International Journal of Scholarly Research in
Science and Technology
Journal homepage: https://srrjournals.com/ijsrst/
ISSN: 2961-3337 (Online)

(RESEARCH ARTICLE)

Effects of spent hydrocarbon on bacteria population

Yusuf UA *, Ekeleme IK, Owuna EJ, Ochai SS and Obiekezie SO

Department of Microbiology, Faculty of Natural and Applied Science, Nasarawa State University, Keffi.

International Journal of Scholarly Research in Science and Technology, 2023, 02(01), 001–009

Publication history: Received on 22 November 2022; revised on 04 January 2023; accepted on 06 January 2023

Article DOI: https://doi.org/10.56781/ijsrst.2023.2.1.0033

Abstract

This study aimed at the effect of spent hydrocarbon contamination on microbial population in soil. Standard microbiological methods were used to determine the total heterotrophic count hydrocarbon utilization, isolation and identification of bacterial and effect of pH and spent hydrocarbon concentration on bacterial. The total heterotrophic bacterial count (THB) ranges from 6.2 ± 0.13 x10^6 to 3.2± 0.10 x10^6 cfu/g. The total hydrocarbon utilizing bacterial count ranges from 3.2 ± 0.13 x10^6 to 1.2± 0.10 x10^6 cfu/g. The bacterial isolated were *Bacillus subtilis, Pseudomonas flourescens, Klebsiella aerogenes* and *Proteus hauseri.* It was observed that *Bacillus subtiliis* had the highest occurrence from location E (50.0%). *Pseudomonas flourescens* from location A and B (33.3%) and *Proteus hauseri* had the highest occurrence from location C (66.6%). The effect of pH on bacterial growth rate analyzed showed that *Bacillus subtilis* had the highest turbidity at pH 6.5 (0.511 ± 0.15 nmm), *Klebsiella aerogenes* had the highest turbidity at pH 7.5 (0.233 ± 0.33nm), *Pseudomonas flourescens* was at pH 6.5 (0.723 ± 0.61 nm) and *Proteus* sp recorded highest turbidity at pH 6.5 (0.373 ± 0.22nm) followed by pH 5.5 (0.237 ± 0.19 nm). The effect of spent hydrocarbon concentration showed that *Bacillus subtilis* recorded highest turbidity at 10% concentration (0.744 ± 0.03 nm), *Klebsiella aerogenes* recorded highest at 10% concentration (0.321 ± 0.21 nm), *Pseudomonas flourescens* was at 10% concentration (0.887 ± 0.23 nm) and *Proteus* sp recorded highest turbidity at 10% concentration (0.378 ± 0.13 nm). From this study it was observed that indigenous bacterial had the ability to utilized the spent hydrocarbon if the pH of the soil is regulated.

Keywords: pH; Concentration; Total heterotrophic; Bacterial; Hydrocarbon; Spent

1. Introduction

Engine oil could simply be defined as a thick mineral liquid applied to a machine or engine so as to reduce friction between the moving parts of the machine [1]. Oil released in to the environment is a well-recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans [2]. Spent/Used engine oil is defined as used lubricating oils removed from the crankcase of internal combustion engines [3]. Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment [4], large amount of them are liberated into the environment when the motor oil is changed and disposed into the soil which is a common practice by motor mechanics and generator mechanics including small scale engine oil sellers along the road. [5, 6]. Spent engine oils contain high percentage of aromatic and aliphatic hydrocarbons, nitrogen, sulphur compounds, and metals (Zn, Pb, Cr and Fe) than fresh oils, some of these metals in used engine oil can dissolve in water and move through the soil easily and may be found in surface water and groundwater [5].

Engine oils are altered during use by vehicles, motor-bikes, generators and other machinery because of the breakdown of additives, contamination with the products of combustion and the addition of metals from the wear and tear of the engine.

* Corresponding author: Yusuf UA

Bioremediation is, therefore, the application of naturally occurring process by which microorganisms transform environmental contaminants into harmless end products [5]. Bioremediation makes use of indigenous oil consuming microorganisms, called petrophiles by enhancing and fertilizing them in their natural habitats. Petrophiles are very unique organisms that can naturally degrade large hydrocarbons and utilize them as a food source [7]. Microbial remediation of a hydrocarbon contaminated site is accomplished with the help of a diverse group of microorganisms, particularly the indigenous bacteria present in soil. These microorganisms can degrade a wide range of target constituents present in oily sludge [8] there by, can be used in cleaning up contaminated sites. Therefor this study focused on effects of spent hydrocarbon on microbial community.

