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Isolation and Identification of Bacteria from Transformer Oil Contaminated Soil

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ARS, MA, Subhanullah and BRK, collected epidemiological data collected samples and wrote the manuscript. Authors IK, MA, NK, Attaullah and Subhanullah helped in manuscript writing and author NUA arranged and performed statistically analysis. Final manuscript was read out and approved by all the authors.

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Original Research Article

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ABSTRACT

Background: Poly chlorinated biphenyls (PCBs) are organic chemicals with toxigenic, carcinogenic affecting human health and the environment using as dielectric fluids in transformers as a cooling and insulating medium containing.
Materials and Methods: Soil samples were collected from transformer oil contaminated soil at different workshops in three different districts of Khyber Pakhtunkhwa i.e. Peshawar, Nowshera and Kohat and were kept at 4°C before analysis.
The samples were subjected to Pure culture isolation through a selective medium (Medium A). After incubation for 24 to 48 hours at 37°C with 1% transformer oil as sole source of carbon, the isolates were examined for their colony size, shape, margin, consistency, opacity, elevation and

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pigmentation; while Gram reaction and cell morphology were examined microscopically. Furthermore the biochemical tests were also done for identification of the bacteria.
Results: A total of 14 isolates were obtained from all the transformer oil contaminated soil samples after examining the samples indicates the bacteria namely *Bacillus, Micrococcus, Pseudomonas, Acinetobacter* and *Staphylococcus* were identified during the current study.
Conclusion: Based on the results of the study, five bacterial species capable of degrading PCBs in transformer oil, from which it was concluded that PCB compounds can be degraded by some microorganisms under aerobic conditions.

Keywords: PCBs; soil; transformer; bacillus; staphylococcus.

1. INTRODUCTION

Poly chlorinated biphenyls (PCBs) are chemical compounds which have been used as dielectric fluids [1] in transformers as a cooling and insulating medium [2] containing organic chemicals with toxigenic, carcinogenic affecting human health and the environment [3]. The high chemical stability and superhydrophobicity of PCBs cause them to bioaccumulate in cells and pass by the food chain [4,5]. Concerning about the environmental fate of contamination by PCBs Transformer oils has increased recently [6] contamination with PCBs still occurs and is of great public concern. This has led the European Union and many other countries to regulate the PCB contents in air, water and sludge. Accordingly, the Stockholm Convention has listed PCBs as priority chemicals for eventual elimination by 2025 and the parties of the Convention must apply environmentally sound management of PCB wastes by 2028 [7]. PCBs are highly toxic, mutagenic and persistent in the environment [8], commercially produced as complex mixtures, starting from the late 1920 which are the man-made contaminants [9].

It is estimated that about 125 million metric tons transformers containing PCBs were in use as of 1999 in USA [10]. Aromatic hydrocarbons and polar compounds with high resistance to degradation [11] and since 1929, polychlorinated biphenyls (PCBs) have been widely used for transformer oils [12].

PCBs are not easily separated from oil because their physicochemical characteristics are very similar to those of mineral oil [8]. The main mixture of PCBs in transformer oils is high chlorinated compounds [13,14]. From 1929 to 1970, 632 million tons of PCBs were produced for several uses about 26% of the total production was used for transformer oil formulation [15]. The actual and potential releases of PCBs seriously threats human beings and ecosystems [16], and also affect the life balance of human beings and other living organisms [17].

It is necessary to improve the treatment methods of transformer oil, reduce their ecological side effects. Different physicochemical methods such as incineration and direct dechlorination for the disposal of transformer oils and destruction of PCBs have been proposed [18].

Bacteria play a basic function in the elimination of waste chemical compounds [5] for PCBs degradation biological methods were applied to solve environmental problems [18] while the number of chlorine atoms per molecule is a critical feature in this method [12]. Highly chlorinated biphenyls are easily degraded under aerobic conditions [19].

The present study was therefore conducted to isolate bacteria from the transformer oil contaminated soil and to identify the transformer oil degrading bacteria through various biochemical tests.

