



Population dynamics and distribution of hydrocarbon utilizing bacteria in Automobile workshops within Uyo metropolis, Akwa Ibom State

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ABSTRACT: Laboratory studies were carried out to assess the bacterial population dynamics and distribution in composite soil samples collected from five (5) different automobile workshops at various locations (Ikpa road, Nwaniba road, Udi street, Idakokpo lane and Mechanic village) within Uyo metropolis. The hydrocarbon utilizing bacteria were isolated and characterized from the soils of automobile workshops using cultural techniques. The total heterotrophic bacterial count (THBC) ranged from 2.5×10^5 to 8.0×10^5 CFU/g of soil sample, while the hydrocarbon utilizing bacterial count (HUBC) ranged from 2.5×10^4 to 4.4×10^4 CFU/g of soil sample. The bacterial isolates which were both Gram positive and Gram negative belonged to the genera; *Bacillus*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Citrobacter* and *Acinetobacter*. The total heterotrophic bacterial count (THBC) and hydrocarbon utilizing bacterial count (HUBC) were higher in Ikpa road automobile workshop and Nwaniba road automobile workshops respectively. *Bacillus* species was found to be present in all the soil samples analysed thus had the highest frequency 5(28%) of occurrence while *Achromobacter* species had the lowest frequency 1(6%) of occurrence. The presence of these organisms in soils contaminated with spent and unspent lubricating oil and their subsequent growth in enrichment medium supplemented with 1% spent lubricating oil suggest their hydrocarbon utilizing potential, hence, their possible use for the bioremediation of soils impacted with lubricating oil. © JASEM

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KEYWORDS: automobile workshops, hydrocarbon utilizing bacteria, bioremediation, enrichment medium, lubricating oil.

Introduction

Automobile workshops may be considered as an integral part of the service industry with significant impact on the environment, resulting from seepage of spent and unspent engine oil leading to environmental pollution (Sathiya-Moothi *et al.*, 2008).

With increasing demand for automobiles especially in developing countries such as in Nigeria, automobile workshops proliferate in major cities and towns, with wastes generated and dumped indiscriminately on every available space; thus contaminating the soil and causing alterations in the microbial populations, distribution and dynamics (Osu and Okereke, 2010). Similarly, petroleum products such as lubricating oil, premium motor spirit, automotive gas oil and kerosene are used daily in automobile workshops and sometimes these fuels are burnt thereby resulting in atmospheric pollution (Owolabi *et al.*, 2013). These materials accumulate and pollute nearby farms as well as surface water via surface run-off and erosion (Aiyesanmi, 2005).

The isolation of high numbers of hydrocarbon utilizing bacteria from soils contaminated with crude oil is a common evidence of the fact that these microbes are capable of utilizing crude oil as their source of carbon and energy (Okerentugba and

Ezeronye, 2003). Ekhaise and Nkwelle (2011) reported high numbers of heterotrophic and hydrocarbon utilizing bacterial counts in soils from major automobile workshops in Edo state, Nigeria. There is an increasing threat to environments impacted with petroleum products and microorganisms play a major role in the degradation of hydrocarbon, thus presenting an alternative way to remediate petroleum product polluted sites (Okpokwasili, 2003) as well as eliminate potential risk or reduce it to acceptable limits. There is however, reliable evidence that autochthonous (indigenous) microorganisms has some advantages over allochthonous microbes in the degradation of hydrocarbons as these organisms are able to develop naturally over the years and are well adapted for survival and proliferation in such environment (Adegbola *et al.*, 2014). This study is therefore aimed at demonstrating the population dynamics and distribution of hydrocarbon utilizing bacteria (HUB) in soil samples from different automobile workshops within Uyo metropolis with a view to optimising their bioremediation potential.

MATERIALS AND METHODS

Study Area and sample collection: The study area comprised of five different automobile workshops specifically located along Ikpa road, Idakokpo lane,

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Nwaniba road, Udi street and Mechanic Village within Uyo metropolis. Composite soil samples were collected aseptically from these workshops using a soil auger at (0-20cm depth). The samples from each of the automobile workshops were bulked and about 200g of each was properly labelled and transported immediately to the microbiology laboratory for analysis.