2. Material and methods

2.1 Methods

2.1.1 Sampling collection

Soil samples were collected from mechanic workshop in Abuja using a method described by Olubunm *et al*, [9]. The soil sample was collected randomly at a depth of 0-30cm from the fallow field in Keffi. They were bulked to form a composite sample and transported in polythene bags to the laboratory, air dried and sieved through a 2mm mesh.

2.1.2 Source of spent engine oil

The spent hydrocarbon was obtained from a mechanic workshop in Abuja, Nigeria. The spent hydrocarbon was dark-brown in colour. It was collected in a clean gallon and transferred to the Department of microbiology laboratory in Nasarawa State University Keffi.

2.2 Isolation of bacteria

The isolation of bacteria was carried out using a technique described by Bada *et al.* [10]. The bacteria were isolated using Nutrient agar and basal medium containing 0.3% yeast extract supplemented with 10% V/V oil substrates. The oil substrates were represent equivalent mixture of Gasoline, Engine oil, Diesel oil and spent engine oil. One (1) gram of the hydrocarbon contaminated soil sample was suspension in a test tube containing 9 ml of sterile water to make the soil suspension. Ten-fold serial dilution was made by transferring one ml of the soil suspension to another test tube containing 9 ml of sterile distilled water. These steps were repeated seven times to obtain a dilution of 10^{-7}. From the fourth test tubes, 0.2 ml of the aliquot were spread on Nutrient agar and basal medium and incubated at 28 ℃ for 24hours. Only culture plates that gave 30 - 300 colonies were counted and expressed in cfu/g and sub-cultured into freshly prepared nutrient agar for further identification.

2.3 Enumeration of Hydrocarbon Utilizing Bacteria (HUB)

Mineral salt medium (MSA) was used for the enumeration and isolation of hydrocarbon utilizing bacteria. The method of Olubunm *et al,* [9] was adapted. The components of the medium in g/l (NaCl 10 g, MgSO 0.4 g, KCl 0.29 g, NaHPO4 1.25 g, KH2PO4 0.83 g, NaNO30.42 g Agar 10 g in 1000 ml distilled water). The Spent engine oil soaked in sterile Whatman (No.1) filter paper and placed aseptically in the dish cover served as carbon source. Thus the hydrocarbon was supplied to the inoculums by vapour-phase transfer. About 1g of the samples was suspended in 9ml of sterile physiological saline. Subsequent 10 fold dilution was made from this initial dilution up to 10-5. About 0.1 ml aliquots of 10-3 - 10-5 dilution were inoculated into molten MSA plates in triplicate. The media was made selective for bacteria by adding nystatin (50 µg/ml) and incubated at room temperature for 5 days. The colonies were counted and expressed in cfu/g and sub-cultured into freshly prepared nutrient agar for further identification.

2.4 Identification of Bacteria isolated from contamination with hydrocarbon soil

Colonies was chosen from each of the cultured plate on the bases of their colonial and morphological similarities. Pure bacterial colonies was identified using Gram staining reaction and biochemical tests Catalase test, Indole, Methyl red, Vorges-Proskauer tests, Nitrate reduction, Urease production, Citrate utilization, and glucose fermentation tests according to the methods Cheesbrough [11].

2.5 Molecular Identification

2.5.1 DNA extraction (Boiling method)

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) were spun at 14000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95 ℃ for 20 min. The heated bactererial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml microcentrifuge tube and stored at -20 ℃ for other downstream reactions as earlier described by Abimiku *et al.,* [12].

2.5.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was lunched by double clicking on the Nanodrop icon. The equipment was initialized with 2 ul of sterile distilled water and blanked using normal saline. 2µl of the extracted DNA was loaded onto the lower pedestal, the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button [12].

2.5.3 16S rRNA Amplification

The 16s rRNA region of the rRNA genes of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 52ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extension, 72ºC for 5 minutes.

2.5.4 Agarose Gel Electrophoresis of the 16SrRNA gene from the bacteria isolates

The agarose gel electrophoretic separation of digested 16S rRNA gene was carried out as described by Abimiku *et al*., [12]. 8µl of PCR products stained with ethidium bromide was loaded into 1.0% (wt/vol) agarose gel wells with a molecular marker run concurrently at 120 V for 30 min. The DNA bands were visualized and photographed under UV light595nm.