2. MATERIALS AND METHODS

2.1 Samples Collection of Soil and Transformer Oil

Soil samples were collected from transformer oil, contaminated soil at workshops in three different districts of Khyber Pakhtunkhwa i.e. Peshawar, Nowshera and Kohat. The samples were taken from the depth of 5 to 10 cm surface and subsurface by the help of the sterile steel rod. These samples were maintained in small covered sterilized plastic boxes having 1 to 2 Kg capacity for soil and then carefully transferred to labeled sterile bags and carried to Microbiological laboratory, Department of Microbiology, Kohat University of Science and Technology Kohat, Pakistan for further processing and were kept at 4°C before analysis.

The transformer oil was collected from the Madina Transformer Workshop, Kohat. This oil

was used as a sole source of carbon in the entire study. It was collected in a sterile airtight bottle and stored in cool and dark place.

2.2 Pure Culture Isolation

For the isolation of pure culture a selective medium (Medium A) was prepared. Medium A contained (g/l): KNO_3, 1; $FeCl_3$, 0.02; $MgSO_4$, 0.2; NaCl, 0.1; $CaCl_2$, 0.1; K_2HPO_4, 1; Yeast extract, 0.05. After autoclaving of the medium, transformer oil was added at 1% (v/v) previously emulsified with an aqueous solution of Triton X-100 (1:1) at 1% (v/v) and agar at 1.5% (w/v).

The pH of the medium was adjusted to 6.3 with HCl (0.1 N). Transformer oil was autoclaved at 121°C for 15 minutes in sealed Erlenmeyer flasks. The oil acted as a source of carbon for the oil degrading bacteria.

Soil samples from various contaminated sites were inoculated by spreading 0.1 ml of the serially dilution (10^{-1}) in Petri dishes with medium A, which were then incubated for 24 to 48 hours at 37°C. After which the colonies were transferred to fresh media with transformer oil as the sole carbon source for further sub culturing. Then these subcultures were repeatedly streaking on Nutrient Agar to gain the pure culture. The isolated pure cultures were stored further processed for their morphological and biochemical characters.

2.3 Preliminary Identification of Pure Isolates

Each isolate was examined for its size, shape, margin, consistency, opacity, elevation, pigmentation of colonies, as well as Gram reaction and morphology of cells as described in Bergey's Manual of Determinative Bacteriology [20]. Biochemical tests were performed with the standard procedure [21] for the isolates, including production of catalase, indole, urease, oxidation fermentation of sugars, methyl red test, Voges Proskauer test and citrate utilization, gelatin utilization/ liquefaction, starch utilization and Casein utilization [22].

2.4 Statistical Analysis

Statistical analysis of the available data was performed by using "STATISTIX", version 9.0, Korean made software. The P value less than 0.05 were considered significant.

3. RESULTS

3.1 Bacterial Isolation

A total of 14 isolates with capacity to degrade PCB compounds were obtained from the soil samples: three isolates (P1P1, P1P2, P1P3) from PESCO regional workshop, G.T Road, Peshawar; two (P2J1, P2J2) from Jawad engineering, Bashir Abad, Peshawar; two (N1A1, N1A2) from Afghan electrical works, Nowshera; three (N2F1, N2F2, N2F3) from Frontier transformer workshop, Nowshera; one (K1P1) from Transformer workshop, Pindi road, Kohat; and three (K2M1, K2M2, K2M3) from Madina transformer workshop, Banuu road, Kohat. The distribution of the isolates indicated that the bacteria able to utilize the hydrocarbons is universal in the soils contaminated with transformer oil.

3.2 Identification of Bacterial Isolates

The cultural, morphological and biochemical characteristics of the PCB degrading isolates were presented in Tables 1 and 2. Based upon these data, the bacteria were identified as *Bacillus* spp. (6 isolates), *Micrococcus* spp. (4 isolates), *Pseudomonas* sp. (1 isolate), *Acinetobacter* spp. (2 isolates) and *Staphylococcus* sp. (1 isolate) (Table 2).

3.3 Presence of Bacteria in Different Soil Samples

Table 2 also summarized that the *Bacillus* spp. were found in all of the sampling areas, Kohat, Nowshera and Peshawar. *Micrococcus* spp. were obtained from Kohat and Peshawar soil samples. *Acinetobacter* spp. were also identified from two regions, Nowshera and Kohat. While *Pseudomonas* sp. and *Staphylococcus* sp. were isolated from only one region. The structure of Polychlorinated biphenyls is composed of a double ring organic compound (Fig 1) [23] which is denatured into simplest form.

