Determination of The Influence of Used Engine Oil on Soil Microbial Community Around Mechanic Workshops

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ABSTRACT
The influence of used engine oil on soil microbial community around a mechanic workshop was determined. Soil samples were collected from 5 different workshops, while the sample obtained from a nearby uncontaminated soil served as control. The Total Heterotrophic bacterial (THB) and fungal (TF) counts degreased throughout the study period, ranging from $3.4x10^5$CFU/g to $5.22x10^4$CFU/g and $8.7x10^4$CFU/g to $4.20x10^3$CFU/g respectfully. The characterization and identification of isolates revealed the presence of the following bacteria;Bacillus spp, *Pseudomonas* spp, *Staphylococcus* spp, *Enterobacteria*spp, *Micrococcus* spp, *Staphylococcus* spp, *Corynebacter*spp, *Lactobacillus* spp and fungi;*Mucor, Rhizopus, Penicillium, Fusarium and Aspergillus, as among the engine oil utilizing microorganisms.Bacillus species has the highest prevalence of 36.40%, followed by Staphylococcus spp with 18.18%. The analysis for utilization of the used engine oil by these organisms on a spectrophotometer revealed that the utilization as shown by the turbidity increased throughout the study period of 0hr to 144hrs, which showed that the microorganisms are utilizing the used engine oil, which indicates that they could be applied for remediation on an engine oil polluted soil.*

Keywords: *Used engine oil, mechanic workshop, impacted soil, turbidity, engine oil utilizing bacteria*

INTRODUTION

Engine oil is a complex mixture of hydrocarbons and other organic compounds, together with some organometallic components [1]that are all made completely from hydrogen and carbon, hence the name "hydrocarbon" [2]. The used engine oil is defined as used lubricating oils removed from the crankcase of internal combustion engines [3]. It is used to grease automobile engine parts so as to keep everything running smoothly. Typical engine oil consists of hydrocarbons having between 18 and 34 carbon atoms per molecule. Before its use, engine oil consists of a complex mixture of hydrocarbons that make up 80 to 90 percent of its volume and performance-enhancing additives that make up 10 to 20 percent of its volume [4].

The quality of engine oil is tested based on its properties to evaluate their suitability and merits for certain service conditions. These properties includes; viscosity, flash point or fire point, cloud point, carbon residue, corrosion, pour point, colour, dilution of crankcase oil, emulsification, oxidation at high temperation, evaporation, sulphur content, specific gravity and neutralization number. Engine oil is normally specified in terms of their performance and viscosity grade ([5], [6]).Abrasives, water, coolants and fuel are major contaminants of engine oil.

As engine oil is used in automobile, it picks up a number of additional compounds from engine wear ([7], [8]). This used lubricating oil obtained after servicing and subsequent draining from automobile and generator engines is called spent engine oil (SEO). The key components of a typical spent engine oil consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene, benzo (a) anthracene, benzo (a) pyrene, and fluoranthene, nitrogen, sulphur compound and metals (Mg, Ca, Zn, Pb).Because of the additives and contaminants, used motor oil disposal can be more environmentally damaging than crude oil pollution ([9], [10], [4]). These additives and contaminants may cause both short and long term effect if they are allowed to enter the environment through water ways or soil ([11], [12]). They cause great damage to soil and soil microflora and create unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH [13]. The disposal of spent engine oil into gutters, water drains, open vacant plots and farms is a common practice in Nigeria especially by motor mechanics.

These additives when exposed to the atmosphere have toxic effects on humans and animals when in contact, and also degrade the land. The spent oil gets to the environment due to discharge by motor and generator mechanics [14] and from the exhaust system used and due to engine leaks [15]. The accumulation of polycyclic aromatic hydrocarbons (PAH) in soil are due to many anthropogenic sources such as cooking plants, solid fuel domestic heating, aircraft exhaust, car exhaust and forest fires [16].

Crude oil being a complex mixture of different compounds, majority of which are very

toxic and carcinogenic for living organisms, its clean up in the water and soil environment is a real world problem. Some of the microorganisms, primarily bacteria which naturally exist in water and soil, are capable of degrading toxic petroleum hydrocarbons under different environmental conditions [17]. The susceptibility of petroleum hydrocarbon to degradation is determined by the structure and molecular weight of the hydrocarbon [18]. Life is composed of series of enzymatic reactions and enzymes carry out most of the reaction in nutrient recycling.

