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Degradation of Askarel (PCB Blend) by Indigenous Aerobic Bacteria Isolates from Dumpsites in Ore, Ondo State.Nigeria.

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Abstract: The removal of toxic industrial products such as polychlorinated biphenyls (PCBs) perchloroethylene (PCE) and trichloroethylene (TCE) in soils has become a daunting and necessary task. These compounds adsorb onto organic matter in the environment, making decontamination using traditional approaches difficult or ineffective. The use of microbes to transform these contaminants to non toxic degradation products is an alternative and imperative approach due to the prevalence of such organisms within the environment. In this study; the use of bacteria in the microbial degradation of polychlorinated biphenyls blend (Askarel) was explored. The notable bacteria isolated from dumpsites in Ore, Ondo state were identified using morphological and biochemical characteristics. These include: *Pseudomonas, Micrococcus, Corynebacteria, Bacillus, Achromobacter* and *Arthrobacter* species. The bacterial isolates potential to utilize Polychlorinated biphenyls blend (Askarel) as carbon source was investigated for twenty-one days period. From the results obtained, there was a general decrease in the pH and increase in mean Optical density (O.D); were the mean pH and O.D readings ranged between (3.08-6.02 and 0.060-0.557) respectively.

Key words: Dumpsite, Polychlorinated biphenyls (PCBs) blend, Microbial degradation, pH, Turbidity,

INTRODUCTION

Generally, one serious manifestation of environmental pollution is the high incidence of recalcitrant pollutants with in the environment. These pollutants accelerate the destruction of natural habitats. Polychlorinated biphenyls (PCBs) and its compounds are examples of these recalcitrant pollutants that are ubiquitous and subject of public debate. According to (ATSDR, 2000) Polychlorinated biphenyls and their compounds/ derivatives have been released to the environment solely by human activity. PCBs are either oily liquids or solids and are colorless to light yellow in colors. They have no known smell or taste. Polychlorinated biphenyls and their derivatives because of their chemical and thermal stability formed the basis for the widespread in closed applications (e.g., capacitor; transformers; heat transfer and hydraulic fluids) and in open-end applications (e.g., flame retardants, inks, adhesives, micro encapsulation of dyes for carbonless duplicating paper, paints, pesticide extenders, plasticizers, polyolefin catalyst carriers, slide-mounting mediums for microscopes, surface coatings, wire insulators, and metal coatings (Durfee 1976; EPA 1976a, 1988c; IARC 1978; Welsh 1995). In most developed countries Polychlorinated biphenyls (PCBs) and their containing compounds are no longer used in the manufacture of new products except under exemption. However these products/ compounds predominantly are redistributed from one environmental phase to another e.g., soil to water, water to air, air to water, sediments to water (Larsson 1985; Swackhamer and Armstrong 1986; Lin and Que Hee 1987; Larsson and Okla 1989; Mackay 1989; Eisenreich *et al.,* 1992). PCBs however may be released to the atmosphere from uncontrolled landfills and hazardous waste sites ; from incineration of PCB-containing wastes sites; leakage from older electrical equipment in use; and improper disposal or spills from PCB-containing wastes (Eisenreich *et al.* 1992; Sakai *et al.* 1993; Boers *et al.,* 1994; Blumbach and Nethe 1996; Hansen and O'Keefe 1996; Wallace *et al.* 1996; Bremle and Larsson 1998). It may also be released to water from accidental spillage of PCB-containing hydraulic fluids; combined sewer overflows (CSOs) or storm water runoffs; and from runoff and leacheates from PCB -contaminated sewage sludge applied to farmland (Gan and Berthouex 1994; Crawford *et al.,* 1995; Gunkel *et al.,* 1995; Shear *et al.,* 1996; Loganathan *et al.*

Corresponding Author: NWINYI, Obinna C., Department of Biological Sciences, College of Science and Technology, Covenant University. KM 10 Idioroko Road, Canaanland, PMB 1023 Ota, Ogun State, Nigeria. E-mail: nwinyiobinna@gmail.com

