International Scholars Journals

Advances in Agriculture and Agricultural Sciences ISSN 2381-3911 Vol. 6 (6), pp. 001-017, June, 2020. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

Biological remediation of polychlorinated biphenyls (PCB) in the soil and sediments by microorganisms and plants

R. O. Anyasi[1]* and H. I. Atagana[2]

[1]Department of Environmental Sciences, University of South Africa, Pretoria-South Africa.
[2]Institute for Science and Technology Education, University of South Africa, Pretoria-South Africa.

Accepted 03 May, 2020

It is a known fact that the environment is suffering from severe contamination as a result of various uncontrolled activities of man and chemicals in the biosphere. This acute and diffuse contamination of air, soil and water by metals, chemicals and metalloids causes wide environmental concerns, which if left unchecked will be detrimental to man and organisms. Biological methods for cleaning of the environment especially the soil have been receiving increasing attention for the past two decades. Bacteria and fungi have been the natural detoxification agent for contaminants in the environment. Recently, research has shown that with the combination of plants and microorganism in the right proportions and technique, detoxification of environmental contaminants will produce a desirable and better result and most importantly the natural environment will not be affected as some of the processes are environmentally friendly. However, the hydrophobic organic molecules such as polychlorinated biphenyls (PCBs) tend to be much less responsive to bioremediation strategies. The wide spread presence of this compound and others in the prominent group known as persistent organic pollutants (POP's), that share common chemical, toxicological and environment properties continues to increase in the environment, even with the various measures taken to control its presence in the environment. This review focuses on the possible trends of remediation of PCBs in the environment and the methodologies applied. It also reviews the merits and demerits of using plants and microorganisms as biological detoxification agents. This will highlight the possible improvement measures on the combination of plants and microorganisms in bioremediation, thereby filling the gap left behind by the conventional methods of remediation with its enormous limitations and disadvantages.

Key words: Bioremediation, phytoremediation, PCB, biodegradation, environmental pollution, rhizodegradation, thermal and chemical/physical process, dechlorination, contaminated soil, oxidative dechlorination/reductive dechlorination.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are representatives of a group of compounds known as "Persistent Organic Pollutants (POPs). This persistence is as a result of the physico-chemical characteristics of the compounds. PCBs are mixtures of aromatic chemicals produced by the chlorination of biphenyls in the presence of suitable catalyst. The chemical formula of PCB can be represented as:

$C_{12}H_{10-n}CL_n$

Where, n is the number of chlorine atom within the range of 1-10. The relative molecular weight of this compound depends on the degree of chlorination.

Physical-chemical properties of PCBs

PCBs are characterised by two linked aromatic rings substituted by 1-10 chlorine atoms. There are about 209 PCB congeners identified as a function of chlorine

numbers and position.

Only twenty nine of these congeners are of environmental interest. Toxicological problems of PCB are associated with its co-planar congeners. The basic structure of PCB according to Wiegel and Wu (2000) is shown in Figure 1. 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 most toxic. It is widely known that ortho substitution increases toxicity. The 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 (Wiegel and Wu, 2000) . The heavy PCBs are honey-like oils. The most highly chlorinated PCBs are waxy and greasy substances. PCBs have a low flash point which is from 140 to 200°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 characteristics properties of the compound. The 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 the same number of chlorine atoms (Borja et al., 2004).

The properties of PCB that leads to their being valuable for industrial applications include chemical inertness, high electrical resistivity and dielectric constancy, thermal stability, non-flammability and acute toxicity. The 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 (that is, PCBs 77, 126, 169 etc.). They tend to have dioxin like properties and are generally among the most toxic congeners (UNEP Chemicals, 1999). PCB health effects on human ranges from the skin conditions to the

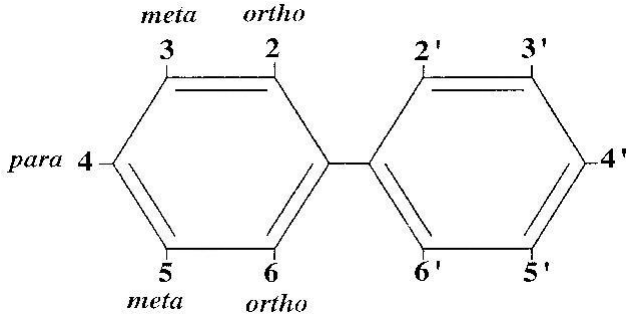

Figure 1. Structural formula of PCB showing the number and location of a Cl group (Wiegel and Wu, 2000).

acute liver damage as a result of man's exposure to the chemical. Animals that eat PCB contaminated food even for a short period of time surfers liver damage and may die (UNEP Chemicals, 1999).

Uses of PCB

For decades, PCBs are extensively used in a range of industrial applications such as transformers oils, dielectrics in capacitors, hydraulic fluids in hydraulic tools and equipment as well as heat exchange liquid. 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 by her huge manufacture of capacitors and transformers (Erickson, 1997).

Sources of PCBs

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., 2004). Majority of the PCBs in the environment finds its way during their manufacture, 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; accidental releases (PCB spills and leaks, transformer fires); and environmental sinks of past PCB

*Corresponding author. E-mail: 41525981@mylife.unisa.ac.za Tel: +27 76 1378545. Fax: 086 5151875.

Abbreviations: PCBs, Polychlorinated biphenyls; **PGPR,** plant growth promoting rhizobacteria; **POP's,** persistent organic pollutant; **TPHS,** total petroleum hydrocarbons; **PAHs,** polynuclear aromatic hydrocarbons.

contamination (Borja et al., 2004).

In water, PCB concentrations are generally higher near human activity and near shorelines. The major source of PCB in surface water is from environmental cycling (that is, from sediments, air and land) . 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 and thousands of times higher than in water because they accumulate in the fish (EPA, 1993a).

PCB also attaches strongly to soil and may remain there for several years. Environmental cycling is expected in disposal and spill sites. 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 (Choi et al., 2009).

Health and environmental effects of PCBs (Toxicity)

PCBs possess dioxin- like toxicity. Toxicity determination for any mixture needs to take into account international toxicity equivalents factor (I-TEF), for example, 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; Pesatori et al., 2003). The first step in toxicity mechanism is mediated by the binding of PCB to the Aryl hydrocarbon (Ah) cellular receptor (Schmidt and Bradfield, 1996; Mukerjee, 1998; WHO, 1998; Okey, 2007). Toxicity of PCBs is said to range from low-moderate. Treated samples of animal show an LD_{50} ranging from 0.5 to 11.3 g/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. Research has shown that the effects on health depend on age, sex, and areas of the body where PCBs are concentrated. Studies have shown that PCBs are carcinogenic in animals, this is because, according to the study of Borja et al. (2006), animals that ate food containing large amount of PCBs for short period of time had mild liver damage and some died. Occupational studies showed some increase in cancer mortality in workers exposed to PCBs (Tsai et al., 2007). It was also found that significant excess cancer mortality at all sites combined and in the gastrointestinal tract in workers exposed to PCBs contain 54 and 42% chlorine. Brown (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 Swedish scientist (Borja et al., 2005), PCBs has anti-estrogens properties that can inhibit calcium deposition during egg shell development, leading to insufficient strong shells and premature lost. Anti-oestrogen effects of PCB may also lead to adverse effects on male reproduction capabilities of birds and animal species.

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.

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. This study shall try to review the physical and chemical treatment option for PCB degradation in the course of this work. However, this section reviews the biological degradation of PCB by plants and microorganisms. 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 leading to a structural change to, or the complete degradation of the target molecule (Borja et al., 2006). 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 the same time. When the products of co-metabolism are ready for further degradation, they can be mineralized, otherwise incomplete degradation occurs. 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. 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.

The water solubility of the compound has a vital role in its degradation. Compounds with high aqueous solubility are easily accessed by microorganisms than those with low solubility. 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 et al., 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 et al., 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.

Bioremediation has its advantage in that it can be done on site or off site. This is referred to as the *in situ* and *ex situ* remediation. 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 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 and Ahmed, 2003).

