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Effect of Spent Engine Oil Contamination on Bacteria and Fungi in the Soil in Karu, Federal Capital Territory Abuja, Nigeria

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Abstract

Spent engine oil is released into the environment in large quantities during manual oil-changing activities, especially in automobile and generator workshops, where it is often dumped on open ground, farmlands, and water bodies, causing soil and water pollution. This study presents the findings of the Effect of Spent Engine Oil Contamination on Bacteria and Fungi in Soil in Karu FCT. Soil samples were collected from five mechanic workshops (Sites A–E) by randomly sampling the top 0–5 cm with a corer in triplicates, after which samples from each site were augered, bulked, placed in labelled polythene bags, and transported to the laboratory. Uncontaminated soil serving as the control sample was collected from a clean area adjacent to Site C where no visible spent engine oil contamination was observed. The results of the study revealed that total heterotrophic bacteria ranged from $6.2 \pm 0.13 \times 10^6$ cfu/g at Site B to $3.2 \pm 0.10 \times 10^6$ at Site E, while fungi ranged from $5.6 \pm 0.25 \times 10^4$ at Site A to $0.9 \pm 0.00 \times 10^4$ at Site E. Total hydrocarbon-utilizing bacteria were highest at Site D ($3.2 \pm 0.13 \times 10^6$) and lowest at Site E ($1.2 \pm 0.10 \times 10^6$), while hydrocarbon-utilizing fungi peaked at Site B ($1.8 \pm 0.56 \times 10^4$) and were lowest at Site E ($0.2 \pm 0.00 \times 10^4$). Isolates identified included *Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Aspergillus fumigatus, Mucor racemosus, and Rhizopus stolonifer*, with *B. cereus* showing up to 50.0% occurrence at Site E and *Aspergillus fumigatus* up to 66.6% at Site A. pH studies showed highest bacterial growth at pH 6.5 for *B. cereus* (0.511 ± 0.15 nm) and *P. aeruginosa* (0.723 ± 0.61 nm), while fungi such as *A. fumigatus* (4.51 ± 0.25 g/L) and *R. stolonifer* (6.14 ± 0.32 g/L) grew best between pH 6.5 and 7.5. Hydrocarbon concentration experiments recorded highest turbidity or biomass at 10% oil for all organisms, with P. aeruginosa reaching 0.887 ± 0.23 nm and *R. stolonifer* 6.88 ± 1.23 g/L, while 20% concentration consistently reduced growth. This study showed that spent engine oil significantly alters soil microbial composition by reducing general microbial populations while enriching hydrocarbon-utilizing bacteria and fungi capable of adapting to polluted environments.

Keywords: Spent engine oil, Karu, Contamination, hydrocarbon-utilizing fungi, Bacteria

1. Introduction

Increase in the use of automobile engines and machines has resulted into rise in use of lubricating (engine) oil hence increase in rates of environmental contamination by used engine oil (Muhammad *et al.,* 2022; Abioye *et al.,* 2012).Used engine oil contains harmful substances such as heavy metals, aliphatic and aromatic hydrocarbons, Benzenes as well as sulphur and nitrogen. These substances cause negative effects to the soil and soil micro flora such as abridged growth rate and reproduction, destitute health and mutagenesis, thus alter population dynamic, disrupt tropical relations and edifice of natural groups within ecosystems ((Muhammad *et al.,* 2022).

The spent oil is generated when the engine oil is exposed to high temperature and mechanical stress as the engines perform their function (Agarry *et al.,* 2010). Spent oil is disposed into the environment in high amount during manual oil changing operation either intentionally as in the case of automobile and generator mechanics or accidentally through spill. The oil is dumped into water bodies, farmlands and open vacant plots use as mechanic workshops hence polluting both soil and water (Ikhajiagbe and Anoliefo, 2011). Osubor and Anoliefo (2003) conveyed that approximately 20 million gallons of spent oil are accumulated every year from mechanic workshops across Nigeria and disposed carelessly into the environment. They further reported that oils are release into the milieu from the exhaust systems during engine operations as well as engine leaks.

