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Response of Resident Bacteria of a Crude Oil-Polluted River to Diesel Oil

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Abstract: The response of twelve bacterial species previously isolated from sediment and water of a crude oil-polluted river were assessed in terms of ability or inability to grow in the presence of 0.5% (v/v) of diesel oil and potential to degrade the diesel oil. Tolerance to diesel oil (maximum toleratable concentration [MTC]) was determined using media dilution technique. Potential to degrade the oil was indicated by ability to grow on the diesel oil as sole carbon source. Four of the bacterial species: *Enterobacter aerogenes*, *Proteus vulgaris*, *Serratia marcescens* and *Staphylococcus aureus* were unable to grow in the presence of 0.5% (v/v) of diesel oil (Diesel-sensitive bacteria: DSB). The remaining eight: *Acinetobacter calcoaceticus*, *Aeromonas* sp., *Alcaligenes paradoxus*, *Bacillus licheniformis*, *Flavobacterium lutescens*, *Micrococcus luteus*, *Pseudomonas fluorescens* and *Vibrio paraheamolyticus* showed tolerance to varying concentrations of the oil (Diesel-tolerant bacteria: DTB). *Alcaligenes paradoxus*, *Aeromonas* sp., *B. licheniformis*, and *P. fluorescens* showed potential to degrade the diesel oil (Diesel-degrading bacteria: DDB). The significance of the responses of the bacteria and prospects of their use in monitoring crude oil related pollution were discussed.

Key words: Crude oil · River · Diesel oil · Nigeria

INTRODUCTION

In the last two decades there have been increased public concerns on the adverse effect of oil exploration on the environment. The toxic effects of crude oil and refined petroleum oils on plants, animals, humans and the environment are devastating[1]. Oil pollution persistence and its transport in water, subsoil and groundwater aquifers is monitored to predict impacts, assess the impacts and audit such effects with a view to mitigate the impacts. Environmental monitoring of petroleum hydrocarbons pollution range from specific methods, such as the use of radio-active labeled compounds to general methods including quantifying gross contamination and evaluating the extent of change caused in the environment by the presence of the pollutant[2]. However, evaluating the effect of the pollutant on the natural biodiversity in the environment appears to be the realistic alternative in developing countries, based on simplicity of procedure and cost involved.

Generally, the concept of bio-monitoring involves the use of organisms to develop cause-and-effect models. The objective is to draw correlation between the presence of certain organisms and the conditions of the environment [3]. Predictions from the microbial community response to pollution stress could be used to investigate the persistence of a pollutant in the environment. Microbial community structure analysis is premised on the assumption that pollution stress will simplify a complex community by eliminating the more sensitive species and also increase the disproportion in numbers of individuals per species[4]. Enumeration and identification of populations of particular microbial species can indicate pollution or some distortions in nutrient balance has occurred in an environment.

Incidence of spills resulting from auto crash involving trucks carrying refined petroleum products (petrol, kerosene, diesel, etc) are common in Nigeria. The spilled products are not recovered but find their way to streams and rivers in addition to sinking into soil. The impact of these kinds of spills on microbial components of the ecosystem is largely un-assessed. The ecological roles of bacteria make it important that there should be information on the likely effect of such spill. This work

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examines the responses of resident bacterial flora of a tropical crude oil-polluted river to diesel oil. The responses were assessed in terms of ability or inability to grow in the presence of 0.5% (v/v) of diesel oil and potential to degrade the diesel oil.

MATERIALS AND METHODS

The test organisms used were previously isolated from water and sediment of a crude oil-polluted river in Abereke, Ondo State (7°3'W 4°35'E). The concentrations of total petroleum hydrocarbon (TPH) in the samples were determined using gravimetric method as described by Standard Method [5]. Tolerance to diesel was assessed by the ability of organisms to grow on nutrient agar into which diesel oil (0.5% v/v) was incorporated; these bacteria were designated "Diesel-tolerant bacteria" (DTB). Potential to degrade diesel was assessed by ability to grow on diesel mineral agar (DMA) prepared by adding 0.5% (v/v) of diesel as sole carbon source to mineral salt medium described by Ogunseitan [6] and Eniola [7]; these bacteria were designated "Diesel-degrading bacteria" (DDB).

The pure cultures of the isolates were standardized as described by Ilori and Amund [8]. Flasks of nutrient broth, containing varying amounts of diesel oil (0.5, 0.7, 1.0 and 2.0% [v/v]), were inoculated with 1ml of the standardized pure cultures of the isolates. The inoculated media and controls (positive and negative controls) were incubated on the orbital shaker (120 rpm) at room temperature (26±2°C) for 5 days. Populations of the resulting culture were enumerated by plating on nutrient agar and incubated at 37°C for 48 hours. Populations greater than or equal to 90% of initial counts were taken as indicative of tolerance.

