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Microbiological and Physicochemical Evaluation of Oil-polluted Soil from Major Auto Mechanic Shops in Port Harcourt Metropolis, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author WJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author APP managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The capability of microorganisms to utilize spent oil in polluted soil from some major auto mechanic workshops in Port Harcourt metropolis as the sole source of carbon and energy was studied. Soil samples were collected from three (3) Auto mechanic workshops (Mile 3 Mechanic Village (N 4º48' 34.07", E 6º59' 10.17"), Ikoku Mechanic Workshop (N 4º47' 54.28", E 6º59' 36.42") and Elekahia Zone H Mechanic Workshop (N4º49' 11.62", E 7º1' 16.58") in Port Harcourt, Rivers State, Nigeria from depths of 0–30 cm, 30–60 cm and 60–90 cm at the same spot from each station. Soil samples were analysed microbiologically and physicochemically using standard methods. The control sample was obtained behind the Biology Building of Rivers State University, Port Harcourt. The results of the total heterotrophic bacterial and fungal counts showed that the microbial load was high at a depth of 0 to 30 cm for all the samples analysed including the control. With 60-90 cm depth, low microbial counts were obtained. The total heterotrophic bacterial counts from the three mechanic workshops ranged from 6.8 X 10^8 to 2.3 X 10^9cfu/g while the total heterotrophic fungal counts

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ranged from 1.3 X 10^6 to 8.0X 10^6 cfu/g. The spent oil-utilizing microbial populations ranged from1.0 X 10^5 to 3.0 X 10^6cfu/g and1.0 X 10^5 to 8.0 X 10^5 cfu/g for the bacterial and fungal counts respectively. Five spent oil utilizing bacterial isolates of the genera, *Pseudomonas*, *Klebsiella*, *Bacillus*, *Micrococcus* and *Proteus* and five fungal isolates of the genera, *Penicillium*, *Candida*, *Rhizopus, Fusarium* and *Aspergillus* were obtained from spent oil-polluted soil in this study. The pollution index of the different physicochemical parameters obtained from the different sample stations was as follows: Electrical Conductivity (EC): Mile 3 had the highest with the mean of 12.50 ±0.0-13.20±0.0µs/cm, while Ikoku had the lowest with mean of 10.99 ± 0.0-11.50 ± 0.0 µs/cm. Ikoku had the highest pH level with mean of 7.0 ± 0.0- 8.5 ± 0.0 while Mile 3 had the lowest pH level with mean of 6.5 ± 0.0-7.59 ± 0.0. Ikoku had the highest Total Organic Carbon (TOC) with mean of 17.55 ± 0.0-20.60 ± 0.0 mg/kg while Elekahia had the lowest with the mean of 16.99 ± 0.0-18.0 ± 0.0 mg/kg. Ikoku had the highest Total Hydrocarbon Content (THC) with mean of 38,862 ± 0.0-40,500 ± 0.0 mg/kg while Mile 3 had the lowest with the mean of 20,550 ± 0.0-30,000 ± 0.0 mg/kg. From the Pollution Index Analysis, Ikoku Auto Mechanic Workshop is more contaminated with Spent oil than Mile 3 and Elekahia.

Keywords: Spent oil; polluted soil; microbial isolates; automechanic workshops; physicochemical parameters.

1. INTRODUCTION

Motor mechanics indiscriminately discharge spent oil on the soil where they carry out the repairs of automobiles. This indiscriminate discharge of spent oil is a major non-point source of oil pollution to the environment. This creates a serious monitoring and control challenge as mechanic workshops and mechanic villages spring up every day and everywhere without plan and policy for management of waste and protection of the environment [1]. One particular area where such indiscriminate discharge is perpetrated is Ikoku in Port Harcourt where spent oil is discharged on soil, drains and hard floors. From these sources, they can be dispersed as a result of rain or water flowing through the drains. Spent oil which is made up of engine oil and lubricating oil is the non-volatile fraction of crude oil. Crude oil is made up mainly of hydrocarbons; metals such as iron, nickel, copper and vanadium are also present [2]. Engine oil also contains additives such as amines, phenols, benzene, calcium, zinc, barium, magnesium, phosphorous, sulfur and lead [3]. Oil-polluted soils are of environmental concern because they are unsuitable for agricultural and recreational purposes and are potential sources of surface and groundwater pollution.