The chemical impurities in spent engine oil are toxic and can cause cancer and mutation in living cells (Muhammad *et al.,* 2022; Ajao *et al.,* 2011). These chemicals seep into the water table, contaminate the ground water and subsequently get into the human body through the food web from plants (Adams *et al.,* 2014). The spent engine oil floats as a scum on the surface hence prevent sunlight and oxygen from penetrating the water thereby affecting water animals like fishes, frogs, crabs and water plants (Agarry and Oladipupo, 2012; Adams *et al*., 2014). Pollution of soil by spent oil results in immobilizations of soil nutrient, loss of water-retaining capacity, low pH, reduced soil catalyse enzyme action, as well as inhibition of nitrate reductase activity of plants; thus, soil dependent activities such as agriculture are affected (Imam *et al.,* 2011).

Contaminations of exposed vacant spaces and farmland with petroleum product and grease is nowadays widespread than crude oil spill especially in the municipal areas (Osaigboro *et al*, 2013). The present study therefore, aimed at evaluating the effect of spent engine oil contamination on microorganisms in the soil in Karu FCT.

2. Materials and Methods

2.1 Study Area

The study area was carried out in Federal Capital Territory, Abuja, Nigeria. Abuja is located in the centre of the country. Federal Capital Territory is located between latitude $9^{o}4'$N of the equator and longitude $7^{o}29'$E and situated on an altitude of 360m above sea level

2.2 Sample Collection

The study sites were 5 different mechanic workshops, designated as Site A, Site B, Site C, Site D, and Site E, situated in different locations in Karu FCT. Apart from visual observation, the attendants at the auto-mechanic workshops were asked questions pertaining to the sites with spent engine oil contamination. The sites with the spent engine oil contamination had characteristic of black colour. Samples were collected at the different sites with a corer to the depth of 0–5 cm in triplicates using random sampling technique from each mechanic workshop, augured and bulked together and put in well labelled black polythene bags and transported to the laboratory for analysis.

Uncontaminated soil serving as the control sample was collected adjacent to site C, a clean area within the same study environment in Karu

FCT where there was no visible spent engine oil contamination. The samples were subjected to microbiological analyses. Used motor engine oil was also collected from one of the mechanic workshops as the fresh engine oil sample as described by Yusuf *et al*. (2023).

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 Yusuf *et al.* (2023) and Agu *et al.* (2014) was adapted. The components of the medium in g/l (NaCl 10 g, MgSO 0.4g, KCl 0.29g, $NaHPO_4$ 1.25g, KH_2PO_4 0.83g, $NaNO_3$ 0.42g Agar 10g in 1000ml 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 0.1ml aliquots of10^{-3} - 10^{-5} dilution was inoculated into spent engine oil soaked in sterile Whatman (No.1) filter paper on MSA plates in triplicate. The media was made selective for bacteria byadding nystatin (50 µg/ml) and incubated at room temperature for 5 days. The colonies waere counted and expressed in cfu/g and sub-cultured into freshly prepared nutrient agar for further identification.

2.4 Identification of Bacteria isolated

Colonies were chosen from each of the cultured plate on the bases of their colonial and morphological similarities. Pure bacterial colonies were identified using Gram staining reaction and biochemical tests according to the methods Yusuf *et al*. (2023).

2.4.1 Gram Staining Examination

The Gram staining technique were carried out as described by Yusuf *et al*. (2023) with some modifications; A small portion of cultural organism was transferred onto a clean grease-free glass slide, and emulsified in a drop of distilled water until a thin homogeneous film is obtained, then the wire loop was` re-sterilized and the thin homogeneous film was allowed to air-dry, and heat-fixed by passing through the flame. The slide was then flooded with crystal violet for 1 minute, and then rinsed with distilled water. The stain was again flooded with Lugol's iodine for one minute, and rinsed with distilled water and then decolorized, rapidly with acetone alcohol until no more colour appeared to flow from preparation and rinsed appropriately with distilled water. The stain was then counter-stained with neutral red for one minute, and rinsed with distilled water and allowed to air dry and viewed microscopically using x100 oil immersion objective. Gram positive organism retains the dark blue colour inferred by the iodine/crystal violet complex, while Gram negative organisms appears red; maintaining the colour of the secondary dye.