Microbiological Analysis: Ten (10) grams of each soil sample was diluted in 90ml of sterile distilled water in a conical flask to get the aliquot, and a ten-fold serial dilution was carried out.

Enumeration of Total Heterotrophic Bacteria Total heterotrophic bacteria was enumerated according to methods described by Ghazali *et al.* (2004). Standard pour plate technique was employed to determine the total heterotrophic bacteria count (THBC) in duplicates. Molten nutrient agar (20ml volume) that has cooled to about 45°C was aseptically added to the petri dishes and swirled gently to mix then allowed to set. The replicate plates were incubated invertedly at 25°C for 24-48 hours. The plates were observed for growth and recorded accordingly.

Isolation of Hydrocarbon Utilizing Bacteria: The methods of Kastner *et al.* (1994) was adopted with slight modifications. Molten mineral salt agar (supplemented with 1% spent lubricating oil) that has cooled to about 45°C was aseptically added to the

petri dishes. The lubricating oil served as the only source of carbon and energy. The plates were swirled gently to mix, allowed to set and incubated invertedly for 24-48 hours at 25°C. The colonies that developed were enumerated and recorded as total hydrocarbon utilizing bacteria. The distribution of these isolates in soil samples from the various automobile workshops as well as their frequency of occurrence was also recorded.

Characterization and Identification of bacterial colonies: The different colonies that developed after incubation were subcultured twice, to obtain pure culture. The final isolates were then maintained on properly labeled nutrient agar slants. The isolates were characterized and identified according to the methods of Holt *et al.* (1994).

Screen Test for hydrocarbon utilizing bacteria: The isolates were screened for their ability to utilize hydrocarbon using the vapour phase transfer methods of Itah and Essien (2005) with slight modifications. Molten mineral salt agar (20ml volume) that has cooled to about 45°C was aseptically added to petri dishes containing aliquot and swirled gently to mix then allowed to set. Whatman 1 filter paper soaked respectively in spent and unspent lubricating oil was placed on the lids of the petri dishes. The plates were covered and incubated invertedly for 5 to 7 days at 25°C.

RESULTS AND DISCUSSION

Table 1: Total heterotrophic bacteria and hydrocarbon utilizing bacterial count

Sample location	Total heterotrophic bacterial count (cfu/g)	Total hydrocarbon utilizing bacterial count (cfu/g)
AW1	$8.0 \times 10^5 \pm 2.45$	$2.5 \times 10^4 \pm 0.29$
AW2	$3.9 \times 10^5 \pm 0.61$	$4.4 \times 10^4 \pm 0.56$
AW3	$4.0 \times 10^5 \pm 0.45$	$3.0 \times 10^4 \pm 0.06$
AW4	$4.2 \times 10^5 \pm 0.29$	$2.8 \times 10^4 \pm 0.15$
AW5	$5.7 \times 10^5 \pm 0.08$	$3.0 \times 10^4 \pm 0.06$

Note: values are mean \pm standard deviation of duplicate determinations

KEY: AW1=Ikpa Road Automobile Workshop
AW2=Nwaniba Road Automobile Workshop
AW3=Udi street Automobile Workshop
AW4=Idakokpo lane Automobile Workshop
AW5=Mechanic Village Automobile Workshop

Table 2: Bacterial distribution in the soil samples

Sample location	Bacterial isolates
AW1	<i>Bacillus</i> sp, <i>Micrococcus</i> sp, <i>Citrobacter</i> sp
AW2	<i>Micrococcus</i> sp, <i>Bacillus</i> sp, <i>Acinetobacter</i> sp, <i>Flavobacterium</i> sp
AW3	<i>Acinetobacter</i> sp, <i>Micrococcus</i> sp, <i>Bacillus</i> sp
AW4	<i>Citrobacter</i> sp, <i>Bacillus</i> sp
AW5	<i>Flavobacterium</i> sp, <i>Micrococcus</i> sp, <i>Citrobacter</i> sp, <i>Bacillus</i> sp, <i>Achromobacter</i> sp, <i>Acinetobacter</i> sp

KEY: AW1=Ikpa Road Automobile Workshop
AW2=Nwaniba Road Automobile Workshop
AW3=Udi street Automobile Workshop
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Table 3: Screen test for hydrocarbon utilizing ability of each bacterial isolate