1997; Pham and Proulx 1997; Durell and Lizotte 1998; O'Connor *et al.* 1990; Gan and Berthouex 1994; Gutenmann *et al.*,1994; Liberti *et al.,* 1992; McLachlan *et al.,* 1994; Morris and Lester 1994; Ohsaki and Matsueda 1994; Alcock *et al.* 1995). Polychlorinated biphenyls can enter the body by inhalation or ingestion or by direct contact with the skin. According to Safe (1992), PCBs have the capacity to accumulate in different stages of the food chain were it is toxic and carcinogenic. The trace quantities present in the body mostly are as a result of ingestion through the food chains. Polychlorinated biphenyls accumulate in fatty tissues and can be transmitted in breast milk and across the placenta. Polychlorinated biphenyls alter the functioning of major body systems such as the immune, hormone, nervous, and enzyme systems; thus, Polychlorinated biphenyls affect a wide variety of body organs and functions. From research findings, people exposed in the workplace, have skin conditions, such as acne and rashes. This may occur in people exposed to high levels of PCBs. Furthermore, studies on workers exposed to polychlorinated biphenyls reveal symptoms such as irritation of the nose and lungs, gastrointestinal discomfort, changes in the blood and liver, depression and fatigue. Workers in places were transformers are repaired and maintained, are more predisposed to the effects of this recalcitrant pollutant.

According to research reports of Aoki, (2001) on workers around PCB contaminated environment, it was found out that most workers have specific kinds of cancer such as cancer of the liver and biliary tract. Other symptoms include dermal and ocular lesions, irregular menstrual cycles and a lowered immune response, fatigue, headache, cough, and unusual skin sores. Additionally, in children, there were reports of poor cognitive development. From the studies on the dynamics of the health effects of PCBs in children and mothers who were exposed to PCBs there has been reports of oestrogen in breast cancer cells and this can enhance breast carcinogenesis (Darbre *et al.,*2008).

Due to the adverse potential to human welfare and environment, there is therefore an urgent need for development of bioremediation strategies for the removal of polychlorinated biphenyls and their containing compounds from contaminated sites. From studies, the potential of polychlorinated biphenyls and their containing compounds to be degraded or transformed in the environment depends on the degree of chlorination of the biphenyl molecule as well as on the isomeric substitution pattern. Biodegradation of these compounds have been demonstrated under both aerobic and anaerobic conditions (Chen *et al*. 1988; Sugiura 1992; Thomas *et al*., 1992; Dowling *et al.,* 1993; Fava *et al.,* 1993; Gibson *et al*., 1993; Haluska *et al.,* 1995) and anaerobic conditions (Brown *et al*., 1988; Pardue *et al.* 1988; Rhee *et al*., 1989; Abramowicz 1990, Anid *et al.,* 1993). In 1973 Ahmed and Focht reported aerobic degradation of PCBs by two species of *Achromobacter*. From previous studies, several aerobic microorganisms are able to biodegrade certain, less chlorinated polychlorinated biphenyl compounds (Bedard *et al*., 1987, kohler *et al.,* 1988; Sylvestre and Fateux, 1982; Furukawa *et al*., 1992).

PCB-degrading bacteria are found among the gram negative genera *Pseudomonas, Alcaligenes, Achromobacter, Burkholderia, Comamonas, Sphingomonas, Ralstonia* and *Acinetobacter* and among the Gram-positive genera *Rhodococcus, Corynebacterium* and *Bacillus* (Furukawa, 2000; Borja *et al*., 2005; Pieper, 2005 Adebusoye *et al*., 2008). These bacteria have an enzyme, biphenyl 3,4- dioxygenase enabling them to attack some of the double *ortho*-substituted congeners (Bedard *et al*., 1986, 1987; Bopp, 1986). Theoretically, the

biological degradation of PCBs should result in CO_2, chlorine and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting compound. The biphenyl metabolic enzymes encoded by the bph gene cluster are responsible for the attack on PCBs (Abramowicz, 1990; Furukawa, 2000). In Nigeria particularly in most emerging urban centers such as Ore, in Ondo State Nigeria, PCB-containing items such as adhesives, transformers, large ,high and low voltage capacitors, liquid –cooled electrical motors, hydraulic systems, heat –transfer system ,fluorescent light ballasts, electromagnets, liquid filled cables, gasketing and dampening belts, voltage regulators, vacuum pumps, microwave ovens and transformer oils are dumped at any dumpsites without recourse to the health implications. These problems are further compounded by the inadequate capacity, administrative and legislative framework to ensure effective management and disposal of these PCB containing items; with a view of reducing or eliminating health and environmental risks. Thus the disposal of these PCB wastes items remains a major challenge as there is inadequate capacity to carry out an environmentally sound disposal programme in the nation. The overall need is for complete PCB degradation however partial solutions such as lowering bioaccumulation potential and lipophilicity of this pollutant can be reached by assessment of the biodegradation potential of indigenous bacterial species since most of the research carried out to date are from temperate soils. From the reports of Adebusoye *et al*., (2008) it is clear that tropical microbes may possess fascinating metabolic diversity and novel catabolic properties for real time degradation of Polychlorinated biphenyls and their compounds. Thus to contribute towards providing solution to this national menace author seeks to investigate the potentials of aerobic bacterial isolated from ore dumpsite in degrading askarel a (PCB blend).

