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Review

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

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The environment is suffering severe contamination as a result of various uncontrolled activities of man and chemicals in the biosphere. This widespread contamination of air, soil and water by metals, chemicals and metalloids causes environmental concerns, which if left unchecked will be detrimental to man and other organisms. Biological methods for the cleaning of the environment especially our soil have been receiving increasing attention especially in the past two decades. Bacteria and fungi have been the natural detoxification agents 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 our natural environment may not be affected as some of the processes are environmentally friendly. However, hydrophobic organic molecules such as polychlorinated biphenyls (PCBs) tend to be much less responsive to bioremediation strategies. The wide spread presence of this compounds and other persistent organic pollutants (POPs), that share common chemical, toxicological and environment properties continues to increase in the environment, even with the various measures taken to control its presence. This review focuses on the possible trends in the remediation of PCBs in the environment and the methodologies applied. It also compares plants remediation as well as microorganisms' degradation as biological detoxification agents of the compound. This will highlight the possible improvement measures on the combination of plants and microorganisms in bioremediation, thereby filling the gap left by the conventional methods of remediation with its limitations and disadvantages.

Key words: Bioremediation, phytoremediation, (PCB), biodegradation, environmental pollution, rhyzodegradation, dechlorination, contaminated soil.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are Persistent Organic Pollutants (POPs). Their persistence in the environment 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;
$C_{12}H_{10-n}CL_n$ (Erickson, 1997)

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.

Physico-chemical properties of PCBs

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

About twenty nine of these congeners are of environmental interest. Toxicological problems of PCB

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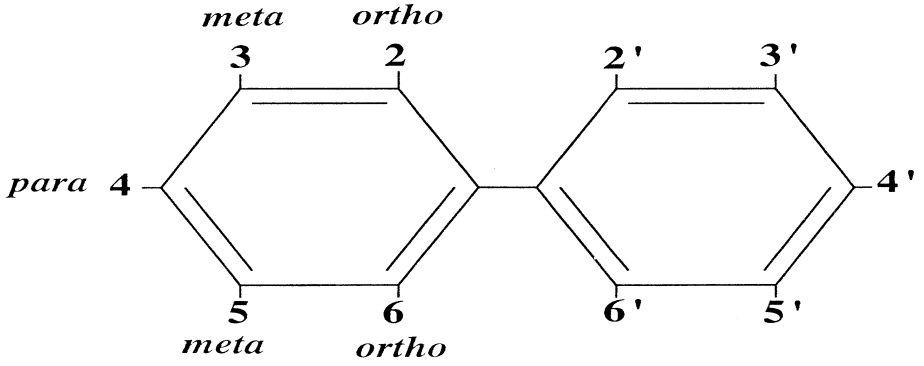

Figure 1. Structural formula of PCB showing the number and location of a Cl groups (Wiegel and Wu, 2000).

are associated with its co-planar congeners, and the basic structure of PCB according to Wiegel and Wu (2000), is as 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 the most toxic. It is known that *ortho* substitution increases toxicity, therefore 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 viscous 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 ranging from 140^{o}C to 200^{o}C, but most of them have no flash points according to standard tests (Wiegel and Wu, 2000). Its vapour is invisible and has a very strong odour; this is one of the characteristic 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 6mg/l for monos-, and about 0.007mg/l for the octas. Strangely, decachlorinated biphenyls though with high chlorine content, have a double solubility in water than the octachlorinated counterparts. This solubility is known to vary among congeners of same number of chlorine atoms (Borja et al., 2005).

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 low acute toxicity (Hutzinger, 1974). The toxicity of PCB varies considerably among congeners. The coplanar PCBs are known as non-*ortho* PCBs because they are not substituted at the ring positions to the other ring, (i.e. PCBs 77, 126, 169 etc.). They tend to have dioxin like properties, and are generally among the most toxic congeners (UNEP Chemicals, 1999). PCB health effects on human ranges from the skin conditions to the 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).

Sources of PCBs

No evidence has supported the natural occurrence of PCBs although they continue to exist in many environmental matrices (Borja et al, 2004). Majority of the PCBs in the environment finds its way during their manufacture, usage as well as during disposal (EPA, 1996). Human activities influence the concentration of PCBs especially close to shorelines and in water (Borja et al., 2005), this could be attributed to human occupation as well as the use of PCB-containing products. The major source of PCB in surface water is from environmental cycling (i.e. 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 bioaccumulate in the fish (EPA, 1993a). PCBs are versatile and synthetic chlorinated compounds, though its production was banned years ago, they are still contained in most of the finished products that are used by man. PCB attaches strongly to soil and may remain there for several years as a result of its lipophilicity it is for this reason that environmental cycling is expected in disposal and spill sites. Another possible source of PCB exposure is the workplace, these occurs during the course of maintenance and repair of PCB transformers, accidents, fires and spills (Yang et al., 2006). It also occurs during the disposal of PCB-containing materials by breathing contaminated air and by making contact with materials

containing PCBs (Borja et al., 2005). 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 (ATSDR, 2000).

PCB release into the environment

PCBs are largely generated as by-products of other chemical production meaning that the compound is synthetically generated. Dating from 1929 until 1970s, they were commercially produced as complex mixtures (Erickson, 1997), such production were stopped in some of the producing countries in 1970s because of the harmful effect of the compound. However, production continued in some areas till 1984 (Erickson, 1997). Each country of production of PCBs adopted different methods and name to the compound, for example, In USA, Monsanto Industrial Chemicals Company, produced PCBs as Aroclor; in Germany, Bayer produced as Clophen; Caffaro produced as Phenoclor in Italy; Japan as Pyralene by Kanegafuchi Chemical Company; Kanechlor in France by Prodelec; Fenchlor in Czechoslovakia by Chemko; and Delor in USSR by Sovol. During the period of production especially in the US, about 571,000 metric tons (1,250x106 pounds), were produced and or were used in the United States (Erickson, 1997; Hamlin, 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 could be granted to individual practitioners for use with optical microscopy and for research and development (EPA 1998u). However, production of PCBs in Europe, USA and Canada ceased years ago as a result of its toxicity and persistence (Pross et al., 2000).

The release of exotic PCB-materials into the environment rarely occur as a result of the fact that PCBs are no longer being manufactured or imported in large quantity (ATSDR, 2000). Rather the compounds are predominantly redistributed from one compartment to the other (e.g. soil to water, water to air, and sediments to water) (Eisenreich et al., 1999; Larsson and Okla, 1989). Thus, the majority of PCB in the air results from volatilization of PCBs from soil and water. Some PCBs may be 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.

Health and environmental effects of PCBs

About twenty nine of PCB congeners are of environmental interest when considering its toxicity. Toxicological problems of PCB which are associated with its co-planar congeners have been studied extensively *in vitro* and *in vivo* using animals as well as well as humans that were exposed to the compound through occupation or perhaps incidents for example the Yusho incident of Japan in 1968, the Hudson River incident in the US in 1979, the New Bedford Harbor incident also in the US, in the 1970s, and in the Great Lakes incident of 1988 in Canada (Erickson, 1997). Lethality, toxicity on reproduction, growth inhibition, porphyria, immunotoxicity, induction of enzyme, hepatotoxicity, endocrine effects, neurotoxicity, thymic atrophy, dermal toxicity, carcinogenicity and other biochemical responses have all been implicated in almost all the multiple PCB studies in the laboratory. This laboratory exposure is subject to some questions as a result of the purity of the compound. However, the possibility of other POPs present in the studies, make the assignment of the observed effects to PCBs liable to criticism (Safe, 1992). The toxicological effects of PCBs relates directly with their structures. The most important of the congeners is that containing no ortho-chlorine substituents or single ortho chlorine substituents which can assume a co-planar configuration with shapes similar to 2,3,7,8-TCDD (Erickson, 1997). The congeners of PCB that are toxic carry between 5-10 chlorine atoms, mostly in the para and meta positions. Meanwhile, the congener's that substitute at the 3, 4-ortho positions are considered the most toxic. It is known that ortho substitution increases toxicity, therefore properties of every PCB congeners depend entirely on the degree of its chlorination.Polychlorinated biphenyls possess dioxin- like toxicity. Toxicity determination for any mixture usually take into account international toxicity equivalents factor (I-TEF), e.g. 3,3',4,4'-tetrachlorobiphenyl has I-TEF of 0.0001, and 3,3',4,4',5-pentachlorobiphenyls has I- TEF of 0.1. Recorded effects of PCB toxicity include dermal toxicity, immune-toxicity, reproductive effects and tera-toxicity, endocrine disruption and carcinogenicity (WHO, 1998). The first step in PCBs toxicity mechanism is mediated by the binding of PCB to the Aryl hydrocarbons (Ah) cellular receptor (Mukerjee, 1998, WHO, 1998, Anonymous, 2000). Toxicity of PCBs is said to range from low to moderate (WHO, 1998). Treated samples of animal show a Lethal Dose (LD50) ranging from 0.5 g/kg to 11.3 g/kg of body weight. Most of the effects are as a result of repetitive or chronic exposure (Schmidt and Bradfield, 1996).

