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Microbial Determination of Hydrocarbon Polluted Soil in Some Parts of Niger State, Nigeria

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Abstract

Spent engine oil management in Nigeria is not well supervised and the indiscriminate discharges into the soil drains and water of the pollutants. This has attendant implications on soil and water quality, contamination on soil ecosystem alters soil biochemistry, immobilizes nutrients. The study was designed to determine the microbial load and identify some of the microorganisms present in the hydrocarbon polluted soil were *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus megaterium, Pseudomona aeroginosa, Aspergilus flavus, Aspergilus fumigatus, Aspergilus niger, Fusarium oxysporium* and *Penicillium notatun.* Minna, had more bacteria counts within the rhizosphere of the plants found within the vicinity of the automobile workshop with the Mean count of $1.42\times10^7\pm2.41\times10^7$ cfu/g, while Bida had the lowest bacterial count in the Automobile workshop $1.56\times10^5\pm2.13\times10^5$ cfu/g when compared to Minna and Suleja. For fungi, Bida had the highest count with a mean of $7.10\times10^6\pm1.20\times10^7$ cfu/g while Minna had the lowest count with a mean count of $1.02\times10^6\pm1.72\times10^6$ cfu/g in relative terms to Suleja, Bida and Tegina. *Bacillus subtilis* had the highest frequency of occurrence of (37.50%) while *Staphylococcus epidermidis* had the lowest occurrence (9.38%), in soil remediated with *M. officinalis* alone *Bacillus subtilis* had the highest frequency of occurrence (42.85%) and *Staphylococcus epidermidis* had the lowest frequency of occurrence (4.54%). That of fungi, *A. niger* had the highest frequency of occurrence (47.61%) while the fungi with the least occurrence was *P. notatum* (4.76%) with *F. oxysporium* with occurrence of 9.52% (Table 4), in soil remediated with *M. officinalis* alone (SP1), *A. niger* had the highest frequency of occurrence (52.17%) while *P. notatum* had the lowest frequency of occurrence (4.34%), in soil remediation. *A. niger* had the highest frequency of occurrence (45.83%) while *P. notatum* had the lowest frequency of occurrence (4.16%), soil polluted with 50cl of spent engine oil. *A. niger* had the highest frequency of occurrence (50.00%) while *P. notatum* and *A. flavus and F. oxysporium* had the lowest frequency of occurrence (11.11%). This *Bacillus subtilis, Bacillus megaterium, Pseudomona aeroginosa, Aspergilus niger* and *Aspergilus flavus,* showed great potential to withstand, tolerate and degrade spent engine oil (SEO), hence can used for biodegradation of hydrocarbon.

Keywords

Microorganism, Soil, Contamination

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1. Introduction

Spent engine oil can enter the environment through improper disposal by automobile mechanics when servicing cars. The increase in the number of vehicles in Nigeria has necessitated a higher production and use of engine oil (EO). This has subsequently given rise to the generation of large quantities of spent engine oil (SEO) at the time of servicing the vehicles. This SEO is considered as ordinary waste by majority of

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workers of the automobile workshops in Nigeria, who dispose this off on soil surface [1]. Spent engine oil management in Nigeria is not well supervised hence the indiscriminate discharge into the soil drains and sometimes open water. This has attendant implications on soil and water quality, contamination on soil ecosystem alters soil biochemistry, immobilizes nutrients and creates oxygen tension [2]. Spent engine oil contains impurities in the course of usage and handling; toxic and harmful substances such as benzene, lead, cadmium, polycyclic aromatic hydrocarbons (PAHs), zinc, arsenic, polychlorinated biphenyls (PCBs) etc. which are hazardous and detrimental to the soil and the surrounding environment [3-5].