MATERIALS AND METHODS

Chemicals and Reagents:

All chemicals and reagents were of analytical grade. The (PCB blend) Askarel was generously provided by Power Holding Company of Nigeria. All other chemicals and reagents were obtained from Sigma Chemicals Co Ltd England.

Site Selection and Sample Collection:

The site selected was ore in Ondo state of Nigeria Ore is a growing town characterized with a fast growing population. It is also a major link connecting to the South Eastern Nigeria. Soil Samples for microbial isolations were randomly collected (up to 12cm) from dumpsites at Ore. The site had wastes of several PCB items. Soil samples, weighing about 250g were taken from dumpsites then transferred into conical flasks and stored in cool ice packs for transfer to the laboratory for further work. The physical examination of the soil shows a mixture of sand loamy composition..

Fig.1: Map showing Ondo state Nigeria.
Source: Google.com

Isolation and Enrichment of Aerobic Indigenous Bacterial Strains:

Askarel degraders' indigenous to the soils were enriched and isolated using an Askarel (PCB blend) Minimal salt medium without nitrogen and carbon. It comprises the following minimal salts in 1 liter distilled water, K_2HPO_2, 7.0g, KH_2PO_4, 3.0g, $MgSO_4.7H_2O$, 0.1g yeast extract, Askarel (Carbon source) as sole carbon source and energy; and incubated at 25^{o}C for a period of three weeks. The pH was adjusted to 6.0. Incubation at 37^{o}C is lethal to many soil bacteria. When growth had occurred, the enrichment culture was transferred to fresh Askarel minimal salt medium using about 10% of inoculum from the previous enrichment and incubated at 25^{o}C. This procedure was repeated for three successive transfers. Pure cultures were isolated from enrichments by plating out on nutrient agar. Discrete single colonies were selected and inoculated on Mineral media sprayed with Askarel. The process was repeated severally to obtain pure cultures capable of growth on PCB blend. (Liu *et al.,* 2002, Nwinyi *et al.,* 2008).

Identification of the Isolated Bacterial Strains:

The pure bacterial strains were identified on the basis of their morphological and biochemical tests. The pure cultures of the bacterial isolates were subjected to various morphological and biochemical characterization tests such as color, shape, elevation, consistency , margin , Catalase test, MRVP (methyl red-voges proskauer test), fermentation of sugars, kovacs citrate, indole, hydrolysis of starch, and sensitivity tests. In order to determine the identity of bacteria isolates, results were compared with standard references of Bergey's Manual of Determinative Bacteriology 2nd edition (Buchanan and Gibbon, 1974; Olutiola *et al*., 1991).

Determination of Growth Profile in Different Concentration of Askarel Blend:

The isolates were inoculated into different concentrations, 5µl, 10µl, 15µl and 20µl askarel minimal broth. This was done to determine the potential degradation/ transformation of askarel and its utilization as a carbon

source. The culture was then incubated at room temperature 25^{o}C for a period of three weeks. The Growth was monitored by measuring weekly the turbidity at 540nm using standardized Hanna H198703 Turbidimeter and pH by Hanna microprocessor pH meter.

Results:

Table 4.1: Shows cultural and morphological characteristics of bacteria isolates capable of degrading askarel.

