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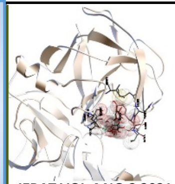

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Isolation and Characterization of Hydrocarbon-degrading Bacteria in Soils of Mechanical Workshops in Maiduguri, Borno State

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ABSTRACT

The study was conducted to determine the distribution of hydrocarbon utilizing bacteria in spent engine oil (SEO) contaminated soil. Five mechanical workshops within Maiduguri Metropolis. Five bulk soil sample comprising of one each of the five sites; the sites are Leventis Area on Kashim Ibrahim Way, Damboa Raod, Boiler (commonly called Bola) near Maiduguri Monday Market, Ngomari, on Kano Road, and 1000 Housing Estate, also on Kano Road. Nutrient agar was used, or isolation and enumeration total heterotrophic bacteria and Mineral salt agar was used for the isolation and enumeration of hydrocarbon utilizing bacteria. The result shows THB ($92.0x10^4$) at Damboa road (DR) to as higher and ($78.8x10^4$) at Bola area (BL) while HUB ($2.0x10^4$) at Damboa road (DR) and as high as ($9.3x10^4$) at Bola area (BL). The bacterial species isolated were species of *Bacillus licheniformis, Bacillus subtilis, Bacillus coagulans, Bacillus alvei, Bacillus cereus, Bacillus lentus* other are *Pseudomonas aeruginosa, Klebsiella pneumonia. Bacillus licheniformis,* and *Bacillus subtilis* are the most occurring bacterial isolates identified. The ability of those bacterial isolate to degrade hydrocarbon buoyantly will help in remediation of oil polluted environments.

INTRODUCTION

Human activity has had an increasing impact on many ecosystems in the last several years, as seen by the numerous changes that have occurred. As a result, many individuals have begun to recognize the need of safeguarding ecosystems and assessing the harm that pollution causes. Recent years have seen an increase in study on oil pollution because of the increasing incidence and risk [1–3,2,4,5]. There is a corresponding rise in demand for energy for transportation, residential usage, and industrial use as the human population grows. Since the 1950s, fossil fuels have been the primary source of energy.

There has been a noticeable rise in soil pollution across the world due to the increased use of petroleum and its derivative products such as gasoline, diesel, and motor oils. In both industrialized and developing countries, the environmental effect of petroleum exploration, production, refining, and transportation is a serious problem. Liquid oil has a devastating impact on the marine environment as well as on soil and plant life [3,6–9]. A number of factors, including blowouts, tank leaks, and waste disposal, all contribute to oil spills [10,11]. Roadside sales of lubricating oil in Nigeria, for example, are a major contributor to soil pollution by lubricating oil because of the casual ways that automobile and generator owners discard leftover lubricating oil [12–20]. A widespread practise among motor technicians and generator owners is to dispose of motor oil in gutters, water drains, open unoccupied plots, and farms, which releases large volumes of engine oil into the environment [20]. In addition, the exhaust system releases oil into the atmosphere when the engine is running and when there are leaks. Unlawful dumping of spent motor oil is a serious environmental threat with far-reaching consequences [2,20–24]. Crankcase oil flow from automobiles is a major source of oil pollution in Buea, Cameroon, according to a similar assessment by Akoachere et al. [25]. In India's Pudukkottai district and Nigeria's Gwagwalada region, studies by Ugoh and Moneke [26] found evidence of soil contamination caused by the discharge of spent motor oil. Poor aeration caused by the presence of spent motor oil in the soil results in an unsuitable environment for life in the soil. Used motor oil and heavy metals have been discovered to impact soil biochemistry, which includes changes in soil microbiological features such as

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pH, Oxygen availability and nutrition supply [27,28,28–31]. We are not aware of any study that has attempted to isolate and identify bacteria present in the used motor oil polluted soil environment in Maiduguri. Soil samples polluted with spent motor oil were the focus of the current investigation.

MATERIALS AND METHODS

Study area

The study was conducted in Maiduguri Metropolis. Maiduguri is located in the North-easter part of Nigeria at coordinates 11°50'N Latitude and 13°09'E Longitude. The average monthly temperature ranges from 24°C in December and January to 42°C in March and April. It has a tropical climate with distinct dry season which starts in November and ends in April, and a distinct wet season which starts in May and ends in October. Maiduguri is the capital of Borno State; the inhabitants are mainly civil servants, farmers, fishermen, and petty traders [32].

The study sites are five different mechanical workshops in Maidudguri metropolis located at Bola Workshop near the Post Office, Anthony Mechanical workshop along Dambo Road, Leventis Mechanical Workshop Near West-End, Ngomari Workshop near Maiduguri International Airport, and 1000 Housing Estate along Kano Raod.

Media used

The media used for this research were: Nutrient Agar (NA) for enumeration and isolation of bacteria; Mineral Salt Media- a modified Bushnell and Haas (BH) medium for isolation of hydrocarbon utilizing bacteria. Spent engine oil was used as carbon source in the BH medium [20].