Absorption of PCBs by human and animals is through the skin, the lungs, and the gastrointestinal tract. Once inside the body, they are transported through the blood stream to liver and to various muscles and adipose tissue where they accumulate. Research studies has demonstrated that the effects of PCBs on health depend on age, sex, and areas of the body where there are concentrated. Borja et al, (2005) has shown carcinogenic

effects of PCBs in animals. They demonstrated and show mild liver damage and occasional death in animals that ate food containing large amount of PCBs (Schmidt and Bradfield, 1996). Occupational studies show 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 percent chlorine. Brown (1987), found overwhelming mortality from cancer of the liver, gall bladder, and biliary tract in capacitor manufacturing workers exposed to Aroclors 1254, 1242, and 1016 (Schmidt and Bradfield, 1996; EPA, 1997). ATSDR-TP., (1993) found significant excess malignant melanoma mortality in workers exposed to Aroclors 1216 and 1041. PCBs have also been implicated as a cause of mass mortality in seabirds. The effects of this compound on the environment first came to limelight in the late 1960s, after the introduction of PCBs (Borja et al., 2005; Hatamian-Zarmi et al., 2009). According to a study by a 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

PCBs can affect the productivity of phytoplanktons and the composition of phytoplankton communities. Phytoplankton is the primary source of food to 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 food source containing PCBs (Borja et al., 2005).

Biological PCB transformation

As was earlier mentioned in this paper, 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. However, this section reviews the biological degradation of PCB by plants and microorganisms. Presently, the process of putting biochemical capabilities of microorganisms into use has become the technique of interest in the bioremediation of contaminated soil (Semple et al., 2001). 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., 2005). Recent improvement on bioremediation technique has increased the existing clean-up processes currently available for the restoration of contaminated sites. It can be done either in-situ or ex-situ (Idris and Ahmed, 2003). This biological technique relatively depends on the breaking down of indigenous micro flora, providing enabling conditions for growth and biodegradation (Semple et al., 2001). 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. The process of cometabolism depends on the secondary substrate as the source of carbon and energy for microorganisms when the target pollutant is transformed (Borja et al., 2005). When the products of co-metabolism are ready for further degradation, they can be mineralized, otherwise incomplete degradation occurs. This results in the formation and accumulation of metabolites that are toxic than the present molecule hence, requiring greater number of microorganisms which make use of the new substance as their source of nutrients (Furukawa et al., 1985).

The optimization of bioremediation is dependent on many environmental factors, and the rate of optimization depends on the present environmental condition (Borja et al., 2005). These factors are:

(a). the structure of the compound.

(b). the presence of foreign substituent and their position in the molecule.

(c). Solubility of the compound and

(d). Concentration of the pollutants (Furukawa et al., 2005).

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; Basharudin, 2008). 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. Microorganisms are found to access compounds that has high aqueous solubility than the low ones (Basharudin, 2008; Borja et al., 2006). For the PCBs, highly chlorinated congeners are very insoluble in water. This could account for the resistance of highly chlorinated PCB congeners to biodegradation. The concentration of pollutants plays a major role in biodegradation (Borja et al., 2005). 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).

Some other factors affecting biodegradation include-temperature, pH, inhibitory substance acceptors and microbial interactions. All these help to bring about the unpredictable nature of biodegradation (Dobbings, 1995). 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. The technique 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; Basharudin, 2008). Bioremediation has limitations as well; to start with is the fact that some chemicals are not amenable to bioremediation, for instance, heavy metals, radionuclides and some chlorinated compounds. Metabolism of contaminants by micro-organisms sometimes produces toxic metabolites. Bioremediation however, is a scientifically intensive procedure which should be tailored towards site specific conditions. Therefore, it is imperative that treatability study should be encouraged before actual clean-up of the site (Boopathy, 2000). Some of the questions that one should be nurturing when considering bioremediation technique however, are: is the contaminant in question 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 provided with answers by carrying out site characterization and by treatability studies (Aken et al., 2010).

Bioremediation could be on site or out of site 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 is less controllable and less effective.

Various studies have reported long term accumulation of PCBs in soils and sediments as well as its continuous bioaccumulation in food chains (WHO, 1976). Toxicity of POPs is said to be comparable to that of other halogenated aromatics and this implies numerous dysfunctions in the body of organisms (Pesatori et al., 2009). Human exposure to POPs for example dioxins is exclusively almost from food intake, especially from fish, meat and dairy products. Unusually high exposure of the POPs following for example accidental/occupational exposure, together with experiments in laboratory animals, have shown the effects of dioxins on health to include developmental and reproductive toxicity, effects on immune system and carcinogenicity (Allsopp et al. 1998). Even more disturbing are findings from recent studies which shows that the concentration of various POPs in human tissue of general population (of industrialize countries) are already at – or near -those levels where the health effects may occur.

The detection of PCB in blood, adipose tissue, breast milk and other tissue samples from the population indicate widespread exposure to PCBs from the environmental sources. People who live near hazardous waste site where PCBs have been detected may be exposed primarily by consuming contaminated fish from adjacent water bodies and by breathing air that contains PCB (Fitzgerald et al. 2001).

The release of exotic PCB-materials into the environment rarely occur as a result of the fact that PCBs are no longer being manufactured or imported in large quantity (ATSDR, 2000). Rather PCBs are predominantly redistributed from one compartment to the other (e.g. soil to water, water to air, and sediments to water) (Eisenreich et al. 1999; Larsson and Okla 1989). Thus, the majority of PCB in the air results from volatilization of PCBs from soil and water. Some PCBs may be 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 1995), and some other means.

This ability of PCBs to constantly persist in the atmosphere requires a more environmentally friendly alternative dissipation method having been failed by the conventional methods of incineration. The environmentally friendly alternative mentioned above is bioremediation.

Bioremediation involves the use of biological means to destroy, transform or deactivate environmental contaminants as to protecting potential sensitive receptors (ENTACT. www.entact.com/26/03/11). 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. Bioremediation is sometimes used to attack contaminants like PCBs that are degraded by bacteria.

Various forms of bioremediation technique include:

- Land farming
- Bioventing
- Biosparging
- Bioslurping
- Phytoremediation
- In situ/ex situ remediation

I shall elaborate only on those that are within the scope of this review.

In situ remediation technique

In situ remediation is a phenomenon used to treat pollutions on site devoid of significant disturbance. It is a biological technique which incorporates the use of either physical extraction, biological activities, chemical modification or other processes to remove, stabilize or degrade pollutants in soil and groundwater. In *in-situ* processes, result is usually accomplished by the introduction of exotic substances into the site.