2.6 Preparation of experimental medium.

Medium preparation was carried out as described by Olubunm *et al.* [9], mineral salt medium containing g/l (NaCl 10 g, MgSO 0.4 g, KCl 0.29 g, NaHPO4 1.25 g, KH2PO4 0.83 g, NaNO30.42 g) supplemented with spent hydrocarbon and thoroughly mixed was used as medium for this study. Four experimental conical flasks were used. The control conical flask with spent hydrocarbon was also set up. The experiment was set up in four replicates.

2.7 Effect of pH on utilization and growth of bacteria

The effect of pH was carried out following a method described by Olubunm *et al,* [9]. One hundred (100ml) milliliter of mineral salt medium mixture was transfer into different conical flasks. The pH ranges will be adjusted to 5.5 6.5 and 7.5 for degradation using 1.0 N HCl to adjusting the pH. The experiment lasted for 4 weeks and was incubated at ambient temperature. The utilization and growth of bacteria was determined by using **Uv-**spectrophotometer at OD 600nm.

2.8 Effect of concentration on utilization and growth of bacteria

Effect of concentration was carried out following a method described by Chaˆıneau *et al*. [13]. The concentration of the spent hydrocarbon was adjusted to 20%, 15% and 10% concentration in the mineral salt medium. The experiment lasted for 4 weeks and was incubated at ambient temperature. The utilization and growth of bacteria was determined by using Uv**-**spectrophotometer at OD 600nm.

3. Results

3.1 Total heterotrophic bacteria population count from the contaminated soil

The heterotrophic bacteria population count from the contaminated soil is as give in Table 1. The highest heterotrophic bacteria count was obtained from location B (6.2 ± 0.13 x10^6) followed by location D (5.2± 0.07 x10^6), location C (4.8 ± 0.03 x10^6), location A (4.1 ±0.90 x10^6) and the least was from location E (3.2± 0.10 x10^6).

3.2 Total Hydrocarbon Utilizing Bacterial and fungi count

The total hydrocarbon utilizing bacterial count is as given in Table 2. The highest Hydrocarbon Utilizing bacterial was obtained from location D (3.2 ± 0.13 x10^6) followed by location B (2.2± 0.07 x10^6), location A (2.1 ±0.90 x10^6), location (1.8 ±0.03 x10^6) and least location E (1.8 ± 0.03 x10^6) and the least was from location E (1.2± 0.10 x10^6).

Table 1 Total Heterotrophic bacteria population counts in the contaminated soil

Location	106 CFU/g THB
A	4.1 ±0.90 x106
B	6.2 ± 0.13 x106
C	4.8 ± 0.03 x106
D	5.2± 0.07 x106
E	3.2± 0.10 x106

Key: location A- E= Different mechanic workshop

Table 2 Total Hydrocarbon Utilizing Bacterial and fungi count

Sample	10^6 CFU/g THB
A	2.1 ±0.90 x10^6
B	2.2± 0.07 x10^6
C	1.8 ± 0.03 x10^6
D	3.2 ± 0.13 x10^6
E	1.2± 0.10 x10^6

3.3 Identification of bacterial isolated from hydrocarbon contaminated soil

Table 3 Cultural, Morphology and Biochemical characteristic of Bacteria isolated

Cultural Morphology	Gram Reaction	Biochemical characteristic				Sugar fermentation			Inference
		Cat	Ox	In	Nit	Fru	Mal	Glu	
Milksh, circular, coarse, flat, convex, entire and opaque	-	+	-	-	+	+	-	+	*Bacillus subtilis*
smooth elevated colonies and cocci on NA	+	-	-	+	-	-	+	+	*Klebsiella aerogenes*
smooth none elevated colonies green pigment on NA	-	-	-	-	+	-	-	+	*Pseudomonas flourescens*
Grey colored with a shiny surface and entire margin; mucoid or rough	+	-	+	-	+	+	-	+	*Proteus hauseri*

KEY: NA- nutrient agar, Cat-catalase, Nit- nitrate, Ox-oxidase, In-indole, Glu- glucose, mal – maltose, Fru – fructose

