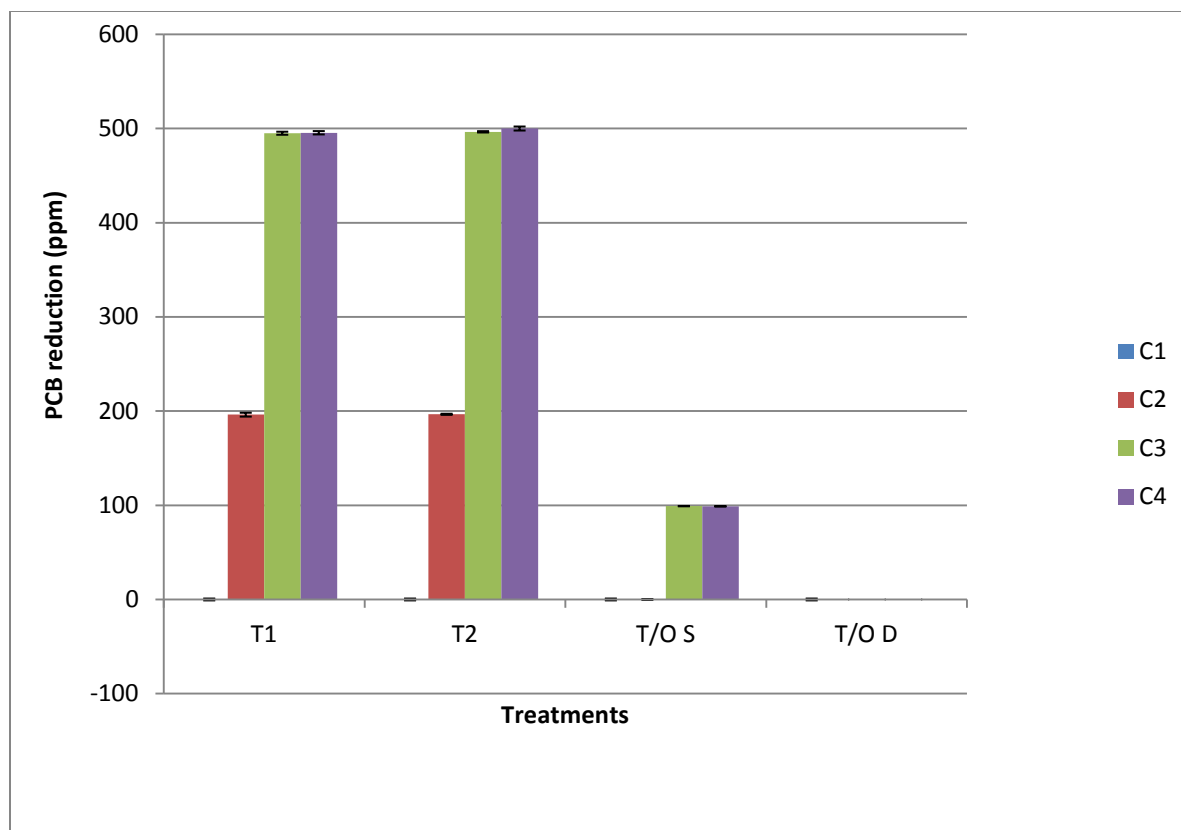
Figure 4.6: Relationships between initial PCB concentration and reduction of PCB *by C. odorata* in treated control soil samples. (Values are means of three replicates), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T$_1$=1254, T$_2$=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

Inferring from Figures above, at 100 and 200 ppm, T$_2$ (98.2/197.2 ppm) was greater than T$_1$ (97.9/197 ppm), but when the treatment concentration was increased to 500 ppm, T$_1$ became higher than T$_2$ (494.5 and 494 ppm) respectively. However, T/O $_S$ was zero at 100 ppm, but 99.7 and 99.1 at 200 and 500 ppm respectively while T/O $_D$ was zero at all concentration. In treated control samples final PCB concentration in T$_2$ were 196.6 and 496.5 ppm for C2, and C3 respectively, which was higher in the entire control treatments than T$_1$ (196.2 and 495 ppm) for C2 and C3 respectively. C4 in both T$_1$ and T$_2$ has the same concentration which is equal to the initial concentration (500 ppm). Final concentration of PCB at T/O $_S$ was greater in C3 than in C4 irrespective of the fact that both treatments had equal concentration (100 ppm) of PCB initially. No detection of PCB was made at the entire control treatments at T/O $_D$ even though the treatments have equal initial PCB concentration as T/O $_S$. From this results, it can be deduced that in Aroclor 1254, increase in initial concentration leads to relative reduction of PCB. However, the

reverse was the case in Aroclor 1260 where increase in initial concentration causes a synonymous increase in relative reduction. That is to say that at lower concentration of Aroclor 1254, PCB reduction was higher than the value at an increased concentration. But increase in concentration of Aroclor 1260 causes an increase in PCB reduction.

4.4.3 Relationships between initial soil PCB concentration and percentage of PCB absorbed by *C. odorata* plant.

The relationships between initial soil PCB concentration and the percentage of PCB absorbed by *C. odorata* are presented in Figures below.
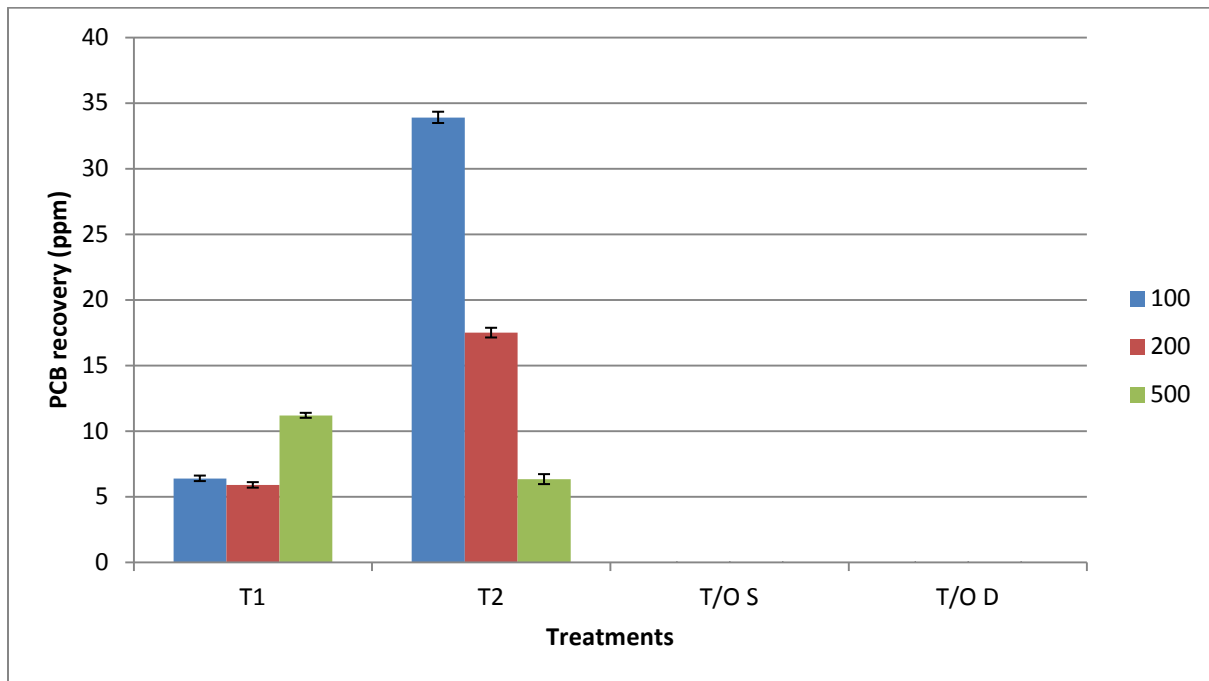
Figure 4.7: The relationship between initial soil PCB concentration and Percentage of PCB absorbed by plants. (Values are means of three replicates), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4
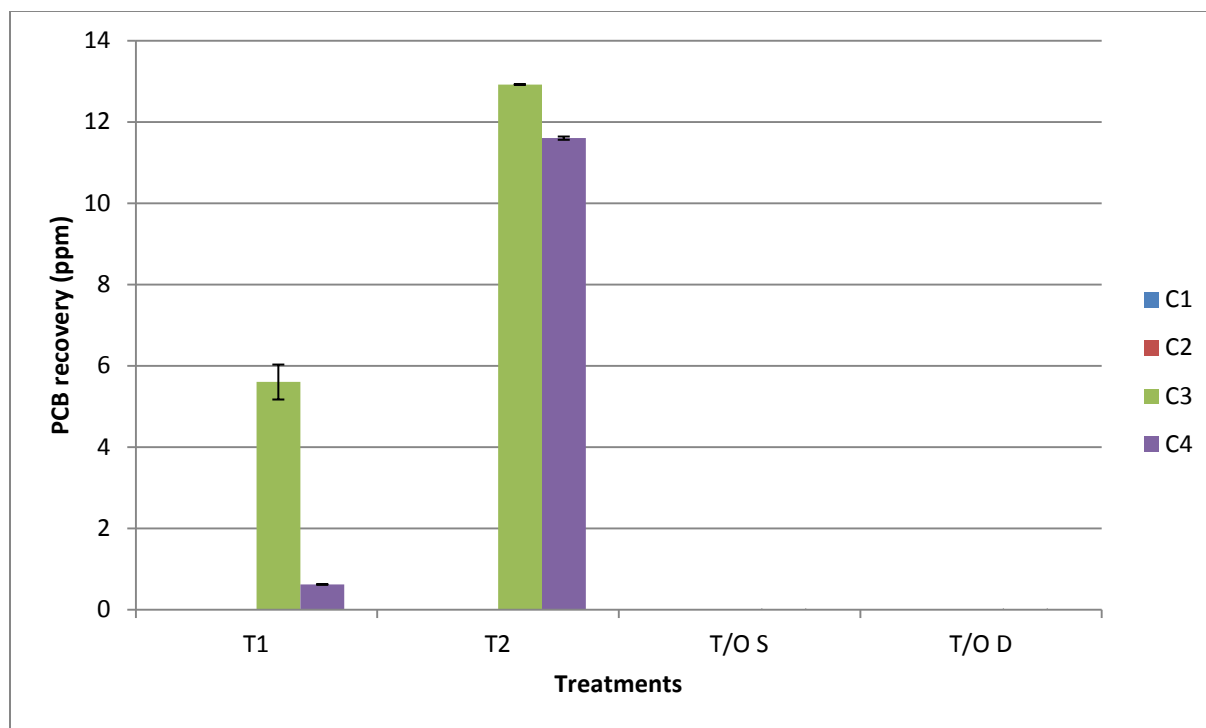
Figure 4.8: The relationships between the initial soil PCB concentrations and Percentage of PCB absorbed in treated control samples (Values are means of three replicates), T/O_D=Direct transformer oil, T/O_S=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