4. DISCUSSION

Aerobic biodegradation is one of the means used by microorganisms for the removal of unrelenting organic pollutants from the soil [24]. Several microorganisms have been isolated which can degrade PCBs in an aerobic situation [25]. First of all Furukawa studied microbial degradation of PCBs in 1983 [26].

Table 1. Characteristics of the bacterial isolates

Isolate	Cellular characteristics					Colony morphology						
	Size	**Shape**	**Gram's reaction**	**Motility**	**Spore**	**Size**	**Shape**	**Margin**	**Elevation**	**Color**	**Texture**	**Opacity**
P1P1	Small	Rods	Positive	M	SF	Large	Round	Irregular	Flat	Milky	Smooth	Opaque
P1P2	Small	Cocci	Positive	NM	NSF	Small	Round	Entire	Slightly Raised	White	Smooth	Opaque
P1P3	Big	Rods	Positive	M	SF	Large	Round	Irregular	Flat	Milky	Rough	Opaque
P2J1	Big	Rods	Positive	M	SF	Large	Round	Irregular	Flat	Milky	Smooth	Opaque
P2J2	Small	Cocci	Positive	NM	NSF	Pin Point	Round	Entire	Flat	Greenish	Smooth	Opaque
N1A1	Small	Rods	Positive	NM	NSF	Small	Round	Entire	Flat	Creamy	Smooth	Opaque
N1A2	Small	Rods	Negative	NM	NSF	Small	Round	Entire	Flat	Creamy	Smooth	Opaque
N2F1	Small	Rods	Positive	M	NSF	Large	Round Mucoid	Entire	Raised	Milky	Smooth	Opaque
N2F2	Small	Staphylococci	Positive	NM	NSF	Small	Round	Irregular	Flat	Greenish	Smooth	Translucent
N2F3	Small	Rods	Positive	M	NSF	Pin Point	Round	Entire	Flat	Creamy	Smooth	Opaque
K1P1	Big	Rods	Positive	M	SF	Large	Round	Entire	Flat	Brownish Creamy	Smooth	Opaque
K2M1	Small	Cocci	Positive	NM	NSF	Large	Irregular	Irregular	Flat	Browny	Concentric	Opaque
K2M2	Small	Cocci	Positive	NM	NSF	Small	Round	Entire	Raised	Yellowish	Smooth	Opaque
K2M3	Small	Cocco bacilli	Negative	NM	NSF	Large	Irregular	Lobate	Flat	Greenish	Rough	Opaque

Table 2. Identification of the bacteria isolated from transformer oil contaminated soils in different sites, based upon their biochemical characters

Isolate and identification*	Biochemical test#													
	0x	Cat	Cit	H_2S	Man	PA	TSI	Sta	Cas	Gel	MR	VP	U	DNase
Bacillus spp.														
K1P1	-	+	-	-	-	+	y.b	+	+	+	-	-	-	-
N2F1	-	+	-	-	+	-	r	+	+	+	+	-	-	-
N1A2	-	+	+	-	+	-	y.b	-	-	+	-	-	-	-
P1P1	-	+	-	-	-	-	y.b	+	+	+	+	-	-	+
P1P3	+	+	+	-	-	-	y.b	+	+	+	+	-	-	+
P2J1	-	+	-	-	-	-	y.b	+	+	+	+	-	-	-
Micrococcus spp.														
P2J2	+	+	+	+	-	+	ND	-	+	+	+	-	+	+
P1P2	-	+	+	-	-	-	y.b	-	+	+	-	+	-	+
K2M2	-	+	+	-	-	+	r	-	+	+	-	-	-	-
K2M1	+	+	+	-	-	+	r	-	+	+	-	-	-	-
Pseudomonas sp.														
N2F3	+	+	+	-	-	-	r	-	+	-	-	-	-	-
Acinetobacter spp.														
N1A1	-	+	-	-	-	-	r	-	-	-	-	-	-	+
K2M3	+	+	+	-	-	+	r	-	+	-	-	-	-	-
Staphylococcus sp.														
N2F2	-	+	+	-	+	-	r	-	-	-	-	-	-	-