Isolate Code	Morphology	Gram's reaction	Motility
A1	Growth moderate, filiform, flat, smooth, cream-colored, mucoid and opaque.	Gram positive rods	Non-motile
A2	Growth moderate, filiform, flat, smooth, cream-colored, mucoid and opaque.	Gram positive rods	Non-motile
A3	Growth moderate, filiform, flat, smooth, cream-colored, mucoid and opaque.	Gram positive rods	Non-motile
A4	Growth scanty, filiform, no pigmentation and translucent	Gram positive short rods	Non-motile
B1	Growth scanty, filiform, no pigmentation and translucent	Gram positive short rods	Non-motile
B2	Growth scanty, greyish, granular, translucent growth, with irregular margin.	Gram positive rods	Non-motile
B3	Growth abundant, filiform, smooth, green pigmentation, and translucent.	Gram negative rods.	Motile
B4	Growth abundant, filiform, smooth, green pigmentation, and translucent.	Gram negative rods.	Motile
C1	Growth abundant, filiform, smooth, green pigmentation, and translucent.	Gram negative rods.	Motile
C2	Growth moderate, thin, translucent becoming opaque, whitish.	Gram positive rods	Motile
C3	Growth moderate, thin, translucent becoming opaque, whitish.	Gram positive rods	Motile
C4	Growth moderate, filiform, smooth and translucent	Gram negative rods.	Motile

Table 4.2: Shows results of biochemical characteristics of bacteria isolates capable of degrading askarel.

Isolate	Citrate test	ureas test	Catalase test	Growth in Stach		Indole	Methyl red	Growth	Sugar fermentation test				Most probable
				5%Nacl	Hydrolysis	test	test	at pH 6.0	Maltose	Gulcose	Sucrose	Lactose	orgnism
A1	+	-	+	+	+	-	-	+	-	-	-	-	*Arthrobacter spp*
A2	+	-	+	+	+	-	-	+	-	-	-	-	*Arthrobacter spp*
A3	+	-	+	+	+	-	-	+	-	-	-	-	*Arthrobacter spp*
A4	+	+	+	+	-	-	-	+	-	A	-	-	*Micrococcus spp*
B1	+	+	+	+	-	-	-	+	-	A	-	-	*Micrococcus spp*
B2	+	-	+	+	+	-	-	+	A	A	A	A	*Corynebacterim spp*
B3						-	-	+					*Pseudomonas spp*
B4	-	+	+	+	+	-	-	+	-	-	-	-	*Pseudomonas spp*
C1	-	+	+	+	+	-	-	+	-	-	-	-	*Pseudomonas spp*
C2	-	-	+	-	+	-	-	+	-	A	AG	-	*Bacillus lentus*
C3	-	-	+	-	+	-	-	+	-	A	AG	-	*Bacillus lentus*
C4	-	+	+	+	+	-	-	+	-	-	-	-	*Achromobacter pestifer*

Key:
+ = positive.
- = negative.
A = acid but no gas production.
AG = acid and gas production.

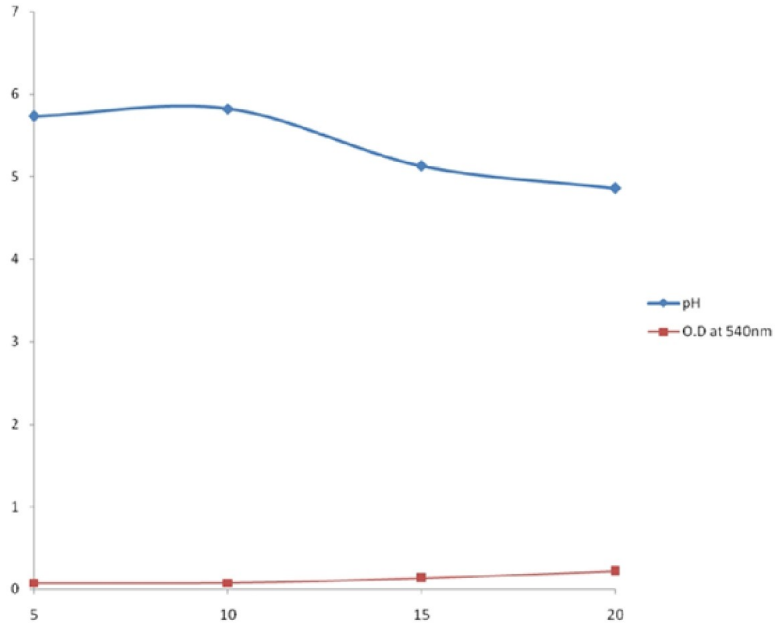

Fig.4.2: Growth profile [Optical density (■) and pH (♦) of *Micrococcus* spp incubated at room temperature in 5µl, 10µl, 15µl and 20µl concentrations of askarel for 1-21 days.