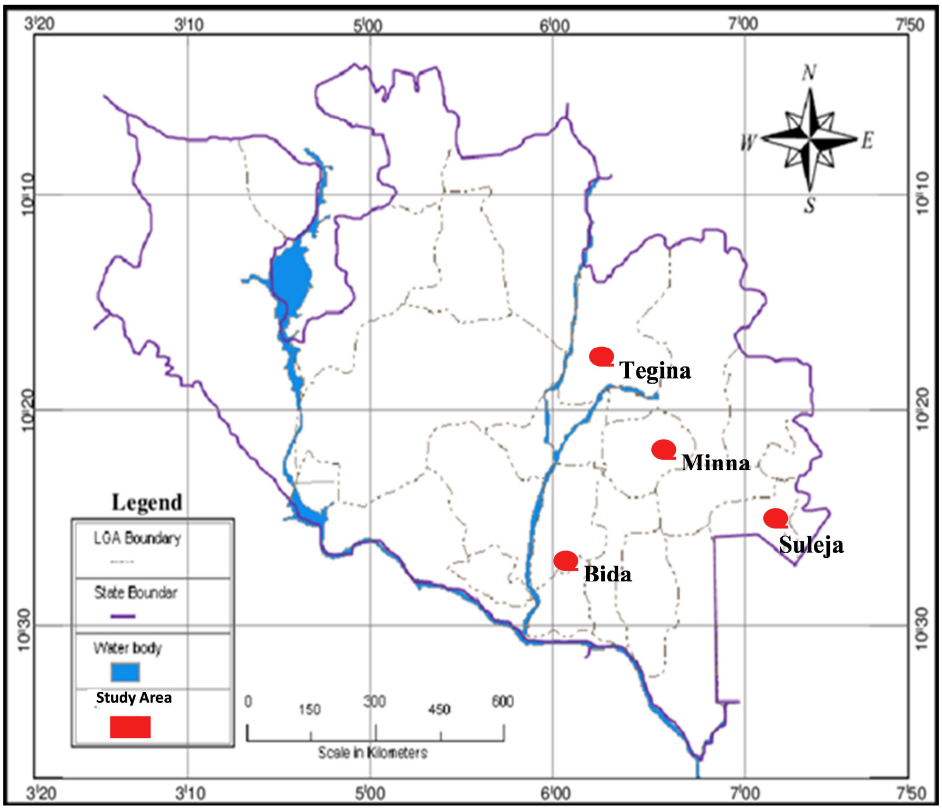
With increasing number of automobile workshops in Niger State, and many more under construction, and the increasing number of vehicles being serviced or repaired at the automobile workshops, it can therefore be established that the amount of SEO from vehicles in Niger State is on a steady increase. In recent time government of some North Central States, including Niger State is evacuating automobile workshop from some areas to make room for the sighting of sporting centers and more lucrative ventures. The problem is the restoration of such sites and call for the immediate action. The aim of this study was to determine the microorganisms found in the hydrocarbon polluted soil in some parts of Niger state.

2. Materials and Methods

2.1. Description of Study Sites

Four different study sites were used for this study namely, Minna, Bida, Suleja and Tegina in Niger State, Nigeria Niger State lies on latitude 80° to 11° 30' North and Longitude 03° 30' to 07° 40' East. The State is bordered to the North by Zamfara State, West by Kebbi State, South by Kogi State, South West by Kwara State, North-East by Kaduna State and South East by the Federal Capital Territory (FCT). The State also has an International Boundary with the Republic of Benin along Agwara and Borgu Local Government Areas (LGAs) to the North West. Land mass is 76, 469.903 Square Kilometers which is about 10% of the total land area of Nigeria out of which about 85% is arable (Niger State Bureau of Statistics, 2012). The majority of the populace in the State (85%) are farmers while the remaining 15% are involved in other vocations such as white collar jobs, business, craft and arts.

Niger State experiences distinct dry and wet seasons with annual rain fall varying from 1,100mm in the northern parts to 1,600mm in the southern parts. The maximum temperature (usually not more than 34°C) is recorded between March and June, while the minimum is usually between December and January. The rainy seasons last for about 120 days in the northern parts and about 150 days in the southern parts of the State [6].



Source: Department of Geography, Federal University of Technology Minna.

Figure 1. Map of Niger State Showing Study Area.