Bioremediation could be *ex situ* or *in situ* depending on whether the soil is taken out from its source or not. *Ex situ* remediation include: 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 other hand include: 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 excavation and transportation costs but it 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., 1998).

Release of PCBs to the environment

From 1929 until 1977, especially in the United States of America, 99% of all PCBs used by US industries were manufactured by Monsator chemical company at a production facility in Sauget, Illinois (Durfee, 1976; IARC, 1978). 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 manufacture,

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, 1998u).

Since PCBs were no longer manufactured or importedin large quantities, significant released of newly manufactured or imported materials to the environment does not occur. Rather PCBs were predominantly redistributed from one compartment to the other (that is, soil to water, water to air, and sediments to water) (Eisenreich et al., 1999; Larsson, 1985; Larsson and Okla, 1989). Thus, for example, 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) and some other means. This ability of PCBs to constantly persist in the atmosphere requires a more environmentally friendly alternative dissipation method, as science has been failed by the conventional methods of incineration. The environmentally friendly alternative stated here is bioremediation.

Bioremediation is the use of biological mechanisms to destroy, transform or immobilize environmental contaminants in order to protect potential sensitive receptors (Bioremediation Discussion Group, 2006). It could also be referred to as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition. It may be employed in order to attack specific contaminants such as chlorinated pesticides that are degraded by bacteria or a more general approach may be taken. Such approach includes; oil spills that are broken using multiple techniques including the addition of fertilizers to facilitate the decomposition of crude oil by bacteria. Various forms of bioremediation technique include:

1. Land farming
2. Bioventing
3. Biosparging
4. Bioslurping
5. Phytoremediation
6. *In situ/ ex situ* remediation

Bioremediation is a required option especially where sediments are contaminated with PCBs. However, thermal and chemical processes have always been the method use to decontaminate highly polluted sites until bio-technology offered a more economically friendly alternative for diffuse pollution. The aim of all thermal, chemical/physical methods of remediation is to change the chemical environment in a way that prevents the transport of toxic substances to other elements of the soil system; examples can be given by the transport of pollution to plants, to ground water, or to soil organisms. Such preventive measures may include decreasing mobility change of chemical constitution or any of the factors on which has been elaborated by various researchers.

Thermal process

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

1. Soil conditioning
2. Thermal treatment and
3. 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 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 low temperature range of 350-550°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°C and dioxins are destroyed (Koning et al., 2000). Thermal remediation techniques can be used to remediate a lot of compounds including: TPH, BTEX, PCBs, 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, Gioia et al., 2006). Also, most thermal processes are high technology procedure which requires 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.

Chemical/physical process

Chemical/physical processes is sometimes referred to as pump and treat process. The pump and treat process pumps 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 includes:

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).

$$C_2HCl_3 \text{ (TCE)} + 3H_3O_2 \longrightarrow 3HCl + 2CO_2 + 2H_2O$$

Ozone destruction of toxic contaminants takes place in the following manner:

$$C_2HCl_3 + O_2 + H_2O \longrightarrow 3HCl + 2CO_2$$

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.

Vapour 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 vapour extraction (Van Deuren et al., 2002). In vapour extraction, complete decontamination of the soil is rarely achieved.

In situ remediation technique

Bioventing

This is the only bioremediation technique that allows the treatment of unsaturated soil but this technique is disadvantaged in that it is not effective if the water table is within several feet from the surface (Van Deuren et al., 2002, Gioia et al., 2006). Bioventing uses a vacuum enhanced soil vapour extraction system, as a result of the soil pressure gradient which causes a flow of oxygen into the subsurface thereby triggering aerobic contaminant decomposition process. Sometimes it involves the addition of nitrogen salt by sprinkling a nutrient solution on top soil or by injection above the contaminated soil zone (Held and Dorr, 2002). Sufficient airflow is important in the design of bioventing system. Low permeability as well as low temperature hinders the effectiveness of bioventing.

Biosparging

This is the injection of atmospheric air into the aquifer. Biosparging is used in both saturated and unsaturated soil zones hence was designed to augment for the shortcomings of bioventing process meaning that reduction of energy consumption is reduced (Held and Dorr, 2002). The injection of air into the aquifer results in small channels for the air to move to the unsaturated soil zone. Therefore, in order to form the necessary numerous branches in these channels, the air must be pulsed into this soil. This then result in volatile contaminants being transported to the unsaturated zone. Finally, soil vapour extraction is then used to extract the volatile vapours and then treat them at the surface. In order for biosparging to be effective, the sparge point must be below the contamination zone because air always flows upwards. The up flow of air will form an influence cone; the degree of branching and the angle of the cone are determined by the amount of air pressure during the injection. The disadvantage with biosparging is that it must be combined with a physical method before the process can be completed and it's effective and therefore less economical. According to the case study done at the Damoder valley in Eastern India as was done by Gogoi et al. (2002), biosparging was effective at removing 75% of contaminants present within one year period. According to him, the first results were obtained in the field but it was later enumerated using laboratory tests and computer programs.

The aforementioned techniques of bioremediation are only effective if the soil being treated is homogenous. If a remediation area has non –homogenous soil, it may be the best to consider passive treatment techniques. Passive treatment involves applying treatment technique at the end of contamination plume. The passive treatments are – activated zones, bioscreens, reactive walls and reactive trenches (Koning et al., 2000). These passive treatments enable autochthonous microbial population as the nutrients injected into the system through the walls to the surface acts as stimulants. The techniques are only effective if the hydraulic conductivity is the same in the activated zone as it is in the surrounding aquifer (Held and Dorr, 2002). The question remains how much one can afford to spend in order to increase the effectiveness of the remediation technique.

The promise of phytoremediation as was shown by the work of various schools of thought could be an answer to these questions.

PHYTOREMEDIATION OF PCBS

Polychlorinated biphenyls (PCBs) are xenobiotic

compounds of great concern widely spread in the environment. A review of literatures indicates that PCBs are not leachable in soils and that they are readily absorbed by soil constituents. It appears that lower chlorinated PCBs are less absorbed and thus slightly mobile in soils. There have also been reports of absorption of PCBs by plants, but in very low amounts as PCBs appears to have some effects on photosynthesis and respiration in plants (Toro et al., 2006). Also contradictory evidence ensures thus; while some studies report that there is little or no active transport, others showed evidence of an active uptake and translocation. According to Quiping et al. (1992), an investigation into the possible effects of PCB congeners in tomato and barley plants, showed a lack of active transport or metabolism of PCBs. From the study, 95% of the injected PCBs were retrieved from stem section within 5 cm of point of introduction after 55 days. PCB is said to be thermally and chemically stable and are also recalcitrant to biodegradation. Their strong absorption to organic matter leads to bioaccumulation in the food chain (Safe, 1992; Dietz and Schnoor, 2001). Using different mechanisms, anaerobic consortia of microorganisms, as well as aerobic bacteria, are able to attack PCBs. But according to Toro et al. (2006), actual site PCB contaminated soil is often limited by their poor content of autochthonous pollutant-degrading microorganisms. Here, inoculation was propounded to be the solution for a successful bioremediation. This inoculation can be done by direct introduction of complex microbial systems such as compost or sludge or the use of plant microbial interaction in their symbiotic relationship.

Thus, phytoremediation can be defined as the use of plants to dissipate organic compounds like PCB from the soil (Ferro et al., 1994; Dietz and Schnoor, 2001; Mackova et al., 1997). It is an in situ technique which is most suited for sites where other remediation options are not cost effective, low- level contaminated sites, or in conjunction with other remediation technique. Deep rooted trees, grasses, legumes, and aquatic plants all have application in the phytoremediation field. The ultimate aim of this review is to highlight microbiological bioremediation and phytoremediation technologies that have been proven successful in the remediation of PCB. It will then compare the bio/phytoremediation with other conventional thermal and physical/chemical methods of remediation of PCBs.