Bioventing-

This bioremediation technique that allows only the treatment of unsaturated soil. It is an *in situ* remediation technology that uses indigenous microbes to biodegrade organic constituents which were adsorbed to the soil especially in the unsaturated zones (www.sci.ccny.cuny//bioventing). Bioventing is mostly used in the remediation of petroleum products because it induces airflow as a provision of oxygen which promotes the biodegradation of the pollutants. Some factors referred to as site characteristics support the practicability of bioventing technique, they are:-

(a) Intrinsic permeability-this means that there should be enough oxygen supply to the system. The air flow rates also should be in 1 order of magnitude less with corresponding less pressure.

(b) There should be enough microbial presence.

(c) Supply of appreciable sources of energy which depends on whether the microbes and system is heterotrophic, autotrophic, aerobic, anaerobic or facultative.

(d) Optimal soil pH which ranges between 6 and 8, moisture content of 40-60%, soil temperature between 10^{o}C and 45^{o}C, enough nutrient concentration and also the depth to groundwater.

Advantages of bioventing include- the system makes use of readily available equipments which are easy to install, minimal disturbance to its site of operation, short time of treatment, less expensive, ability to combine with other technologies etc. But this technique is disadvantaged in that it is not effective if the water table is within several feet from the surface, high constituent concentration usually toxic to microbes, requires certain site conditions, and cannot achieve low clean-up standards (Van Deuren et al., 2002). 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, 2000). Therefore sufficient airflow is important in the design of bioventing system. Low permeability as well as low temperature hinders the effectiveness of bioventing.

Biosparging-

Biosparging is an in situ remediation technique which exploits and stimulates the use of indigenous microbes to degrade organic contaminants in soil. This is the injection of atmospheric air into the aquifer to stimulate the activity of microorganisms by increasing oxygen dissolution which by so doing enhances biodegradation of the contaminants present in the soil (www.remedios.uk.com). 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, 2000). 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 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 (EPA, 1994). 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. Advantages of biosparging range from those of bioventing and also support biodegradation of components rather than just through volatilization of bioventing. However, constituent adsorbed to soil in the unsaturated zone could be treated by biosparging (EPA, 1994). The disadvantage with biosparging is that it must be combined with a physical method before the process can be completed and it is however, expensive and therefore less economical. It can only be used in environment where air sparging is suitable. It has the inability to understand the interaction between complex chemicals in addition to physical and biological process. Lack of field and laboratory data to support design consideration, and potential for inducing migration of constituents also hampers the effectiveness of biosparging. According to the case study done at the Damoder valley in Eastern India as was reported by Gogoi, et al. (2002), biosparging was effective at removing 75% of contaminants present within one year period. From this study, the first results were obtained in the field but, it was later enumerated using laboratory tests and computer programs.

The earlier mentioned techniques of bioremediation are only effective if the soil being treated is homogenous. If a remediation area has non –homogenous soil, it may be 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 activate 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, 2000). The question remains how much money one can afford to spend in order to increase the effectiveness of the remediation techniques. The promise of phytoremediation notwithstanding, as was shown by the work of various schools of thought could be an answer to these questions.

Phytoremediation

Phytoremediation is a recent development in green technology that uses plants to remedy soils, sediments, surface water and ground water, when contaminated with metals, organics and radionuclides (Alkorta and Garbisu, 2001).

Phytoremediation is an effective, environmentally friendly and inexpensive means of remediating soil (Wiltse et al., 1998; Alkorta and Garbisu, 2001). It is a more cost effective method than the conventional mechanical and chemical methods of removing hazardous compounds from the soil (Bhandry, 2007). Apart from these, phytoremediation is a natural, aesthetically pleasing low-cost technology which is socially accepted by surrounding communities and regulatory agencies as a potentially elegant and striking technology (Chekol et al., 2004). 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-phototoxic metabolites.
2. Releasing exudates and enzymes enhancing microbial activity and biochemical transformations (Mackova et al., 1997).
3. Enhancement of mineralization in the rhizosphere.

There is a suggestion that plant enzymes released into the environment have a significant catalytic effect (Cunningham et al., 1997). 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 on the other hand, can be degraded by aerobic bacteria via a well-documented pathway (Abramowicz, 1995) to chlorobenzoates. Degradation of PCBs by fungi has been described (Aken et al., 2010) and the data obtained have shown many similarities with bacteria aerobic degradation (aerobic process, inability to degrade higher chlorinated PCBs, etc) (Borja et al., 2005).

Phytoremediation is a word derived from Greek prefix “phyto” which means plant, and latin suffix “remedium” meaning to clean or restoring (Cunningham et al., 1997; Hamlin, 2002.). 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 (Borja et al., 2005). The primary motivation for the development of phytoremediative technologies according to Chaudhry et al. (2005), is the potential for low-cost remediation. Phytoremediation, though a recent invention, its practice therefore is not common (Prasad et al., 2003). Research for treating radionuclide-contaminated waters using semi-aquatic plants existed in Russia at the down of the nuclear era (Strek and Weber, 1982; Smith et al., 2007). A good number of plants have the ability to accumulate large amount of metals in their tissue when grown in a metal containing soil without any symptoms of toxicity (Panwar et al., 2010). The first researcher to suggest this on his study using hyper accumulators on his study in the phytoremediation of metal polluted sites was Chaney (1993).

Direct benefits of phytoremediation

Phytoremediation is an *in situ*, solar driven technique, which limits environmental disturbance and reduces cost (Smith et al., 2007). Moreover, it is particularly well suited to 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., 1997). 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 biotechnology, especially in Canada (Carrillo-Castaneda et al., 2001).

Plant samples can be harvested and used as indicators

of 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 (Quiping et al., 1992). 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). For this reason, microbial communities in the rhizosphere may be able to biodegrade wide variety of organic contaminants. Finally, using existing agricultural practice in a contaminated site and application of phytoremediation could be done at ease (Haritash and Kaushik, 2009).

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., 1997). The use of plant in remediation efforts stabilizes the soil, thus preventing erosion and direct human exposure (inhalation of soil particles carried by the wind (Carrillo-Castaneda et al., 2001). Phyto-remediation eliminates secondary air- or water- borne waste, for example, the accumulation of PAHs from the atmosphere (Bock et al., 2002). 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 which serve as sinks for the green house CO_2 (Schnoor et al., 1995). Reduction of noise level from industrial sites is achieved because phytoremediation is less noisy than the other reclamation alternative. Another indirect benefit of phytoremediation is that the growth of high hardy plants gives room for growth of lower ones also (Germida et al., 2002; Frick et al., 1999).

Limitations of phytoremediation

Petroleum hydro-carbon contamination must occur at shallow depths for phytoremediation to be effective. There is generally, decrease in root diversity with depth as most plants do not have high root depths like the trees (Frick et al., 1999: Germida et al., 2002). Consequently, as depth increases beyond one or two metres, relatively, immobile contaminant- those that cannot migrate to the plant roots during water uptake, increasing and are unlikely to be affected by phytoremediation. Phytoremediation requires more than annual planting seasons for site clean-up hence, slower than *ex-situ* methods (Frick et al., 1999; Germida et al., 2002). Because it is slow, phyto-remediation 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 soil particles or organic matter, it may not be available to plants or microbes for degradation (Olsen et al., 2003). Environmental conditions like soil texture, pH, salinity, oxygen availability, temperature and level of non-hydrocarbon contaminants (e.g. 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., 1997).

Bioremediation is a required option especially where sediments are contaminated with PCBs (Furukawa and Fujihara, 2008). However, thermal and chemical processes have always been the method used to decontaminated highly polluted sites until biotechnology offered a more economically friendly alternative for diffuse pollution (Furukawa et al., 2004). 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 are 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 it has been elaborated by various researchers (Aken et al., 2010).