The cultural, morphological and biochemical characteristics of bacterial is as shown in Table 3. where Milksh, circular, coarse, flat, convex, entire and opaque colonies, Gram negative, catalase positive, oxidase negative, indole negative, nitrate positive and fructose positive, maltose negative and glucose positive were suspected to be *Bacillus subtilis,* smooth elevated colonies and cocci on NA, catalase negative, oxidase negative, indole positive, nitrate negative and fructose negative, maltose positive and glucose positive were suspected to be *Klebsiella aerogenes,* smooth none

elevated colonies green pigment on NA, gram negative, catalase negative, oxidase negative, indole negative, nitrate positive and fructose negative, maltose negative and glucose positive were suspected to be *Pseudomonas flourescens.*

Figure 1 shows the Gel electrophoresis of bacterial species: 1 and 2 represent *Bacillus subtilis* and *Klebsiella aerogenes*. N represent control, 3 and 4 represent *Pseudomonas flourescens* and *Proteus hauseri* at molecular weight of 1500basepair (bp). The phylogenic tree of the molecular identified bacterial is as given in figure 1, 2, 4 and 4 showing relatedness of the hydrocarbon utilizing bacterial.

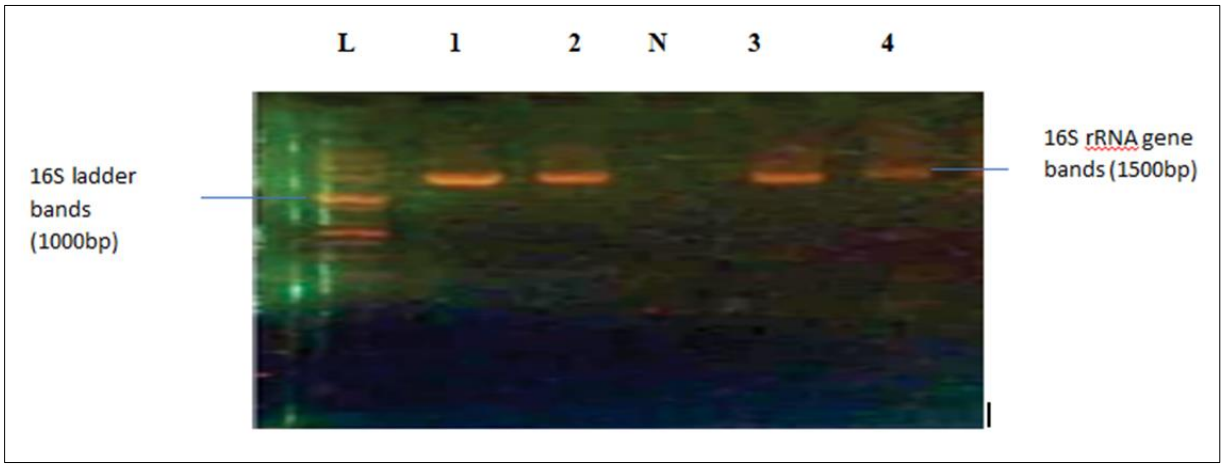

Figure 1 Gel electrophoresis of bacterial species: Lanes 1 and 2 represent isolate *Bacillus*

subtilis and *Klebsiella aerogenes* respectively. Lane N represent control. Lanes 3 and 4 represent *Pseudomonas flourescens* and *Proteus hauseri* respectively. At molecular weight of 500basepair (bp)

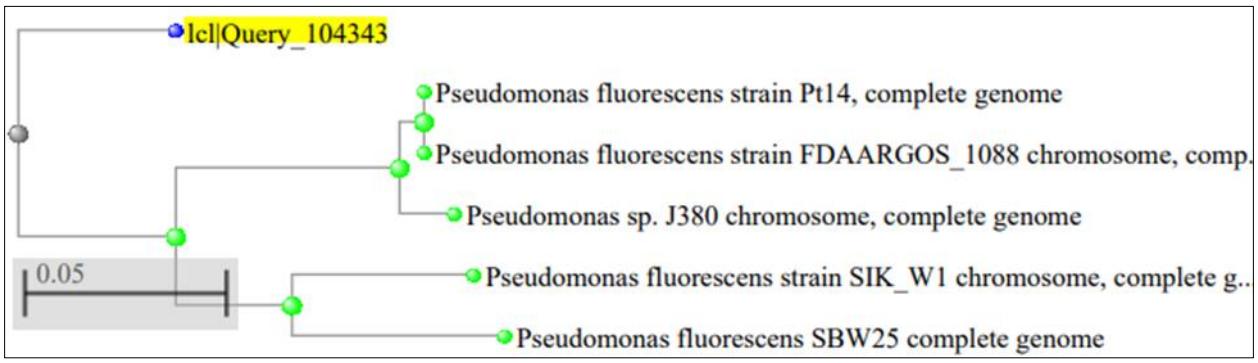

Figure 2 Phylogenic tree showing relatedness of *Pseudomonas fluorescens* isolated

