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Bacterial Community Dynamics of Diesel - Polluted Soil and Their Bioremediation Potential

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Abstract
Bacterial community dynamics of diesel-polluted soil was carried out using the vapour phase transfer technique. The highest mean log of total heterotrophic count was 9.19 ± 0.04 log_{10}cfu/g from pristine soil (control) and least mean log count was 5.32 ± 0.09 log_{10}cfu/g from a diesel contaminated soil. There was no statistical difference (P>0.05) in the total heterotrophic count of soil samples. The highest mean log count of diesel degrading bacteria was 8.86 ± 0.07 log_{10}cfu/g while the least mean log count was 4.83 ± 0.03Log_{10}cfu/g for the control. There was a significant difference in the mean count of diesel degrading bacteria (P<0.05). The bacteria isolated from diesel chronically polluted soil were of the *genera Bacillus sp., Staphylococcus aureus, Micrococcus sp., Corynebacterium sp., Alicagenes sp., Flavobacterium sp., Pseudomonas sp., Arthrobacter sp., Acinetobacter sp., Streptococcus sp., and Klebsiella sp.* These organisms were screened for their ability to degrade diesel. All the organisms were able to grow in diesel at different rates with *Pseudomonas sp., Bacillus sp. and Micrococcus sp.* showing heavy growths. These bacteria screened for their ability to degrade diesel can be employed as seeds for bioremediation of diesel polluted soil.
Keywords: Bacteria, bioremediation, diesel fuel pollution, soil.

1. Introduction

Diesel is a complex hydrocarbon fuel made up of a mixture of alkanes and aromatic compounds. It is reported frequently as a soil contaminant, often spilled from storage tanks, generator sets, pipelines or accidental spills (Bhasheer *et al.,* 2014; Palanisamy *et al*., 2014).

Diesel fuel contamination is one of the most dangerous pollution factors known today. It can cause a threat to the environment. It is very feared by environmentalists as it is very hard to control if it gets out of hand (Teli *et al.,*2013). The accumulation of such petroleum hydrocarbons in the environment can cause serious problems, negatively affecting the stability of many ecosystems and can also cause difficulties for animals and human health. Diesel fuel spills on agricultural land generally reduce plant growth due to its toxic effects on plants (Lawson *et al.,* 2013).

Bioremediation is a process that utilizes the capability of microorganisms to degrade toxic waste **(**Karthika *et al.,* 2014**)**. Biodegradation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contaminants, has been established as an efficient, economic, versatile and environmentally sound treatment

(Singh and Lin, 2008). Bioremediation is considered the best approach for restoring diesel fuel-contaminated soils in that the technology is cost effective and environmentally favorable (Vasquez *et al.*, 2009).

To obtain an efficient diesel fuel degrading bacterial consortium and monoculture, the knowledge of the diversity of the microbial community present in the soil contained with the diesel oil, its metabolic features and capacity to degrade diesel oil is of paramount importance (Margensin and Schinner, 2001).

In this investigation, indigenous bacteria which degrade diesel were isolated, characterized and identified and further screened for their diesel degradation efficiency.

2. Materials and Methods

2.1Sample Collection

Diesel contaminated soil samples were collected from three diesel generator houses where the soil had been chronically polluted by diesel. Pristine soil sample not contaminated by diesel served as the control. The samples were collected using a sterile spatula and put into sterile plastic bags. They were placed in an ice chest and sent to the laboratory for analysis within 24 h.

2.2 Sample Processing

Ten-fold serial dilutions of the soil samples were prepared using 0.85 % sterile physiological saline. From each dilution of 10^{-3} to 10^{-6}, aliquots were aseptically plated on sterile Nutrient agar.

2.3 Enumeration of Heterotrophic and Diesel Degrading Bacterial Counts.

The total heterotrophic bacterial count was performed using Nutrient agar (Oxoid). The medium was prepared according to manufacturer's specifications. Aliquots (0.1ml) of the serially diluted soil samples were plated out in duplicates on Nutrient agar plates using spread plate method. The plates were incubated at 35°C for 24 to 48 h as described by Ukaegbu-Obi and Mbakwem (2015). The total diesel fuel degrading bacteria were isolated using the spread plate method on Mineral Salt agar. The mineral salt agar plates were also inoculated in duplicates. The plates were incubated at 35^0C for 3-5 days (Palanisamy *et al.,* 2014). The colonies that formed were enumerated and expressed as Colony Forming Unit per gram soil (CFU/g). The diesel served as carbon and energy sources for the diesel degraders. The counts of diesel degraders were further calculated and expressed as a percentage of the total heterotrophic bacterial population.

2.4 Isolation, Characterization and Identification of Diesel Fuel Degraders.

Pure stock cultures of diesel degrading bacterial isolates were examined for colonial appearance and used to carry out the following tests: gram staining, motility, catalase, citrate utilization, indole, hydrogen sulphide production, methyl red – Voges Proskauer, oxidase, sugar fermentation tests. Confirmatory identities of the bacteria were made using the Bergey's Manual of Determinative Bacteriology (Holts *et al.,* 1994).

2.5 Screening of Diesel Fuel Degraders

The isolates were tested for diesel fuel degradation capabilities in mineral salt broth medium. The bacterial isolates were tested for their ability to degrade diesel fuel using the turbidity

method as described by Ukaegbu-Obi and Mbakwem-Aniebo (2014). The bacterial isolates were cultured in nutrient broth and incubated at 28±2^{0}C for 24 h. Aliquot (0.1ml) of the young culture in nutrient broth grown was inoculated into each test tube containing 9.9ml of sterile mineral salt broth and 0.1ml of crude oil. A control test tube containing 9.9ml of sterile mineral salt broth plus 0.1ml of crude oil remained uninoculated. The tubes were incubated at room temperature for 7 days. The growth of the inocula was determined by visual observation of the mineral salt broth turbidity, as compared with the uninoculated control tube according to Ukaegbu-Obi and Mbakwem-Aniebo (2014).