RHIZO/PHYTODEGRADATION

This section reviews the interactions of pollutants with soil looking at the effectiveness of remediation of the contaminated soil using rhizodegradation technology of microorganisms and phytoremediation. This method is based on the combination of microbial and plant growth process to enhance biomass accumulation, particularly plant roots in the soil, and thus, accelerates the remediation kinetics (Dowling and Doty, 2009). The processes used are 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., 2004, 2001). Combining two or more techniques for the remediation of persistent contaminants like PCBs can overcome many of the limitations that exist for individual technologies. For example, in phyto-remediation, many plant species are quite sensitive to contaminants, including TPH (Huang et al., 2004; Burd et al., 1998). 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. Furthermore, in most contaminated soils, the population of microorganisms is depressed so that there are not enough bacteria either to facilitate contaminant degradation or to support plant growth (Carrillo- Castaneda et al., 2001; Siciliano and Germida, 1997).

For effective remediation of variety of environmental contaminants, it is advantageous to use multiple techniques or process to accelerate remediation kinetics and to increase plant and microbial biomass (Huang et al., 2001; Carrillo-Castaneda et al., 2001; Siciliano and Germida, 1997; Glick, 2003). In the use of double or multi-process remediation, both PGPR and specific contaminant degrading bacteria was found to be vital for successful remediation (Huang et al., 2004, 2001; Burd et al., 1998; Carrillo-Castaneda et al., 2001; Siciliano and Germida, 1997; Ajithkumar et al., 1998, Newman and Reynolds, 2004). For organic contaminants, the use of bacteria as a pre-treatment that consume organics in the soil can promote remediation process (Shann and Boyle, 1997; Walton et al., 1994). Various bacteria are able to rapidly metabolize some readily available compounds; these include TPH/PCB consuming bacteria that have been used on soils (Huang et al., 2001; Mackova et al., 1997, Newman and Reynolds, 2004). Remediation process commences hence lowering the toxicity of the compounds to plants when used prior to phytoremediation. Furthermore, there are bacteria called plant PGPR that increases the plant tolerance to TPHs and massive biomass accumulation (Glick, 1995, 2003). They work by preventing stress ethylene synthesis and providing auxins to the root (Glick et al., 1998). The result is a much greater biomass (especially roots) and therefore a faster remediation (Glick et al., 1998; Glick and Holguin, 1998).

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 deconta-mination 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 soil was from Imperial Oil land farm site in Sania, Ontario, Canada. The actual environmental contaminated and aged soils often behave differently than laboratory-spiked soils with respect to remediation.

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. Therefore, the highly hydrophobic, recalcitrant TPH fractions were remediated from the soil whereby after a second 4-month, the system removed 90% of TPHs from the soil. Phytoremediation was unable to remove only about 50% of TPHs in the same period. Therefore the key elements for successful phyto-remediation were the use of plants species that can proliferate in the presence of high levels of contaminants and strains of PGPR that increase plant tolerance and accelerate plant growth in heavily contaminated soil.

The use of microorganism, both anaerobic and aerobic, is the only known process to degrade PCBs in the soil systems or aquatic environments. Anaerobic bacteria possess characteristics that are well adapted to pollutants with high carbon concentration because of the diffusion limitation of oxygen in high concentration systems (Dobbins, 1995). The environment of anaerobes is conducive to reductive transformations where chlorine is displaced by hydrogen (McEldowney et al., 1993). 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).

Anaerobic transformation of chlorinated organic compounds involves reductive dehalogenation where the halogenated organic compounds serve as the electron acceptor (Morris et al., 1992); the halogen substituent is replaced with hydrogen (Quensen et al., 1990).

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

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 (Mohn et al., 1992), 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. The typical phenomenon that is common to the dehalogenators includes:

1. Aryl reductive dehalogenators function in a syntrophic communities and may be dependent on such a community.
2. This aryl reductive dehalogenation is catalysed by enzymes that are inducible.
3. There is exhibition of distinct substrate specificity by this enzyme.
4. The aryl dehalogenators derive metabolic energy from reductive dehalogenation. Hence micro-organisms with these sorts of 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 depends on the makeup of the active microbial community. This tends to be influenced by the factors of the environment like presence of carbon source, hydrogen or other electron donors, the presence or absence of electron acceptors other than PCBs, temperature and pH (Borja et al., 2005).

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 by J. Borja et al. (2005), it showed that the sediments has a high proportion of the mono- and di- congeners and a reduction of the congeners. 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 the proportions of mono- and di-chlorobiphenyls increased considerably (Quensen 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 syntrophic communities attacking PCB at different

positions with specificity for PCB dechlorination (Rhee, 1999).

According to Quensen et al. (1990), dechlorination of Aroclors 1242, 1254, and 1260 by microorganisms in a particular sediments and Aroclors 1242 and 1260 in another sediment, the rate of dechlorination in the second sediments by microorganisms was similar for Aroclors 1242 and 1248. This is an indication of extensive dechlorination from the meta-plus para- positions within the 8weeks of incubation leaving ortho- substituted, mono- and di-chlorobiphenyls to predominate. Aroclor 1254 was dechlorinated at a somewhat lesser rate with 63% of the chlorines in the meta- plus para—positions in 25weeks. And for Aroclor 1260, only 15% of the meta-para- positions were removed even after 50weeks. The compound that predominate from the dechlorination of Aroclors 1242,1248, and 1254 were 2-chlorobiphenyls, 2,2-chlorobiphenyls and 2,6-chlorobiphenyl while Aroclor 1260 followed a somewhat different pattern where 2,5,-2',5'-chlorobiphenyl was the major product (Harkness et al., 1993; Quensen et al., 1990).

Dechlorination of Aroclor 1242 in the second sediments by microorganisms was less extensive compared to the microorganisms in the first sediments, which have 46% of the meta- plus para- chlorine removed even after 16 weeks. Contrasting, dechlorination of Aroclor 1260 was more rapid than with the first inoculums. Quensen et al. (1990) attributed this difference in the dechlorination activities to the previous exposure of microorganisms to the particular Aroclor present at the site. Sonzogeny (1990) also reported a dechlorination pattern in a river believed to be contaminated with Aroclor 1248 and 1254. From his investigation, it was observed that congener profiles shifted from higher chlorinated to the lower chlorinated ones. The meta- and para- chlorinated congeners were depleted more than the ortho congeners.

With microorganisms, the use of organic substrate as electron donors has also been shown to increase the rate of dechlorination of Aroclor 1242 (Nies et al., 1990). 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 ortho- 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 (Nies et al., 1990).

The presence of potential electron acceptors also affects the removal of chlorine atoms as reported by Morris et al. (1992). According to the work, electron acceptors compete with halogenated compounds for reducing potential and the compounds impose different selective pressures on growing communities. Sulphate and bromoethane sulphate was found to inhibit dechlorination but it is enhanced by carbon dioxide and nitrate as electron acceptors.

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. Another dechlorination type of reaction was reported by William WA. (1994). According to the study, the dechlorination was in sequence in six trichlorobiphenyls with all chlorides in one ring. The slurries of each reagent of the trichlorobiphenyls were incubated just according to the work of Sonzogni (1990) while removing the chlorines between two other chlorines. Here, dechlorination of every trichlorobiphenyls occurred in the first segment with all meta- and para-chlorines removed but no ortho-dechlorination was observed. In contrast, only one chlorine, meta- or para- was removed in the second and last slurries (Van Dort et al., 1997).

Priming the indigenous microorganisms in the segment was seen to cause and sustain meta- dechlorination of Aroclor 1260. 2,3,4,5,6-pentachlorobiphenyl, 2,3,4,6-tetrachlorobiphenyl and 2,3,6-trichlorobiphenyl were used to stimulate dechlorination. The targets of this reaction were hexa- and octa- chlorobiphenyls and converted them to tetra- and penta- chlorobiphenyls containing mostly ortho- and para- chlorines (Van Dort et al., 1991).

The enrichment of these microorganisms from sediments with 2, 3, 4, 5, 6-pentachlorobiphenyls resulted in a sequential meta- and para- dechlorination of Aroclor 1260. The hexa- and nona- derivatives in the segment were reduced from 66.3% to only 16.7 molecular percentages through meta-chlorine removal from serial transfers of actively dechlorinating slurries. The enrichment however, tested para- dechlorination that causes further conversion of the meta- dechlorination products to tri- and tetra- chlorobiphenyls (Bendard et al., 1996).