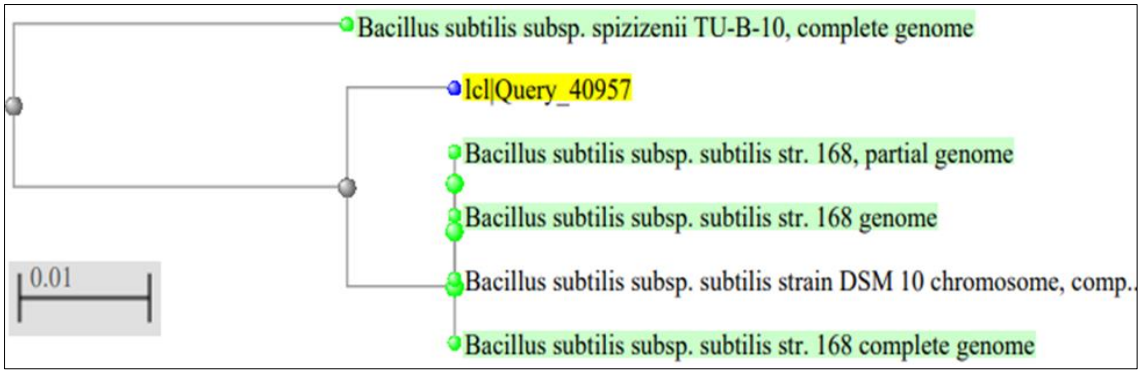

Figure 3 Phylogenic tree showing relatedness *of Bacillus subtilis* isolated

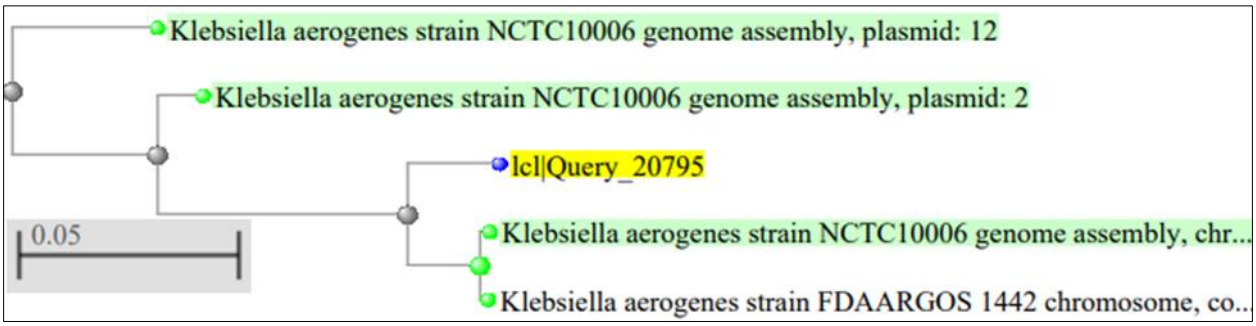

Figure 4 Phylogenic tree showing evaluation relatedness of *Klebisella aerogenes*