Spent oil has a relatively large amount of hydrocarbons including the highly toxic polycyclic aromatic hydrocarbons [4]. Contamination of soil with spent lubricating oil is increasing due to global increase in the usage of petroleum products. Environmental pollution with petroleum and petrochemical products has attracted much attention in the recent decades. The presence of various types of automobiles and machinery has resulted in an increase in the use of lubricating oil. Spillage of used motor oils such as diesel or jet fuel contaminates our natural environment with hydrocarbons [5]. Release of hydrocarbons into the environment whether accidentally or due to human activities is the main cause of water and soil pollution [6].

Crude oil is physically, chemically and biologically harmful to soil microorganisms because it contains many toxic compounds in relatively high concentrations (e.g. polycyclic aromatic hydrocarbons, benzene and its substituted cycloalkane rings) [7].

Generally, petroleum hydrocarbon compounds bind to soil components and are difficult to remove or degrade [8]. The disposal of spent oil into open vacant plots, farms, gutters and water drains is an environmental risk [9].

Since spent oil is liquid, it easily migrates into the environment and eventually pollutes either water or soil [10].

Contamination of soils with spent oil leads to a significant reduction of soil moisture [11]. As the usage of petroleum hydrocarbon products increase, soil contamination with diesel and engine oil is becoming one of the major environmental problems andas a result, environmental pollution with used engine oil continues to generate interest [4]. Used motor oil contains more metals, toxic and carcinogenic polycyclic aromatic hydrocarbons (PAHs). Thus,

it constitutes a potential threat to humans, animals and vegetation [12]. Theaim of this study was toassess the physicochemical and microbiological analyses of oil-polluted soil from Major Auto Mechanic Shops in Port Harcourt Metropolis, Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in the following locations: Mile 3 Mechanic Village (N 4º48' 34.07'', E 6º59' 10.17''), Ikoku Mechanic Workshop (N 4º47' 54.28'', E 6º59' 36.42'') and Elekahia Zone H Mechanic Workshop (N4º49' 11.62'', E 7º1'16.58'') in Port Harcourt, Rivers State, Nigeria.

2.2 Sample Collection

The samples were obtained from three (3) Auto mechanic workshops in Port Harcourt, Rivers State, Nigeria. Soil samples were collected from depths of 0 – 30 cm, 30 – 60 cm and 60 – 90 cm at the same spot from each station with the use of a core borer. The control sample was obtained from behind New Biology Building of Rivers State University, Port Harcourt. The soil samples were labelled as shown below:

Station 1: Mile III Mechanic Village
Station 2: Ikoku Road Auto Mechanic Workshop
Station 3: Elekahia Zone H Extension Mechanic Village
Control: Behind New Biology Building, RSU

2.3 Sample Preparation and Analysis

2.3.1 Soil samples

The soil samples collected were stored in polyethene bags following standard procedures [4].

2.4 Microbiological Analysis

2.4.1 Total heterotrophic bacterial and fungal counts

The samples were microbiologically analysed using spread plate method. About 1 g of the soil samples were serially diluted. An aliquot was plated on nutrient agar and incubated at 37ºC for 24hrs and Sabouraud dextrose agar at 27ºC for 72-120 hrs. The bacterial and fungal counts were thereafter enumerated.

2.4.2 Isolation and characterization of isolates

Pure isolates of bacteria were obtained usingstreak platemethod on nutrient agar and stored (4°C) using agar slants. Pure isolates of fungi were transferred with an inoculating needle on Sabouraud dextrose agar and stored (4°C) in peptone and glucose. Bacterial colonies were identified by morphological and biochemical techniques using [13] and fungal colonies were determined macroscopically and microscopically.

2.4.3 Petroleum utilizing bacteria and fungi

Mineral salt agar (MA) was the medium used and was formulated for isolation of both hydrocarbons utilizing bacteria and fungi. The medium had the following composition: K_2HPO_4 (0.5 g), $MgSO_4$ $.7H_2O$ (0.3 g), NaCl(0.3 g), $MnSO_4.H_2O$ (0.2 g), $FeSO_4.6H_2O$ (0.02 g), $NaNO_3$ (0.03 g), $ZnCL_2$ (0.3 g) and Agar (15 g), 1000 ml of distilled water.