Primary and enriching effects of several PCB congeners on the dechlorination of Aroclor 1260 are believed to be mediated by two populations of PCB dechlorination that destructs and with different specialities (Kuipers et al., 1999). Different enrichment cultures (BK24 and BK27) enriched from marine sediments with a history of PCB contamination was able to dechlorinate four octachlorobiphenyls and three nonachlorobiphenyls. The single congeners each with a concentration of 50 ppm were added separately to the microbial enrichment culture. In about 16weeks, all the seven congeners were reductively dechlorinated.

Temperatures and pH has significant effects on the growth of the microorganisms and catalytic activity of the enzymes (Wiegel et al., 2000). According to studies, Aroclor 1260 was marginally dechlorinated at 8-34°C and at 50-60°C with an optimal temperature of 18-30°C, but at 8-34°C and 50-60°C, flanked meta-dechlorination occurred whereas unflanked para- chlorines was observed to occur only between 18 and 34°C. The optimal temperature for 2, 3, 4, 6-chlorobiphenyls for the overall chlorine removal was 20-27°C, unflanked ortho-dechlorination was observed at 8-30°C (Wiegel and Wu, 2000). Wiegel and Wu (2000), proposed a temperature-dependent route of microbial reductive dechlorination of spiked 2, 3, 4, 6-chlorobiphenyl in woods ponds. On the other hand, the overall optimal pH for chlorine removal was at 7.0-7.5. The flanked meta-dechlorination occurred at pH 5.0-8.0, unflanked para-dechlorination at pH 6.0-8.0 and ortho-dechlorination at 6.0-6.5. At pH 7.0 and 15°C, ortho-dechlorination dominated, whereas at 18 and 25°C, unflanked para-dechlorination outpaced the other dehalogenation reactions. The optimal pH for overall chlorine removal was at 6.0-7.5. All laboratory investigations of microbial PCB dechlorination have been carried out using sediments slurries, both as a source of microorganism and matrix (Brown et al., 1987). These investigations show that microorganisms with different characteristics specificities for PCB dechlorination existed in PCB-contaminated sites. The microbial population present in the sediments have distinct dehalogenating enzymes, each exhibiting a unique patter of congener selectivity resulting in various patterns of PCB dechlorination (Alder et al., 1993). However, a similarity between degradation patterns exists. The para- and meta-substituted congeners are more commonly degraded than ortho-substituted congeners. Only as few ortho-substituted congeners have been reported biodegradable (Fish et al., 1994) . Moreover, anaerobic degradation has most commonly been observed under methanogenic conditions. This may lead one to conclude anaerobic reductive dechlorination occurs under methanogenic conditions, if not inhibited by sulphate-reducing conditions. Sulphates have a higher affinity for electrons than the chloroaromatics (Alder et al., 1993).

Anaerobic PCB dechlorination reduces the potential risk and potential exposure to PCBs. According to Moore (1991), carcinogenic potential of PCBs correlates with total chlorine levels. PCBs with two para, two or more meta, and no ortho substituent exhibit dioxin-like activities (Safe, 1992). PCB congeners like 3,4,5,3',4'-, and 3,4,5,3',4',5'-chlorobiphenyl exhibit strong binding to the dioxin receptor. The preferential loss of meta- and para-chlorines catalyzed by anaerobic dechlorination results in dramatic reductions in the levels of coplanar, dioxin-like congeners in the PCB mixtures (Quensen III et al., 1992). The decrease in risk is manifested in two ways:

1. Lightly chlorinated congeners produced by dechlorination can be readily degraded by indigenous bacteria.
2. Dechlorination significantly reduces bio concentration potential of the PCB mixtures through conversion to congeners that do not significantly bioaccumulate in food chain (Moore et al., 1991).

AEROBIC BIODEGRADATION OF PCB

Sparsely chlorinated PCB congeners which form as a result of dechlorination of higher congeners are substrates for aerobic bacteria (Cookson, 1995; Kuipers et al., 1999). Aerobic oxidative destruction involves two clusters of genes. 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 –degradation of bacteria is biphenyl or monochlorobiphenyls. During utilization of biphenyls, a yellow meta-ring cleavage product is formed as observed in most studied bacteria like the *Pseudomonal* sp. (Boyle et al., 1992) and *Micrococcus* sp. (Benvinakatti et al., 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 (Benvinakatti et al., 1992) . 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 also serve as an indicator of degrading enzymes. Ahmed and Focht (1972) reported that two species of Achromobacter are capable of growing on biphenyls and 4-chlorobiphenyl. The degradation of PCB by *Myocardial* sp. and *Pseudomonas* sp. increased upon addition of biphenyls. Clerk et al. (1979) described the enhanced co-metabolism of Aroclor 1242 in the presence of acetate using mixed cultures of *Alcalegenes Odurans,* A. Dentrificans and an unidentified bacterium. Focht and Brunner (1978) observe the increased mineralization of Aroclor 1242 by Acineto bacteria strain P6 by addition of biphenyls and 4-chlorobiphenyl. These microorganisms also co-metabolised Aroclor 1254 in the presence of biphenyl, Furukawa et al. (1978) reported same.

In a recent study, an anew bacterium, *Janibacter*, MS3-O_2 was isolated from soil (Sierra et al., 203). It was interesting to note that the degradation of Aroclor1242 was significantly higher in the liquid medium without biphenyl (70-100% after 7 days) . 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. The 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 lower bioavailability of PCBs and the interactions with the indigenous soil microorganisms (Sierra et al., 2003).

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 (Ahmad et al., 1991; Yagi et al., 1980).

The complete degradation of PCB requires various microbial strains with specific congener preferences. In addition, the position and number of chlorines on the molecule can influence the rate of the first oxygenate attack? Unterman et al. (1988) proposed a mechanism for the oxidation of PCB by *A. eutrophus, P. putida*, and a *Corynebacterium* sp.; *A. eutropus* and *P. putida* bacterium strain degrade tetrachlorobiphenyl via 2,3- attack while *corynebacteria* degrades the compound via 3,4- attack.

In the study conducted by Kumancova et al. (2003), using cells of *Pseudomonas* SP. to immobilize an SIRAN carrier then Furukawa et al. (1979), degradation of individual congeners (2,4,4'-trichlorobiphenyl, 2,2',5-trichlorobiphenyl, 2,2',5,5'-tetrachlorobiphenyl, 2,2',4,5'-tetrachlorobiphenyl and 2,2'5,6'-tetrachlorobiphenyl) with biphenyl as growth substrate showed a common metabolic pathway starting with oxidation at the 2,3-position of the less chlorinated ring. The degradation for 2,4,4'-trichlorobiphenyl, a 2,3-deoxygenase attack of the less chlorinated ring was the primary reaction used by *Pseudomonas* sp., resulting in the formation of the yellow metabolite 3-chloro-2-hydroxy-6-oxo-6- (2,4-dichloro-biphenyl) 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 indicate that *Pseudomonas* sp. 2 was capable of dehalogenating PCBs (Komancova et al., 2003). The ability of the bacterial strain to dehalogenate PCBs was confirmed by the degradation of 2, 2', 5, 6'-tetrachlorobiphenyl (Komancova et al., 2003). Degradation for this compound was via 2, 3-deoxygenase attack and the products formed corresponded to (based on molecular weights) 4-(2, 5-dichlorophenyls)-oxobutanoic acid, and two other compounds, 2-chloro-3-(2, 5-dichlorophenyls)-2-acrylic acid and monochloro-acetophenone, were also detected. These products are consistent with 3, 4-dioxygenase attack (Komancova et al., 2003); the bulkiness of the chlorine atoms prevents access to the enzyme's active site. Furthermore, the chlorine atoms may prevent oxygenation if they occupy the carbon positions that are most susceptible to the oxygenase attack (Bedard et al., 1986). The ortho positions are also the most resistant to microbial attack (Bedard et al., 1990)

Some aerobic bacteria have the capacity to degrade highly chlorinated PCB congeners using different initial oxygenase reaction involving 3, 4-hydroxylation instead of 2, 3-hydroxylation. This is the case for *P. Putida* LB400 (Bedard et al., 1987), and *P. Testosterone* B356 (Albro et al., 1981). In *P. testosterone* the oxygenase attack was always on the ortho- and meta-carbon while for *P. putida*, the oxygenase attack on PCB congeners with chlorine atoms on both rings was usually accompanied by para-or ortho-dehalogenation of the molecule.