Phytoremediation of PCBs

PCBs are exotic compounds of note which spreads widely in the environment (Toro et al., 2006). A review of literatures indicates that PCBs are not leachable in soils and that they are readily adsorbed by soil constituents. It appears that lower chlorinated PCBs are less adsorbed and thus slightly mobile in soils. There have also been reports of absorption of PCBs by plants, but in very low amounts as PCBs, they appear to have some effects on photosynthesis and respiration in plants (Toro et al., 2006). As a result, 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 of 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

reported to be thermally and chemically stable and is also recalcitrant to biodegradation (Singh and Wards, 2004). The strong binding of PCB molecules to soil organic matter tends to its bioaccumulation into the food chain (Seeger et al., 1997). Using different mechanisms, anaerobic consortia of microorganisms, as well as aerobic bacteria, PCBs can be remediated in the soil. But according to Toro et al. (2006), actual site of 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.

Phytoremediation is referred to as the use of plants to dissipate organic compounds like PCB from the soil (Ferro et al. 1994). 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 bioremediation technologies with plants and microorganisms that have proven successful in the remediation of PCB. It will then compare the two mechanisms, describing the positive impacts of combining the two processes in the remediation of PCBs

RHIZO/PHYTODEGRADATION OF PCBS

The rates of removal of pollutants in bioremediation are usually slower than those that can be achieved by the conventional methods. This is purely shown in remediation by plants in which its growth depends on some environmental factors. Therefore, the need arises for finding ways to enhance the entire scope and rate of bioremediation in order to propel them as a competitive commercial technique (Chaudhry et al., 2005). PCBs are hydrophobic hence sorbs strongly to soil particles, rendering it to have biotransformation property. The compounds are poorly taken up by plants tissues, but in the rhizospheres, microbes play a dominant role in their remediation. They have been many reports of recent, showing significant increase in the reduction of PCBs in soil with different plants grown in it as compared to unplanted soil (Chaudhry et al., 2005; Gerhardt et al., 2009).

This section reviews the interactions of plants and microorganism in a rhizosphere, looking at the effectiveness of remediation of PCB-contaminated soil with microorganism and plants and explaining the differences between the two. It will also throw more light on the combination of the two techniques using rhizodegradation technology of microorganisms and phytoremediation of plants.

Degradation of PCBs by microorganisms

Recalcitrance of PCBs to biodegradation by microbes was as a result of its chemical stability (Furukawa and Fujihara, 2008). Just as higher chlorine constitution increases chemical stability and lowers water solubility; it makes higher chlorinated congeners more resistant to remediation. Metabolism of PCBs is usually unfavourable energetically, thus requiring additional source of carbon to aid its co-metabolism.

PCBs are regarded as POPs, however; their degradation by microbes has been well reported (Pieper and Seeger, 2008; Borja et al., 2005; Field and Sierra-Alvarez, 2008; Vasilyeva and Strijekova, 2007). There are two known metabolic pathways of microbes in PCB: aerobic and anaerobic, these depends on the degree of chlorination of the congener, the types of microbes involved as well as the redox conditions (Borja et al., 2005: Aken et al., 2010)

Rhizoremediation is based on the combination of microbial and plant growth process to enhance biomass accumulation, particularly plant roots in the soil, and thus, accelerating the remediation kinetics. In a natural environment, most of the demerits to remediation can be amended by the dynamic synergy existing between plants root and its associated microbes. This is because the effects of microorganisms around the root of a plant and the plants ability to withstand soil contamination could be more closely related than previously thought. The actions of the microbes in and around the root seem to render the environment favourable for the co-metabolism of toxic chemicals that abound in the soil (Chaudhry et al., 2005). Microbial transformation is not usually driven by energy need, but a quest for reduced energy. This is to enable secretion of root exudates that serve as energy source to microorganisms. Thus root exudates stimulates microbes and therefore aids degradation of phytotoxic compounds available as nutrients (Walton and Anderson, 1990; Shann et al., 2001). The processes used include land farming, inoculation with contaminated degrading bacteria and growth of plants with plant growth promoting rhizabacteria (PGPR). The rhizo/phytodegradadtion was found to increase the overall rate of PAH remediation in creosote contaminated soil (Huang et al., 2001, 2004). Combining two or more techniques for remediation of persistent contaminants like PCBs, can overcome many of the limitations that exist for individual technologies. For example, in phytoremediation, many plant species are quite sensitive to contaminants, including TPH (Huang et al., 2004; Bock 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. Sometimes, the number of microorganisms in contaminated soil is affected by inhibition, thereby not having enough bacteria to aid degradation or even to support plants growth (Idris and Ahmed, 2003).

For effective remediation of variety of environmental contaminants, it is advantageous to use multiple techniques or process to accelerate remediation kinetics and increase plant and microbial biomass (Huang et al., 2001; Carrillo-Castaneda et al., 2001; Gerhardt et al., 2009). In the use of double or multi-process remediation, both PGPR and specific contaminant degrading bacteria was found to be vital for successful remediation (Huang et al., 2001, 2004; Bhandary, 2007; Carrillo-Castaneda et al., 2001). For organic contaminants, use of bacteria as a pre-treatment that consume organics in the soil can promote the remediation process (Shann et al., 2001; Walton and Anderson, 1990). Various bacteria are able to rapidly metabolize some readily available compounds consuming bacteria that have been used on soils (Huang et al., 2001; Gerhardt et al., 2009). This will start the remediation process and can lower the toxicity of the compounds to plants when used prior to phyto-remediation. Further, there are bacteria called PGPR that increases the plant tolerance to organics and massive biomass accumulation (Gogoi et al., 2002). They work by preventing stress ethylene synthesis and providing auxins to the root (Gioia et al., 2006). The result is much greater in biomass (especially roots) and therefore fastens remediation (Gioia et al., 2006).

In a study by Huang et al., (2004), a series of laboratory experiments were carried out to determine the effectiveness of multi-process remediation for decontamination of creosote-spiked soil. The system consists of land farming, inoculation of degrading bacteria and plant growth with PGPR. In a 4-month period, the multi-process remediation removed more 50% 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. Actual environmentally 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. About 90% of the total recalcitrant TPH was remediated from the soil after the second 4-months (Huang et al., 2005). Phytoremediation alone was unable to remove only about 50% of TPHs in the same period. Therefore, rhizo-remediation provides the key elements for successful remediation, with the use of plants specie which proliferates in the presence of high levels of contaminants, and strains of PGPR that increase plant tolerance to accelerate plant growth in heavily contaminated soil.

The use of microorganism, both anaerobic and aerobic, is the only known process that is able to degrade PCBs appreciably in the soil systems or aquatic environments (Mackova et al., 2007).

Anaerobic PCB-dechlorination.

PCB congeners that contain four or more chlorine substituent undergo anaerobic reductive dechlorination (Aken et al., 2010). This is an energy yielding process in which PCBs serves as the electron acceptor for the oxidation of organic substrates. Anaerobic bacteria possess characteristics that are suited for high carbon-concentration pollutants because of the limitation in oxygen diffusion in a high concentration system (Borja et al., 2005; Basharudin, 2008). A predominant anaerobe environment is conducive for the reductive transformation resulting in the displacement of chlorine by hydrogen (Brazil et al., 2005). These compounds that are dechlorinated are however substrates for oxidative attack of the anaerobes. 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 (Bedard and Haberl, 1990; Boyle et al., 1992).

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

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

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

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

in their energy metabolism. The typical phenomenon that is common to the dehalogenators includes:

a. Aryl reductive dehalogenators function in a syntrophic communities and may be dependent on such a community.
b. This aryl reductive dehalogenation is catalysed by enzymes that are inducible.
c. There is exhibition of distinct substrate specificity by this enzyme.
d. Aryl dehalogenators obtain their metabolic energy from reductive dehalogenation (Borja et al., 2005). 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 which 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 (Mackova et al., 2010).

For every anaerobically mediated dechlorination of PCB, the significant evidence was dependent on the observed modification of the substance in the sediments devoid of oxygen. When the distribution patterns of PCB in both the anaerobic sediments and commercial mixtures introduced to the river were compared by J. Borja and his group in (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 proportions of mono- and di-chlorobiphenyls increased considerably (Quensen III et al., 1990).