2.2. Enumeration of Bacteria

Bacteria were enumerated by spread plate technique, by inoculating 0.1ml of serially diluted sample onto Nutrient agar (NA) plates. Spent engine oil (SEO) degrading bacteria were enumerated on spent engine oil agar (SEOA) (1.2g KH_2PO_4, 1.8g K_2HPO_4 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 0.01g $FeSO_4.7H_2O$ and 20g agar per liter at pH 7.4; supplemented with 0.1% SEO). The Nutrient agar plates were incubated at 35±2°C for 24-48 hours while the SEOA plates were incubated at 30±2°C for 5-7 days as described by Ijah, Safiyan and Abioye [7]. Colonies, which appeared on the plates, were counted and expressed as colony forming units per gram of soil (CFU/g). Pure cultures were obtained by repeated sub-culturing and maintained on agar slants for further characterization and identification [8, 9].

2.3. Enumeration of Fungi

A 0.1ml aliquot of appropriate dillutions of SEO polluted soil sample was inoculated into replicate Petri dishes containing Sabouraud dextrose agar and incubated for 3-5days at room temperature. An oil agar medium was used for isolation of SEO utilizing mycoflora, and incubated at room temperature for 9days as reported by [10]. Colonies formed were counted and expressed as cfu/g. Pure cultures were obtained by repeated sub-culturing and maintained on agar slants for further characterization and identification.

2.4. Characterization and Identification of Bacteria

The bacterial isolates were identified based on the Taxonomic Schemes of [11] and characterized using standard biochemical tests. Gram staining and the biochemical tests were carried out using morphological and biochemical techniques using the taxonomic scheme of Bergey's manual of determinative bacteriology [8, 9]. Some of the tests carried out were;

2.5. Characterization and Identification of Fungi Isolates

The fungi isolates were characterized based on the color of aerial and substrate hyphae, shape and kind of asexual spores, presence of foot cell, sporangiophore, conidiophores, and characteristics of spore head. A small portion of mycelial growth were carefully picked and placed in a drop of lactophenol cotton blue on a slide and covered with cover slip. After microscopic examination, the fungi isolates were identified by comparing their characteristics with those of known taxa using the schemes of [12] and [9].

2.6. Screening of Bacterial Isolates for Potential to Utilize Spent Engine Oil

Selected bacterial isolates were grown separately in nutrient broth at 37°C for 24h. The utilization of spent lubricating oil as the sole carbon source and energy by the selected bacterial isolates were determined using the mineral salt medium (MSM) of [13]. Five millilitres (5ml) of mineral salts medium were dispensed in each bottle containing 0.05ml of spent lubricating oil. After sterilization at 121°C for 15 minutes, the bottle was allowed to cool before being inoculated with 0.1ml of Nutrient broth grown culture bacterial isolates. The bottles were incubated at room temperature (28±2°C) for 10 days according to the methods of [14]. The growth of the organism in the oil medium at the end of incubation was determined by the use of spectrophotomer to examine the degree of turbidity of the oil medium.

2.7. Determination of Total Petroleum Hydrocarbon (TPH)

This was determined gravimetrically by diethyl ether cold extraction method of [15]. Ten (10) grams of soil sample was weighed into 250ml capacity conical flask and 50ml of diethyl ether was added, and shaken for 30minutes in an orbital shaker. The diethyl ether extract was filtered with Whatman No. 1 filter paper, the liquid phase of the extract was measured at 420nm wavelength using spectrophotometer. The TPH in soil was estimated with reference to the standard curve. TPH data were fitted to first- order kinetics model of [16] and [17].

$$y = ae\text{-}kt$$

Where: y represents the residual hydrocarbon content in soil (g kg-1), a stands for the initial hydrocarbon content in soil (g kg^{-1}), 'k' is the biodegradation rate (d-1) and 't' is time (days). The model the biodegradation rate and half-life of hydrocarbons within the soil. Half-life was then calculated using the model of [16] as Half life = ln(2)/k. This model was using the assumption that the degradation rates of hydrocarbons positively correlate while using hydrocarbon pool size in the soil.