2.4.4 Enumeration and isolation of petroleum utilizing bacteria

The mineral salt medium of Williams and Wilcox [14] was used for the enumeration of petroleum utilizing bacteria and fungi. The mineral salt agar used for enumeration of hydrocarbon utilizing bacteria was amended with 250 mg of Amphotericin B sold as fungi zone. The medium was sterilized by autoclaving at 121°C for 15psi before dispensing into sterile Petri dishes. An aliquot (0.1 ml) of the 10^{-4} dilution was inoculated unto the gelled mineral salt agar (MSA) in duplicate. Sterile filter paper (Whattman no. 1) saturated with 2 ml crude oil was placed inside the cover of the Petri dish. The Petri dish was closed, inverted and incubated at 28°C for 5-7days. The filter paper saturated with crude oil served as a sole source of carbon [15]. Colonies formed in the duplicate plates were counted and the mean values were recorded in cfu/g. Bacterial isolates growing on MSA were sub-cultured into a fresh nutrient agar plate. Pure cultures were preserved with 10% (v/v) glycerol suspension [4].

2.4.5 Enumeration and isolation of petroleum utilizing fungi

Enumeration of total culturable petroleum utilizing fungi was done using mineral salt agar (MSA). The compounded medium for hydrocarbon utilizing fungi was amended with 250 mg of tetracycline to inhibit the growth of

hydrocarbon utilizing bacteria [16]. An aliquot from 10^{-3} was inoculated in duplicate by spread plating and inverted over sterile filter papers moistened with 2 ml crude oil, which were placed on the cover of the Petri dishes. The plates were inverted and incubated at 28°C for 5-7 days [17].

2.4.6 Physicochemical analyses of soil

The pH of the soil samples was determined using a pH meter (Jenway 3051) in 1:1 soil solution in distilled water in accordance with the Manufacturer's instructions andthe temperature using a thermometer of a range, 0 – 100ºC. The carbon organic content, total nitrogen content, potassium content and available phosphorous were determinedusing standard methods. Conductivity was determined using a conductivity meter (PW 9504 Philips, USA) witha cell constant of 1.2. The total hydrocarbon content of the soil was extracted using n-hexane: dichloromethane solvent systems (1:1) [18].

2.5 Statistical Analysis

The Parameters obtained from the various stations were analysed using one-way ANOVA with SPSS package version 25 and the means were separated using the Duncan multiple range test of the different samples.

3. RESULTS

3.1 Microbiological Results

3.1.1 Total Heterotrophic Bacteria (THB)

The total heterotrophic bacterial (THB) counts in Station A (Mile Three Mechanic Village) for 0-30, 30-60 and 60-90 depths were as follows: 1.71 x 10^9, 9.0 x 10^8 and 6.8 x 10^8 ,Station B (Ikoku Road Auto-mechanic Workshop) for 0-30, 30-60 and 60-90 depths were as follows: 2.3 x 10^9, 2.0 x 10^9 and 1.35 x 10^9 and Station C (Elekahia Zone H Extension Auto-mechanic Workshop) for 0-30, 30-60 and 60-90 depths were as follows: 1.78 x 10^9, 1.18 x 10^9 and 8.3 x 10^8while the control(Behind New Biology Building, Rivers State University, Port Harcourt) for 0-30, 30-60 and 60-90 depths were as follows: 2.1 x 10^9, 1.07 x 10^9 and 6.8 x 10^8, respectively. The total heterotrophic fungal (THF) counts in Station A for 0-30, 30-60 and 60-90 depths were as follows: 6.1 x 10^6, 2.9 x 10^6 and 1.6 x 10^6, Station B, 4.8 x 10^6, 1.8 x 10^6 and 1.3 x 10^6 and Station C, 1.7 x 10^6, 1.3 x 10^6 and 8 x 10^5 while the control, 2.5 x 10^6, 2.2 x 10^6 and 1.5 x 10^6 respectively. Ranged from 7.2 x 10^6 – 9.2 x 10^6. The Petroleum Utilizing bacterial (PUB) counts in Station A for 0-30, 30-60 and 60-90 depths were as follows: 1.8 x 10^6, 6 x $10^5$and 4 x 10^5, Station B, 3.0 x 10^6, 2.0 x 10^6 and 1.3 x 10^6 and Station C, 1.8 x 10^6, 1.1 x 10^6 and 8 x 10^5while the control, 3.0 x 10^5, 1.0 x 10^5 and 0, respectively. Ikoku had the highest Petroleum Utilizing Bacteria with mean of 6.11±0.0 - 6.47±0.0 cfu/g, while Elekahia had the lowest mean of 5.60±0.0 - 6.25±0.0 cfu/g.