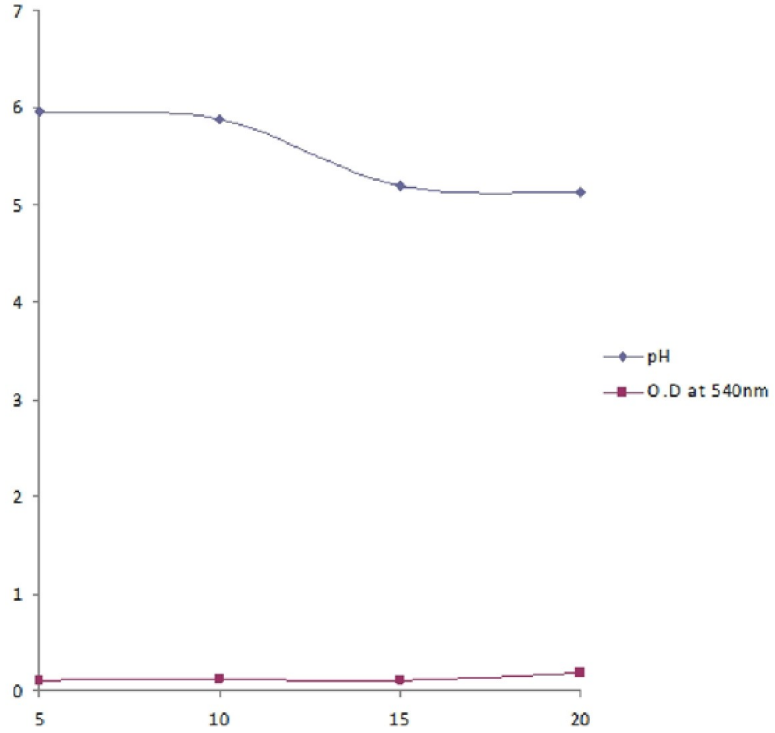

Fig.4.3: Growth profile [Optical density (■) and pH (♦) of *Corynebacterium* spp incubated at room temperature in 5µl, 10µl, 15µl and 20µl concentrations of askarel for 1-21 days.

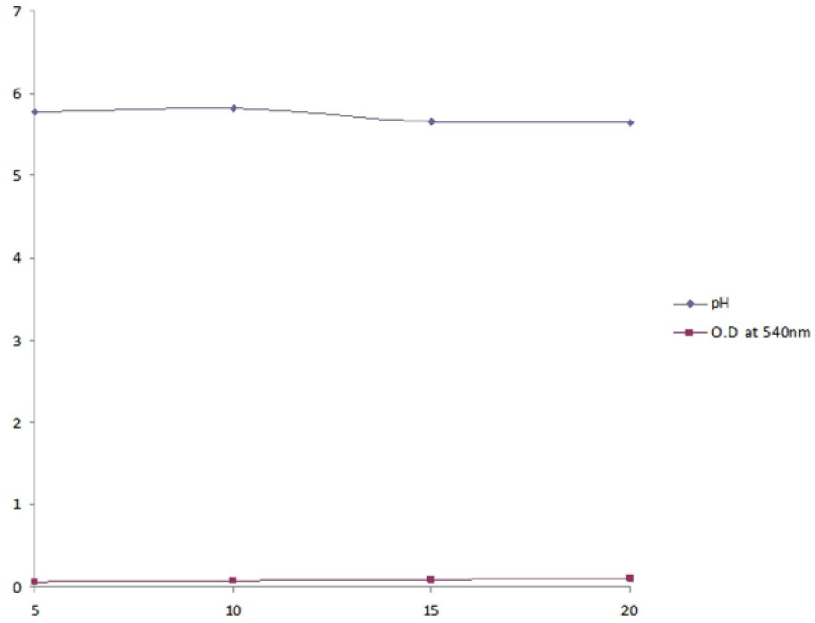

Fig.4.4: Growth profile [Optical density (■) and pH (♦) of *Pseudomonas* spp incubated at room temperature in 5µl, 10µl, 15µl and 20µl concentrations of askarel for 1-21 days.