2.4.2 Biochemical tests

The following biochemical test was carried out on the suspected bacteria species isolates: Catalase test, Indole, Methyl red, Vorges-Proskauer tests, Nitrate reduction, Urease production, Citrate utilisation, and glucose fermentation tests.

2.4.3 Isolation of Fungi

Fungi isolation was carried out using a method described by Agu *et al,* (2014). Four replicate samples from each oil contaminated soil was withdrawn in every three days for the enumeration and identification of fungi. 1g of oil-contaminated samples were weighed and poured into 9ml of sterile distilled water and mixed thoroughly. 10-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, 0.2ml of the aliquot was spread on Potato Dextrose Agar (PDA) and basal medium and incubated at 28^{o}C for 7daysfor fungi determine the loads of Total Heterotrophic Fungi (THF). The PDA was fortified with lactic acid for fungi after sterilization to avoid bacterial contamination.

2.4.4 Identification of Fungal

Identification of fungal strains were carried out as described by Agu *et al.* (2014). Identification was based on microbiological standard procedure using cultural and morphological characteristics. The cultural characteristics were determined by their appearance on culture plates while the morphological features were determined microscopically using lactophenol cotton blue staining technique. where lactophenol cotton, blue strain was dropped on a clean grease free microscope slide, a small portion of mycelium or colony from the fungi culture plate was dropped on the lactophenol cotton blue with aid of mounted needle, the mycelium was spread well with the two mounted needles and covered with cover slip. The slides were then viewed under the microscope at x40 and x100 lens. The images were identified with reference to the work and fungi standard chart.

2.5 Preparation of Experimental Medium

Medium preparation was carried out as described by Yusuf *et al.* (2023), mineral salt medium containing g/l (NaCl 10g, MgSO 0.4 g, KCl 0.29 g, $NaHPO_4$ 1.25 g, KH_2PO_4 0.83 g, $NaNO_3$ 0.42 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 three replicates.

2.5.1 Effect of pH on Utilization and Growth of Bacteria and Fungi

The effect of pH was carried out following a method described by Yusuf *et al.* (2023). One hundred (100ml) millilitre of mineral salt medium mixture was transfer into different conical flasks. The pH ranges were 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.

2.5.2 Effect of Concentration of Spent oil on Growth of Bacteria and Fungi

Effect of concentration was carried out following a method described by Yusuf *et al.* (2023) and Chaˆıneau *et al.* (2015). The concentration of the spent hydrocarbon was adjusted to 20%, 15% and 10% concentration in the mineral salt medium. The experiments lasted for 4 weeks and were incubated at ambient temperature. The utilization and growth of fungi and bacteria were determined by using Uv-spectrophotometer at OD 600nm.

2.5.3 Effect of Temperature on Utilization and Growth of Bacteria and Fungi

Effect of temperatures was carried out following a method described by Anjana and Meenal, (2009). One hundred (100) ml of the degradation media were transferred into different conical flasks and the degradation media were incubated at 28^{o}C, 32^{o}C, 35^{o}C and 38^{o}C. The utilization and growth of fungi and bacteria was determined by using Uv-spectrophotometer at OD 600nm.

2.6 Total Utilization of Spent Hydrocarbon

Total utilized spent hydrocarbon content of the degradation media samples was determined using UV Visible spectrophotometer methods according to the toluene extraction method (Yusuf *et al.* 2023; Agu *et al*., 2022) and Sonication water bath methods. Fifteen miligram (15ml) of each of the sample were transferred into a test tube, and then 1ml of 60ug/ml of 1-chlorooctadecane surrogate standard was added. Then 30 millilitres of dichloromethane (extraction solvent) was added to extract oil in the soil. After shaking vigorously in water bath for 5hrs, the mixture was allowed to stand for 60 minutes and then filtered through Whatman No.1 filter paper fitted with cotton wool and sodium sulphate into a clean beaker washed with methylene chloride. Residue was then washed with 20ml extracting solvent and then filtered through funnel. The extracted oil was transferred to vial and placed on a UV Visible spectrophotometer chamber for analysis. The amount of waste lubricating oil degraded was calculated by subtracting the weight of residual waste lubricating oil from weight of the initial

waste lubricating oil, divided by the weight of the initial waste lubricating oil and then multiplied by 100.