The Petroleum Utilizing Fungal (PUF) counts in Station A for 0-30, 30-60 and 60-90 depths were as follows: 5 x 10^5, 3 x 10^5 and 1 x 10^5, Station B, 8 x 10^5, 3 x 10^5 and 2 x 10^5 and Station C, 7 x 10^5, 7 x 10^5 and 2 x 10^5 while the control, 5 x 10^5, 2 x 10^5 respectively. Mile 3 had the highest Petroleum Utilizing Fungi with mean of 5.30±0.0- 5.90±0.0 cfu/g while Ikoku had the lowest with the mean of 5.00±0.0-5.69±0.0 cfu/g.

Table 1 shows the morphological and biochemical Identification of Petroleum Utilizing Bacteria isolated from the different stations such as *Proteus*, *Pseudomonas*, *Micrococcus*, *Klebsiella* and *Bacillus species.*

Table 2 shows the microscopic and macroscopic identification of Petroleum Utilizing fungi isolated from the different stations such as *Aspergillus*, *Rhizopus*, *Fusarium*, *Penicillium* and *Candida species.*

Table 3 shows the Physicochemical Parameters from Samples A, B, C and D. The pollution index of the different physicochemical parameters obtained from the different sample stations were as follows: Electrical Conductivity (EC): Mile 3(A) had the highest value with mean of 12.50 ± 0.0 - 13.20 ± 0.0µs/cm, while Ikoku (B) had the lowest mean of 10.99 ± 0.0 - 11.50 ± 0.0 µs/cm. Ikoku had the highest pH level with mean, 7.0 ± 0.0-8.5 ± 0.0, while Mile 3 had the lowest pH level with mean, 6.5 ± 0.0- 7.59 ± 0.0. Ikoku had the highest TOC with mean of 17.55 ± 0.0-20.60 ± 0.0 mg/kg, while Elekahia (C) had the lowest with the mean of 16.99 ± 0.0-18.0 ± 0.0 mg/kg. From the Pollution Index analysis, Ikoku Auto Mechanic Workshop was more contaminated than all the other stations.

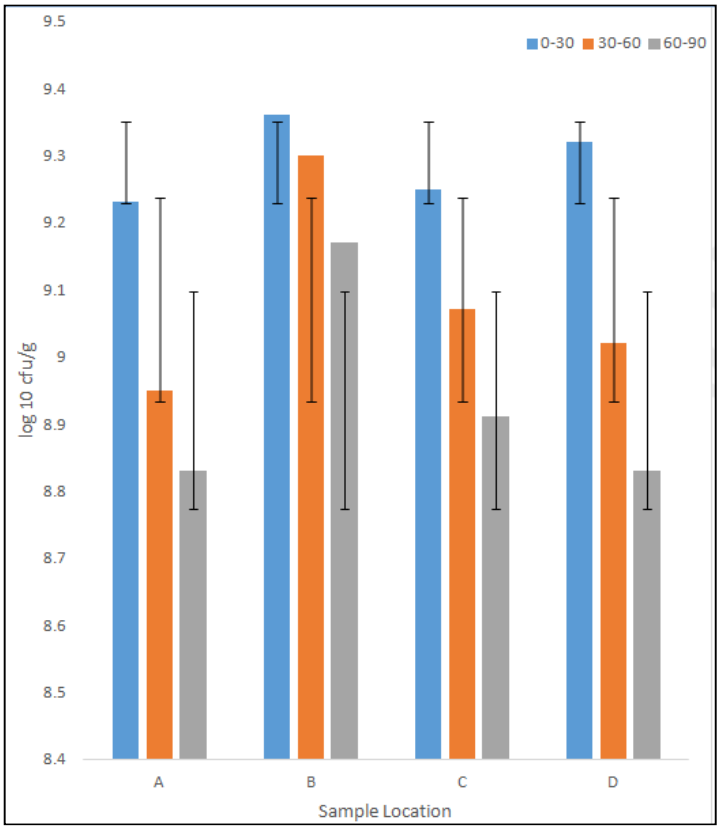

Fig. 1. Total heterotrophic bacterial counts in different sample locations
Key: A = Mile 3, B = Ikoku, Sample C = Elekahia, D = Control