RESULTS

The total petroleum hydrocarbon in the water and sediment averaged 85 mg/l and 400 mg/kg , respectively. The response statuses of the twelve (12) bacterial species are shown in Table 1. Three categories were recognized: four of them (*Enterobacter aerogenes*, *Proteus vulgaris*, *Serratia marcescens* and *Staphylococcus aureus*) were Diesel-sensitive bacteria (DSB), while the remaining eight: *Acinetobacter calcoaceticus*, *Aeromonas* sp., *Alcaligenes paradoxus*, *Bacillus licheniformis*, *Flavobacterium lutescens*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Vibrio paraheamolyticus* were Diesel-tolerant bacteria (DTB). *Alcaligenes paradoxus*, *Aeromonas* sp., *B. licheniformis*, and *P. fluorescens* were able to grow with the diesel as sole

Table 1: Response Status of the Bacterial species to Diesel Oil

	Growth Response Status		
Source of organism	Diesel-Sensitive Bacteria [Unable to tolerate 0.5% (v/v) diesel oil]	Diesel-Tolerant Bacteria [Able to tolerate 0.5% (v/v) diesel oil]	Diesel-Degrading Bacteria [likely to degrade diesel oil]
Water	*Enterobacter aerogenes* *Proteus vulgaris* *Staphylococcus aureus*	*Acinetobacter calcoaceticus* *Alcaligenes paradoxus* *Aeromonas* sp. *Bacillus licheniformis* *Pseudomonas fluorescens* *Flavobacterium lutescens* *Vibrio paraheamolyticus*	*Alcaligenes paradoxus* *Aeromonas* sp. *Bacillus licheniformis* *Pseudomonas fluorescens*
Sediment	*Enterobacter aerogenes* *Serratia marcescens* *Staphylococcus aureus*	*Acinetobacter calcoaceticus* *Alcaligenes paradoxus* *Aeromonas* sp. *Bacillus licheniformis* *Pseudomonas fluorescens* *Flavobacterium lutescens* *Micrococcus luteus* *Vibrio paraheamolyticus*	*Alcaligenes paradoxus* *Aeromonas* sp. *Bacillus licheniformis* *Pseudomonas fluorescens*

Table 2: Tolerance of Bacterial Species to Different Concentrations of Diesel Oil

Bacterial Species	Maximum Toleratable Concentration (MTC) (v/v)
Alcaligenes paradoxus	NI
Aeromonas sp.	NI
Acinetobacter calcoaceticus	1.0
Micrococcus luteus	0.7
Pseudomonas fluorescens	NI
Vibrio paraheamolyticus	0.7
Bacillus licheniformis	NI
Flavobacterium lutescens	NI
Enterobacter aerogenes	0.5
Proteus vulgaris	0.5
Staphylococcus aureus	0.5

Tolerance is indicated by recovery of 90% of initial cell population.

NI: Not inhibited by range of concentration used.

carbon source; they were designated: Diesel-degrading bacteria (DDB). Those that could tolerate the diesel varied in their extent of tolerance (Table 2).

DISCUSSION

The total hydrocarbon concentrations in the water body were generally lower than the values reported for many rivers and coastal waters in Nigeria, especially in the Niger-Delta region [9]. This could be because this area experiences fewer oil spills or oil installations vandalization. The petroleum hydrocarbon concentrations however, fall within the range of values that have adverse public health impact [10]. They are also higher than the maximum tolerable concentration (MTC) for most aquatic organisms; and would be able to kill or inhibit the growth of microorganisms, microalgae and juvenile forms of aquatic animals especially in coastal waters [11, 12] Walker and Colwell [13, 14] suggested that the presence of petroleum hydrocarbon in high concentration is one of the major factors influencing microbial diversity and succession in polluted sediments and water bodies.

Presence of petroleum hydrocarbons has been reported to influence the diversity, distribution and population of microorganisms in an environment [15]. The inability of some of the bacteria (e.g. *E. aerogenes*, *S. marcescens* and *P. vulgaris*) to grow on diesel oil may be group specific. Ensley *et al*. [16] have related the inability of many of the *Enterobacteriaceae* to utilize hydrocarbons to lack of membrane-bound, group specific oxygenases and mechanisms for optimizing contact between the microorganisms and hydrocarbons. The inability of *Micrococcus luteus* and *Vibrio paraheamolyticus* to tolerate 1.0 and 2.0% (v/v) diesel oil may not be unconnected with the inhibitory effect of high concentrations of diesel oil. The tolerance of high concentrations of diesel oil by some bacteria is probably because they possessed the capacity to mineralize or transform some component [17]. *F. lutescens* demonstrated a strong tolerance of the diesel oil; however, it could not grow with the diesel oil as sole carbon source.

It is possible to use some of the bacterial species within the microbial community as sensitivity index to various concentrations of petroleum oils in the environment. Thus a comprehensive assessment of bacterial response to oil pollution would be useful to design simple oil pollution models. This would provide useful information about pollution carrying-capacity of an environment and serve as a tool in rapid environmental impact monitoring and assessment of oil pollution.

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