Laboratory studies in the dechlorination of commercial mixtures of PCB showed that the rate and extent of dechlorination is inversely proportional to the degree of chlorination and dechlorination was said to be associated with synthropic communities attacking PCB at different positions with specificity for PCB dechlorination (Rezek et al., 2007).

According to the work of Quensen et al, (1990), dechlorination of Aroclors 1242, 1254, and 1260 by microorganisms in a particular sediments and Aroclors 1242 and 1260 in other sediment. The rate of dechlorination in the second sediments by micro-organisms was similar for Aroclors 1242 and 1248.

This is indicative of extensive dechlorination from the meta-plus para- positions within his 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 (Held and Dorr, 2000; Zeeb et al., 2008).

Dechlorination according to Quensen, 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 16weeks. 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.

With microorganisms, the use of organic substrate as electron donors has also been shown to increase the rate of dechlorination of Aroclor 1242 (Newman and Reynolds, 2004). Even separate addition of glucose, acetone, methanol and acetate has almost the same pattern of dechlorination for each substrate, but the extent and rate of dechlorination were different. The rate of dechlorination was decreasing and greatest with methanol, glucose, acetone while acetate has least. As usual, dechlorination occurred primarily on the meta-and para- position of the highly chlorinated congeners resulting in the accumulation of less-chlorinated, primary 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 (Newman and Reynolds, 2004).

When Iron II sulphate ($FeSO_4$) was added to PCB-contaminated sediments, an almost complete meta- plus para- dechlorination of Aroclor 1242 was discovered (Borja et al., 2005). According to the study, while $FeSO_4$ was stimulating the growth of sulphate reducing organism responsible for PCB dechlorination, Fe^{2+} reduced the sulphide bioavailability and toxicity through the formation of an insoluble FeS precipitate. The appreciable loss of meta- and para- chlorines catalysed anaerobic dechlorination leads to preferential reductions in the level of coplanar, dioxin-like congeners in the PCB mixtures (Abramowicz, 1995; Borja et al., 2005; Mackova et al., 2010).

The decrease in risk is manifested in two ways:

1. Sparsely chlorinated congeners produced as a

result of dechlorination can be degraded by indigenous bacteria (Borja et al., 2005).
2. Dechlorination significantly reduces bioconcentration potential of the PCB mixtures through conversion to congeners that do not significantly bioaccumulate in the food chain (Magae et al., 2008).

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

Aerobic biodegradation of PCB

Sparsely chlorinated PCB congeners which are formed as a result of dechlorination of the higher congeners are substrates for aerobic bacteria (Komancova et al., 2003). Those PCB congeners undergo cometabolic aerobic oxidation which is mediated by an enzyme deoxy-genases, bringing about a ring opening, hence completing mineralization of the molecule (Kohler et al., 1989; Vasilyeva and Strijakova, 2007; Furukawa and Fijihara, 2008). A lot of bacterial strains are implicated in oxidative degradation of PCBs; among them are *Pseudomonas, Burkholderia, Comamonas, Rhodococcus,* as well as *Bacilus* (Aken et al., 2010). Obviously, chlorine numbers per molecule and its placement are important factors in aerobic biodegradation (Furukawa et al., 2004). PCB congeners with three or less chlorine atoms per molecule are easily degraded, but ones with more are recalcitrant, therefore requires reductive dechlorination prior to oxidative mineralization (Aken et al., 2010). PCB-destruction in the presence of oxygen involves two gene clusters (Borja et al., 2005). The first one enables transformation of PCB congeners to chlorobenzoic acid and the second involves degradation of the chlorobenzoic acid. A common growth substrate for PCB-degradating bacteria is biphenyl or monochlorobiphenyls. During utilization of biphenyls, a yellow *meta*-ring cleavage product is formed as observed in most studied bacteria like the *Pseudomonas* 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 (Bianucci et al., 2004). Since PCBs persists more at increasing chlorination of the congeners, aerobic biodegradation involving ring cleavage is restricted to the lightly chlorinated congeners.

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

In a recent study, a new bacterium, *Janibacter*, MS3-O2, was isolated from soil (Mackova et al., 2007). It was interesting to note that the degradation of Aroclor1242 was significantly higher in the liquid medium without biphenyl (70 to 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. Comparison of the result obtained in the soil and that of the liquid shows that the degradation was less efficient in the soil because of the effects of lower bioavailability of PCBs and its interactions with the surrounding soil microorganisms (Mackova et al., 2007).

Several studies on the microbial degradation of commercial PCBs show that certain patterns of chlorine substitution seriously hinder PCB degradation. For lightly chlorinated PCB congeners, a sequential enzymatic step involved in the degradation was however developed (Seeger et al., 1997).

The complete degradation of PCB requires various microbial strains with specific congener preferences (Mackova et al., 2007). In addition, the position and number of chlorines on the molecule can influence the rate of the first oxygenate attack. Mackova et al. (2007), proposed a mechanism for the oxidation of PCB by *Alcaligenes euterophus*, *Pseudomonas putida* and a *Corynebacterium* sp. *Alcalegenes odorans*, *A. euterophus* and *P. putida* bacterium strain degrade tetrachlorobiphenyl via 2,3- attack while C*orynebacteria Sp.,* 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, and Furukawa et al. (1986), 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 by 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-dichlorobiphenyl) hexa-2,4-dienoic acid; and a final product, 2,4-dichlorobenzoic acid. The congener, 2,2',5,5'-tetrachlorobiphenyl was degraded via 2,3-dioxygenase attack, with the formation of 2,5-dichlorobenzoic acid and trichlorobiphenyl. The identified metabolites indicate that *Pseudomonas* sp. 2 was capable of dehalogenating PCBs (Komancova et al., 2003). The degradation of 2,2',5,6'-tetrachlorobiphenyl confirms the ability of bacteria strain to dehalogenate PCBs (Borja et al., 2005). Degradation for this compound was via 2,3-deoxygenase attack and products formed corresponded to (based on molecular weights) 4-(2, 5-dichlorophenyls)-oxobutanoic acid. Two other compounds, 2-chloro-3-(2,5-dichlorophenyls)-2-acrylic acid and monochloroacetophenone, were also detected. These products are consistent with 3,4-dioxygenase attack (Komancova et al., 2003).

Furukawa (1994) summarised the relationship between chlorine substitution and the microbial breakdown of PCBs as follows:

1. The rate of degradation of PCBs is inversely proportional to the increase in chlorine substitution (Borja et al., 2005).
2. PCBs containing two chlorine in the *ortho*- position of a single ring (2, 3, 6-) and each ring (2, 2') shows a striking resistance to degradation.
3. PCBs which have all of its chlorine on its single ring degrades much faster than those with same number on double rings (Borja et al., 2005; Bhandari, 2007).
4. PCBs having two chlorines at the 2,3- position of one ring such as 2,3,2',3'-, 2,3,2',5'-,2,4,5,2',3'-chlorobiphenyls are susceptible to microbial attack when 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.

ANAEROBIC-AEROBIC TRANSFORMATION OF PCBS

There have been a lot of studies on aerobic bacteria PCB-degradation (Borja et al., 2006). It was observed that only PCB congeners 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 there have been few reports on the aerobic degradation of penta- and hexa-chlorobiphenyls (Brazil et al., 1995; Borja et al., 2005).

There were also various studies on the transformation of PCBs using anaerobic bacteria eluted from PCB-contaminated sediments. There are reports of preferential *m~eta*- and *para*-chlorine removal from highly chlorinated PCB congeners under aerobic means producing lesser chlorinated congeners that can biodegrade aerobically (Borja et al., 2005). The biotransformation pattern above is commonly found among halogenated aromatic compounds (Aken et al., 2010).

Macek et al. (2002) reported a sequential anaerobic-aerobic treatment of PCBs 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 availability of microorganism capable of degrading tri- and tetra-biphenyls was shown in the aerobic microcosm experiment (Borja et al., 2005).