From Figures above, at 100 ppm, percentage of PCB absorbed by *C. odorata* was 6.4 in T_1, 33.9 in T_2, zero in T/O_S and T/O_D respectively. But at 200 ppm, the percentage dropped to 5.9 in T_1, 17.5 in T_2, while T/O_S and T/O_D were still zero. However, at 500 ppm, the percentage absorbed PCB in T_1 was higher than that of T_2 (11.2 and 6.35) respectively, T/O_S and T/O_D were zero (Figure 4.7 above explains). With the treated controls also, the percentage of PCB absorbed at T_1 in C3 and C4 were 5.6 and 0.62 respectively, but percentage absorption increased at T_2 with C3 and C4 having 12.92 and 11.60 respectively. C1 and C2 were zero at T_1 and T_2 even with the initial concentration of 200 ppm of T_1 and T_2 respectively. PCBs were not recovered in the entire control samples at T/O treatments irrespective of the concentration of the oil although the oil was amended with initial 100 ppm of PCB (See Figure 4.8 above).

4.4.4 PCB recovery in plant tissues of Aroclor and T/O treatments.

The concentration of PCB in different plant tissues among different concentrations of Aroclor and T/O are represented in Table 4.5 below.

Treatments	Init. Soil PCB (µg/g)	Residual Soil PCB (µg/g)	Root Dry weight biom. (g)	Root Absd. Root PCB (µg/g)	Root Total root PCB (µg)	Stem Dry weight biom. (g)	Stem Absd. Stem PCB (µg/g)	Stem Total stem PCB (µg)	Leaf Dry weight biom. (g)	Leaf Absd. Leaf PCB (µg/g)	Leaf Total leaf PCB (µg)	Total Total plant biom. (g)	Total Total plant PCB (µg)	TLF	RF / BAF
T_1 100	100	97.9 ±0.56	7.25 ±0.25	0.22 ±0.10	1.6	10.87 ±1.07	BC	0	10.93 ±0.15	ND	0	29.05	6.4	N/A	0.022
T_1 200	200	197 ±1.90	3.37 ±0.12	0.52 ±0.13	1.75	11.02 ±0.47	ND	0	3.55 ±0.18	0.13 ±0.03	0.46	17.94	11.7	0.25	0.032
T_1 500	500	494.5 ±1.63	6.61 ±0.27	2.70 ±0.60	17.85	7.43 ±0.31	0.26 ±0.07	1.93	3.09 ±0.09	0.30 ±0.06	0.93	17.13	55.8	0.21	0.065
T_1 C1	0	BC	3.16 ±0.12	ND	0	3.55 ±0.17	ND	0	4.03 ±0.09	BC	0	10.74	N/A	N/A	N/A
T_1C2	200	199.2 ±0.67	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T_1C3	500	495 ±0.39	4.72 ±0.28	1.45 ±0.32	6.84	7.82 ±0.29	0.46 ±0.34	3.6	2.21 ±0.15	ND	0	14.75	28.2	0.32	0.038
T_1C4	500	495.5 ±0.61	3.72 ±0.27	0.07 ±0.03	0.26	5.29 ±0.20	ND	0	2.11 ±0.14	0.21 ±0.07	0.44	11.12	3.1	0.32	0.006
T_2 100	100	98.2 ±0.31	5.91 ±0.09	1.16 ±0.13	6.86	9.39 ±0.23	0.37 ±0.04	3.47	6.83 ±0.48	ND	0	22.13	33.9	0.32	0.38
T_2 100	200	197.2 ±0.12	4.45 ±0.81	1.51 ±0.08	6.72	5.98 ±1.48	0.91 ±0.05	5.44	4.43 ±0.81	0.19 ±0.03	0.84	14.84	35	0.73	0.012
T_2 100	500	494 ±0.19	3.09 ±0.10	2.81 ±0.32	8.68	1.57 ±0.12	1.35 ±0.14	2.12	2.97 ±0.08	ND	0	7.63	31.7	0.48	0.083
T_2 C1	0	BC	8.32 ±0.15	BC	0	10.97 ±0.30	BC	0	3.90 ±0.17	BC	0	23.19	N/A	N/A	N/A
T_2 C2	200	199.6 ±0.21	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T_2 C3	500	494.5 ±0.39	2.82 ±0.17	3.42 ±0.27	9.64	7.59 ±0.11	1.38 ±0.10	10.47	3.05 ±0.09	ND	0	13.46	64.6	0.43	0.096
T_2 C4	500	500 ±0.07	3.54 ±0.17	3.04 ±0.25	10.76	6.21 ±0.32	1.71 ±0.08	10.62	2.49 ±0.20	ND	0	12.24	58	0.56	0.095
T/O_D 100	100	BC	1.04 ±0.12	BC	0	2.92 ±0.10	BC	0	2.30 ±0.13	BC	0	6.26	N/A	N/A	N/A

T/O $_D$ 200	100	BC	0.34 ±0.07	BC	0	2.65 ±0.08	BC	0	2.02 ±0.19	BC	0	5.01	N/A	N/A	N/A
T/O $_D$ 500	100	99.6 ±0.12	0.20 ±0.05	BC	0	1.42 ±0.34	BC	0	1.12 ±0.25	BC	0	2.74	N/A	N/A	N/A
T/O $_D$ C1	0	0	1.83 ±0.36	BC	0	3.72 ±0.25	BC	0	3.64 ±0.06	BC	0	9.19	N/A	N/A	N/A
T/O $_D$ C2	100	100 ±0.00	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T/O $_D$ C3	100	99.5 ±0.21	0.26 ±0.04	BC	0	1.64 ±0.32	BC	0	1.42 ±0.06	BC	0	3.32	N/A	N/A	N/A
T/O $_D$ C4	100	99.7 ±0.07	0.19 ±0.06	BC	0	1.50 ±0.11	BC	0	1.34 ±0.05	BC	0	3.03	N/A	N/A	N/A
T/0 $_S$ 100	100	BC	6.24 ±1.24	0.10 ±0.07	0.62	3.07 ±0.08	BC	0	1.64 ±0.05	BC	0	10.95	N/A	N/A	N/A
T/0 $_S$ 200	100	99.7 ±0.14	4.77 ±0.20	0.11 ±0.07	0.52	2.36 ±0.35	BC	0	1.27 ±0.20	BC	0	8.40	N/A	N/A	N/A
T/0 $_S$ 500	100	99.1 ±0.07	0.98 ±0.10	ND	0	1.41 ±0.02	ND	0	0.81 ±0.06	ND	0	3.20	N/A	N/A	N/A
T/0 $_S$ C1	0	BC	6.40 ±0.56	BC	0	3.83 ±0.31	BC	0	1.83 ±0.08	BC	0	12.06	N/A	N/A	N/A
T/0 $_S$ C2	100	99.1 ±0.24	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
T/0 $_S$ C3	500	99.1 ±0.31	1.88 ±0.24	ND	0	1.54 ±0.04	ND	0	0.77 ±0.09	ND	0	4.19	N/A	N/A	N/A
T/0 $_S$ C4	100	98.9 ±0.32	1.22 ±0.09	ND	0	1.30 ±0.02	0.09 ±0.03	0.12	0.63 ±0.06	ND	0	3.15	N/A	N/A	N/A

Init=Initial, Absd=Absorbed, biom=Biomass, TLF=Translocation factor, RF/BAF=Remediation factor/Bioaccumulation factor, T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C3=Control 3, C4=Control 4, BC=Below calibration, N/A=Not applicable, ND=Not detected, NP=Not planted.

Bioremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

From Table 4.5 above, it is evident that absorption of PCB occurred mostly at the root tissues of the plant. For example, in T_1 at 100 ppm, the concentration of PCB in the root was found to be 0.22 ppm with a total root biomass of 7.25 g, resulting to a total root PCB of 1.6 µg. Although the stem and leaf biomass were above 10 g respectively which was higher than what is obtained in the root, the total PCB in both the stem and leaf was still insignificant compared to what was recovered in the below ground tissues. However, total PCB in plants tissues of T_2 was relatively higher than that obtained in T_1 although highest recovery was obtained at the root of T_1 at 500 ppm treatment (17.85 µg). No PCB recovery was obtained at the untreated control (C1) at both T_1 and T_2 respectively. There was no PCB detected in the T/O $_D$ plant tissues but sparing reduction occurred in the soil PCB concentration, this could be attributed to natural attenuation. In T/O $_S$ treatments, PCBs was found only at the root tissue at the 100 and 200 ppm treatments (0.62 and 0.52 µg) respectively. There was no recovery at the above ground tissues of the T/O $_S$ treatments.

4.4.5 Relationship between initial soil PCB and final concentrations of specific pollutants after six weeks of treatment with *C. odorata*.

Effects of initial concentrations of specific Aroclor and T/O in soil to their final concentrations after treatments with *C. odorata* were evaluated and are presented in the figures below.

4.4.5.1 Relationship between initial and final soil PCB concentrations in T_1 (1254)

The relationship between the initial and final soil PCB concentrations of treatments in T_1 is presented in Figure 4.9 below.
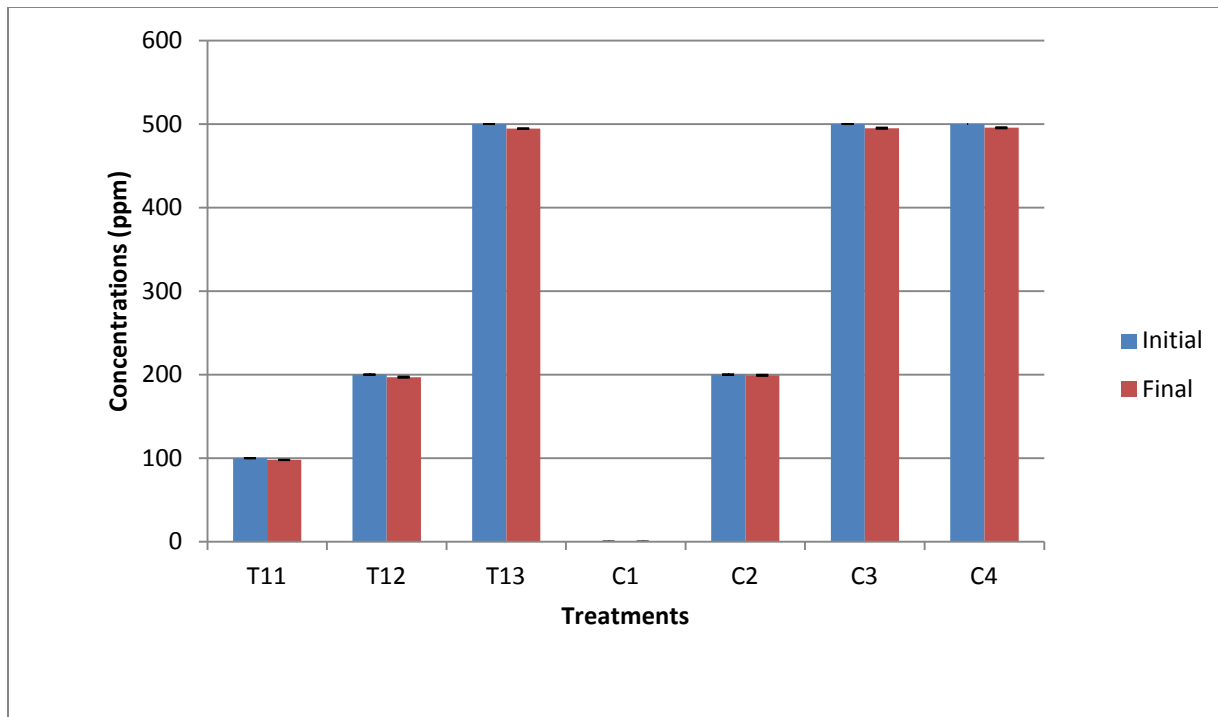
Figure 4.9: Relationships between the initial and final soil PCB concentrations at T_1 (values represents the mean of three replicates), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

From Figure 4.9 above, the highest reduction in PCB concentration was in the 500 ppm concentrations as observed in $T_1$3, C3 and C4 where there was reduction of PCB of about 5.5, 5.0 and 4.5 ppm respectively. Lower initial concentrations of 100 and 200 ppm had reductions of 2.1 and 3.0 ppm respectively. However, the control sample without plant (C2) only reduced by 0.8.

4.4.5.2 Relationship between initial and final soil PCB concentrations in T_2 (1260)

The relationship between the initial and final soil PCB concentration of treatments in T_2 is presented in Figure 4.10 below.
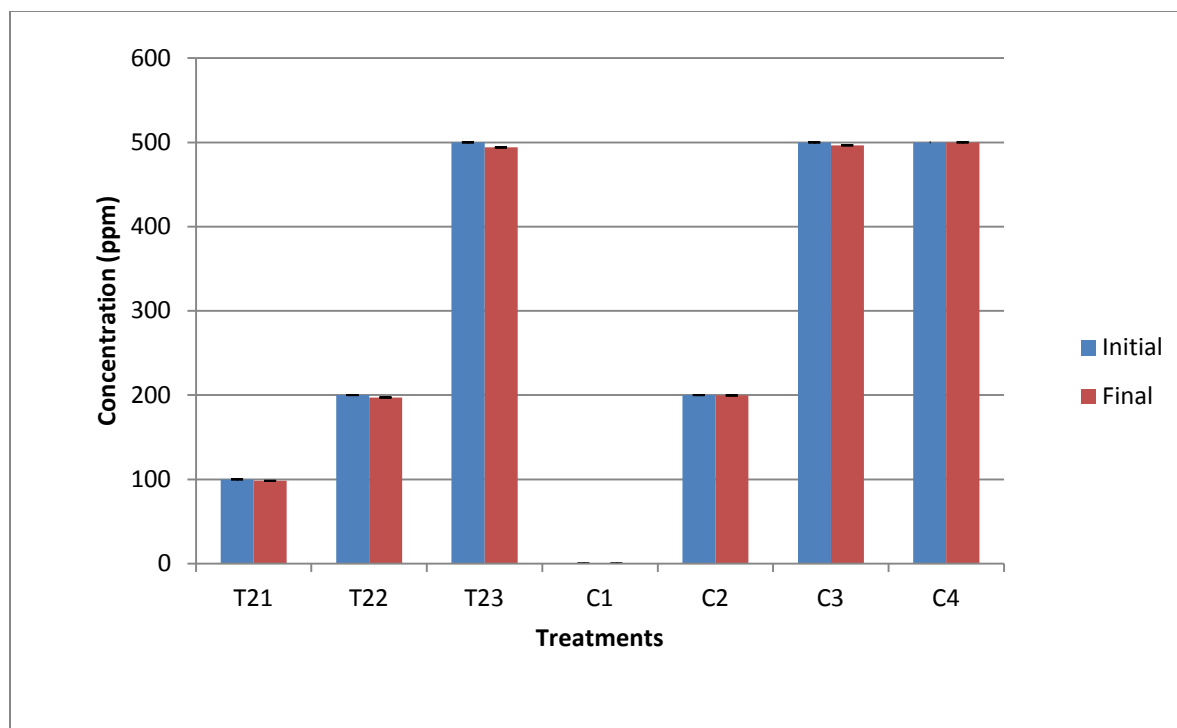
Figure 4.10: Relationships between the initial and residual soil PCB concentration at T_2 (values represents the mean of three replicates), T/O $_D$=Direct transformer oil, T/O $_S$=Suzuki transformer oil, T_1=1254, T_2=1260, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

From Figure 4.10 above, the same variation of PCB reduction as seen in T_1 was observed but the rate was lower in T_2. 1.8, 2.8 and 6.0 ppm of PCB were reduced at 100, 200 and 500 ppm concentration of T_2 respectively. 0.4 and 3.5 ppm were the reduction in soil PCB of C2 and C3 respectively. Residual soil concentration of C4 remain unchanged even with the absorption of traces of PCB in the plants tissue, this could be that such absorption was not enough to cause an appreciable reduction in the soil concentration.

4.4.5.3 Relationship between initial and final PCB concentrations in T/O $_D$ treatments.

The relationship between the initial and final soil PCB concentration in the T/O $_D$ treatments is presented in Figure 4.11 below.

Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*
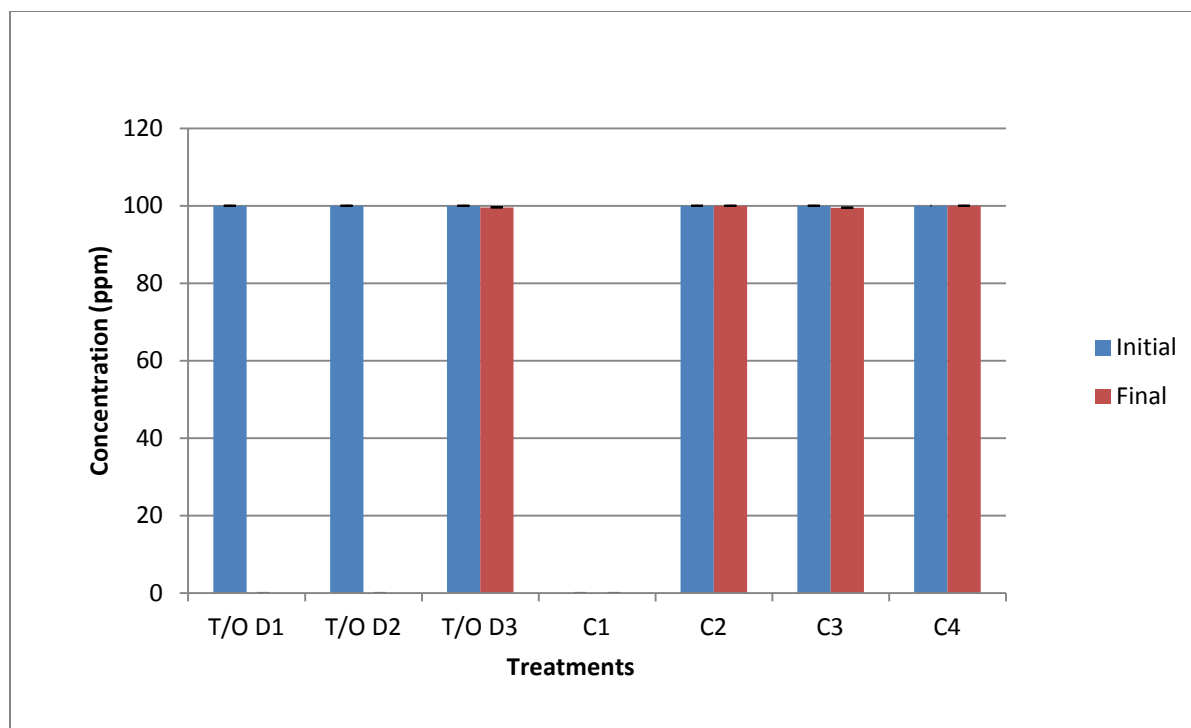
Figure 4.11: Relationships between the initial and final soil PCB concentration at T/O $_D$ (values represent the mean of three replicates), T/O $_{D1}$=Direct transformer oil 1, T/O $_{D2}$=Direct transformer oil 2, T/O $_{D3}$=Direct transformer oil 3, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

From Figure 4.11 above, the residual concentration of the soil PCB in the co-contaminated samples with transformer oil were minimal in the entire treatments. At 100 ppm T/O (T/O $_{D1}$) and 200 ppm T/O (T/O $_{D2}$), no PCB was detected, at 500 ppm T/O (T/O $_{D3}$) however, final PCB concentration of 99.6 ppm was detected showing a reduction of 0.4 ppm from the initial concentration. There was 0.5 and 0.0 ppm reduction of PCB from the initial concentrations at C3 and C4 respectively. C2 showed no reduction in PCB concentration from the initial. This result showed a PCB reduction of less than one (< 1) from the initial soil PCB an indication that T/O has adverse effect to the plants survival that would have aided in absorption of a proportionate quantity of PCB.

Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

4.4.5.4 Relationship between initial and final PCB concentrations in T/O $_S$ treatments.

The relationship between the initial and final PCB concentration in the T/O $_S$ treatments is presented in Figure 4.12 below.
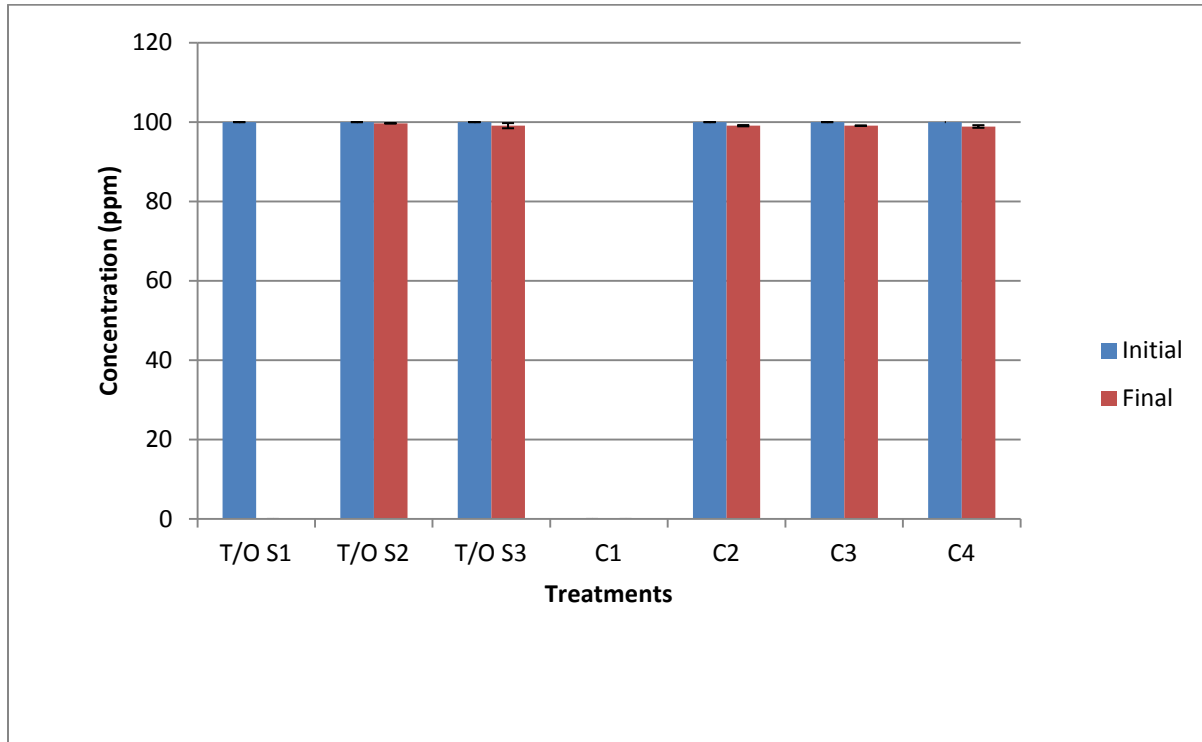
Figure 4.12: Relationships between the initial and residual soil PCB concentration at T/O $_S$ (values represent the mean of three replicates), T/O $_{S1}$=Suzuki transformer oil 1, T/O $_{S2}$=Suzuki transformer oil 2, T/O $_{S3}$=Suzuki transformer oil 3, C1=Control 1, C2=Control 2, C3=Control 3, C4=Control 4

From Figure 4.12 above, final soil PCB concentration in T/O $_S$ was greater than what was obtained in T/O $_D$ treatments, the reason being that the soil culture method adopted slightly reduced the adversity of T/O to plants growth hence sparse plants growth was ensured which brought about the slight absorption of PCB by the plants as seen in Table 4.7 above. Reduction from the initial soil PCB concentration was 0.0, 0.3 and 0.7 ppm at 100, 200 and 500 ppm initial soil T/O concentrations respectively. Reductions were also 0.9, 0.9 and 1.1 at C2, C3 and C4 respectively.

Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

CHAPTER FIVE

Discussion

5.1 Effects of different concentrations of Aroclor and Transformer oil on growth of *C. odorata*.

The result of the effect of different concentrations of Aroclor and Aroclor co-contaminated in transformer oil showed a percentage growth reduction on *C. odorata* plants as the concentration of the contaminant is increased. In T/O $_D$ treated samples, the percentage increase in length of the plant were relatively low. The value decreases as the concentration of T/O was increased, hence reduced to a value less than zero in the 500 ppm contamination (-1.03). This means that the phytotoxicity of T/O to *C. odorata* was lethal at high concentrations hence the plant could not survive the duration of the experiment. The relative effects of 200 ppm of treatments was lower to *C. odorata* than the corresponding values in 100 ppm, while it was higher compared to the corresponding 500 ppm treatments. Olusola and Anslem (2010), reported on the death of plants grown on crude oil polluted soil two weeks after pollution compared to the luxuriant growth of the same plant in unpolluted control soil after the same time interval. Nwazue, (2011) and Agbogidi (2011) also reported on the negative interaction observed between crude oil level and weight gained in plant seedling. This could be explained from the inhibitive effect of oil on the nutrient content as well as the physico-chemical parameters of soil. The plants in T/O $_S$ maintained a somewhat increased percentage growth in the entire treatment concentrations than T/O $_D$ with significant difference in the growth in each concentration at p = 0.05. There is much sense in arguing that the condition under which T/O $_S$ experiment was carried out was favorable to the plants than in T/O $_D$ (refer to 3.4.1(2)). Since the condition under which the plants were grown in T/O S were favourable to the attainment of result, it therefore encouraged in other phytoremediation stidy involving oil. T$_1$ and T$_2$ plant samples had relatively high percentage growth compared to the T/O samples with values significantly different (P < 0.05) from that of the oil amended sample. T$_1$ and T$_2$ values were not significant with each other (P > 0.05) and were also similar to the percentage growth trend in C1 plant samples where the soil was not