3. Results and Discussion

3.1. Total Aerobic Heterotrophic Bacterial (THB) Counts of Soil from Automobile Workshops

The result reveals that the automobile workshop in Minna, had more bacteria counts within the rhizosphere of the plants found within the vicinity of the automobile workshop with the Mean count of $1.42\times10^7\pm2.41\times10^7$ cfu/g and Tegina had the lowest bacteria counts that was found within the rhizosphere with a mean count of $6.75\times10^6\pm1.15\times10^7$ cfu/g when compared to the mean count of Bida and Suleja (Figure 1). Statistical analysis revealed that there was a significant difference ($p<0.05$) across the column of the different location. The result of the bacterial count found in the automobile workshops vicinity revealed that Suleja had the highest number bacteria found within their vicinity and the mean count was $8.10\times10^6\pm1.38\times10^7$ cfu/g while Bida had the lowest bacterial count within the automobile workshop vicinity (Figure 1) with a mean of $4.73\times10^6\pm8.03\times10^6$ cfu/g when compare to Tegina and Minna. Statistical analysis revealed that there was significant difference ($p<0.05$) across the column i.e different locations. The result of bacterial count found within the various workshop showed that there counts were generally low when compared to the counts from both rhizosphere and the automobile workshop vicinity count, the mean bacterial count of the automobile workshop was more in Tegina ($2.37\times10^6\pm4.01\times10^6$ cfu/g), while Bida had the lowest bacterial count in the Automobile workshop ($1.56\times10^5\pm2.13\times10^5$ cfu/g) when compared to Minna and Suleja (Figure 1). Statistical analysis revealed that there was no significant difference ($p>0.05$) across the column which means there was no significant difference in the bacterial count from the various automobile workshops.

The high number of bacteria found within rhizosphere soil of plants within the vicinity of the automobile workshop in Minna might be as a result of favorable physical and chemical components of the soil that supports their growth and development and availability of growth nutrients which enables both obligate and facultative organisms to survive and degrade the spent engine oil within the root region of the plant, which can also be a major pointer that the pollutant (SEO) might have not seeped too deeply into the soil, the plants found might have certain compounds that supports their proliferation, also the soil type and its physicochemical parameters might be a major factor too.