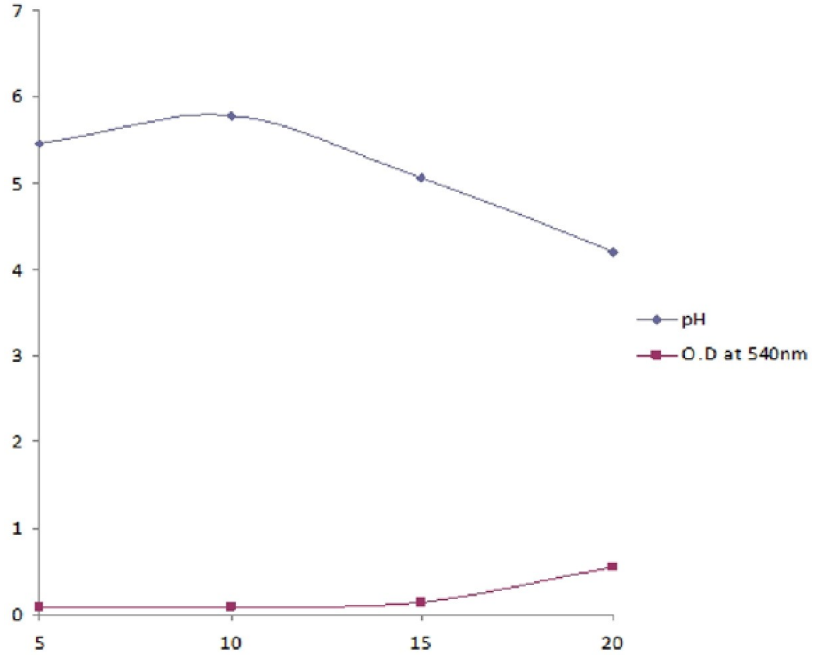

Fig. 4.5: Growth profile [Optical density (■) and pH (♦) of *Bacillus lentus* incubated at room temperature in 5µl, 10µl, 15µl and 20µl concentrations of askarel for 1-21 days.

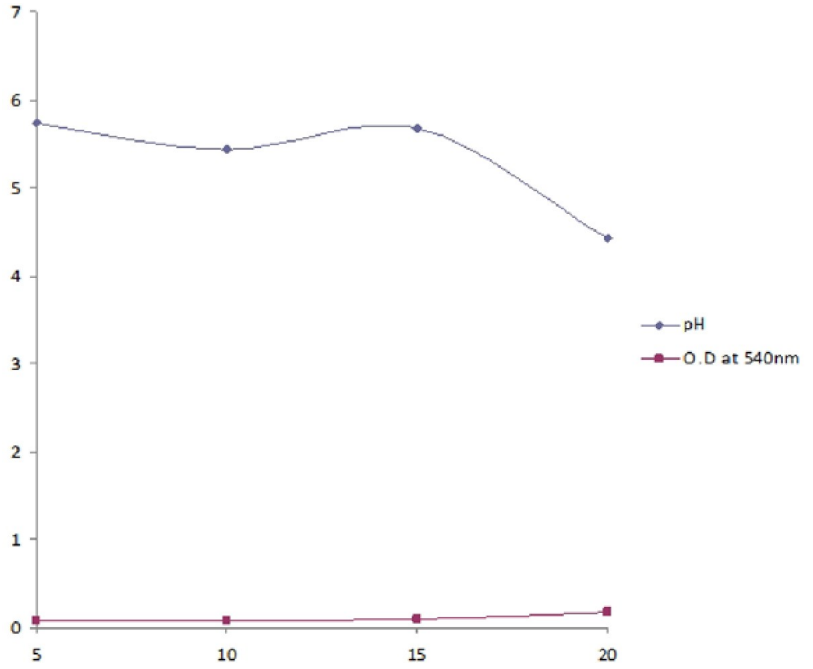

Fig.4.6: Growth profile [Optical density (■) and pH (♦) of *Achromobacter pestifer* incubated at room temperature in 5µl, 10µl, 15µl and 20µl concentrations of askarel for 1-21 days.