Furukawa (2004) summarised the relationship between chlorine substitution and the microbial breakdown of PCBs as follows:

1. The degradation rate of PCBs decreases as chlorine substitution increases.
2. PCBs containing two chlorine in the ortho- position of a single ring (that is, 2, 3, 6-) and each ring (that is, 2, 2') shows a striking resistance to degradation.
3. PCBs containing all chlorines on a single ring are generally degraded faster than those containing the same number on both rings.
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.

Sequential anaerobic-aerobic transformation of PCBs

The biodegradation of PCBs by aerobic bacteria had been well studied. It was observed that only lightly chlorinated PCB congeners, those with four or less chlorine atoms, was degraded. Highly chlorinated PCB congeners, those with five or more chlorine atoms, remain bio refractory 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. Under anaerobic condition, highly chlorinated PCB congeners have been found to be reductively dechlorinated through a preferential meta-and para- chlorine removal, producing less chlorinated congeners that are amenable to aerobic biodegradation. This biotransformation pattern appears to be common among halogenated aromatic compounds (Fathepure et al., 1991). Rogers and Julia (1999) reported a sequential

anaerobic-aerobic treatment of PCB s in the soil microcosms. Results of the batch soil-slurry microcosm showed dechlorination of several hexachlorobiphenyl to penta- and tetra-chlorobiphenyl by indigenous microorganisms. The aerobic microcosm experiment demonstrated the presence of microorganisms capable of degrading the tri- and tetra- chlorobiphenyl (Montgomery et al., 1994).

Both aerobic and anaerobic metabolism modes transform PCBs. Different microorganisms show preferential attack on PCBs resulting in different patterns of degradation. 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.

CHALLENGES OF THE DEGRADATION OF PCBS

Much work has been directed towards a better alternative technology for PCB destruction in the environment. The conventional method of incineration, despite its high effectiveness tends to be very expensive and sometimes produces undesirable end-products like polychlorinated dibenzo furans/dioxins (PCDF/Ds), which is as result of incomplete combustion.

For the past 2 decades, many remediation technologies for PCBs have been proposed and some are already in use commercially, but till date there have not been any of the methods that has gained wide acceptance like the old incineration methods. This has been because of the following reasons:

1. None of the alternative technology has been certified to be applicable to all PCB contaminated media.
2. There is no certainty on the by-products of 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.

The aforementioned factors have somehow posed threats to researchers and government agencies by their effort in trying to come up with an alternative technology than incineration. There is need for an extensive review of the extent of PCB problem of each country for an appropriate technology to suffice. Also, the complexity of the microbial process which is used to degrade these complexities of compounds should also 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 work of microorganisms.

PHYTOREMEDIATION

Phytoremediation is an emerging green technology that uses plants to remediate soil, sediments, surface water, and ground water environment contaminated with toxic metals, organics, and radionuclide's (Pradhan et al., 1998).

Phytoremediation is an effective, non-intrusive and inexpensive means of remediating soils (Wiltse et al., 1998). It is a more cost effective method than the conventional mechanical and chemical methods of removing hazardous compounds from the soil (Bollag et al., 1994). Apart from these, phytoremediation is a natural, aesthetically pleasing low-cost technology which is socially accepted by surrounding communities and regulatory agencies as a potential elegant and beautiful technology (Ensley, 1997). Phytoremediation of contaminated soils offers an environmentally friendly, cost effective, and carbon neutral approach for the cleanup of toxic pollutants in the environment. Plants with abilities to hyper accumulate heavy metals, uptake volatile organic compounds and sequester pollutants have been proposed as a solution to the treatment of toxic contamination *in situ*. Plant remediates organic pollutants by:

1. Direct uptake of contaminants, their conversion and accumulation of non-phytotoxic metabolites.
2. Release of exudates and enzymes stimulating microbial activity and biochemical transformations.
3. Enhancement of mineralization in the rhizosphere.

There is suggestion that plant enzymes released into the environment have a significant catalytic effect (Cunnigham et al., 1995). After screening of freshwater sediments, it was shown that five specific enzymes-dehagenase, nitroreductase, peroxidise, laccase and nitrilase were of plant origin. Though, there has been scarce detailed description of enzymatic reactions leading to the degradation of PCBs in plants. But the metabolic pathways of PCB degradation in microbial cells has been intensively studied; this showed that bacterial degradation occurs via two main routes, highly chlorinated PCB congeners can be dechlorinated under anaerobic conditions to form less chlorinated ones which are more susceptible to aerobic degradation. Lower chlorinated PCBs can be degraded by aerobic bacteria via a well-documented pathway (Abramowicz, 1990) to chlorobenzoates. Degradation of PCBs by fungi has been described (Yadav et al., 1995) and the data obtained have shown many similarities with bacteria aerobic degradation (aerobic process, inability to degrade higher chlorinated PCBs, etc.) (Dowling and Doty, 2009).

Phytoremediation is a word formed from the Greek prefix "phyto" meaning plant and the Latin suffix "remedium" meaning to clean or restore (Cunningham et al., 1997). The term actually refers to a diverse collection of plants-based technologies that use either naturally

occurring or genetically engineered plants for cleaning contaminated environment (Paul et al., 2007).

The primary motivation for the development of phytoremediative technologies according to Ensley (2000) is the potential for low-cost remediation. Although the term, phytoremediation is a relatively recent invention but the practice is not. Research for treating radionuclide-contaminated waters using semi-aquatic plants existed in Russia at the down of the nuclear era (Salt et al., 1995a; Cherian and Margarida, 2005). Some plants which grow on metalliferous soils have developed the ability to accumulate massive amounts of the indigenous metals in their tissues without exhibiting symptoms of toxicity (Beker et al., 1991). Chaney (1983) was the first to suggest using these "hyperaccumulators" for the phytoremediation of metal polluted sites.

Direct benefits of phytoremediation

Phytoremediation is an *in situ*, solar driven technique, which limits environmental disturbance and reduces cost (Shimp et al., 1993). Moreover, it is particularly well suited for the treatment of large areas of surface contamination, especially where other methods may not be cost effective (Schnoor, 1999). In general, both public and government regulators look favourably upon phytoremediation because it involves exploiting the natural ability of the environment to restore itself (Cunningham et al., 1996). There has been a wider support from the public on the use of plants for remediation. This was cited at a series of public focus group meetings to gauge public perceptions and awareness of environmental applications of bio-technology especially in Canada (Mclutyre and Lewis, 1997).

Plant samples can be harvested and used as indicators to the extent of remediation or conversely contamination. Similarly, a field of plants may serve as a direct, visual bioassay (Shimp et al., 1993). There is also the potential to grow various phytoremediator species together on the same site in an attempt to simultaneously remediate various contaminants, including salts, metals, pesticides, and petroleum hydrocarbons. Plants help to contain the region of contamination by removing water from soil, thereby keeping the contaminants from spreading or confining them within or near the root system (Shimp et al., 1993). Some wetland plants can transport oxygen to the rhizosphere under conditions that may otherwise limit the amount of oxygen available to soil microorganisms, as in the case in soils and sediments saturated with water or contaminated with oil (Schnoor et al., 1995) . Microbial communities in the rhizosphere may be able to biodegrade wide variety of organic contaminants. Finally, phytoremediation may be applied with relative ease using existing agricultural practices at the contamination sites (McIntyre and Lewis, 1997).

Indirect benefit of phytoremediation

Phytoremediation leads to improvement of soil quality by improving soil structure (aggregates and pads), increasing porosity/aggregation and therefore water infiltration, providing nutrients (nitrogen fixing legumes), and accelerating nutrient cycling, and increasing soil organic carbon (Schnoor et al., 1995; Cunningham et al., 1996). The use of plant in remediation efforts stabilizes the soil, thus preventing erosion and direct human exposure (that is, inhalation of soil particles carried by the wind (McIntyre and Lewis, 1997). Phytoremediation eliminates secondary air- or water-borne waste, examples is the accumulation of PAHs from the atmosphere (Simonich and Hites, 1994). It also has the potential to eliminate green house gas emission because it does not require the use of pumps or motors that give off green house gases and plants used in phytoremediation may serve as sinks for the green house CO_2 (Tsao, 1999). Reduction of noise level from industrial sites is achieved because phytoremediation is less noisy than the other reclamation alternative. Another indirect benefit is that the growth of certain hardy plants in a contaminated soil can allow for the growth of other less hardy plants.