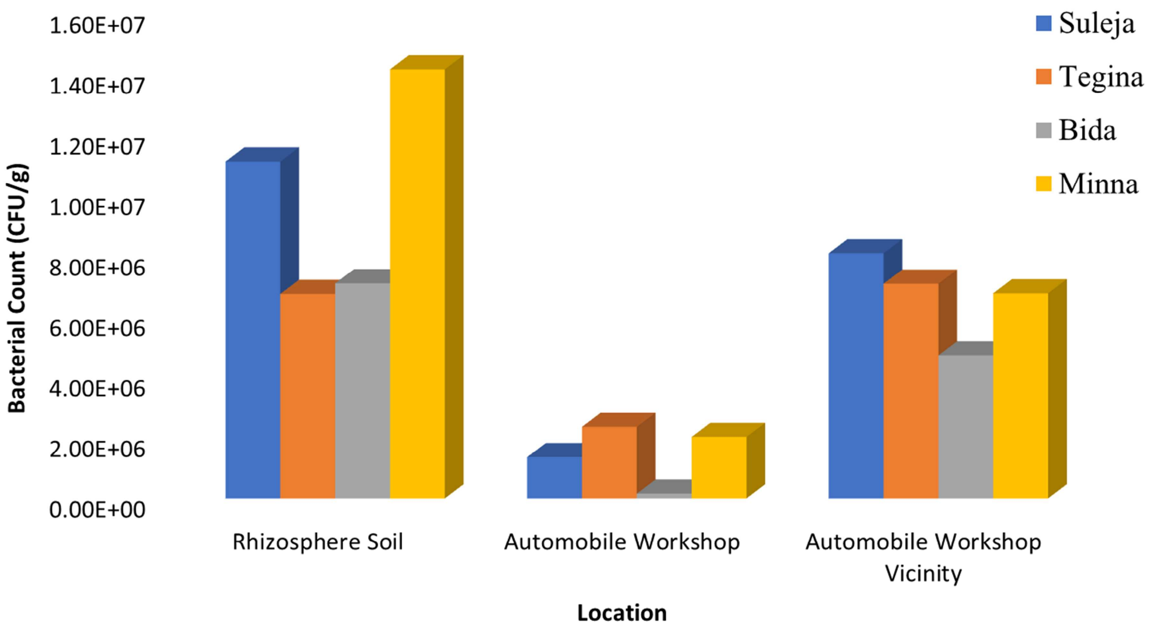


Figure 2. Total aerobic heterotrophic bacterial (TBH) counts of Soil from various Automobile Workshops.

3.2. Total Fungal Counts of Soil from Automobile Workshops

The result from the total fungal count within the rhizosphere soil showed that Bida had the highest count with a mean of ($7.10\times10^6\pm1.20\times10^7$ cfu/g) while Tegina had the least count ($1.70\times10^6\pm2.86\times10^6$ cfu/g) when compared to Minna and Suleja (Figure 2). Statistical analysis revealed that there is significant difference ($p<0.05$) across the column with different location. Automobile workshop vicinity had a relatively lower number of fungal count when compared to the rhizosphere soil, Suleja had the highest fungal count with a mean of ($1.68\times10^6\pm2.87\times10^6$cfu/g) while Minna had the lowest count with a mean ($1.02\times10^6\pm1.72\times10^6$ cfu/g) in relative terms to Suleja, Bida and Tegina (Figure 2). Statistical analysis revealed that there was no significant difference ($p>0.05$) across the column that is, between Suleja, Tegina, Bida and Minna. The total fungal count of soil within the automobile workshop had the lowest fungal count when compared to the rhizosphere soil and the automobile workshop vicinity, with Bida having the highest count with a mean of ($1.36\times10^6\pm2.26\times10^6$ cfu/g) while Bida and Minna having the lowest count (Figure 2) with a mean count ($3.41\times10^5\pm5.71\times10^5$ cfu/g). Statistical analysis showed that there was no significant difference ($p>0.05$) across the column.

The general decrease in fungal count across all the soil samples collected from the rhizosphere, automobile workshop vicinity and the automobile workshop might be as a result of the effect of the SEO that might inhibit the growth of strict aerobic organism hence the only hydrocarbon degrading fungi will survive, due to the high growth rate of bacteria within the same soil, some bacteria has the ability to produce compounds that inhibit the growth and proliferation of fungal, the pH of the soil heavily polluted with SEO has shown to be acidic and thus only few fungi can survive in such environments. The limited amount of nutrients within the automobile workshop soil can also lead to competition for the limited supply hence only the once with adaptive and protective mechanisms will grow.