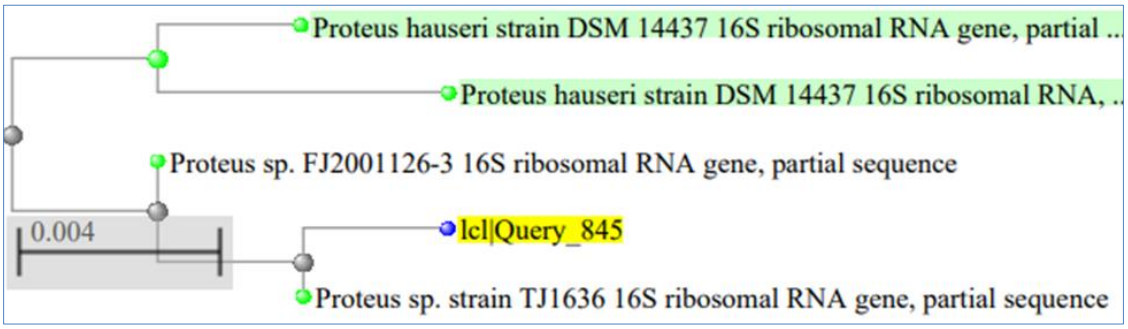

Figure 5 Phylogenic tree showing evaluation relatedness of *Proteus hauseri*

3.4 Occurrence of bacteria from spent hydrocarbon contaminated soil

Table 4 shows the frequency occurrence of bacterial isolated from soil contaminated with spent hydrocarbon. *Bacillus subtiliis had* the highest occurrence from location E (50.0%) followed by location A (33.3%) and location C (16.6%). *Klebsiella aerogenes* had highest occurrence from location B (33.3%) and least was from location A (16.6%). *Pseudomonas flourescens* had the highest occurrence from location A and B (33.3%) and the least from location D and E (16.6%) and *Proteus* sp had the highest occurrence from location C (66.6%) and least from location D (33.3%).

3.5 Effect of pH on bacterial growth on spent hydrocarbon medium

The effect of different pH on the turbidity measurement (OD600nm) on growth rate of bacterial in spent hydrocarbon medium is as given in Table 5. *Bacillus subtilis* had the highest turbidity at pH 6.5 (0.511 ± 0.15 nm) followed pH 7.5 (0.423 ± 0.11nm) and the least was at pH 5.5 (0.303 ± 0.10 nm). *Klebsiella aerogenes* had the highest turbidity at pH 7.5 (0.233 ± 0.33nm) followed by pH 6.5 (0.183 ± 0.10 nm) and at pH 5.5 (0.117 ± 0.40 nm). The highest turbidity by *Pseudomonas flourescens* was at pH 6.5 (0.723 ± 0.61 nm) followed by at pH 7.5 (0.711 ± 0.16 nm) and at pH 5.5 (0.514 ± 0.72 nm) and *Proteus* sp recorded highest turbidity at pH 6.5 (0.373 ± 0.22nm) followed by pH 5.5 (0.237 ± 0.19 nm) and at pH 7.5 (0.208 ± 0.13 nm). While the control turbidity was 0.97 ± 0.00 nm

3.6 Effect of concentration on bacterial growth on spent hydrocarbon medium

The effect of different concentration on the turbidity measurement (OD600nm) on growth rate of bacterial in spent hydrocarbon medium is as given in Table 6. *Bacillus subtilis* recorded highest turbidity at 10% concentration (0.744 ± 0.03 nm) followed by after 15% (0.743 ± 0.13 nm) and 20% (0.327 ± 0.10 nm). From *Klebsiella aerogenes* the highest was obtained after at 10% concentration (0.321 ± 0.21 nm) followed by 15% (0.302 ± 0.31 nm) and at 20% (0.212 ± 0.03 nm). The highest turbidity recorded by *Pseudomonas flourescens* was at 10% concentration (0.887 ± 0.23 nm) followed by 15% concentration (0.753 ± 0.11 nm) and at 20% (0.414 ± 0.52 nm). *Proteus* sp recorded highest turbidity at 10% concentration (0.378 ± 0.13 nm) followed by 15% concentration (0.753 ± 0.11 nm) and at 20% concentration (0.414 ± 0.52 nm).

Table 4 Frequency occurrence of bacteria isolated from contaminated soil with spent hydrocarbon

Location	No. sample	*Bacillus subtilis* (%)	*Klebsiella aerogenes* (%)	*Pseudomonas flourescens* (%)	*Proteus* sp (%)
A	6	2(33.3)	1(16.6)	2(33.3)	0(00)
B	6	0(00)	2(33.3)	0(00)	0(00)
C	6	1(16.6)	0(00)	2(33.3)	4(66.6)
D	6	3(50.0)	0(00)	1(16.6)	2(33.3)
E	6	0(00)	0(00)	1(16.6)	0(00)

Table 5 Effect of pH on turbidity of bacterial growth in spent hydrocarbon medium

Bacterial isolates	Control	Optical density (600 nm) on pH		
		5.5	6.5	7.5
Bacillus subtil	0.97 ± 0.00	0.303 ± 0.10	0.511 ± 0.15	0.423 ± 0.11
Klebsiella aerogenes	0.97 ± 0.00	0.117 ± 0.40	0.183 ± 0.10	0.233 ± 0.33
Pseudomonas flourescens	0.97 ± 0.00	0.514 ± 0.72	0.723 ± 0.61	0.711 ± 0.16
Proteus hauseri	0.97 ± 0.00	0.237 ± 0.19	0.373 ± 0.22	3.208 0.13