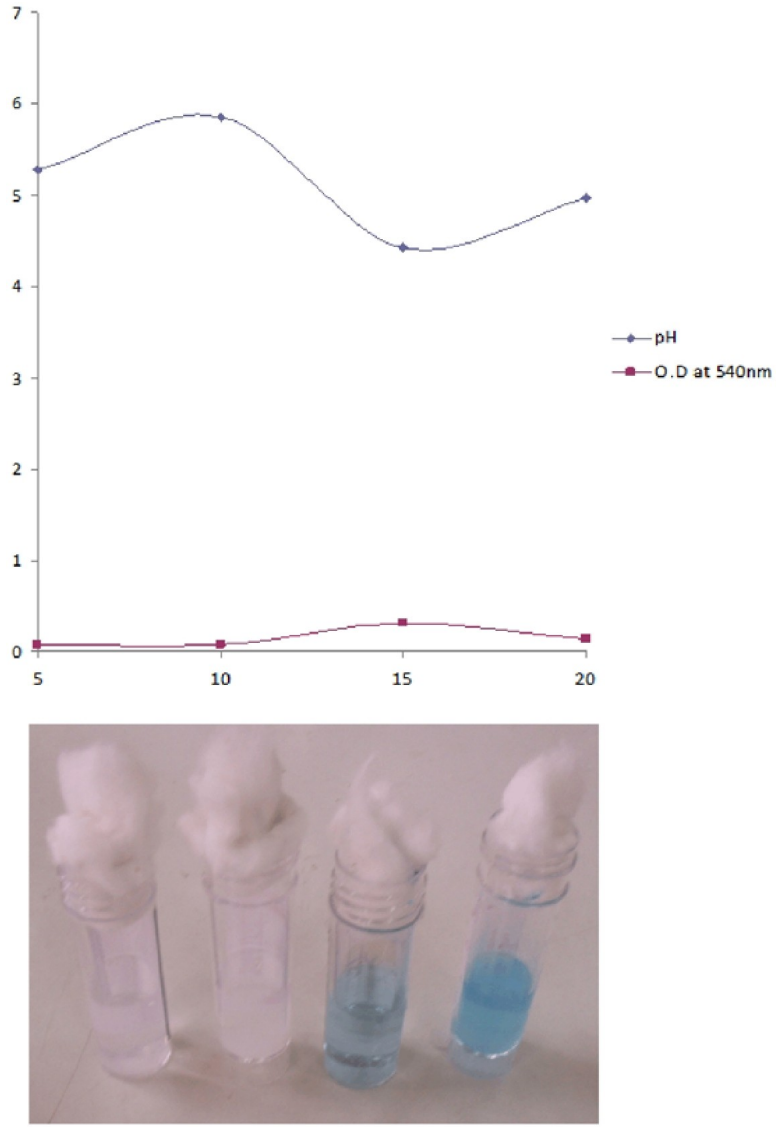

Plate 1: Showing PCB blend minimal salt medium and the *Pseudomonas* isolates.

Discussion:

A number of microorganisms in soil can use PCB blend as sole carbon and nitrogen source. Several microorganisms in particular bacteria have been implicated in utilization of PCBs. Bacterial species of *Pseudomonas* (Gibson *et al.*, 1993), *Arthrobacter* (Furukawa and Chakkrabarty, 1982), *Achromobacter* (Masse *et al.,* 1984), *Bacillus* (Masse *et al.,* 1984), and *Corynebacterium* (Bedard *et al.,*1986) have been shown to be able to degrade PCBs.In this study, six soil microorganisms of bacterial species capable of degrading askarel (PCB blend) were isolated. The include: *Arthrobacter spp. Micrococcus spp, Corynebacterium spp, Pseudomonas spp, Bacillus lentus* and *Achromobacter spp*. They all were able to utilize askarel as their sole carbon source but at different concentrations of 5µl, 10µl, 15µl and 20µl. Growth was observed by measurement of pH and turbidity. From literature, utilization of organic pollutants as carbon source can be evaluated by an increase in turbidity and decrease in pH. The decrease in pH with an increasing turbidity is in agreement with the previous knowledge about microbial degradation (Nwinyi *et al*, 2008). Most bacteria degrade PCBs via a major biphenyl pathway that is initiated by the attack of an enzyme, 2, 3-biphenyl deoxygenase (2, 3- BDO) and followed by a meta-1, 2-fission. This enzyme cleave the 2, 3 carbons of the PCBs (Hickey and Focht, 1990).

Theoretically, the biological degradation of PCBs should result in CO_2, chlorine and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage

and oxidation of the resulting compound. From our finding, it is observed that *Arthrobacter spp* was able to utilize the askarel (PCB blend) in the minimal salt medium as its carbon source at all concentrations with turbidity values of 0.0074, 0.077, 0.135 and 0.218 at concentration of 5µl, 10µl, 15µl and 20µl respectively.