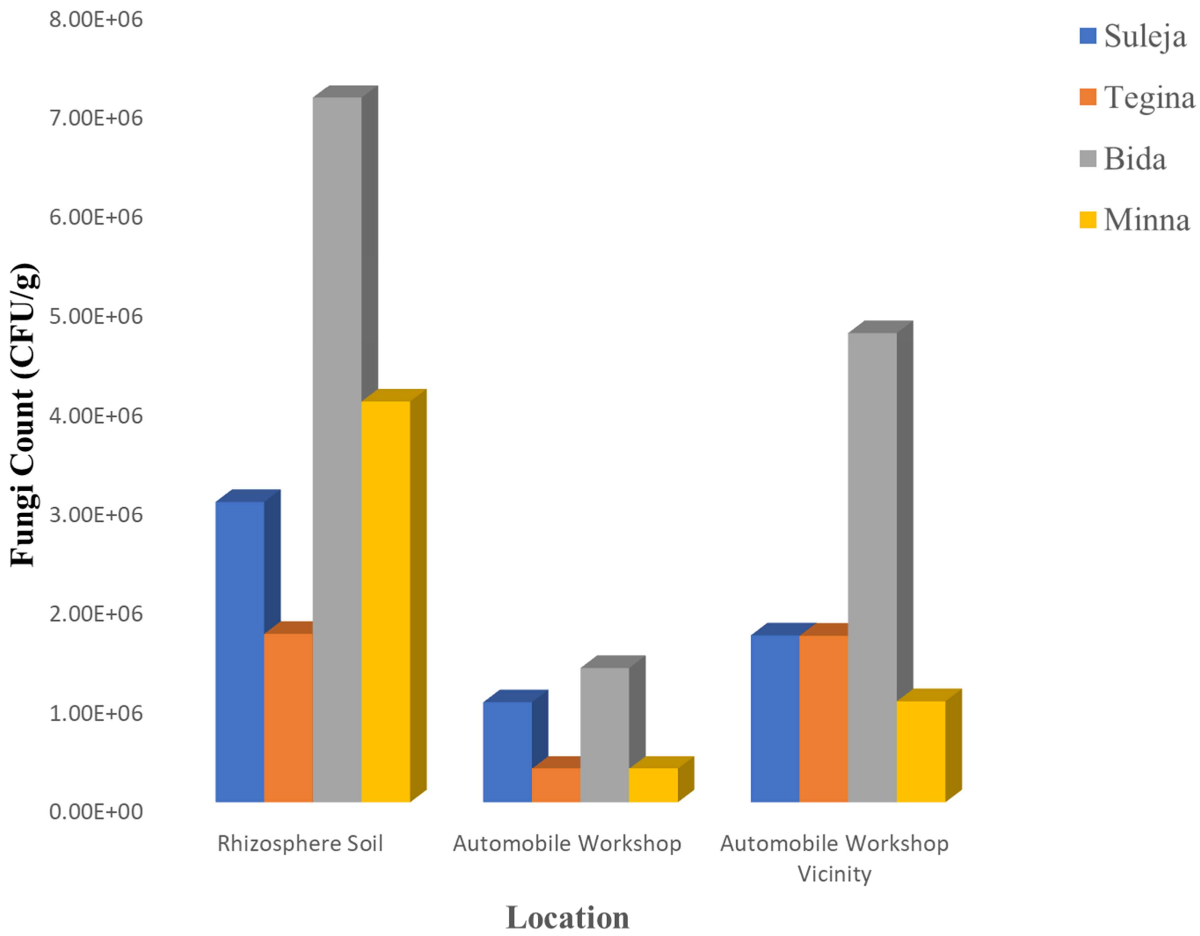


Figure 3. Total Fungal counts of Soil from various Automobile Workshops.

3.3. Identification and Frequency of Occurrence of Microorganisms in the Experimental Setup

3.3.1. Bacteria

Bacterial isolated from the experimental setup were Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeroginosa, and Staphylococcus epidermidis (Table 1). In soil alone (SA) Bacillus subtilis had the highest frequency of occurrence of (37.50%) followed by Bacillus megaterium with occurrence of 21.87% (Table 2) while Staphylococcus epidermidis had the lowest occurrence (9.38%), in soil remediated with M. officinalis alone (SP1) Bacillus subtilis had the highest frequency of occurrence (42.85%) and Staphylococcus epidermidis had the lowest frequency of occurrence (4.54%) also similar patterns of results was observed in soil remediated with U. lobata alone (SP2) Bacillus subtilis had the highest frequency of occurrence (50.00%) while Staphylococcus epidermidis had the lowest frequency of occurrence (4.54%), soil polluted with 50cl of spent engine oil and remediated with M. officinalis (SP1 50) Bacillus subtilis had the highest frequency of occurrence (44.44%) while Staphylococcus epidermidis and Staphylococcus aureus had the lowest frequency of occurrence (11.76%), soil polluted with 70cl of spent engine oil and remediation. Bacillus subtilis had the highest frequency of occurrence (36.36%) These microorganisms have been reported by many researchers to play a major role in hydrocarbon degradation. [18-21].

Table 1. Morphological and BiochemZical Characteristics of Bacterial Isolates.