treated. However, plants in untreated control had 43% growth rate which was high compared to the treated samples but, maintained the same trend with the 100 ppm of T_1. This evidently implies that only the treatment with 500 ppm of PCB concentration has inhibitive effects to the growth of *C. odorata,* such inhibition was not pronounced as it only began to impact on the plants from the sixth week of contamination. Thus may have been caused by the depletion of the nutrient in the soil as organic pollution has been reported to be synonymous with the reduction of soil nutrients (Minai-Tehrani, 2008). In PCB co-contaminated in transformer oil, growth inhibition to *C. odorata* was evident in all the concentrations (100, 200 and 500 ppm) respectively, this was shown by the change in vigor of the plants at the first week of growth in the treated soil. Relative results were reported by previous studies with other plant species (Merkl *et al*., 2004; Low *et al*., 2010; Iwata *et al*., 1974). Presence of oil in soil reduces the nutrient content of the soil limiting their availability for absorption by plants (Winter *et al*., 1976; Wang and Lau, 1985; Gill *et al*., 1992; Merkl *at al*., 2004; Minai-Tehrani, 2008; Edema *et al*., 2009; 2011). Percentage growth rate was higher in S_1 than S_2 mostly in 100 and 200 ppm of Aroclor and T/O respectively, but was sporadic in the 500 ppm of Aroclor and T/O respectively. High concentration of oil in soil have been reported to recede the growth of plants as well as in biomass accumulation as the later is known to impact on plant yield, this is one of the properties of any phytoremediation plant (Dominguez-Rosado *et al*., 2004). *Chromolaena odorata* responded the same way to the inhibition of oil as reported in other study which was even lethal at high concentration (Doty, 2008; Muratova *et al*., 2009; Atagana, 2011b). The plant survived the lower concentration (100 and 200 ppm) of PCB in the soil, but at 500 ppm concentration, *C. odorata* responded to the inhibitive effect of the pollutant evident at the sixth week of the experiment. Since *C. odorata* could withstand the inhibition of PCB as it could for other pollutants (Singh *et al*., 2009; Tanhan *et al*., 2011; Atagana, 2011a/b), it then present the plant as a good candidate for the remediation of PCB-contaminated soil.

5.2 Effect of different concentrations of Aroclor and Transformer oil on change in plants biomass.

Presence of water in plants signifies presence of nutrient and these aids plants growth and repleshment (De Rough, 1991). High change in biomass from wet to dry is an indication of high water content in plants, which signifies growth of such plant in a growth supportive environment. Therefore, increased change in biomass is a good indicator for plants phytoremediation ability; hence *C. odorata* displayed such good phytoextraction ability in Aroclor treatments. Meanwhile, the presence of organic pollutants in soil is known to cause a lot of adversities to plants, a good example being that when a plant is growing in an organic contaminated soil, transpilation pull is reduced by the closure of stomatal wall reducing evaporation of water from the plants (Tanhan *et al*., 2011). However, cumulative water use seems to be more sensitive than plant growth to PCB. This indirectly means that the effects on plant growth by pollutants may be indirectly related to its water use and may reflect on transpiration (Strek and Weber, 1982). This was shown in this study by the effect of treatment concentrations on the change in plants biomass from weight to dry infering a significant difference ($P < 0.05$) between T/O treatments (T/O $_D$ and T/O $_S$). At a concentration of 100 ppm of T/O, mean percentage change in biomass of *C. odorata* was 41 and 63 % in T/O $_D$ and T/O $_S$ respectively. This difference in biomass change was lower as the concentration of the oil is increased and such decrease in percentage biomass change at increase in concentration was very proportionate. The values obtained in biomass measurement was synonymous with what was reported by Atagana, (2011b) in a study using *C. odorata* to decontaminate used engine oil in soil under greenhouse conditions. This however explains the fact that the plants ability to thrive in oil contaminated soil decreases as the concentration of the oil increases, evidence of inhibition of plants growth as well as biomass accumulation as was reported by past studies (Udo and Fayemi, 1975; Xu and Johnson, 1995; Hosler, 1999; Merkl *et al*., 2004; Diab, 2008; Muratova *et al*., 2009; Atagana, 2011b). In Aroclor treated samples, percentage change in biomass at T_1 and T_2 was not significant from each other ($P > 0.05$) although a decrease in their value was observed as the concentration was increased from 100 to 200 to 500 ppm; percentage biomass changes were 83 and 82 % respectively in 100 ppm treatment, 81%

in the 200 ppm and dropped to 74 and 73 % respectively in the 500 ppm treated samples. This reduced trend though was not significant (P > 0.05), but agrees with the report of Aslund *et al*, (2007), on a study of the phytoremediation abilities of three selected plants on Aroclor contaminated soils. The study reported that phytoremediation appeared to be enhanced at lower concentration of PCBs supporting the work of Zeeb *et al*, (2006). Control 1 in this study maintains an absolute high percentage biomass change (89) while Control 3 and 4 maintained almost equivalent values like the Aroclor treated samples. The two soil samples had almost equal proportion of values as seen in Figures 4.1.1-4.2.3 and Tables 4.1.1 and 4.1.2 in chapter 4. Percentage biomass change in T/O contaminated samples was significantly different from Aroclor treated samples (P < 0.05). Anoliefo (1991); Baker (1970); and De Jong (1980), have reported on the positive relationship between extent of reduction in growth of *C. odorata* and the concentrations of crude oil applied. This simply means that the effect of transformer oil negatively impacted on the ability of *C. odorata* to retain water, a reason behind the inability of the plant to survive the duration of the experiment. It also explains why PCB was not detected in T/O treated plants. This means that the lethality of transformer oil especially on the Transformer oil direct treated plants samples (T/O $_D$) was not out of place, but a clear indication of the inhibitive effect of oil to plants. However, there is room for improvement as was shown by the the Suzuki amended method of treatment. Since *C. odorata* could survive the T/O $_S$ treatments and was able to recover traces of PCB in its root, and then there is possibility of exploiting such method for more effective remediation of soil PCB co-contaminated in transformer oil. It could also be employed in the remediation of soil polluted by other other oil containing pollutants for example PAHs for effective results.

5.3 Effects of different concentrations of PCB and Transformer oil on mature leaves per plant (MLPP).

Mature leaves in each plants per treatment in the greenhouse study was done by manual counting of the number of live leaves at each given time. Multiplication of leaves by plants in a particular habitat is an indication of luxiriant growth. The leaf is one of the most important parts of the plant because of the

functions that plants use it to perform. It contains various features that enable them to perform such functions like the external waxy cuticle used mostly for photosynthesis, the epidermal cell, epidermis, vascular tissues, mesophyl and the chloroplast. All these add together to make up the leaf of a plant. An important function of the leave in a plant is production of food for plant through the energy it gets from sunlight; this is known as photosynthesis (www.emc.mericiopa.edu). Photosynthesis is carried out in the chloroplast of the leave, giving the plants leave it's usually green colour (chlorophyll). Leave of *C. odorata* varies in colour when it is growing in an environment that possesses growth supportive enabling nutrients. Leave colour could range from light to middle green colour (Luwum, 2002). The greener the colour of the leaf, the more supportive the nutrient are to the growth of the plants in a soil. *Chromolaena odorata* and its perculiar multi-stemmed nature with ovate leaves occuring in pairs within the stems possesses multiples leaves per plants most especially when it is growing in a favourable environment (Lowum, 2002). Low concentration of oil in this study at direct T/O treatment caused a slight increase in MLPP within the six weeks of experiment. But the inhibitive effect of T/O to soil as well as plants only allowed a decrease in leaf numbers at higher concentration of the oil, the reason why the leaves of *C. odorata* shrank towards the sixth week of study. Growth of plants is usually complimented by a synonymous increase in leaf number, but such phenomenon rescinds at adverse conditions. Both S_1 and S_2 used in the greenhouse maintained a somewhat similar trends in the numbers of their individual mature leaves but, they have their perculiarities as a result of the physical characteristics of the two soil types.

Soil are known to behave different to plant it habours although *C. odorata* is been reported to be a verssatile plants in terms of its nutrient requirement (Barman *et al*., 2000). Hence the differences in leaf numbers as well as in colour is not unconnected with the difference in soil types even when equal measure were applied to soil to support equal growth in tte entire treatment.The results obtained especially in samples co-contaminated with T/O therefore is in aggreement with the fact that exposure of plants to a concentration higher than what it can tolerate may cause chlorosis of the leaves, plants dehydration, stunted growth and also death (Udo and Fayemi, 1975; Xu and Johnson, 1995; Merkl *et al*.,

2004). Strek and Weber (1982), reported about 47% inhibition of soybean by 1000 ppm of Aroclor 1254 resulting to the molten of the leaves, this was a confirmation to the earlier report of Weber and Mrozek (1979) on the toxicity of the plant at high concentration of PCB. According to Sinclair *et al*, (1977) also, inhibition of plants by PCB is as a result of its inhibitive effect to oxygen evolution. The whole precentage increase in mature leaves per plant in a specific plant sample among the two soil types were almost not significant ($P \leq 0.05$) within each other but, for certain occassions as can be found in Table 4.2.3.1-2 and Figures 4.3.1-4.3.2. It can therefore be adduced that measuring the leaf conditions of plant in phytoremediation studies in an organic contaminated environment could be used as a sure way to determining the adversity of the contaminant to the plant. Two soil samples used in the greenhouse study maintained the same trend in leaf number within different contamination of the contaminant just that in S_2, changes in colour as a result of inhibition of the contaminants occurred faster in its samples. That was not out of place because soil 2 was described as sandy-loam containing larger pores which would enable easier movement of solutes in the soil. Therefore, plants in higher concentrated contamination in S_2 were expected to dry up must faster than S_1 counterparts. Biotic factors for example soil and rainfall difference, usually do not affect the distribution of *C. odorata* (Caldwel, 2000; Delfino, 2002; Majam, 2002). This means that much relativity was expected in the plants grown among the two soil types used in this greenhouse experiment, hence either of the two soil types could be used in this kind of study.