$$HR\ (\%) = \frac{(\text{Weight of oil before remediation}) - (\text{Weight of oil after remediation})}{\text{Weight of oil before remediation}} \times 100$$

Where
HR = Hydrocarbon utilization

3. Results

3.1 Total heterotrophic bacteria and fungi population counts in the contaminated soil

The heterotrophic bacteria and fungi population counts in the contaminated soil are as given 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). While the highest fungi were observed from location A (5.6 ± 0.25 x10^4) followed by location C (3.6± 0.10 x10^4), location C (3.1 ± 0.05 x10^4) and the least was obtained from location E (0.9± 0.00 x10^4).

3.2 Total Hydrocarbon Utilizing Bacterial and fungi count

The total Hydrocarbon Utilizing Bacterial and fungi 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). While highest Hydrocarbon Utilizing fungi was obtained from location B (1.8 ± 0.56 x10^4) followed by location C (1.5 ± 0.42x10^4), location A (1.3± 0.16 x10^4) and least was from location E (0.2 ± 0.0x10^4).

Table:1 Total Heterotrophic bacteria and fungi population counts in the contaminated soil

Location	10^6 CFU/gTHB	10^4 CFU/gTHF
A	4.1 ±0.90	5.6 ± 0.25
B	6.2 ± 0.13	3.1 ± 0.05
C	4.8 ± 0.03	1.9 ± 0.10
D	5.2± 0.07	3.6± 0.10
E	3.2± 0.10	0.9± 0.00
Control site	7.0± 1.11	6.8± 1.10

Table 2: Total Hydrocarbon Utilizing Bacterial and fungi count

Sample	10^6 CFU/gTHB	10^4 CFU/gTHF
A	2.1 ±0.90	1.3± 0.16
B	2.2± 0.07	1.8 ± 0.56
C	1.8 ± 0.03	1.5 ± 0.42
D	3.2 ± 0.13	1.1 ± 0.13
E	1.2± 0.10	0.2 ± 0.0
Control site	5.0± 0.13	4.2± 1.10

3.3 Identification of bacteria and fungi isolated from hydrocarbon contaminated soil

The cultural, morphological and biochemical characteristics of bacterial and fungi is as shown in Table: 3 and Table: 4. where Milkish, 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 cereus* , 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 pneumoniae,* 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 aeruginosa.*

While fungi isolate which Colonies are fast growing, grey green on the surface with yellow on the reverse side, microscopic view showed Chains of round condia with conidial heads in the form of compact columns. Phialides uniserate, concentrated on the upper surface of the vesicle were suspected to be *Aspergillus fumigatus, fungi.* Colonies, very rapid growth with wooly texture.Color is grayish on the surface and reverse is pale, on microscope are Non septate broad hyphae, branched sporangiophores with round sporangiospores. Stolons and rhizoids are absent were *Mucor racemosus.*

3.4 Occurrence of Bacteria and Fungi from Spent Hydrocarbon Contaminated Soil

Table: 5 shows the frequency occurrence of bacterial isolated from soil contaminated with spent hydrocarbon. *Bacillus cereus had* the highest occurrence from location E (50.0%) followed by location A (33.3%) and location C (16.6%). *Klebsiella pneumoniae* had highest occurrence from location B (33.3%) and least was from location A (16.6%). *Pseudomonas* aeruginosa had the highest occurrence from location A and B (33.3%) and the least from location D and E (16.6%) and *Proteus mirabilis* had the highest occurrence from location C (66.6%) and least from location D (33.3%).