Limitations of phytoremediation

Petroleum hydrocarbon contamination must occur at shallow depths for phytoremediation to be effective. Though some plants such as trees may have root systems that can extend to a depth of 60 m, most plants do not produce roots to anywhere near this depth and root diversity generally decreases with depth (Cunnigham et al., 1996) . Consequently, as depth increases beyond one or two metres, relatively immobile contaminant-those that cannot migrate to the plant roots during water uptake are increasingly unlikely to be affected by phytoremediation. Phytoremediation is slower than *ex situ* methods, typically requiring several seasons for site clean-up (McIntyre and Lewis, 1997). Because it is slow, phytoremediation is not an appropriate solution where the target contaminant presents an immediate danger to human health or the environment. If the contaminant is bound tightly on the soil particles or organic matter, it may not be available to plants or microbes for degradation (Otten et al., 1997). Environmental condi-tions like soil texture, pH, salinity, oxygen availability, temperature and level of non- hydrocarbon contaminants (for example, metals), must all be within limits tolerated by plants. However, plants will not grow if concentrations of the target contaminant are too high, therefore phytoremediation of the target contaminants will not proceed unless the soil is pre-treated to reduce phytotoxicity or a resistant plant species is selected (Cunningham et al., 1996).

Phytoremediation and PCB

A review of the literature indicates that polychlorinated biphenyls (PCBs) are not leachable in the soils and that they are readily absorbed by soil sediments. It appears that lower chlorinated PCBs are less absorbed and slightly mobile in the soils. Total organic matter content of the soils seems to be more important than total clay content or total surface area in explaining adsorption of PCBs by soil. There have been various work on the effect of PCBs on plants, the results of this work indicated that plants absorb PCBs but in a very slow amount. PCBs therefore appear to have some effects on photosynthesis and respiration in plants (Strek et al., 1982).

Effects of PCBs on plants

The inhibition of plants growth due to PCB effects has been well documented by Kiel et al. (1998). This report, documented mainly for algae denoted several deductions in algae cell numbers at a general low level (0.3-10 ppm) of PCBs in aqueous solution. They had been scarce reports of the growth inhibition to higher plants. Mahanty and Fineran (1976) reported the complete internal disorganisation of chloroplasts in the front cells of an aquatic plant *Spirodela oligorrhiza* (Kurtz), Hegelm exposed to 5 ppm Aroclor 1242. Weber and Mrozek (1999) reported malformations on newly developed soybean leaves on plants growing in 1000 ppm Aroclor 1254 applied to soil. Reduction in plants height and fresh weight was observed for soybean, beets and pigweed *Amaranthus refloflexus* L. but only fresh weight reductions were reported for Fescue (Strek and Weber, 1980). At 1000 ppm rate of Aroclor 1254, soybean growth was inhibited by about 47%. However, cumulative water use seems to be more sensitive than plant growth to PCB. This indirectly means that the effects on plant growth may be indirect, following the effects which may reflect on transpiration.

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 (Moza et al., 1976). The former route is probable 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 fallout is unlikely to occur to any great degree because the chemical may absorb to the outer surface of the plants and may not be truly present inside the plant. The cuticle contains many lipophylic compounds in which the PCB could effectively 'dissolve', limiting further internal migration. 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 studiers 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 and Mrozek, 1979).

The work done by Iwata and Gunter (1976) was reported by Strek et al. (1982). According to the report, they grew plants on soil treated with PCBs (Aroclor 1254 measured in ppm). Data input on the work included isotope, number of chlorines per biphenyl, plant part analysed, PCB content in ppm, PCB content of the soil at planting and at harvest and also growth period. Plant species that were used include carrot, fescue, reddish, soybean, spruce, sugarbeet, tomatoes and lastly unidentified weeds. The species according to Strek et al. (1982) were planted in soils fortified with 0.046-100 ppm. At harvest, the concentration of PCBs at soil levels ranged from 0.040-76 ppm. Although, wide range of experimental condition was ensured, several factors were responsible for the trend in plants uptake of the contaminant. The amount of uptake was generally low ranging from 0.0016-13.9 ppm, and averaged 1.241ppm over all data. According to the information aforementioned, a suggestion was made that PCB content of plant was dependent on the PCB concentration in the soil. Hence from the result, the bio concentration factor (BCF) at both planting and harvest period was found to be significant (PR>F< = 0.0001) for both soil sampling time. Therefore in every study of PCB phytoremediation by plants, it must consider the fact that PCB availability and mobility in the soils depends on the clay and organic contents of the soil as well as temperature. Differences in plants species were also an important factor, with carrot containing an average amount of 2.52 ppm, fescue averaging 1.67 ppm while the unidentified weeds averaging 1.52 ppm. Tomatoes contained the least amount averaging 0.0023 ppm, and this could be according to Wallnofer and Koniger (1994) as a result of application of extremely low rates of PCB of between 0.046-0.062 ppm.

The importance of biphenyl metabolites in plants has often been overlooked. In a variety of plants the main metabolites which have been isolated appears to be mono- and dehydroxylated biphenyls. This however agrees with the study of some researchers on PCB.

In the work of Smith et al. (2007), he conducted a greenhouse study to evaluate the effects of plants growth on PCB congeners found in Aroclor 1260. Here Smith and his fellow scholars added an organic amendment (starch straw) in order to hasten the degradation. The source of soil was a river bank and the texture of the sediment was silt-loam which on analysis has the following (61% silt, 5% clay, 34% sand). The source of PCB was a transformer oil containing Aroclor 1260. The plants used include river bulrush (wetland sp.) (*Seirpus*

fluviatilis), eastern gamagrass (terrestrial sp.) (*Tripsacum dactyloides*), lake sedge (wetland sp.) (*Carex aquatalis*) and praire cord grass (wetland sp.) (*Spertina pectinata*). 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 0.1mg/l in solution). Significant differences between treatments were observed for the percentage loss among the penta- to the hepta- chlorobiphenyls.

Carex aquatilis with amendment had significant higher percentage loss than *C. aquatilis* without amendment, SD. pectinata with amendment and *T. dactyloides* with amendment; Mulberry (*Morus rubra*) with amendments was reported to have significantly higher percentage loss than in *Sotalia fluviatilis* with amendment. Euliss (2005) reported that highly chlorinated PCBs found in Aroclor 1260 require reductive dechlorination as the first step in remediation, and this process require a treatment with low transpiration and high soil water content. According to him, the reductive dechlorination would lead to the accumulation of less chlorinated congeners that were possibly lost to aerobic microorganisms during the aerobic stage of the work. The result of this study however is in agreement with Quensen et al. (1990), who noted that aerobic mineralization of PCBs is limited to PCBs with 5 or fewer chlorines. According to Smith et al. (2003) of the congeners he monitored, only one had five chlorine present (2, 3', 4, 4', 5'-pentachlorobiphenyls). This also was the congener with the smallest number of chlorine detected in significant amount in Aroclor 1260 and one of the 2 congeners to show significant differences in response to treatment. Quensen et al. (1990) did not find any evidence for the aerobic degradation of Aroclor 1260 and did not detect 2, 3', 4, 4', 5'-pentaclorobiphenyls. During the dechlorination of Aroclor 1260, penta-, tetra-, tri- and dichloro biphenyls accumulate (Bedard et al., 1996; Van Dort et al., 1997). Examining the chlorine distribution of the PCB compound monitored in this study, the 2,3,3',4,4',5,5'-heptachloro-biphenyls could lose one chlorine from a meta position and become2,2',4,4',5,5'-hexachlorobiphenyls, which is another 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 (Nies and Vogel, 1990) and could explain why percentage loss of the 2,3',4,4',5'-pentachloro biphenyls was not large. In sediments maintained at water saturation, reductive dechlorination is likely with possible accumulation of less chlorinated PCBs (Brown and Wagner, 1990; Quensen et al., 1990). Therefore, using plant species that remove water from the sediments and introducing oxygen into the rhizosphere through aerenchyma could greatly stimulate the removal of lower-chlorinated PCBs from the environment but would have far less impact on higher chlorinated congeners. Tang and Myers (2002) achieved a 40% reduction of PCB in dredged sediments.