5.4 Effect of different concentrations of Aroclor and Transformer oil to the increase in root length of *C. odorata*.

When a plant triumphs in its aerial growth, it is complemented by an equal synonymous elongation and multiplication of the root system. Therefore increased root length is an indication of an optimal growth of plant in a given habitat. Root length of *C. odorata* in the entire greenhouse study which was measured on the day of harvest by measuring the percentage change in root length from the day of contamination was a good indicator to the physiological characteristics of the plant. In this study, percentage change in roots of *C. odorata* in T/O $_D$ treatment was low which progressively decreases as the concentration of the oil was

increased. In the T/O $_S$ samples, the values were relatively higher than in T/O $_D$ but, still maintained the same trend along higher concentration of the treatments, as seen in T/O $_D$ treated sample value of less than zero. In S_2 of T/O $_S$, the percentage change in root length were all values greater than one but, have percentage values less than zero as the concentration increases. The values of the percentage change in root length within the Aroclor amended soil samples were relatively higher than those in the T/O amended soil. Hence values of Aroclor treated samples were significantly different from the values of the T/O treated samples (P < 0.05). According to the study of Wiltse *et al*, (1998), increased root biomass which were as a result of root length increase leading to increased surface area of the root, lead to an increase in rhizosphere volume. This however means that root biomass is also important indicator in organic contaminant remediation process (Brandt, 2002). Reduced root length resulting to low biomass increase of the root could lead to reduced rhizosphere volume and thus will have impact in the root surface of the plant towards the contaminants. Increased shoot biomass was suggested by Ficko *et al*, (2010), for optimization of phyoremediation of PCBs which synonymously increased the amount of the contaminant removed by the shoot tissues. Such increased shoot biomass lead to an increase in root biomass enabling the adsorption of the contaminants in the root. This study presented an average shoot to root ratio range of 4:1 to 13:1. The ratio fell within the range reported by one of the first studies on phytoremediation using a field tobacco plants (Gler, 1940). The study reported that plants with low PCB concentration could still extract a valuable quantity of PCBs with a large shoot biomass. The values of the percentage change in root length of the plants as used in this greenhouse experiment is presented in Table 4.3.1-4.3.2 as well as in Figure 4.4.1.1-4.4.2.3. The progressive reduction in the measured parameters of *C. odorata* grown in a soil treated with different concentrations of Aroclor and T/O should be attributed to changes in soil condition as a result of hydrophobicity of PCB and T/O which interferes with nutrient and water uptake as well as gaseous exchange (Smith *et al*., 1989; Anoliefo and Edobgai, 2001).

5.5 Phytoremediation ability of *C. odorata* on PCB-contaminated soil.

In the Aroclor amended soil treatments, PCB concentrations within 200 ppm treatments were not phytotoxic to *C. odorata* as the plant was able to complete the growth duration of the experiment in those treatments or that the plant was able to manage such effects. At 500 ppm PCB concentrations therefore, *C. odorata* was slightly affected by phytotoxicity of the pollutant towards the sixth week of growth in the treated soil, although it completed the experimental period. PCB contamination between 0-260 µg/g has been reported not to be phytotoxic to various plants tested for its phytoremediation ability, but higher concentration of PCB above this range was seen to cause stress to the plants (Weber and Mrozek, 1979; Zeeb *et al*., 2006; Ficko *et al*., 2010). The response shown by *C. odorata* towards 500 ppm of PCB may have been the cause of the stress.

However, total PCB concentrations found in the tissues of *C. odorata*, ranges from 3.1 to 64.6 ppm, the value was seen to increase as the concentration of the treatment was increased. This is in agreement with the study of Pinsker, (2011), which reported that initial soil PCB has a great effect on the amount of PCB absorbed by plants, its translocation as well as the concentrations of the residual PCB in the soil at the end of a phytoremediation study. There was percentage reduction of PCB concentration from 0.01 to 4.2 which is appreciable when compared with the mean reduction of PCB per month of other plants species that were used for various PCB phytoremediation studies. The mean reduction were reported to be in the range of 0.1-14.8% (Dzantor *et al*., 2000; Dzantor and Woolston, 2001; Checkol *et al*., 2004; Chen *et al*., 2005, 2009, 2010; Mackova *et al*., 2009; Teng *et al*, 2010; Xu *et al*., 2010; Ficko *et al*., 2010). Although total PCB concentration in the plant tissues of transformer oil co-contaminated with PCB samples was not applicable as a result of the adversity of the oil, there was reduction in the amount of PCB in soil at the end of the experiments. Such effect was also observed in the unplanted control samples and could be attributed to natural attenuation and perhaps other parameters not measured.

In control sample tested to study the effect of volatilization (C3), the rate of reduction of soil PCB as observed in the mean final PCB in that treatment was low compared to other treatments, an indication that

volatilization plays a part in the removal of PCB from the environment. Such reduction was also not significant compared to the reduction on the treated and planted samples (P > 0.05). This is in agreement with Aslund *et al*., (2008), which reported that the primary uptake pathway of PCB into plants should be root uptake and possible translocation and consistent with other studies on other POP uptake in plants (Mattina *et al*., 2000; White, 2001; White *et al*., 2006). Control 4 (C4) was also tested to study the effect of microorganisms in the soil to the reduction of soil PCB by deactivation of the effects of microbes using commercial bleach. It was demonstrated that microbial effects has an important role in the remediation of PCB contaminated soil. Such demonstration was only possible in the Aroclor amended samples as mean percentage reduction recorded was 0.01 and 0.9 in 1260 and 1254 respectively. In T/O treated samples however, percentage reduction of PCB in C4 was higher than what was observed in the treated planted samples. The reason behind this in-balance could be that commercial bleach's reaction with oil resulted to an organic amendment that aided the remediation of the contaminated soil. Borja *et al*, (2005) reported a stimulating effect of $FeSO_4$ on PCB-contaminated sediments leading to almost complete dechlorination of Aroclor 1242. This was possible because of the reductive properties of the compound. (The above inference is liable for further study to be able to unravel such effect). Chekol *et al*, (2004) reported an enhanced PCB removal through uptake by plants as a result of the interaction between plants and microorganism. This is in agreement with the work of other scholars on the influence of microbes on uptake of PCB by plants (Leigh *et al*., 2006; Mackova *et al*., 2007, 2009; Xu *et al*., 2010; Anyasi and Atagana, 2011). From this result therefore, it is evident that *C. odorata* is a good candidate for uptake of PCB from a contaminated soil and such effect could be enhanced with soil ammendements (bioaugumentation) that aids microbial presence in the rhizosphere.

5.5.1 Root and shoot PCB concentration of *C. odorata* after six weeks of growth in PCB-contaminated soil.

Plants ability to accumulate PCB in its root has been reported as the first stage in any phytoextraction phenomenon, therefore substitutes of the compound in close contact with the root of plants are of great

importance. In this study, greater amount of PCB found in the plants tissue were concentrated in the roots, it could be as a result of the diffuse root system of the plant an importance feature of any phytoremediation plant. Total root concentrations of PCB were reported to be in the range of 0.26-17.85µg/g (Table 4.5). Increased root concentration of PCB leads to a synonymous increase in bioaccumulation factors (BAF) also referred to as remediation factors (RF). Bioaccumulation factor determines plants ability to accumulate and concentrate a greater quantity of PCBs than the surrounding soil. This phenomenon is important as it provides an idea on how to measure the ability of plants to draw PCB towards the roots when it absorbs water and nutrients from the soil. The measurement of BAF in *C. odorata* was in range of 0.01 to 0.4 which is greater than what was observed with Alfalfa by Zeeb *et al*., (2006). To date, average BAF of previously used phytoremediation plants is as reported thus: 0.06 reported for nine different plants (Low *et al*., 2010; Zeeb *et al*., 2006), 0.42- 0.53 by plants of Cucurbit family (Aslund *et al*., 2007/8). White *et al*., (2006) reported an average BAF of 0.21 using Zucchini plants, and the least BAFs (0.0004) was reported by Zeeb *et al*., (2006) for soybean and sedge. From this, it can be explained that *C. odorata* was able to draw PCBs towards its root with its BAF value within the range of measured value of BAF of other PCB phytoremediation plants. Meanwhile, plants with higher amount of PCB in its root typically have a higher shoot concentration when compared with other plants. Shoot concentrations are usually much less than root concentrations due to the characteristics of PCB which make phytoremediation difficult to be successfully accomplished. However, remedies that take advantage of higher root concentrations shall be useful for these high concentrations of PCBs in the roots. Furthermore, concentrations of PCB in plants shoot are not a good indicator of what plant species removed the most PCBs out of the soil because plants have different biomasses (Ficko *et al*., 2010; Pinsker, 2011). Direct comparison of concentrations is not sufficient to compare different plants' ability to remove PCB from soil until the biomasses are included. Including biomass of plants will therefore determine the amount of PCB extracted into the plant and how much was therefore removed in a given area (Pinsker, 2011).