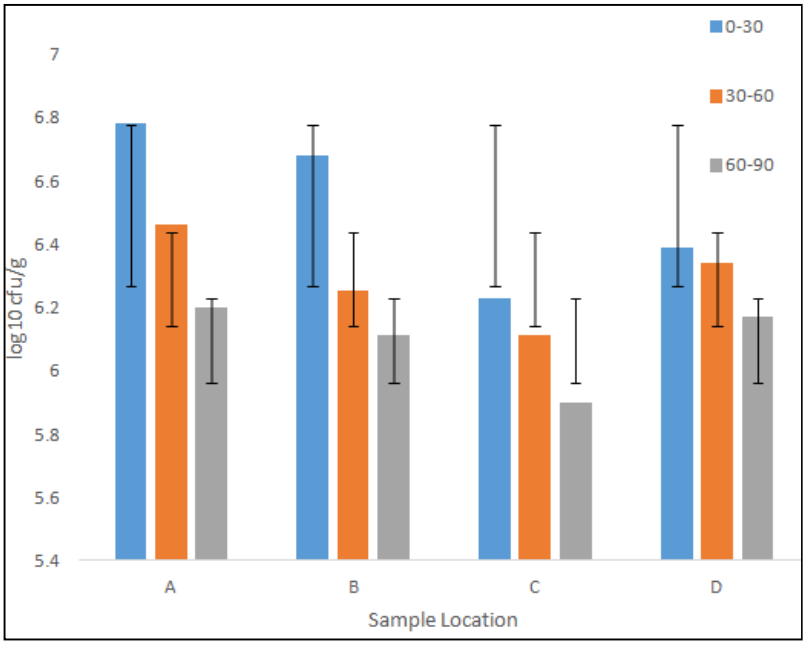

Fig. 2. Total heterotrophic fungal counts in different sample locations
Key: A = Mile 3, B = Ikoku, Sample C = Elekahia, D = Control

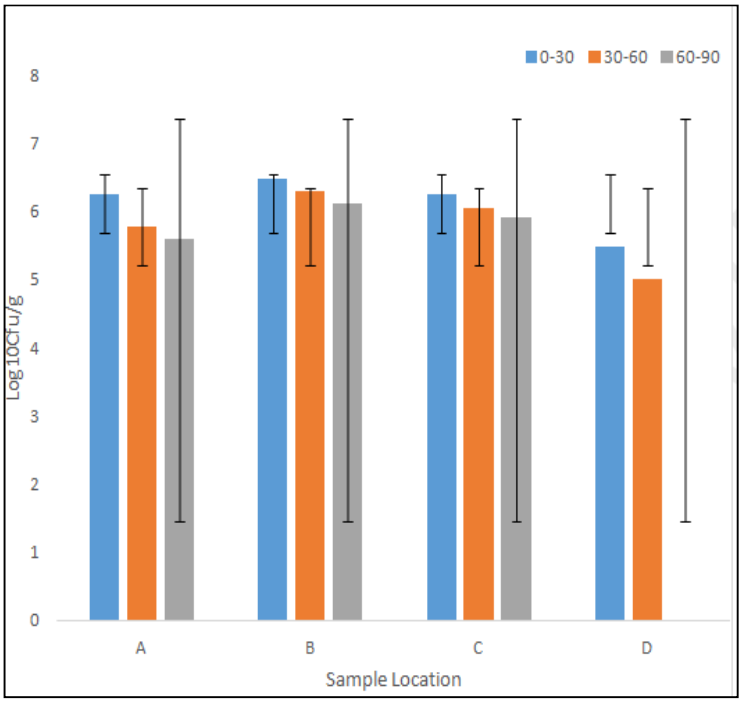

Fig. 3. Petroleum utilizing bacterial counts in different sample locations
Key: A = Mile 3, B = Ikoku, Sample C = Elekahia, D = Control