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 are 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. But to some extent this can be addressed through interactions with the natural micro flora associated with plants; both endophytic bacteria and rhizosphere bacteria have been shown to have the potential to degrade organic contaminants in association with plants. However, these disadvantages as this review has stated, can be amended by employing means that uses rhizo-phytodegradation.

Vicinity of plants root is the preferred environment for microorganisms. Approximately 1.2×10^{11} cells/cm^3 live within a distance of less than 1 mm to the roots, whereas only 1.3×10^{10} at a distance of 2 cm (Paul and Clark, 1989). About 5- 10% of the root surfaces are covered with bacteria. Roots live in symbiosis with fungal mycorrhiza. Their mycelium is again covered with bacteria through soil (Karlson et al., 1995). Besides forming a habitat for microorganisms, plants roots also provide nutrients, for example, sugars, in exchange for phosphates (fungi) or nitrogen (N_2-fixation). Mulberries, *M. 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 soil –born pollutants for example, saponins, proteins and enzymes. Spectacular was the findings that roots and xylem exudates of zucchini (cucurbitaceae) solubilise PCDD/F (Hulster et al., 1994), probably by protein (Neumann et al., 1996). However, plants hyper accumulating lipophilic compounds have not yet been detected but with microorganisms, reasonable result is ensured.

The combined effort of microorganisms and plants on PCB was seen in report of the work of Dzantor et al. (2001), in which they enhanced the dissipation of Aroclor 1248 using substrate amendment in the rhizosphere soil. In this work, Dzantor and his group used two plants species-reed canary grass (*Phararis arundinaceae* L.) and the legume, flat pea (*Lathyrus silvestris* L.). According to the work, these plants were among the crops that showed inherent potentials for stimulating PCB dissipation in previous greenhouse experiments (Dzantor et al., 2000). In the course of this work, Dzantor, tested other legume; burr medic (*Medicago polymorpha*), for potentials to enhance PCB dissipation. The reason for this was that Medics are well characterized family, hence may serve as model system for elucidating and manipu-lating the process that can improve rhizodegradation

beyond the currently observed inherent capabilities of selected plant species.

Moisture replacement systems (MRS) were used in this study for plant growth. The result of this study indicated an overall PCB recovery in unamended, unplanted soil to be high and higher than the recoveries in unamended soils that were planted with selected crops. After about 100 days, 69% of the initially added PCB was recovered in unplanted soil, compared to 65, 59, and 54% of initial additions in soils planted with flat pea, red canary grass and burr medic respectively. In spite of a definite trend towards enhanced PCB dissipation compared to unplanted soil, especially in burr medic rhizosphere, however, the differences were not statistically significant at $P<0.05$. This was attributed in part to analytical difficulty and consequent variability that was encountered during the experiment.

The experiment described here were based on the assumption that supplies of organic residue containing inducers for PCB degradation could enhance degradation in rhizospheres that harbour competent degraders already.

The work of Mehmannavaz et al. (2002) agrees with the aforementioned postulates as well. According to him, the effect of plants-microbe- soil interaction on the biotransformation of PCBs in a rhizosphere soil ensured an appreciable depletion, loss or change in PCB levels; these could be attributed to either direct or indirect biotransformation, bio translocation or adsorption of the contaminants due to the presence of alfalfa and or rhizobial inoculations. Mehmannavaz et al. (2002) reported that the present of alfalfa plants increased the transformation/depletion of PCB congeners as compared to bio augmentation alone; however, alfalfa alone was the most effective treatment, according to his study. Bio augmentation of the soil significantly increased the hardness of the soil and thus, may have indirectly slowed the growth of alfalfa plant. The decrease in productivity and growth of plants and PCB transformation in the contaminated soil suggested that PCB as or their bacterial products may have been slightly phototoxic due to increased biotransformation, bioavailability or solubility. However, the difference in plant growth and PCB depletion in bio augmented and non bio augmented treatments may have been related to both the bacterial augmentation and the soil hardness. He 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.

CONCLUSIONS

Plants plays a very useful role in dealing with persistent organic pollutants (Companella et al., 2002), therefore if properly exploited in its relationship with microorganism could give us an alternative to the complete remediation of PCB from soil and sediments.

Schnoor and his co-workers evaluated applicability of phytoremediation (Schnoor et al., 1995; Schnoor, 1997). They found out that the technique is most successful when top soil is polluted with chemicals being either degraded in the rhizosphere or effectively taken up by plants for too high pollutants concentrations, toxic effects may occur, and phytoremediation therefore is restricted to lower medium contaminated level. There is need for phytoremediation to be used in combination with an alternative treatment method for hot spots (Schnoor, 1997).

REFERENCES

Agency for toxic substances and disease registry (1993). Toxicology profile for selected PCBs, TP-92/16, pp. 103-108.

Bioremediation Discussion Group (2006). IPCC guidelines for National Greenhouse Gas Inventories. IPCC report, 5: 1-8.

Borja J, Marie TD, Auresenia J, Gallardo S (2005). Polychlorinated biphenyls and their biodegradation. Process Biochem., 40: 1999-2013.

Borja JQ, Auresenia JL, Gallardo S (2006). Biodegradation of polychlorinated biphenyls using biofilm grown with biphenyl as carbon source in fluidized bed reactor. Chemosphere, 64: 555-559.

Boyle AW, Silvin CJ, Hassett JP, Nakas JP, Tanenbaum SW (1992). Bacterial PCB biodegradation. Biodegradation, 3: 285-298.

Bremle G, Larsson P (1998). Uptake of PCBs in fish in a contaminated river system-Bioconcentration factor measured in the field. Environ. Sci. Technol., 29: 2010-2015.

Brown J, Bendard DL, Brennan MJ, Carnahan JC, Feng H, Wagner RE (1987). Polychlorinated biphenyl dechlorination in aquatic sediments. Sci., 236: 709-712.

Carrillo-Castaneda G, Munos JJ, Peralta-Videa JR, Gomez E, Tiemannb KJ, Duarte-Gardea M, Gardea-Torresdey JL (2001). Phytoremediation. Adv. Environ. Res., 6: 391.

Cherian S, Margarida OM (2005). Transgenic Plants in Phytoremediation: Recent Advances and New Possibilities. J. Environ. Sci. Technol., 39(24).

Choi H, Veriansyah B, Kim J, Kim D, Lee YW (2009). Recycling of transformer oil contaminated by polychlorinated biphenyls (PCBs) using catalytic hydrodechlorination. J. Environ. Sci. Health, A: 494-501.

Dietz AC, Schnoor JL (2001). Advances in Phytoremediation. Environ. Health Perspect., 109: 163-168

Dobbins DC (1995). Biodegradation of pollutants. Encyclopeadia of environmental biology, 1, Academic Press Inc., p. 128.

Dobri AB (2009). How to Write a Good Review Article. www.yahoosearch.com . 26/10/2009.

Dowling DN, Doty SL (2009). Improving phytoremediation through biotechnology. Curr. Opin. Biotechnol., 20: 204-206.

Dzantor EK, Chekol T, Vough LR (2000). Feasibility of using forage Grasses and Legumes for Phytoremediation of Organic Pollutants. J. Environ. Sci. Health, A35 (9): 1645-1661.

Dzantor EK, Woolston J (2001). Enhancing Dissipation of Aroclor 1248 Using Substrate Amendment in Rhizosphere Soil. J. Environ. Sci. Health, A36(10): 1861-1871.

Eisenreich SJ, Jeremiason JD, Peterson MJ, Beaty KG, Hecky R, Elser JJ (1999). Biochemical cycling of PCBs in lakes of variable tropical status. A paired-lake experiment, Limnol. Oceanogr., 44(3 part 2): 889-902.