Sample collection

Digging with a hoe and transferring the soil sample straight into sterilised containers were the methods used to gather soil samples at each session. At each repair shop, samples were taken at five different locations. They were subsequently taken to the microbiology laboratory at the University of Maiduguri for testing.

Isolation and enumeration of Bacteria

Bacterial load of the soil samples were enumerated by making tenfold dilution of the samples collected from the soil samples. The test tubes with 10^8 and 10^9 were covered or corked and incubated at 30°C in an incubator. Using a dropper pipette, 0.025 ml of each dilution was inoculated on NA and BH (containing 0.5% v/w SEO) agar surfaces. The plates were incubated at 30⁰C for 24h and 48h respectively. Colony formation was used to determine viable and hydrocarbon-degrading bacterial counts in the samples. In terms of colony forming units per gramme of soil, the result was calculated using the number of counts and dilutions employed ([19].

Pure isolates were obtained by repeated subculture on fresh NA. Pure isolates were maintained on agar slants for further characterization and identification.

Characterization and identification of isolates

Pure isolates of bacteria were identified based on colonial, morphological and biochemical characteristics following the guidelines outlined by Prescott and Harley [33].

RESULTS AND DISCUSSIONS

Heterotrophic bacterial counts were generally higher (92.0 x 10^4 CFU/g) Damboa road (78.8 x 10^4 CFU/g) lower in Bola (**Table 1**). Hydrocarbon utilizing bacteria ranged as high as (9.3 x 10^4 CFU/g) in Bola to as low as (2.0 x 10^4 CFU/g) in Damboa road (**Table 2**).

Table 1. Enumeration of total heterotrophic bacteria and hydrocarbon-utilizing bacteria.

S/No.	Sample Sites	THB (cfu/g)	HUB (cfu/g)
1	LT	84.0 x 10^4	3.0 x 10^4
2	DR	92.0 x 10^4	2.0 x 10^4
3	BL	78.8 x 10^4	9.3 x 10^4
4	NG	84.0 x 10^4	2.4 x 10^4
5	OHE	84.0 x 10^4	4.3 x 10^4

Keys: THB=Total Heterotrophic Bacteria, HUB=Hydrogen Utilizing Bacteria, CFU/G=Colony Forming Unit/Gram LT=Leventis, DR=Damboa Road, BL= Bola, OHE=One thousand Housing Estate, NG=Ngomari

Table 2. Distribution of hydrocarbon utilizing bacteria in the five sites and their percentage occurrence.

Isolates	Sites					
	LT	DR	BL	NG	OHE	% Occurrence of Isolates at each site
Bacillus licheniformis	+	+	+	+	+	100
Bacillus subtilis	+	+	+	+	+	100
Bacillus coagulans	+	+	+	+	+	100
Pseudomonas aeruginosa	+	-	+	+	+	80
Klebsiella pneumonia	+	+	-	+	+	80
Bacillus alvei	+	+	+	+	-	80
Bacillus cereus	+	+	+	-	+	80
Bacillus lentus	+	+	+	-	+	80

Keys: += Present. - = Absent, LT= Lake chad, DR= Damboa road, BL= Bola, NG= Ngomari, OHE= One thousand housing.

DISCUSSIONS

The total heterotrophic bacterial count as shown in the table above is higher in Damboa road with (92.0 x 10^4 CFU/g) which is the site with little or less spent engine oil contaminations as the workshop there used constructed equipment for the collection of the used engine oil and this show that the lower the level of the contamination the greater the population of heterotrophic bacteria. Whereas the least total heterotrophic bacterial counts as encountered at Bola the densely populated area with different workshops and the level of contamination there is very high as the used engine oil were disposed anyhow to the soil which we assume suppress the growth heterotrophic bacteria to be lower showing (78.8 x 10^4 CFU/g) the finding here agrees with that of Ugoh and Moneke [26].

The hydrocarbon utilizing bacterial count also shows a similar findings to that of Ugoh and Moneke 2011 where higher counts of (HUB) are enumerated at the densely polluted area of Bola (9.3 x 10^4 CFU/g) which shows that the bacteria are able to utilized spent engine oil as their source of carbon and grow efficiently in that contaminated soil whereas lower counts were enumerated at Damboa road workshop (2.0 x 10^4 CFU/g) which is having lower or little disposed engine oil in the soil there.

The hydrocarbon utilizing bacteria isolated are mostly o the genus of *Bacillus* the species identified are *Bacillus licheniformis, Bacillus subtilis, Bacillus coagulans, Bacillus alvei, Bacillus cereus, Bacillus lentus* other are *Pseudomonas aeruginosa, Klebsiella pneumonia* as shown in Table 2. *Bacilli are* soil bacteria known to produce spores and can withstand adverse condition their mostly isolated in contaminated soil as recorded by [19,34–42].

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COMPETING INTEREST

The authors declare no competing interest

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