Both aerobic and anaerobic metabolism modes transform PCBs. The difference in the pattern of degradation of PCB was as a result of preferential attack by different microorganisms (Mackova et al. 2007). 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.

PHYTOREMEDIATION AND PCB

The inability of PCBs to be leached in soil has been reviewed by literatures. PCBs are also reported to be readily absorbed by soil sediments (Strek and Weber, 1982). These indicated the difficulty in the removal of PCBs from the soil. It appears that lower chlorinated PCBs are less absorbed and slightly mobile in the soils. Meanwhile, 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 those work indicated that plants absorb PCBs, but in a very slow amount. PCBs therefore appear to have some effects on the photosynthesis and respiration in plants (Strek and Weber, 1982).

Reports on the potential of plants for phytoremediation of PCBs started during the late 70s and early 80s (Aken et al., 2010). From then, a lot of significant advances have been made to elucidate the potentials of plants and microbes for the metabolism of PCBs. Some processes are known to be involved in phytoremediation of PCBs; they are rhizoremediation, phytoextraction and phytotransformation.

Rhizoremediation

PCBs are hydrophobic as earlier stated hence possesses high affinity for soil particles. They are therefore taken up into the plants tissues sparingly. However, microorga-

nisms in the rhizosphere play a dominant role in the biodegradation of PCBs (Aken et al., 2010). Reports have continued to show significant improvement in the reduction of PCBs in a soil planted with different types of plants as compared with non-planted controls (Campanella et al., 2002; Chaudhry et al., 2005; Gerhardt et al., 2009; Wood et al., 2000). The mechanisms by which plants can stimulate microorganism activity in the soil to enhance the biodegradation of PCBs include:

(a) The release of organic compounds like sugar, amino acids and organic acids by plants root used as electron donor support for either aerobic or anaerobic metabolism of chlorinated compounds. In certain instances, microbial aerobic degradation consumes energy, resulting in anaerobic processes which is usually favourable for PCB dehalogenation (Chaudhry et al., 2005).
(b) Extracellular enzymes that cause transformation of PCBs leading to further microbial metabolism are secreted by plants (Fletcher et al., 1995).
(c) Microbial degradation of PCBs are speed up by inducers which are secreted by plants, however, Hedge and Fletcher (1996) reported that *Burkholderia xenovorans* LB400 and its activity as a PCB degrader was induced by plants phenolic exudates.
(d) The effects of plants root increases the permeability of the soil and also oxygen diffusion in the rhizosphere. These induce microbial oxidative transformation by certain enzymes (Chaudhry et al., 2005).
(e) Growth factors are also known to be secreted by plants (Campanella et al., 2002).
(f) Organic acids and molecules that act as surfactants comes from the roots, they therefore help to mobilize PCBs, making them more susceptible to plants tissues (Chaudry et al., 2005).

A lot of articles have elucidated the importance of root exudates on the activities of microbes in the soil and also on biodegradation of PCBs (Fletcher and Hedge, 1995). These exudates which are made up of water soluble and insoluble compounds in addition to the volatile components, enable the acquisition of minerals by plants thereby stimulating the growth of microbes in the rhizosphere (Chaudhry et al., 2005). Other factors affected by this synergistic effect of the root exudates include pH change, water flux and oxygen availability in the rhizosphere. Plants significantly increase the removal of PCBs from soil (Aken et al., 2010). There was a report on the interaction of the difference in treatment in the degradation of Aroclor 1242 in soil (mackova et al., 2007). In all the reports, degradation of higher molecular weight PCBs in the soil was a significant observation as compared to non-vegetated control; hence, the conclusion that plants enhances PCB degradation (Aken et al., 2010). Plants perform this task through oxygen diffusion increase, infiltration amendment and through enrichment of microbes perhaps during genetic engineering (Brazil et al., 1995).

With the use of several plants in the phytoremediation of PCBs, there was less than 38% recovery as compared to 80% and above recoveries in non-planted controls (Chekol et al., 2004). It was also shown that increased soil enzymatic activities by plants were correlated with PCB-degradation level (Chekol et al., 2004). Another factor that enhances removal of PCBs from a contaminated soil is soil amendment (Smith et al., 2007). According to the author, organic amendment brings about oxygen consumption that is needed to achieve anaerobic dechlorination of PCBs (Aken et al., 2010). Molecular biology has been used also to develop a tool used to locate PCB-degraders in the roots of plants growing in a soil contaminated by PCB (Hogan et al., 2004; In Aken et al., 2010)

Uptake of PCBs by plants

Prediction of uptake of organic pollutants by plants depends on the octane rating of the pollutant (Schnoor, 1999). Based on this model, only moderately hydrophobic compounds ranging from 0.5 to 4.5 log K_{ow}, would be absorbed and consequently translocated within plant tissues. Obviously, the effectiveness of uptake of PCBs by plants with its K_{ow} ranging from 4.5 (the monos) to 8.2 (the decas) will be expected to decrease synonymously with the degree of its chlorination.

In the phytoextraction study done by Zeeb and his co scholars (2006), there were variable concentration of Aroclor 1260 in root tissues, and lesser concentration in the shoot. According the study, those PCBs with higher concentrations ranges from the tetras to the hexachlorobiphenyls. But the heptas and the nonas were also detected in minute quantities. This result however, counteracts the prediction based on octane rating of the pollutant and suggests the possibility of higher chlorinated PCBs taken up within plants tissues. In another development, Liu and Schnoor (2008), discovered that selected monos-tetrachloro PCBs were absorbed to plant roots, but only the lower chlorinated ones got translocated to the aerial parts of the plants. Also, in a field trial by Aslund et al. (2008), there was an increase in PCB concentration within stem and leaves of pumpkin plants, considering the time of exposure, but the concentration in the root remained unchanged (Aslund et al., 2008). Therefore, the authors inferred that transfer of PCBs in plants primarily occur through uptake and translocation, those other mechanisms have negligible effects (Aken et al., 2010).

PCB metabolism by plants

Xenobiotics metabolism by plants is been described as a three way process in the green liver model as represented in the Figure 3. It starts with the activation process consisting of oxidation of PCBs to hydroxylated products which are very soluble and reactive

(Sandermann, 1994; Coleman et al., 1997). The second process involves conjugation of activated compounds with plant molecules. Here, lesser toxic and more soluble compounds are formed. In the final process of sequestration, the conjugates are adsorbed into plant organelles (Sandermann, 1994; Coleman et al., 1997).

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

However, metabolism of PCBs by plants is aided by several enzymes (Mackova et al., 2001; Aken, 2008). These enzymes include oxygenases, peroxidises, oxidases and transferases. Cytochrome P-450 and peroxidises are implicated in initial process of metabolism (Iiarms et al., 2003), commercial horseradish peroxidise (HRP), was used to transform dichloro- and tetrachlorobiphenyls (Chroma et al., 2002 a), and Remazol Brilliant Blue R (RBBR) oxidases with other enzymes were involved in *in vitro* cell culture of plants that was carried out by Chroma et al. (2002b). Recently, Magae et al., (2008) reported dechlorination of biphenyl with extract of a reductase enzyme from *Medicago sativa* and *Zea mays.*

Effects of PCBs on plants

The inhibition of plants growth due to PCB effects has been well documented (Furukawa et al., 2004). This report, documented mainly for algae denoted several deductions in algae cell numbers at a general low level (0.3 to 10 ppm) of PCBs in aqueous solution. There have been scarce reports on the growth inhibition of higher plants. There was also a report on 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 noted for soybean, beets and pigweed *Amaranthus refloflexus L*. But only fresh weight reductions were reported for Fescue (Strek and Weber, 1982). At 1000 ppm rate of Aroclor 1254, soybean growth was inhibited by about 47%. However, 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 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 (Mackova et al., 2007). 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 studies of this nature (using algae and bacteria) will become misinterpreted. Uptake of 14C-labelled PCBs following application on leaves has been demonstrated, although in low amounts (3.2 to 15.5%); the greatest loss probably occurred through volatilization (Weber and Mrozek, 1979)

The work done by Iwata and Gunter (1976) was reported by Strek and Weber (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, redish, soybean, spruce, sugerbeet, tomatoes and lastly unidentified weeds. The species according to Strek et al. (1982), were planted in soils fortified with 0.046 to 100 ppm. At harvest, the concentration of PCBs at soil levels ranged from 0.040 to 76 ppm. Although, wide range of experimental condition was ensured, several factors were responsible for the trend in plants uptake of the contaminant. Amount of uptake were generally low, ranging from 0.0016 to 13.9 ppm, and averaged 1.241 ppm over all data.