Table: 6 shows the Frequency occurrence of fungi isolated from contaminated soil with spent hydrocarbon. Where the highest occurrence of *Aspergillus fumigatus* was observed from location A (66.6%) followed by location D (50.0%), location B (33.3%), and least from location E (16.6%). *Mucor racemosus* had the highest occurrence from location B (50.0%) followed by location A and C (33.3%) and location D (16.6%). The highest occurrence of *Rhizopus stolonifer* was observed from location C and D (50.0%), location E (33.3%) and the lowest was observed from location A and B (16.6%) respectively

3.5 Effect of pH on Bacterial and Fungi 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 7. *Bacillus cereus* 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 pneumoniae* 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 aeruginosa* 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 mirabilis* 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.

The effect of pH on fungi growth rate in spent hydrocarbon medium is as given in Table: 7. *Aspergillus fumigatus* recorded highest dried biomass at pH 6.5 (4.51 ± 0.25 g/l) followed by at pH 7.5 (2.42 ± 0.51 g/l) and at pH 5.5 (2.30 ± 0.14 g/l). *Mucor racemosus* had the highest dried biomass at pH 7.5 (6.23 ± 0.36 g/l) followed by pH 6.5 (5.18 ± 0.15 g/l) and pH 5.5 recorded the least (3.11 ± 0.42 g/l). *Rhizopus stolonifer* had

highest dried biomass at pH 7.5 (6.14 ± 0.32 g/l) followed by pH6.5 (5.72 ± 0.11 g/l) and pH 5.5 (5.11 ± 0.06 g/l) respectively.

Table: 4 Cultural, Morphology and Microscopic characteristic of fungi isolated

Colonial Morphology	Morphological Characteristics	Inference
Colonies are fast growing, grey green on the surface with yellow on the reverse side	Chains of round condia with conidial heads in the form of compact columns. Phialides uniserate, concentrated on the upper surface of the vesicle	*Aspergillus fumigatus*
Colonies have very rapid growth with wooly texture. Color is grayish on the surface and reverse is pale	Non septate broad hyphae, branched sporangiophores with round sporangiospores. Stolons and rhizoids are absent	*Mucor racemosus*
White cottony colony rapidly filling the plate, producing spores	Non septate hyphae with branched sporangiophore, dark sporangia and sporangiospores	*Rhizopus stolonifer*

3.6 Effect of Concentration on Bacterial and Fungi 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: 8. *Bacillus cereus* 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 pneumoniae* 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 aeruginosa* 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 mirabilis* 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).

The effect of concentration on fungi growth rate in spent hydrocarbon medium is as given in Table: 9. *Aspergillus fumigatus* recorded highest dried biomass at 10% concentration (3.45 ± 0.41g/l) followed by 15% (3.41 ± 0.31g/l) and after 7 days (1.62 ± 0.13g/l). *Mucor racemosus* had the highest dried biomass at 10% (5.84 ± 1.03g/l) followed by 15% (5.74 ± 0.13g/l) and at 20% it recorded the least (3.27 ± 0.40 g/l). *Rhizopus stolonifer* had highest dried biomass at 10% (6.88 ± 1.23 g/l) followed by 15% (6.13 ± 1.11 g/l) and at 20% it recorded the least (3.11 ± 1.52 g/l) respectively.

Table: 5. Frequency occurrence of bacteria isolated from contaminated soil with spent hydrocarbon

Location	No. sample	*Bacillus Cereus* (%)	*Klebsiella Pneumoniae* (%)	*Pseudomonas Aeruginosa* (%)	*Proteus Mirabilis* (%)
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 6 Frequency occurrence of fungi isolated from contaminated soil with spent hydrocarbon

Location	No. sample	*Aspergillus fumigatus* (%)	*Mucor racemosus* (%)	*Rhizopus stolonifer* (%)
A	6	4(66.6)	2(33.3)	1(16.6)
B	6	2(33.3)	3(50.0)	1(16.6)
C	6	0(00)	2(33.3)	3(50.0)
D	6	3(50.0)	1(16.6)	3(50.0)
E	6	1(16.6)	0(00)	2(33.3)

Table: 7 Effect of pH on turbidity of bacterial growth in spent engine oil medium