Isolates	Spent lubricating oil	Unspent lubricating oil
<i>Bacillus</i> sp	+++	+++
<i>Flavobacterium</i> sp	++	+++
<i>Micrococcus</i> sp	++	++
<i>Citrobacter</i> sp	+	+
<i>Acinetobacter</i> sp	+++	++
<i>Achromobacter</i> sp	+	+

Key: + represents ≤ 25 cfu/g (poor growth)
 ++ represent 25 – 200 cfu/g (average growth)
 +++ represent > 200 cfu/g (profuse growth)

Table 4: Percentage occurrence of bacterial isolates

Bacterial isolates	Frequency of occurrence	Percentage occurrence
<i>Bacillus</i> sp	5	27.7
<i>Flavobacterium</i> sp	2	11.1
<i>Micrococcus</i> sp	4	22.2
<i>Citrobacter</i> sp	3	16.7
<i>Acinetobacter</i> sp	3	16.7
<i>Achromobacter</i> sp	1	5.6
Total	18	100

The result of this study reveals the occurrence of hydrocarbon utilizing bacteria within automobile workshops in Uyo metropolis. This corroborates the report of Okoh (2003) that hydrocarbon degraders are usually present in large numbers in many oil polluted soil and water compared with uncontaminated environments. Similarly, Ekundayo *et al.* (2012), reported that the presence of microbes in sites impacted with petroleum products suggests their ability to utilise the hydrocarbon as their sole carbon source for growth. These organisms release enzymes capable of breaking down hydrocarbon molecules into simpler absorbable forms (Adekunle and Adebambo, 2007).

Notably, in this study, there was an observable evidence of the impact of lubricating oil on the bacterial population dynamics represented by the relatively low counts of the total heterotrophic bacteria (THB). This, according to Jensen (1975), suggests a toxic or inimical effect of the lubricating oil on the heterotrophic bacterial population. This finding confirms the report of the study by Akoachere *et al.* (2008) that heterotrophic bacterial counts were significantly lower in lubricating oil impacted soils than in non-impacted soils. There was however a relatively high counts of lubricating oil degrading bacterial populations and this supported the reports of Herbert *et al.* (1997) and Michalcewicz (1995) and may be attributed to the supposed stimulatory effect of lubricating oil (substrate) as carbon and energy source. Another study by Walker and Crawford (1997) had shown that a decrease in substrate will

therefore result in a drop in the population of oil degraders.

The distribution and percentage occurrences of the bacterial isolates indicated that *Bacillus* sp had the highest frequency of occurrence. Although, this report was at variance with the work of Adegbola *et al.* (2014) where *Bacillus cereus* had the least percentage occurrence, it however confirmed the reports of other researchers (Okoh and Trejo-Hernandez, 2006; Atlas, 1992) that *Bacillus* sp is one of the most frequently isolated bacteria from hydrocarbon polluted sites.

Bacillus sp, which was predominant in all the five automobile workshops studied also possessed the highest hydrocarbon utilizing capability. This corroborates the work of Udeani *et al.* (2009) who isolated *Bacillus stearothermophilus* from soils of various automobile workshops in Enugu contaminated with 30% and 40% hydrocarbon. This comparatively high tolerance of *Bacillus* species to hydrocarbon may be attributed to their possession of resistant endospores and/or plasmids thus, suggesting their efficiency in the clean-up of hydrocarbon polluted sites (Ghazali *et al.*, 2004). Besides, the biodegradation potential of *Bacillus* species isolated from soils have been reported to be far higher than other bacterial isolates (Del' Arco and de Franca, 2001; Obuekwe and Al Zarban, 1998). This trend was also observed in *Bacillus* sp isolated from crude oil polluted soils in Nigeria (Ilori and Amund, 2000). Other bacteria of environmental significance isolated from these workshops were *Micrococcus*, *Acinetobacter*, *Citrobacter*, *Flavobacterium* and *Achromobacter*.

Conclusion: The isolated organisms, particularly *Bacillus* sp with widespread distribution have been implicated in the biodegradation of environmental pollutants and may establish their possible use in bioremediation. However, to demonstrate their bioremediation capabilities *in situ* as well as optimize their biodegradation profile, further research needs to

be carried out. Molecular approaches would further enable the isolation and identification of non-culturable soil microbes from such hydrocarbon impacted ecosystem.

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