There was also a decrease in pH values (5.73, 5.82, 5.13 and 4.86) indicating breakdown of the askarel and production of acids i.e. chlorobenzoic acids by *Arthrobacter spp.* In the case of *Achromobacter pestifer,* the organism was able to utilize the askarel (PCB blend) in the minimal salt medium as its carbon source at lower concentration of 5µl, 10µl and 15µl. However, it did not perform so well at higher concentrations of 20µl as shown by the O.D values (0.077, 0.085, 0.315 and 0.144) thus indicating that this *Achromobacter pestifer* tolerates relatively lower concentrations of PCB blend. This is also evidenced by the change in pH profile reading (5.27, 5.85, 4.43 and 4.97). This phenomenon can occur as a result of the toxic effect of the by- products of metabolism produced during catabolism of askarel (PCB blend) by the organism, thereby causing death to some, reducing the number of the organism. *Corynebacterium spp* has the lowest ability to degrade PCB blend. This organism utilized PCB minimally, as clearly illustrated by the low values of pH (5.77, 5.82, 5.66, and 5.64) and O.D (0.060, 0.076, 0.082, and 0.110) at concentrations 5µl, 10µl, 15µl and 20µl respectively. This could be because it is a slow growing organism and in the presence of a new compound, i.e. the askarel, it needs to adapt, adjust and synthesize enzyme required for degradation.

It is observed that *Micrococcus* were able to utilize the askarel (PCB blend) in the minimal salt medium as its carbon source at all concentration, hence was able to grow, as seen in its O.D values (0.102, 0.123, 0.103, and 0.181) and also the decrease in pH of the medium (5.96, 5.41, 5.26, and 5.66) at concentrations 5µl, 10µl, 15µl and 20µl respectively. In this study *Micrococcus spp.* may have patterned the same metabolic pathway used by *Achromobacter pestifer* to degrade PCBs blend, hence it exhibited almost similar OD and pH readings. *Bacillus lentus* was the second best organism that degraded the PCB, resulting in a very low pH values (5.73, 5.44, 5.68, and 4.43) and increased turbidity (0.087, 0.080, 0.097, and 0.181) at concentrations 5µl, 10µl, 15µl and 20µl respectively. On comparison to other organisms, *Bacillus lentus* performed best at the 20µl concentration, illustrating that the species thrives better at higher concentrations, as it was also able to rapidly multiply in the 20µl medium.

Pseudomonas spp utilized askarel (PCB blend) at all concentrations but maximally at the highest concentration, resulting in the lowest pH value (4.43) and also increased turbidity O.D at (0.557). *Pseudomonas* spp.performed effectively at higher concentration possibly because it is a gram negative organism equipped with versatile degradative genes. Gram positive bacterial organisms are usually more sensitive than gram-negative ones towards lipophilic toxic substrates, possibly because they lack protection by the outer membrane (Prescott *et al.,* 2002).Furthermore, it was noteworthy that broth containing *Pseudomonas* species changed to blue in appearance (plate 1) . The blue color is as a result of the production of pyocyanin, a pigment produced only by *Pseudomonas*. This color change can be an absolute diagnostic character for *Pseudomonas*, since no other bacterial species have been found to produce this pigment.

Conclusion:

Arthrobacter and *Pseudomonas spp*, were able to utilize the Askarel (PCB blend) in all concentrations, *Bacillus lentus* did so at higher concentrations, where as *Micrococcus* and *Achromobacter pestifer* performed minimally at higher concentrations. *Corynebacterium spp* was unable to utilize the PCB well, as it grew in the medium slightly.

Conversely, these isolated organisms are soil organisms and are prevalent, hence can be used for bioremediation of organic pollutant contaminated soils and sediments with appropriate nutrient adjustment. Since most of these organisms have been researched on, they can be cultivated in large quantities in laboratories and applied on polluted soils. Also, the enzymes responsible for the degradation, i.e., 2, 3-dioxygenase biphenyl can be extracted from these organisms and mass produced for use in biodegradation of PCB containing compounds and derivatives. Relevant government agencies should ensure that old transformers containing PCBs oils should be phased out/ banned from use in the distribution of electricity and proper environmental friendly disposal methods adopted. Further research should be carried out in order to determine the plasmid or gene responsible for conferring the degradative property on these isolates. The gene can be extracted and modified to function better or transferred to indigenous ubiquitous bacterial species, giving them the potential to detoxify PCB containing compounds.

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