Although congener analysis was not within the scope of this study, but from the analysis of the samples using GC-MS, it was mostly the mono, di and tri-chlorobyphenyls of the lower congeners (with predominance of monochlorinates) that was found in the plants tissues, while the pentas and the hexa-chlorobiphenyls were found as the residues in the soil at end of the six weeks experiment. This is an indication that phytoextraction was the probable mode of phytoremediation of PCB-contaminated soil by *C. odorata*. The result of this study however is in agreement with Quensen III *et al*, (1990), which noted that aerobic mineralization of PCBs is limited to PCBs with 5 or fewer chlorines. According to Smith *et al*, (2007) also, of the congeners that was monitored, only one had five chlorine present (2, 3', 4, 4', 5'-pentachlorobiphenyls). During the dechlorination of Aroclor 1260, penta-, tetra-,tri-,and dichloro biphenyls was found to accumulate (Van Deuren *et al*., 2002; Borja et *al*., 2005). Examining the chlorine distribution of the PCB compound monitored in this study, 2,3,3',4,4',5,5'-heptachlorobiphenyls could lose one chlorine from a *meta* position and become 2,2',4,4',5,5'-hexachlorobiphenyls, which is one of the congeners present in Aroclor 1260. This is a likely pathway for reductive dechlorination, because it preferentially removes chlorine from the meta- and *para*- positions (Aken *et al*., 2010) and could explain why percentage loss of the 2,3',4,4',5'-pentachloro biphenyls was not large. When water saturation is maintained in sediment, reductive dechlorination results with accumulation of cell chlorinated PCBs (Smith *et al*., 2009). It could therefore be argued that the lower chlorinated biphenyls found in the plants tissues were as a result of the effect of microbes on higher chloro PCBs found in Aroclor 1254/60 anaerobically.

5.6 Plants responses within the two soil samples and Aroclor behavior in the soil.

Prior to analysis of plant and soil samples, recovery studies using the standard Aroclor samples was carried out, and the range were between 95 and 99 % and linearity of response ranged from 0.997 to 0.999 for Aroclor 1254 and 1260 respectively, with relative standard deviation of less than 2 %. Inferences drawn from the ability of *C. odorata* grown in an Aroclor contaminated soil and soil treated with transformer oil co-contaminated with Aroclor to remediate the soil after six weeks of growth in the

contaminated soil showed little variations, but no significant difference within the variations amongst the two soil samples was found ($P > 0.05$) (Table 3.1). Although one would have expected a more improved growth and subsequent remediation of the contaminant in S_1 (clay soil) at the end of the experiment as it contained higher percentage weight of total organic carbon (TOC) (7.0) than S_2 (sandy-loam soil) with 0.5. TOC is implicated in successful remediation of organic contaminants as it enables the release of root exudates of plants which aids the remediation processes (Anyasi and Atagana, 2011). This has therefore proven the fact that *C. odorata* is a versatile weed in its choice of soil and therefore should be encouraged in any PCB-contaminated soil. Sprout culture method adopted in this study for the remediation of T/O-treated soil by *C. odorata*, did gave positive evidence on the translocation of organic chemicals through plants root without direct contamination of the plant either by volatilization of the compound from the atmosphere or by direct contact with pollutant tested in the soil (Suzuki *et al*., 1977). The favourable result obtained compared to that in direct treatment is an indication that plants could be used successfully for remediation of any pollutant-contaminated soil irrespective of the concentration and result will be achieved. Therefore there is sence in arguing that both soil samples used in this greenhouse experiment were of equall constituents irrespective of the figures released by the characterized Table 3.1. Meanwhile, since there was much increase in height in the Aroclor treatments than in T/O, it is evident that remediation of the pollutant will be ensured in the former as phytoremediation is known to be synonymous with increase in height and biomass accumulation of phytoremediation plants. Recovery of PCB from Aroclor amended treatments at lower concentration using *C. odorata* favoured T_1 (Aroclor 1254) as recoveries were higher at such concentration. However, higher recoveries shifted towards T_2 (Aroclor 1260) at high initial concentrations. Phytoremediation abilities of plants were reported to be depended amongst other factors on the initial concentration of the contaminant, and such influence really took effect on this study. Therefore toxicity study of plants with contaminant to be remedied should be of interest for an effective phytotremediation projects and is encouraged.

Conclusions and recommendations

This study has been able to demonstrate that *C. odorata* is able to survive the phytotoxicity of PCB, but the effect of T/O was adverse. At the end of six weeks of growth, plants showed a diminished effect in T/O amended soil to the parameters tested. The plants demonstrated a least percentage increase in plants size to the value of -1.0 as was observed in the 500 ppm. In Aroclor amended samples, 45.9, 39.4 and 40.0 % were plants sizes at different concentrations. Such effects were observed in the leaf numbers and root length. Leaf colour also showed an indication to the effects of the contaminants. The control sample has 43.3 % increase in plant size which was not significant among the values in Aroclor treated soils ($P >$ 0.05), an indication that *C. odorata* was able to survive PCB contamination as to remediate it.

From this study, *Chromolaena odorata* demonstrated an ability to reduce the concentrations of PCB in the contaminated soil, these it does by reducing the initial concentration to as high as 4.2 %. Percentage reduction in the concentration was found to decline as the concentration of Aroclor 1254 was in increased but was irregular in the soil samples treated with Aroclor 1260. Reduction of soil PCB concentration was also observed in the transformer oil co-contaminated with Aroclor samples. However, the reduction in the oil treated sample was lower than in the Aroclor samples, but the difference was not statistically significant ($P >$ 0.05). Equally, unplanted control set up as seen in C2 recorded a reduced concentration from the initial contamination, such reduction was however lower than the treated planted samples an indication of plants action in the remediation of the PCB-contaminated soil. Reduction of PCB concentration in soil sample in C2 could be as a result of natural attenuation (Chen *et al*., 2010; Pinsker, 2011). In control sample tested to study the effect of volatilization (C3), the rate of reduction of soil PCB as observed in the mean final PCB in that treatment was low compared to other treatments, an indication that volatilization plays a part in the removal of PCB from the environment. Such reduction was also not significant compared to the reduction of the treated and planted. C4 was also tested to study the effect of microorganisms in the soil to the reduction of soil PCB by deactivation of the effects of microbes using commercial bleach. It was demonstrated that microbial effects has an important role in the remediation of

PCB contaminated soil. Such demonstration was only possible in the Aroclor amended samples as percentage reduction recorded was 0.01 and 0.9 in 1260 and 1254 respectively. In T/O treated samples however, percentage reduction of PCB was higher than what was observed in the treated planted samples. The reason behind this in-balance could be that commercial bleach's reaction with oil resulted to an organic amendment that aided the remediation of the contaminated soil (this is liable for a further study to be able to unravel such effect). At certain instances, the aerial tissues of the plant was found to contain traces of PCB even as the concentration of the soil PCB did not change at the end of the six week of experiment. This is not out of place as there could be several reasons to why such was possible; volatilization may have taken place from the atmospheric PCB either through the embedding soil but was too small to record an effect on the residual concentration of the soil or from cross contamination of the atmosphere since several treatments was made in the same environment (within the greenhouse), though efforts was made to separate the contaminants from each other. This greenhouse study has provided an insight to some fundermental mechanism of phytoremediation with an accumulator plant (*Chromolaena odorata*), however, field trial is needed to give a measure of plant's performance in the real environment as many factors are known to affect plants growth on contaminated soil (Tanhan *et al*., 2007, 2011). Since *C. odorata* has demonstrated the ability to phytoextract PCB in its root, treatment of the plant with contaminant for a longer period could cause the transpiration pull to move the contaminant towards the shoot area of the plant. This will be an added advantage in the sence that C. odorata is a long term competitor and ardent survivor under adverse changing conditions hence could be used for phytoremediation trials for a longer time.

Sequel to the inferences drawn from the analysis of this research study and the conclusion thereof, it is therefore recommended that:

1. *Chromolaena odorata* should be tried with PCB in the field for at least one growth cycle as to be able to dictate the potency of the plant towards remediation of PCB contaminated soil;

2. Density study using *C. odorata* should be employed in further PCB phytoremediation study to determine the number of plants per pot for optimal PCB soil remediation;