Erickson MP (1997). Analytical chemistry of PCBs, 2ND ed., CRC Lewis publishers New York, p. 66.

Ferro AM, Sims RC, Bugbee B (1994). Hycrest crested wheatgrass accerelates the degradation of pentachlorophenol in soil. J. Environ. Qual., 23: 272-279.

Fitzgerald ED, Hwang S, Deres DA, Bush B, Cook K, Worswick P (1998). The association between local fish consumption and DDE,

mirex, and HCB concentration in the breast milk of Mohawk women at Akwesasne. J. Exp. Anal. Environ. Epid., 11: 381-388.
Fletcher JS, Hedge RS (1995). Release of Phenols by Perennial Plant Roots and their Potential Importance in Bioremediation. Chemosphere, 31: 153-158.
Furukawa K, Matsumura F, Tonomura K (1978). Alcaligenes and Acinetobacter strains capable of degrading polychlorinated biphenyls. Agric. Biol. Chem., 42: 543-548.
Furukawa K, Tomisuka N, Kabayashi A (1979). Effect of chlorine substitution on the bacterial metabolism of various polychlorinated biphenyls. Appl. Environ. Microbiol., 38: 301-310.
Furukawa K, Hikaru S, Masatoshi G (2004). "Biphenyl Dioxygenases". Functional Versatilities and Directed Evolution. J. Bacteriol., 186: 5189-5196.
Gioia DD, Bertin L, Zanaroli G, Marchetti L, Fava F (2006). Polychlorinated biphenyl Degradation in aqueous wastes by employing continuous fixed-bed bioreactors. Processs Biochem., 41: 935-940.

Gogoi BK, Dutta NN, Goswami P, Krishna MTR (2002). A case study of Petroleum hydrocarbon contaminated soil at a crude oil site. Adv. Environ. Res., 7(4): 767-782.
Held T, Dorr H (2002). *In situ* Remediation. Biotechnol., 11b: 350-370.
Huang XD, El-Alawi Y, Penrose DM, Glick BR, Greenberg BM (2004). A multi process phytoremediation system (MPPS) for removal of total petroleum hydrocarbons. Environ. Pollut., 130: 400-453.
Huang XD, Glick BR, Greenberg MB (2001). In: Greenber et al., (Eds), Environmental Toxicology and Risk Assessment. ASTM, 10: 271.
IARC (1978). Monograph programme on the evaluation of carcinogenic risks to human. IARC, 18: 43.
Idris A, Ahmed M (2003). Treatment of polluted soil using bioremediation – A review. Faculty of Chemical and Environmental Engineering, University of Putra, Malaysia, pp. 1-18.
Komancova M, Jurcova I, Kochankova L, Burkhard J (2003). Metabolic pathways of polychlorinated biphenyls degradation of *Pseudomonas* sp. 2. Chemosphere, 50: 537-543.
Koning M, Hupe K, Stegmann R (2000). Thermal Process, Scrubbing/ Extraction, Bioremediation and Disposal. Biotechnology, 11b: 306-317.
Larsson P (1985). Contaminated sediments of lakes and oceans as sources of chlorinated hydrocarbon for release to water and atmosphere. Nature, 317: 347-349.
Larsson P, Okla L (1989). Atmospheric transport of polychlorinated hydrocarbon to Sweden 1985 compared to 1973. Atmos. Environ., 16: 1699-1711.
Mackova M, Macek T, Ocenaskova J, Burkhard J, Demnerova K, Pazlarova J (1997). Biodegradation of Polychlorinated Biphenyls by Plant cells. Int. Biodeterior. Biodegrad., 39(4): 317-325.
McEldowney S, Hardman DJ, Wait S (1993). Pollution: ecology and biotreatment. New York. Longman Sci. Tech., pp. 122-128.
Mehmannavaz R, Prasher SO, Ahmad D (2002). Rhizospheric effects of alfalfa on biotransformation of polychlorinated biphenyls in a contaminated soil augmented with *Sinorhizobium meliloti*. Process Biochem., 37: 955-963.
Mukerjee A (1998). Dioxins/PCBs and the food chain. R and H Hall Technical bulletin, Dublin-Ireland, pp. 1-11.
Newman LA, Reynolds CM (2004). Phytoremediation of organic compounds. Curr. Opin. Biotechnol., 15: 225-230.
Okey AB (2007). An aryl hydrocarbon receptor odyssey to the shores of toxicology: The Deichmann Lecture. International Congress of Toxicology-X1. Toxicol. Sci., 98(1): 5-28.
Paul OE, Ana C, Mark J, Nancy MD, Elizabeth AHP-S, Keneth FR (2007). Comparism of Plant Families in a Greenhouse Phytoremediation Study on an Aged Polycyclic Aromatic Hydrocarbon-Contaminated Soil. J. Environ. Qual., 36: 1461-1469.
Pesatori A, Zocchetti C, Guercilena S (2003). Epidemiology/Human Data, Part A: Cancer effects, EPA Review article, Part 2, 7: 1-103.
Quensen JF III, Boyd SA, Tiedje JM (1990). Dechlorination of four commercial polychlorinated biphenyl mixtures (aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol., 56: 2360-2369.
Quiping Y, Puri RK, Kapila S, Yanders AF (1992). Studies on the transport and transformation of PCBs in plants. Chemosphere, 25(7-10): 1475-1479.
Safe S (1992). Toxicology, structure-function relationship, and human and environmental health impacts of polychlorinated biphenyls: progress and problems. Environ. Health Perspect., 100: 259-268.
Schmidt JV, Bradfield CA (1996). Ah receptor signalling pathways. Annu. Rev. Cell Dev. Biol., 12: 55-89.
Shann JR, Boyle JJ (1997). In: Anderson, T., Coats (Eds), Bioremediation through rhizosphere technology. ACS Series, 563, American Chemical Society, Washington, DC, p. 71.
Silvestre M, Sandossi M (1994). Selection of enhanced PCB-degrading bacterial strains for bioremediation: consideration of branching pathways. In: Chaudhry G.R., editor. Biological degredation and remediation of toxic chemicals. New York: Chapman and Hall, pp. 47-73.
Smith KE, Schwab AP, Banks MK (2007). Phytoremediation of Polychlorinated Biphenyls (PCB)-Contaminated Sediments: A Greenhouse Feasibility Study. J. Environ. Qual., 36: 239-244.
Strek HJ, Weber JB (1982). Behaviour of Polychlorinated Biphenyls (PCBs) in soils and Plants. J. Environ. Pollut., 28: 291-312.
Toro SD, Zanaroli G, Fava F (2006). Aerobic bioremediation of an actual site soil historically contaminated by polychlorinated biphenyls (PCBs) through bioaugmentation with a non acclimated, complex source of microorganisms. Microb. Cell Fact., 5: 11.
Tsai PC, Ko YC, Huang W, Liu HS, Guo YL (2007). Increased liver and lupus mortalities in 24-year follow-up of the Taiwanese people highly exposed to PCBs and PDDF. Sci. Total Environ., 247: 216-232.
UNEP (1999). Chemicals guideline for the identification of PCBs and materials containing PCBs, First issue, Inter-organization program for the sound management of chemicals, pp. 562-563.
Van DJ, Lloyd T, Chhetry S, Raycharn L, Peck J, (2002). Remediation technologies screening matrix and reference guide. Fed. Remediat. Technol. Roundtable, 4: 11-54.
Walton BT, Anderson TA (1994). Microbial Degradation of Trichloroethylene in the Rhizosphere: Potential Application for a Biological Remediation of Waste Sites. Appl. Environ. Microbiol., 56: 1012-1016.
WHO Quaterly Report (1998). International toxicity equivalent factor (I-TEF), pp. 201-211.
WHO(1976). Polychlorinated biphenyls and terphenyls. Environ. Health Criteria, Geneva. World Health Organ., pp 201-219.
Wiegel J, Wu Q (2000). Microbial reductive dehalogenation of polychlorinated biphenyls. FEMS Microbiol. Ecol., 32: 1-15.