2.6 Statistical Analysis

The mean and standard deviation of the results were determined. A Student's t-test of significance was used to analyze the diesel polluted and unpolluted soils. All statistical analysis was carried out using Statistical Package for Social Science (SPSS) version 20 (2008).

3. Results and Discussion

The mean counts of heterotrophic and diesel degrading bacteria from this study are shown in Table 1. The genera of bacteria identified were *Bacillus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Corynebacterium* sp., *Alicagenes* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Acinteobacter*sp., *Streptococcus* sp., and *Klebsiella* sp.

Table 1: Mean heterotrophic and diesel degrading bacterial counts of diesel contaminated and pristine soil samples.

	Log10cfu/g	
Sample location	HBC	DDB
Site A	5.32 ± 0.09	8.86 ± 0.07
Site B	6.15 ± 0.02	8.21 ± 0.05
Site C	7.04 ± 0.05	7.54 ± 0.01
Site D (Control)	9.19 ± 0.04	4.83 ± 0.03

Key: A,B,C = Contaminated soil sample D = Pristine soil sample (Control). HBC = Heterotrophic Bacterial Counts DDB = Diesel Degrading Bacterial Counts

Table 2: Screening test for the diesel degrading potential of bacterial isolates

Isolate code	growth concentration	Organism	Isolate code	growth concentration	Organism
ISOA1	++	*Staphylococcus* sp.	ISOB3	+	*Corynebacterium* sp.
ISOA2	+++	*Bacillus* sp.	ISOB4	+++	*Micrococcus* sp.
ISOA3	+++	*Micrococcus* sp.	ISOB5	+++	*Pseudomonas* sp.
ISOA4	+++	*Pseudomonas* sp.	ISOC1	++	*Acinetobacter* sp.
ISOA5	++	*Alicagenes* sp.	ISOC2	++	*Alicagenes* sp.
ISOB1	++	*Arthrobracter* sp.	ISOC3	+	*Flavobacterium* sp.
ISOB2	++	*Streptococcus* sp.	ISOC4	++	*Bacillus* sp.
			ISOC5	+	*Klebsiella* sp.

Key: +++ = Heavy growth, ++ = Moderate growth, + = Little growth.

The extensive use of petroleum products such as diesel fuel leads to the contamination of almost all components of the environment (Bhasheer *et al*., 2014). The estimated costs for the clean-up of contaminated soil with conventional techniques such as incineration and land filling are enormous (Lawson *et al.,* 2013). Bioremediation of these diesel polluted soil is a welcome approach as it is a green technology.

In this study, the bacterial isolates were enumerated as shown in Table 1; the results show that the colony forming units of total heterotrophic bacterial count were higher in the pristine soil sample than in the diesel contaminated samples. This shows that the population of the bacteria genera that grew on diesel contaminated soil was lower. Statistically there was significant difference in the bacterial population of the soil samples ($P<0.05$). Diesel degrading bacterial counts were higher in contaminated soil samples than that of pristine soil sample when cultured on mineral salt medium using diesel as the sole carbon source. This demonstrates that microbial community and structure are adversely affected by chemical pollutants. The pollutants can alter the structure through selection of pollutant degraders. The results obtained in this study showed that the presence of diesel fuel and products of its metabolism may be toxic to some microorganisms resulting in the population reduction.

A total of eleven (11) bacteria genera were isolated. The genera identified were *Bacillus* sp., *Staphylococcus aureus, Micrococcus* sp., *Corynebacterium* sp., *Alicagenes* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Arthrobacter* sp., *Acinteobacter* sp., *Streptococcus* sp., and *Klebsiella* sp. A screening test (Table 2) was carried out to determine the diesel degradation ability of the bacterial isolates. *Micrococcus* sp., *Staphylococcus* sp., and *Bacillus* sp., have been reported to be implicated in the petroleum hydrocarbon utilization (Ujowundu *et al.,* 2011). Teli *et al*. (2009) also reported the degrading ability of *Micrococcus* sp. and *Pseudomonas* sp. Ijah and Antai (1988) reported *Bacillus* sp. as being the dominant bacteria of all petroleum oil utilizing bacteria characterized from chronically polluted soil samples. This is consistent with the present study since *Bacillus* was one of the most dominant bacterial isolates. There is growing evidence that isolates belonging to the *Bacillus* sp. could be effective in cleaning oil spills (Ghazali *et al,* 2004; Karthika *et al.,* 2014).The presence of *Staphylococcus aureus* in this study agreed with that of Gomes *et al.* (2004) who also isolated *Staphylococcus aureus* from a diesel contaminated soil. Andriano *et al.* (2007) isolated *Staphylococcus hominis* from petroleum oil contaminated soil while Stephen *et al*. (2013) reported the isolation of *Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* from diesel contaminated soil.

4. Conclusion

The study showed that the bacterial present in the soil are not restricted to diesel contaminated soil. The screen test for diesel degrading ability of the bacterial isolates proved that all the isolates were capable of degrading diesel. The concentrations of diesel fuel in the soil alter the properties of the bacterial community. Some bacteria genera grow at slow rates in diesel contaminated soil while other genera flourish and grow at heavy rates in diesel contaminated soil.

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