Table 6 Effect of concentration on turbidity of bacterial growth in spent hydrocarbon medium

Fungi isolates	Control	Optical density (600 nm) on concentration		
		20%	15%	10%
Bacillus subtills	0.97 ± 0.00	0.327 ± 0.10	0.743 ± 0.13	0.744 ± 0.03
Klebsiella aerogenes	0.97 ± 0.00	0.212 ± 0.03	0.302 ± 0.31	0.321 ± 0.21
Pseudomonas flourescens	0.97 ± 0.00	0.414 ± 0.52	0.753 ± 0.11	0.887 ± 0.23
Proteus hauseri	0.97 ± 0.00	0.277 ± 0.19	0.373 ± 0.22	0.378 ± 0.13

0. Discussion

Petroleum hydrocarbons are important energy resources and also a major pollutant of the environment when use in different area as source of energy. The effect of spent engine oil contamination on microbial population in Keffi, Nigeria

In this study the total heterotrophic count of bacterial recorded vary from location to location which may be due to the numerous of nutrients, high organic matter concentration and other ecological factors that influence the survival of heterotrophic bacteria and fungi that play significant role in decomposition and recycling of nutrients. However, the relatively low heterotrophic bacterial counts observed in some location that is heavily polluted with spent oil can be credited to the toxic or unfavorable effect of oil contamination, this is similar to study reported by Akomah, and Osayande [14] that high concentration hydrocarbon effect the microbial population of the soil.

The result obtained from screening of spent hydrocarbon tolerance count of bacterial isolated was low to compare to the total heterotrophic count which may be attributed to that most of the bacterial will not survive in heavily contaminated soil because of lack of oxygen circulation in the soil and lack of access to other important nutrient in the soil due to the presence of the spent hydrocarbon. However, the result obtained showed that the different between the total heterotrophic count and hydrocarbon utilizing count was insignificant which advocates that most of the

microorganisms present in the various location sampled have the ability to degrade spent hydrocarbon or withstand the vary concentrations of spent hydrocarbons that comes out from different mechanic workshops and also use them as sole source of carbon this is in agreement with other studies carried by different authors that have reported the ability of bacterial using spent hydrocarbon as sole source of carbon as reported by Ebakota *et al.* [15]; Mandri and Lin [16] and Unimke *et al*. [17].

The different bacterial isolated that was observed to utilize spent hydrocarbon as source of carbon were *Bacillus subtills, Klebsiella aerogenes, Pseudomonas flourescens* and *Proteus* sp these bacterial have been reported to be spent hydrocarbon degrading bacterial which survive in high hydrocarbon contaminated soil and is similar to the study reported by Hassana *et al.* [18]. Similar, the effect of pH on utilization of spent hydrocarbon which was measured at 600nm showed that *Pseudomonas flourescens* and *Bacillus subtills* have the highest growth rate in spent hydrocarbon medium than the other bacterial isolates at various pH used in this study, this is an evidence that these bacterial are the active degraders of hydrocarbon. These bacterial have heavily reported to utilized hydrocarbon in different environment that where contaminated with different amount of hydrocarbon and have been isolated, reported as indigenous organisms in waste engine oil contaminated soil (Arotupin and Ogunmalu, [19]. This suggest that pH of the soil plays a significant role in survivor utilization of the spent hydrocarbon contaminated soil.

1. Conclusion

The result of this study reveals that four species of bacterial isolate were *Bacillus subtilis, Pseudomonas flourescens, Klebsiella aerogenes* and *Proteus hauseri* had the ability to utilized spent hydrocarbon as carbon source, and can be used to clean up soil contaminated with spent hydrocarbon.

Compliance with ethical standards

Acknowledgments

We immensely thank all the staff in Department of Microbiology Laboratory Nasarawa State University Keffi for the help during the bench work and analysis.

Disclosure of conflict of interest

No conflict of interest among the authors.