According to this information, a suggestion was made that PCB content of plant was dependent on the PCB concentration in the soil (Strek and Weber, 1982). Hence from the result, the bioconcentration 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, we 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 having an average of 1.67 ppm, while the unidentified weeds have an average of 1.52 ppm. Tomatoes contained the least amount having an average of 0.0023 ppm, and this according to Borja et al. (2005), could be as a result of application of extremely low rates of PCB of between 0.046 and 0.062 ppm.

The importance of biphenyl metabolites in plants has been neglected (Strek and Weber, 1982). Only the monos- and the dehydroxylated biphenyls seem to be the only metabolites extracted in a variety of plants (Strek and Weber, 1982). This however agrees with the study of some researchers on PCB (Smith et al., 2007; Mackova et al., 2007; Aken et al., 2010).

In the work of Smith et al. (2007), they conducted a greenhouse study to evaluate the effects of plants growth on PCB congeners found in Aroclor 1260. In their study, 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 and 34% sand). The source of PCB was transformer oil containing Aroclor 1260. The plants used include river bulrush (wetland sp.) (*Seirpus fluviatilis*), eastern gama grass (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 of 0.1mg/l in solution). A lot of differences between treatments were observed in the loss among penta-hepta chlorobiphenyls (Smith et al., 2007).

C. aquatalis with amendment had significant higher percentage loss than *C. aquatalis* without amendment, *S. pectinata* with amendment and *T. dactyloides* with amendment; Mulberry (*Morus rubra*) with amendments was reported to have significantly higher percentage loss than *S. fluviatilis* with amendment.

Aken et al. (2010) reported that highly chlorinated PCBs found in Aroclor 1260 require reductive declorination as the first step in remediation, and this process require a treatment with low transpiration and high soil water content. According to them, 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. (2007), of the congeners they 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 was found to accumulate (Van Deuren et al., 2002; Borja et al., 2005). Examining the chlorine distribution of the PCB compound monitored in this study, the 2,3,3',4,4',5,5'-heptachlorobiphenyls could lose one chlorine from a *meta* position and become 2,2',4,4',5,5'-hexachlorobiphenyls, which is one of the congeners present in Aroclor 1260. This is a likely a pathway for reductive dechlorination, because it preferentially removes chlorine from the *meta*- and *para*- positions (Aken et al., 2010) and could explain why percentage loss of the 2,3',4,4',5'-pentachloro biphenyls was not high. When water saturation is maintained in sediment, reductive dechlorination results in accumulation of cell chlorinated PCBs (Smith et al., 2009). Therefore, using plant species that remove water from the sediments and introduce oxygen into the rhizosphere through aerenchyma could greatly stimulate removal of lower-chlorinated PCBs from the environment but would have far less impact on higher chlorinated congeners. Borja et al. (2005) achieved a 40% reduction of PCB in dredged sediments.

The effect of plant action on PCB in the soil according to various studies has been immense, but it is not devoid of demerits. Primarily, due to the fact that plants are autotrophs and not ideally suited for the metabolism and breakdown of organic compounds, 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. The interaction between natural micro floras with plants can help to address this to some extent (Prasad et al., 2010); 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 rhizophyto-degradation.

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} live at a distance of 2 cm (Paul et al., 2007). About 5 to 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 (Paul et al., 2007). Besides forming a habitat for microorganisms, plants roots also provide nutrients, e.g. sugars, in exchange for phosphates (fungi) or nitrogen (N_2-fixation). Mulberries *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 indigenous soil pollutants e.g. saponines, proteins and enzymes. Another spectacular finding was that roots and xylem exudates of zucchini (Cucurbitaceae) solubilises PCDD/F (Held and Door, 2000), probably by protein (Newman and Reynolds, 2004). However, plants hyper-accumulation of lipophilic compounds has not yet been established but with microorganisms, reasonable result is ensured (Hatamian-Zarmi et al., 2009).

The combined effort of microorganisms and plants on PCB was seen on report of the work of Dzantor et al. (2000), in which 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 study, 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 the research, they tested another legume; burr medic (*Medicago polymorpha*), for potentials to enhance PCB dissipation. The reason for this according Dzantor et al. (2001), was that Medics are well characterized family, hence may serve as model system for elucidating and manipulating 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 (Dzantor and Woolston, 2001). 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, as 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 as 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 (Dzantor et al., 2000).

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 (Dzantor et al., 2000). The work of Mehmannavaz et al. (2001), agrees with the earlier mentioned postulates as well. According to them, 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, biotranslocation or adsorption of the contaminants due to the presence of alfalfa and or rhizobial inoculations. Mehmannavaz et al. (2001), reported that the presence of alfalfa plants increased the transformation/depletion of PCB congeners as compared to bioaugumentation alone; however, alfalfa alone was the most effective treatment, according to his study. Bioaugmentation of the soil increased the hardness of the soil in a significant way, and thus may have indirectly slowed the growth of alfalfa plant. The decrease in plant biomass, which however, causes poor PCB transformation in a PCB-contaminated soil, suggests that PCB and their bacteria products are phytotoxic to plants. This phytotoxicity is due to increased biotransformation, bioavailability or solubility (Mehmannavaz et al., 2002). However, the difference in plant growth and PCB depletion in bioaugmented and non-bioaugmented treatments may have been related to both the bacterial augmentation and the soil hardness. The study however, suggested additional studies to confirm these initial findings and to determine the effects of PCB and its product, and of inoculums size on the growth of alfalfa in order to optimise phytoremediation of PCBs in the soil.

DIFFERENCE BETWEEN PCB METABOLISM IN BACTERIA AND PLANTS

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

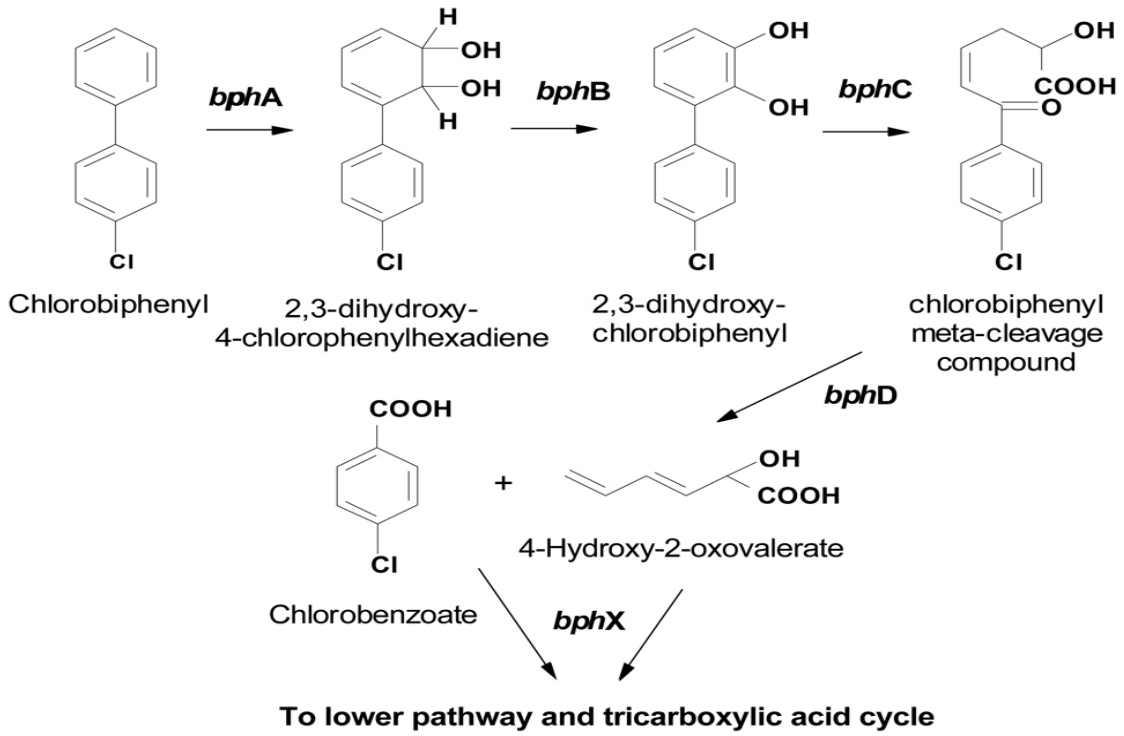

Figure 2. Aerobic bacterial of lower chlorinated PCB catalysed enzymes (*bph*ADCDX) (Furukawa et al., 2004).