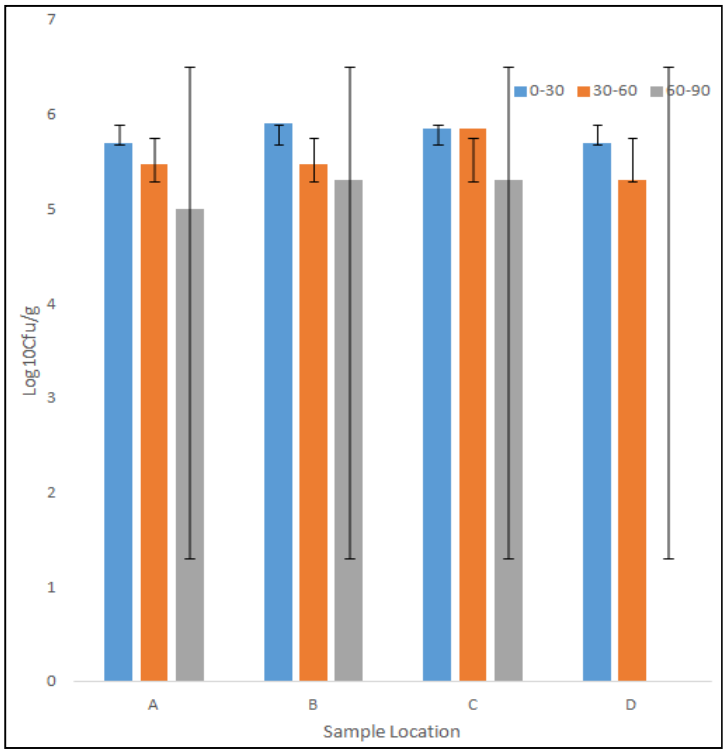

Fig. 4. Petroleum utilizing fungal counts in different sample locations
Key: A = Mile 3, B = Ikoku, Sample C = Elekahia, D = Control

Table 1. Morphological and biochemical identification of petroleum utilizing bacterial isolates

Colonial morphology				Biochemical reaction								Sugar fermentation				
Isolate	Shape	Colour	Surface	Gram reaction	Spore formation	Motility	Coagulase	Catalase	Oxidase	Indole	Methylred	Vogues proskauer	Glucose	Lactose	Sucrose	Probable identification
1.	Rod	Yellowish	Smooth	+	+	-	+	+	-	-	-	-	-	-	-	*Bacillus species*
2.	Rod	Milk	Smooth	-	-	+	+	+	-	-	+	-	+	-	-	*Proteus species*
3	Rod	Greenish	Smooth	-	-	+	+	+	+	-	-	-	-	-	-	*Pseudomonas species*
4.	Rod	Milk	Smooth	-	-	-	+	+	-	+	-	+	+	+	+	*Klebsiella species*
5	Rod	Greenish	Smooth	-	-	+	+	+	+	-	-	-	-	-	-	*Pseudomonas species*
6	Rod	yellow	Smooth	+	-	-		+		_	-	-	+	+	+	*Micrcoccus species*

Table 2. Microscopic and macroscopic identification of petroleum utilizing fungal isolates from sample locations

Isolate no.	Cultural characteristics	Microscopic characteristics	Probable genera
PUF1	Powdery green colonies that is yellow at reserve side	Septate and branched hyphae with conidia in chains	*Aspergillus species*
PUF2	White smooth colonies that are yeast like in appearance	Elongate budding yeast like cells, branched pseudohyphae.	*Candida species*
PUF3	Dense white cottony colonies that is white at the reverse side	Non septate hyphae sporangiospore, rhizooids ovoid brown sporangiospore, sporangium containing columellate	*Rhizopus species*
PUF4	Whitish cottony colonies	Multi-segmented canon shape spore and branched conidiospores	*Fusarium species*
PUF5	Powdery yellowish green dense mycelia and light yellow at the reverse side	Spherical budding yeast like cells	*Penicillium species*