3. Microbiology and molecular analysis should be introduced in further PCB phytoremediation study with *C. odorata*.

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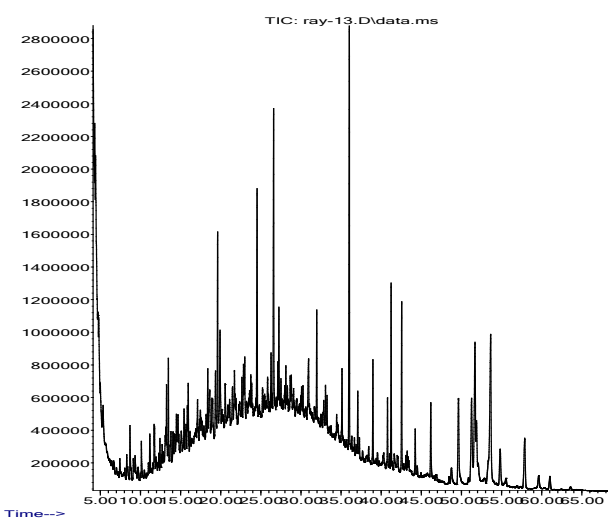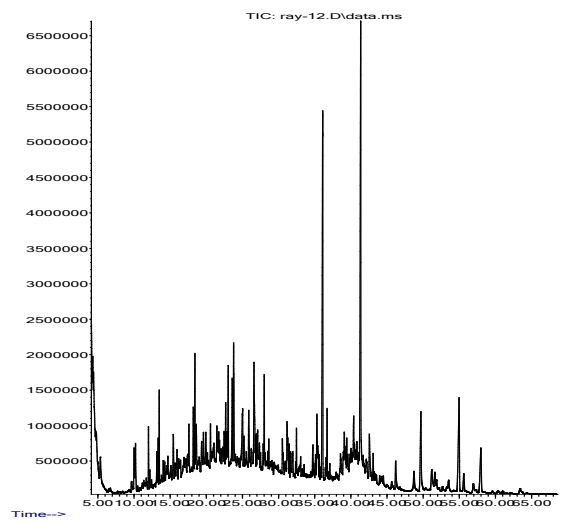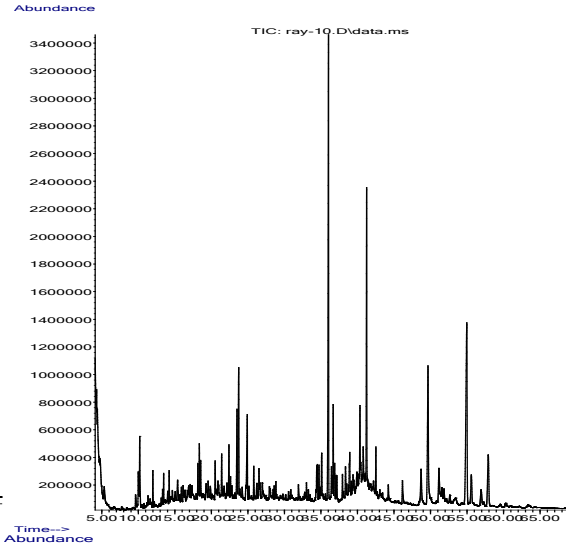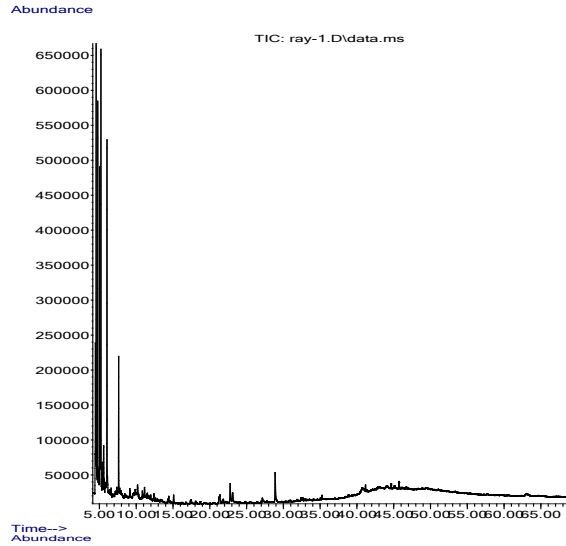
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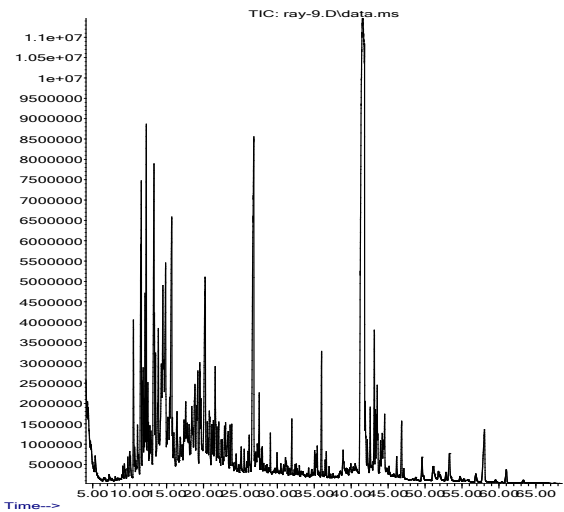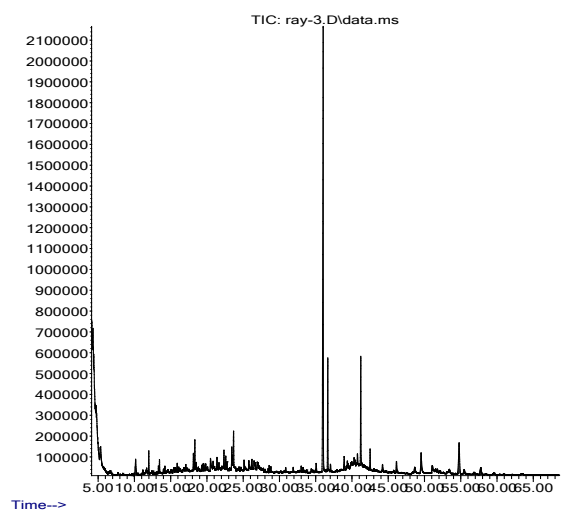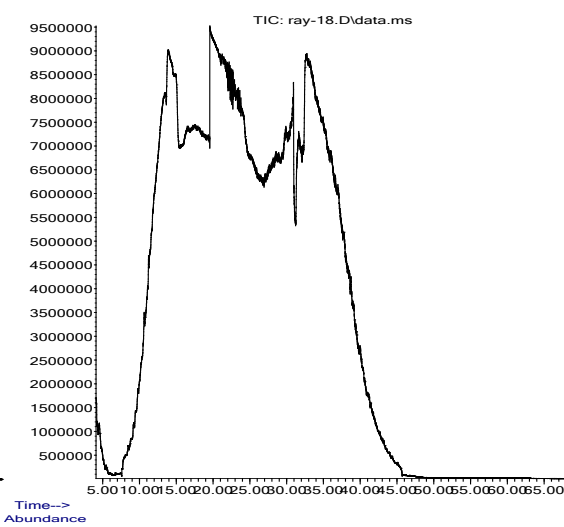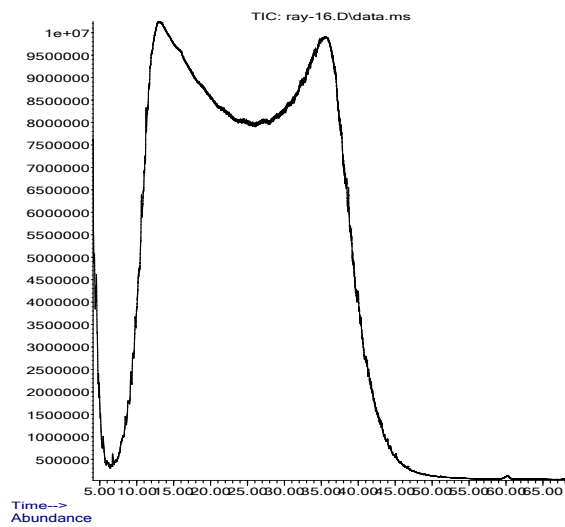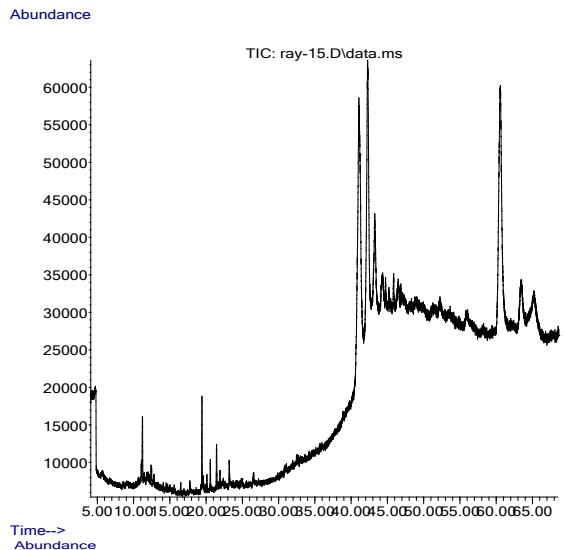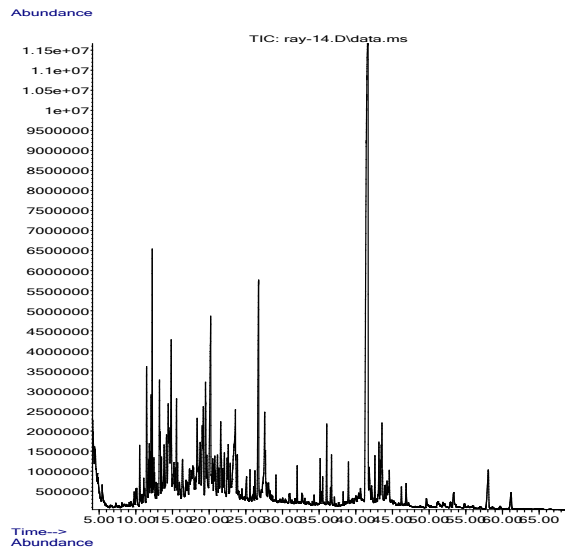
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Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*

Culturing process of the plants

Phytoremediation of polychlorinated biphenyls (PCBs) contaminated soil by phytoremediation with *Chromolaena odorata*