References

[1] Alvarez P. J. J and Vogel T. M (2001) "Substrate interactions of benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries," Applied and Environmental Microbiology, 57 (10) 2981–2985

[2] Nwite, J. N and Alu, M. O. (2015). Effects of different levels of spent engine oil on soil properties, grain yield of maize and its heavy metal uptake in Abakaliki, South Eastern, Nigeria. Journal of Soil Science and Environmental Management 5 (4): 44-51

[3] Okonokhua, B. O.; Ikhajiagbe, B.; Anoliefo, G. O. and Emede, J. O. (2017). The effect of spent engine oil on soil properties and growth of maize (Zea may L). Journal of Applied Sciences Environmental Management. 11 (3): 147 – 152.

[4] Dominguez-Rosado R. E and Pichtel D. (2009). Phytoremediation of contaminated with used motor oil. 1. Enhanced microbial activities from laboratory and growth chamber studies. Environmental Engineering Science, 2, 157-168

[5] Abdulsalam, S., Adefila, S. S., Bugaje, I. M and Ibrahim, S. C. (2012). Bioremediation of soil contaminated with used motor oil in a closed system. Journal of Bioremediation and Biodegradation 3(12): 1-7

[6] Achuba I. and clerke- peretiemo B. O (2008). Effect of spent engine oil on soil catalase and dehydrogenase activities, International Agrophysics 22 pp 1-4.

[7] Al-Majed A.A., Adebayo A.R and Hossain M.E. A (2012) sustainable approach to controlling oil spills. Journal of Applied Sciences Environmental Management, 113: 213-227

[8] Abdulsalam S and Omale A.B (2015) Comparison of biostimulation and bioaugmentation techniques for the remediation of used motor oil contaminated soil, Braz Arch Biol Technol 52: 747-754.

[9] Olubunm F., Olatunde S., Abiodun A and Adetoyi H (2019). Degradation of spent oil contaminated soil using fungi from organic manure. International Journal of Research and Developmen, 4 (10) 36-44

[10] Bada B. S, Tsobaza I. E., Toyin A. A and Tolulope M. O (2019), Degradation of total petroleum hydrocarbon in petroleum productscontaminated soil using pig dung, West African Journal of Applied Ecology, 27(2):1-15

[11] Cheesebrough, M. (2006). Medical Laboratory Manual for Tropical Countries. Cambridge: Cambridge University Press, Pp. 49-97.

[12] Abimiku, R.H., Ngwai, Y.B., Nkene, I.H., Tatfeng, Y. M. (2016). Molecular detection of diarrheagenicpathotypes of Escherichiacoli from diarrheic patients in Keffi, Nigeria. Microbioz Journal of Microbiology and Biomedical Research, 2(3): 1-6.

[13] Chaˆıneau C. H., Rougeux G., Yepr ´ emian C and Oudot J (2015)"Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil," Soil Biology and Biochemistry, 37 (8) 1490–1497

[14] Akomah, O.N and Osayande, A.D (2018). Evaluation of Hydrocarbons Level and Identification of Indigenous Bacteria in Soil from Auto-Mechanic Workshops along Ikokwu Mechanic Village, Port Harcourt, Nigeria. J. Appl. Sci. Environ. Manage. 22 (1) 115-118

[15] Ebakota, O. D; Osarueme, J. O., Nwaeze G.O., Odoligie I., Osazee J. O (2017) Isolation and Characterization of Hydrocarbon-Degrading Bacteria in Top and Subsoil of selected Mechanic Workshops in Benin City Metropolis, Nigeria. J. Appl. Sci. Environ. Manage.21 (4) 641-645.

[16] Mandri, T and Lin, J (2007). Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. African Journal of Biotechnology, 6(1): 23-27.

[17] Unimke A. A., Mmuoegbulam A. O., Bassey I. U and Obot S. E (2017). Assessment of the Microbial Diversity of Spent-Oil Contaminated Soil in Calabar, Nigeria. Journal of Advances in Microbiology 4(4): 1-9.

[18] Hassana, A., Vincent, B.T., Ushuji, O.D., Yakubu, N., Boko, U.H., & Gogo, M.F. (2019). Molecular Identification of Hydrocarbon Degrading Bacteria Isolated from Contaminated Soil of Automobile Mechanic Workshop in Lapai, Niger State., Ind. J. Pure App. Biosci. 7(4), 31-37.

[19] Arotupin, D.J and Ogunmalu, F.E (2012). Screening of Fungal Isolate from Nigerian Tar Sand Deposit in Ondo State for Novel Biocatalysts. Journal of Biological Sciences 12(1): 57-61.