Bacterial isolates	Control	Optical density (600 nm) on pH		
		5.5	6.5	7.5
B cereus	0.97 ± 0.00	0.303 ± 0.10	0.511 ± 0.15	0.423 ± 0.11
K. pneumoniae	0.97 ± 0.00	0.117 ± 0.40	0.183 ± 0.10	0.233 ± 0.33
P. aeruginisa	0.97 ± 0.00	0.514 ± 0.72	0.723 ± 0.61	0.711 ± 0.16
Proteus mirabils	0.97 ± 0.00	0.237 ± 0.19	0.373 ± 0.22	0.208 ± 0.13

Table: 8 Effect of pH on fungi growth in spent engine oil medium

fungi isolates		Dried biomass (g/l)		
		5.5	6.5	7.5
A. fumigatus	0.77 ± 0.00	2.20 ± 0.14	3.11 ± 0.25	2.12 ± 0.51
Mucor racemosus	0.57 ± 0.00	3.10 ± 0.22	4.11 ± 0.12	4.23 ± 0.31
R. stolonifer	0.51 ± 0.01	3.12 ± 0.12	4.22 ± 0.10	4.31 ± 0.01

Table: 9. Effect of concentration on turbidity of bacterial growth in spent engine oil medium

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

Table 10. Effect of concentration on fungi growth in spent engine oil medium

Fungi isolates	Dried biomass (g/) on concentration		
	20%	15%	10%
Aspergillus fumigatus	1.62 ± 0.13	3.41 ± 0.31	3.45 ± 0.41
Mucor racemosus	3.27 ± 0.40	5.74 ± 0.13	5.84 ± 1.03
Rhizopus stolonifer	3.11 ± 1.52	6.13 ± 1.11	6.88 ± 1.23

4. Discussion

Petroleum hydrocarbons are important energy resources and also a major pollutant of the environment when use in different area as source of energy. Effect of spent engine oil contamination on microorganisms in the soil in Karu FCT. In this study the total heterotrophic count of bacterial and fungi 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 unfavourable effect of oil contamination, this is similar to study reported by Akomah, and Osayande (2018) that high concentration hydrocarbon effect the microbial population of the soil.

The result obtained from screening of spent hydrocarbon tolerance count of both bacterial and fungi isolated was low to compare to the total heterotrophic count which may be attributed to that most of the bacterial and fungi 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 and fungi using spent hydrocarbon as sole source of carbon as reported by Ebakota *et al.* (2017); Mandri and Lin, (2007) and Unimke *et al*. (2017).

The different bacterial isolated that was observed to utilize spent hydrocarbon as source of carbon were *Bacillus cereus, Klebsiella pneumoniae, Pseudomonas* sp and *Proteus mirabilis* 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.* (2019). Similarly, the fungi isolated and identified that have the ability to withstand high spent hydrocarbon contamination in the soil were *Aspergillus fumigatus, Mucor racemosus* and *Rhizopus stolonifer* which is in agreement with study reporter by Edna *et al*. (2016).

The effect of pH on utilization of spent hydrocarbon which was measured at 600nm showed that *Pseudomonas* aeruginosa and *Bacillus cereus* 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, 2012). Also, the fungi species isolated recorded varying dried biomass showing different rate of survivor or utilization of hydrocarbon it was observed that *Mucor racemosus* and *Rhizopus stolonifer* recorded high dried biomass. This suggest that pH of the soil plays a significant role in survivor utilization of the spent hydrocarbon contaminated soil.

5. Conclusion

From this study high heterotrophic bacterial and fungi count was recorded from different location of the study. The different bacterial isolated were *Bacillus subtills, Klebsiella pneumoniae, Pseudomonas* aeruginosa and *Proteus mirabilis* and fungi isolated were *Aspergillus fumigatus, Mucor racemosus* and *Rhizopus stolonifer.* Both the bacterial and fungi isolated that was screen for

spent hydrocarbon utilization showed different degree of utilization of spent hydrocarbon. It was also observed that pH and concentrations of hydrocarbon plays significant role in degradation of spent hydrocarbon.

Conflict of Interest statement

The authors declare no conflicts of interest

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