Table 3. Physicochemical parameters from samples A, B, C and D

Sample	Depth (cm)	EC (µs/cm)	Phosphate (Mg/kg)	NH_4 (Mg/100 g)	Sulphate (Mg/kg)	pH	K (Mg/kg)	TN (%)	TOC (Mg/kg)
A	0-30	13.20	5.78	40.00	757.78	7.59	167.40	0.25	18.60
	30-60	13.00	5.35	39.58	725.00	7.00	159.00	0.36	18.00
	60-90	12.50	5.12	36.00	688.56	6.50	150.57	0.48	17.57
B	0-30	11.50	5.08	45.50	688.34	8.50	196.20	1.03	20.60
	30-60	11.00	5.07	40.00	654.89	7.20	176.50	1.00	19.89
	60-90	10.99	4.97	30.89	646.56	7.00	170.99	0.58	19.05
C	0-30	12.50	3.78	35.85	557.78	7.80	193.20	2.89	18.00
	30-60	12.00	3.56	34.76	549.55	6.90	176.50	1.55	17.58
	60-90	11.59	3.35	33.99	539.54	6.70	156.56	1.35	16.90
D	0-30	72.53	4.88	30.50	565.78	6.45	100.40	1.25	20.60
	30-60	72.43	4.07	28.99	540.56	6.40	98.40	1.10	16.57
	60-90	71.00	4.00	29.00	500.99	6.15	76.87	1.00	18.50

4. DISCUSSION

The results of the total heterotrophic bacterial and fungal counts showed that the microbial load was highest at 0 to 30 cm depth for all the samples analysed including the control. At 60- 90 cm depth, low microbial counts were obtained. Samples obtained from Ikoku mechanic workshop (Sample B) had the highest number of microbial load followed by samples A and C. This may be as a result of anthropogenic activities in the various workshops as well as changes in physicochemical parameters of soil which varied in the locations depending on the nature of soil and its constituents which can influence the microbial load.

Petroleum utilizing bacterial and fungal isolates revealed that sample B had the highest number of petroleum utilizing bacterial and fungal isolates followed by samples A and C. The microbial survey of different polluted workshops also revealed that depth has a significant effect on the microbial load because highest number of counts in all the samples were recorded at depth of 0 to 30 cm while lowest counts at 60 to 90 cm. This concurred with results observed by Umanu and Omoikhudu [19] who worked on oil degradation assessment of bacteria isolated in Ota, Nigeria.

About five spent oil utilizing bacterial isolates, namely *Pseudomonas*, *Klebsiella*, *Bacillus*, *Micrococcus*, and *Proteusspecies* and five fungal isolates namely, *Penicillium*, *Candida*, *Rhizopus*, *Fusarium* and *Aspergillus species* were obtained from spent oil-polluted soil in this study. The result is in correlation with the work reported by several authors [20,21] who isolated *Pseudomonas*, *Bacillus*, *Micrococcus* and other bacterial strains from engine oil-contaminated soil. *Pseudomonas*, *Bacillus* and *Rhodococcus species* were isolated from engine oil contaminated soil as reported by Ogunbayo et al. [22]. Some of the fungal isolates have earlier been reported as hydrocarbon utilizers by several authors [23,24]. The result of this study showed that these microorganisms could be used in bioremediation of spent oil contaminated soil from Mile 3 and Elekahia. From the Pollution Index Analysis, Ikoku Auto Mechanic Workshop was more contaminated than all the other stations probably as a result of greater activities of Mechanics in Ikoku compared to the other Auto Mechanic Shops.

5. CONCLUSION

The survey carried out on different automobile mechanic workshops revealed the presence of bacteria and fungi capable of metabolizing spent oil in the studied area. This indicates that mechanic workshops can undergo natural attenuation and clean-up of spent oil over time in the case of minor oil pollution or pollution with similar contaminants. This is however dependent on the subsequent discontinued introduction of these pollutants into the same environment because if the assimilative capacity is exceeded, natural attenuation and clean-up of spent oil by the normal flora may not be effective.

It was also observed in this study that auto mechanic workshops with high concentration of nutrients such as nitrate; phosphate and sulphate have low levels of pollution which means that, application of appropriate concentrations of the essential nutrient sources could accelerate biodegradation of spent oil pollutant in soil. The petroleum utilizing bacterial and fungal isolates obtained in this study could be exploited and used to bio augment oil spill clean-up in similar environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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