while microbes depends on their sequential reactions which is usually activated by various enzymes to transform PCBs, plants uses direct uptake of PCBs, and subsequently transform the contaminant in a non phytotoxic form (Mackova et al., 2007).

The main product of reaction (metabolites) of bacterial degradation pathway of PCBs as shown in Figure 2 is chlorobenzoic acid, while the transformation processes in plant leads to the formation of various hydroxylation products (Figure 3). A good example is shown in the use of plant cells in oxidizing mono- and dichlorinated PCBs into mono- and dihydroxylated biphenyls (Kucerova et al., 2000).

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

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

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 produce undesirable end-products like polychlorinated

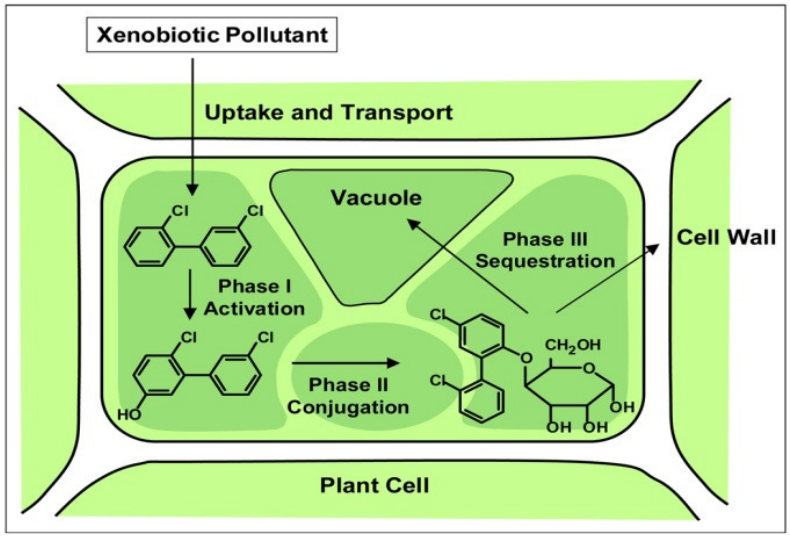

Figure 3. Three processes of the green liver model of xenobiotic metabolism (Aken, 2008).

dibenzo furans/dioxins (PCDF/Ds), which is as result of incomplete combustion that occurs in the process (Borja et al., 2005).

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 conventional methods. This may be 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 (Borja et al., 2005).

The earlier mentioned factors have somewhat posed threats to researchers and government agencies by their effort in trying to come up with an alternative technology than incineration. There was suggestion for an extensive review of the extent of PCB problem of each country for an appropriate technology to suffice (Borja et al., 2005).

Also, the complexity of the microbial process which is used to degrade these complexities of compounds should also be considered. All these and some other factors mentioned earlier gave a need for a more versatile and environmentally friendly method of PCB remediation, a method that can augment the singular actions of micro-organisms or plants on PCBs. Plant-microbial interaction in contaminated soil and the mutual relationship of the level of growth and support in degradation abilities of microbes were already studied and information regarding their cooperative mechanisms are described (Hedge and Fletcher, 1996; Gilbert and Crowley, 1997; Leigh et al., 2004; Biancucci et al., 2004). There is still lack of information on the possible combination of the metabolism of bacteria and plants because of the resultant metabolisation of intermediates and the metabolic products formed in primary degradation of PCBs by the two organisms (Mackova et al., 2007). The particular interest according to the study was directed towards the ability of plants in a PCB contaminated media, which was transforming PCBs initially, resorting to metabolization of chlorobenzoates (bacterial PCB degradation products), and also, to find out if rhizosphere microbes degrading PCBs can transform plants primary metabolites (hydroxychlo-robiphenyls) (Furukawa et al., 1978; in: Mackova et al., 2007). This fear was diffused from the study of Kucerova et al. (1999, 2000, 2004) and Bock et al. (2002), which reported the formation of different hydroxychlorobiphenyls structure, as intermediates of plants PCB metabolisation. The study of Francova et al. (2004), also reported the transformation of commercially available hydroxychlorobiphenyls (found originally as metabolites of single PCB in plants), by bacterial enzymes *in vitro* during isolation from two PCB-degrading bacteria *B. xenovorans* LB400 and *Comamonas testeroni* B356 (Francova et al., 2004). In each step, the products of bacterial PCB pathway were detected after derivatization by GC-MS, and the results confirmed that both enzymes oxygenated hydroxychlorobiphenyls on the non-substituted ring, producing three different metabolites of hydroxychlorobiphenyls. Mackova et al. (2007), revealed that bacterial enzymes of biphenyl operon, isolated from different bacterial PCB degraders LB400 and B356, can degrade mono-substituted hydroxyl- and

hydroxychlorobiphenyls previously identified as products of transformation of plants PCB metabolism (Bock et al., 2002; In: Mackova et al., 2007). They also reported that certain plant species can degrade some chlorobenzoic acids entering the environment as a result of microbial PCB degradation and other means. This report however, created a further possibility of interactions between bacteria and plants in a PCB contaminated environment; it provided more information on the abilities of biological systems to degrade original xenobiotics as well as some of their intermediates and products (Mackova et al., 2007).

CONCLUSIONS

In conclusion, 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 example harnessing the symbiotic relationship between plants and microorganisms in rhizoremediation for hot spots (Schnoor, 1997).

Enhanced rhizosphere biodegradation occurs at the immediate surroundings of the plants roots. Therefore, root exudates released by plants supplies nutrients to microbes that enhances their biological activities and stimulates the degradation of organics. This is done by inducing enzyme system of indigenous bacterial populations (Macek et al., 1999/2002). Both plants and microbes have their limitations with respect to their individual abilities in xenobiotic biodegradation (Mackova et al., 2007). Therefore, the remediation of PCBs from the soil should be facilitated using the synergistic effects of plants and microbes in the rhizosphere (Chaudhry et al., 2005). This co-effect enables them to cope with the toxicity and recalcitrance of the pollutant that would otherwise be difficult for either plants or soil microbes to do alone. In that line also, more research is however important to throw more light into the feedback processes that regulate the interaction of plant and microbes in the root zone of the soil during PCB remediation. The study of Mackova et al, (2007), highlighted on the subject, but suggested that more effort be directed towards proper elucidation of the possibility of interactions between plant and bacteria in a model PCB-contaminated environment. Therefore, Therefore, with the amazing nature of the way plants enriches rhizosphere area of microbial community by the release of compounds which enhances the growth of selective species capable of degrading PCBs. And the ability of the microbes to reciprocate the gesture by providing enabling soil environment for the travail of plants. A better understanding of this synergistic interaction could further be exploited to enable us achieve an effective remediation of PCB contaminated environment. In furtherance, all hands should be on deck by all environmental toxicologists towards achieving an effective solution to PCB contamination.

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