Code	Gram (g)	Shape	Catalyse	Coagulase	Starch Hydrolysis	Oxidase	MSA	Urease	MR	VP	Bacteria Isolate (Identified)
MA_i	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
MA_{ii}	+	Cocci	+	+	-	NA	+	-	+	-	*Staphylococcus aureus*
MB_i	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
MC_i	+	Cocci	+	-	-	NA	+	-	+	-	*Staphylococcus epidermidis*
MC_{Ii}	+	Rod	+	NA	+	-	NA	-	-	+	*Bacillus megaterium*
BA_i	+	Rod	+	NA	-	NA	NA	-	+	-	*Bacillus subtilis*
BA_{ii}	-	Rod	+	NA	-	+	NA	NA	+	-	*Pseudomona aeroginosa*
BB_i	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
BB_{ii}	-	Rod	+	NA	-	+	NA	NA	+	-	*Pseudomona aeroginosa*
BC_i	+	Cocci	+	+	_	NA	+	-	+	-	*Staphylococcus aureus*
BC_{ii}	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
TA_i	+	Cocci	+	+	-	NA	+	-	+	-	*Staphylococcus aureus*
TA_{ii}	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
TB_i	+	Rod	+	NA	+	NA	NA	NA	+	-	*Bacillus subtilis*
TC_i	+	Cocci	+	NA	-	NA	+	-	+	-	*Staphylococcus aureus*
TC_{ii}	+	Rod	+	NA	+	NA	NA	-	-	+	*Bacillus megaterium*
SA_i	+	Rod	+	NA	+	NA	NA	-	+	-	*Bacillus subtilis*
SB_i	+	Rod	+	NA	+	NA	NA	-	+	-	*Bacillus subtilis*
SC_i	+	Cocci	+	+	-	NA	+	-	+	-	*Staphylococcus aureus*
SC_{Ii}	+	Cocci	+	-	-	NA	+	-	+	-	*Staphylococcus epidermidis*

+: Positive, -: Negative, NA: Not Applicable

Table 2. Frequency of occurrence of bacterial isolates in the experimental setup.

Bacteria	SA (%)	SP1 (%)	SP2 (%)	SP1 (50) (%)	SP2 (50) (%)	SP1 (70) (%)	SP2 (70) (%)
Bacillus subtilis	12 (37.50)	7 (42.85)	11 (50.00)	8 (44.44)	5 (29.41)	4 (36.36)	4 (40.00)
Staphylococcus aureus	4 (12.50)	2 (9.52)	2 (9.09)	1 (5.56)	2 (11.76)	2 (18.18)	2 (20.00)
Bacillus megaterium	7 (21.87)	4 (18.18)	4 (18.8)	4 (22.22)	5 (29.41)	3 (27.27)	2 (20.00)
Pseudomonas aeruginosa	6 (18.75)	3 (14.29)	3 (13.63)	3 (16.66)	3 (17.64)	1 (9.09)	1 (10.00)
Staphylococcus epidermidis	3 (9.38)	1 (4.54)	1 (4.54)	2 (11.11)	2 (11.76)	1 (9.09)	1 (10.00)
Total	32 (100)	21 (100)	22 (100)	18 (100)	17 (100)	11 (100)	10 (100)

SP1 50: Soil polluted with 50cl of spent engine oil and treated with *Melissia officinalis*, SP2 50: Soil polluted with 50cl of spent engine oil and treated with *Urena lobata*, SP1 70: Soil polluted with 70cl of spent engine oil and treated with *Melissia officinalis*, SP2 70: Soil polluted with 70cl of spent engine oil and treated with *Urena lobata*.

$$\text{Frequency (\%)} = \frac{\text{Occurrence of individual microorganism}}{\text{Total sum of occurrence of isolate}} \times 100$$