**. For identification, P= 0.0394 <0.05 Significant.*

#. OX: Oxidase, Cat: Catalase, Cit: Citrate, MAN: Mannitol, Coa: Coagulase, Ind: Indole (all isolates are coagulase and indol –ve), PA: Phenylalanine Deaminase, TSI: Triple Sugar Iron, Sta: Starch, Cas: Casiene, Gel: Gelatine, MR: Methyl Reductase, VP: Vogas Proskaeur, U: Urease, r=Red, y.b=yellow blow and ND= not detected, P= 0.000 <0.05

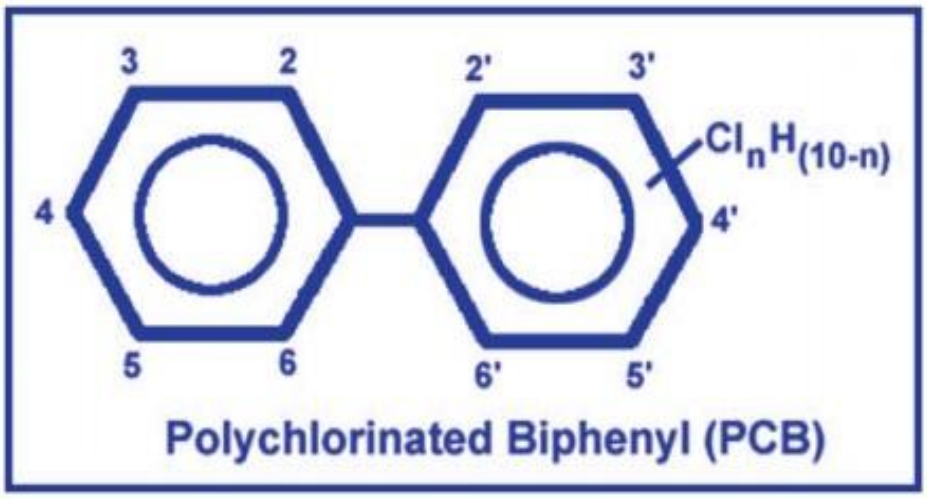

Fig. 1. Structure of polychlorinated biphenyls
Source: Mueller (2009) [23]

PCB-degrading bacterial strains, including gram-negative bacteria such as *Pseudomonas*, *Sphingomonas, Achromobacter, Alcaligenes, Comamonas*, and gram-positive bacteria such as *Corynebacterium, Rhodococcus,* and *Bacillus* have also been described by others [26]. Another researcher reported fifteen bacterial strains using biphenyl as sole carbon and energy source belonged to *Pseudomonas* spp., *Alcaligenes* spp., *Comamonas* spp. and *Ralstonia* spp. [27]. While in the present study, *Bacillus, Micrococcus, Pseudomonas, Acinetobacter* and *Staphylococcus* were identified among the 14 isolates. Among these bacteria, *Pseudomonas*, *Micrococcus Arthrobacter* and *Acinetobacter* have been identified as askarel oil (PCBs blend) degraders in landfill soil samples of Uzogholo, Mgboaku and Auchi in Edo State of Nigeria [24].

In the current study the bacteria were identified on the bases of their cultural, morphological and biochemical tests which are summarized in Tables 1 and 2. The same identification methods were also used in another study in Nigeria during testing the landfill soil samples contaminated with askarel oil (PCBs blend) [24], while from another study stated that PCBs are biodegraded in two general ways; aerobic metabolism via co-metabolism and anaerobically by reductive dehalogenation [28]. In addition, *Rhodococcus* sp., *Corynebacterium* sp., *Archromobacter, Arthrobacter, Bacillus, Alcaligens odorans* and *Alcaligens denitrificans* are studied by different researchers that these bacteria can degrade dichlorobiphenyls [29].

5. CONCLUSION

Based on the results of the study, five bacterial genera capable of degrading PCBs in transformer oil were identified from three sites, which demonstrating an universal presentation of these microorganisms in the oil contaminated environments, and that the oil contion has drived the development of PCB degrading ability in diverse bacteria. The exact species identity of the isolates may be determined by gene sequencing and other molecular techniques in further study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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