3.3.2. Fungi

Table 3 shows the fungal isolates from the experimental setup, the fungi identified were *Aspergillus flavus, Aspergillus fumigates, Penicillin notatum, Aspergillus niger* and *Fusarium oxysporium*. In soil alone (SA) *A. niger* had the highest frequency of occurrence (47.61%) closely followed by *A. flavus* (23.80%) while the fungi with the least occurrence was *P. notatum* (4.76%) with *F. oxysporium* with occurrence of 9.52% (Table 4), in soil remediated with *M. officinalis* alone (SP1), *A. niger* had the highest frequency of occurrence (52.17%) while *P. notatum* had the lowest frequency of occurrence (4.34%), in soil remediation. *A. niger* had the highest frequency of occurrence (45.83%) while *P. notatum* had the lowest frequency of occurrence (4.16%), soil polluted with 50cl of spent engine oil. *A. niger* had the highest frequency of occurrence (50.00%) while *P. notatum* and *A. flavus and F. oxysporium* had the lowest frequency of occurrence (11.11%) most of these fungal have been reported by other researchers to possess the ability to degrade hydrocarbon [22, 21, 23].

Table 3. Cultural and Morphological Characteristics of Fungal Isolate.

Isolates code	Cultural Characteristics	Microscopic Characteristics	Inferences
F1	Green colony with granular surface anda brown reverse coloration on SDA	Septate hyphae, hyaline and coarsely rough conidiophores.	*Aspergilus flavus*
F2	Gray colony with granular surface, white edges and a black reverse coloration on SDA	Septatehyphae, and short smooth-walled conidiophores.	*Aspergilus fumigatus*
F3	Black colony with granular surface and black reverse	Septate hyphae. Dark brown large globose conidial heads.	*Aspergilus niger*
F4	Pink centered white colony with cottony surface anda brown reverse coloration on SDA	Septate hyphae, canoe shaped macroconidia.	*Fusarium oxysporium*
F5	Bluish –green colony with cottony surface, white border and a brown reverse coloration on SDA.	Septate hyphae with unbranchedcnidiophores and secondary branches (metulae).	*Penicillium notatun*

Table 4. Frequency of occurrence of fungi isolates in the experimental setup.

Fungi	SA (%)	SP1 (%)	SP2 (%)	SP1 (50) (%)	SP2 (50) (%)	SP1 (70) (%)	SP2 (70) (%)
Aspergillus flavus	5 (23.80)	7 (30.43)	8 (33.3)	3 (17.64)	4 (22.22)	1 (11.11)	1 (10.00)
Aspergillus fumigates	3 (14.28)	2 (8.69)	3 (12.50)	2 (11.76)	2 (11.11)	2 (22.22)	2 (20.00)
Penicillium notatum	1 (4.76)	1 (4.34)	1 (4.16)	2 (11.76)	2 (11.11)	1 (11.11)	1 (10.00)
Aspergillus niger	10 (47.61)	12 (52.17)	11 (45.83)	7 (41.17)	8 (44.44)	4 (44.44)	5 (50.00)
Fusarium oxysporium	2 (9.52)	1 (4.34)	3 (12.50)	3 (17.64)	2 (11.11)	1 (11.11)	1 (10.00)
Total	21 (100)	23 (100)	24 (100)	17 (100)	18 (100)	9 (100)	10 (100)

SP1 50: Soil polluted with 50cl of spent engine oil and treated with *Melissia officinalis*, SP2 50: Soil polluted with 50cl of spent engine oil and treated with *Urena lobata*, SP1 70: Soil polluted with 70cl of spent engine oil and treated with *Melissia officinalis*, SP2 70: Soil polluted with 70cl of spent engine oil and treated with *Urena lobata*.

$$\text{Frequency (\%)} = \frac{\text{Occurrence of individual microorganism}}{\text{Total sum of occurrence of isolate}} \times 100$$

4. Conclusion

The use of microorganism for biodegradation has been widely encouraged due to low cost and easy to use without leaving residual on the treated soil. This study was able to identify some organism that showed great ability to effectively degrade hydrocarbon. The microorganisms identified were *Bacillus subtilis, Bacillus megaterium*, and *Pseudomonas aeroginosa* for bacteria while the fungi were *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatu*. It is recommended that laws should be enacted to serve as a guide to reduce the proliferations of automobile workshops in a view to reduce the contamination by spent engine oil (SEO) in the ecosystem.

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