MATERIALS AND METHODS

Study site

The study was conducted on soils around mechanic workshops situated at different streets in Calabar South Local Government Area, Calabar, Cross River State, Nigeria. Their locations are on Bassey Street (EkpoAbasi), Effio-Awan Street (New Airport road), EkpeyongAbasi Street (New Airport road), Ekeya Street and a mechanic workshop along Utibe Street (EkpoAbasi).

Sample collection

Top soil samples were collected at surface depth of 0-10cm using a sampling device, hand auger,from sites where used engine oil is poured. Samples were collected at 5 different sites at each mechanic workshop. The area had a characteristic black color, with hard surface. Soil samples were transferred directly into clean and sterile containers. Also, control samples were collected from non-contaminated nearby farms at each of the sites, as control. All samples were then carefully transferred to the Cross River University of Technology, Microbiology laboratory for analysis.

Enumeration of Total Heterotrophic Bacterial (THB) Counts

Samples were enumerated by making ten-fold dilutions of the soil samples from 10^1 to 10^{10}. 0.1ml of Dilutions 10^2,10^4 and 10^6 were transferred unto solid nutrient agar plates. A clean sterile spreader was then used to spread the inoculum evenly on the medium. The plates were inoculated in triplicates. The inoculated plates were incubated at 35^oC for 24 hours and subsequently monitored for growth. The colonies of the isolates were counted using a colony counter and the heterotrophic bacterial counts of the contaminated and uncontaminated samples were compared. Isolated colonies were obtained by sub-culturing[19].

Examination of total fungal (TF) count

Spread plate method was used for enumeration of total fungal count. A 0.1ml of the suspension of the sample was plated in triplicates onto Sabouraud Dextrose agar plates. They were then incubated for 72hours. After which colonies were counted and recorded [19].

Isolation, characterization and identification of bacterial isolates

Pure cultures from the soil contaminated with used engine oil were isolated by plating 1.0 ml of the diluted samples onto Mineral Salt (MS) agar. The bacterial isolates were presumptively identified by means of macroscopic, microscopic and some biochemical characterization following the procedures of Cheesbrough[20].

Isolation and identification of fungal isolates

The fungal isolates were identified by morphological characteristics and microscopic examination. Among the characteristics used were colonial characteristics such as surface appearance and colour of the colonies. Microscopy examination revealed the type of hyphae i.e. septate or aseptate, and the vegetative mycelia and appropriate references were then made.

Assessment of the bioutilization of the used engine oil

This was done using Mineral Salt agar. The Mineral Salt medium consist of 2g of Ammonium chlorides (NH_4Cl) 2g of Magnesium chloride ($MgCl_2$), 2g of Potassium phosphate (K_2HPO_4). Twenty milliliter(20ml) of the mineral salt medium was poured into test tubes and over laid with 1ml of engine oil and cocked.The test tubes were kept for 5days (120 hours) and their turbidity examined at interval of 0hours, 48hours, 96hours and 144hours using spectrophotometer.

RESULT

The result of the total heterotrophic bacterial counts is as shown on table 1. The counts obtained showed that the highest total heterotrophic bacterial counts of $3.40x10^5$ CFU/g was obtained from Sample 1, with the Control having a count of $4.45x10^8$CFU/g. The lowest count of heterotrophic bacterial, $5.22x10^4$CFU/g, was obtained from Sample 6, with a Control count of $2.20x10^7$CFU/g. The Table 2showed the total fungal (TF) counts (CFU/g).The highest count of $8.7x10^4$ CFU/g was obtained from Sample 1, with aControl count of $9.69x10^6$ CFU/g while Sample 6 had the lowest count of $4.20x10^3$ CFU/g, with the Control recording $1.10x10^5$ CFU/g.

The biochemical identification and characterization process (Table 3) revealed the presence of *Bacillus* spp, *Pseudomonas* spp, *Staphylococcus* spp, *Enterobacteria*spp, *Micrococcus* spp, *Corynebacterium*sppand *Lactobacillus*spp. The Table 4 showed that *Bacillus*spphad the highest percentage occurrence of 36.40%, followed by *Staphylococcus*spp (18.18%), *Pseudomonas*spp, *Enterococcus*spp, *Micrococcus*spp, *Corynebacterium*spp and *Lactobacillus*spp had the lowest percentage occurrence of 9.10% each. The colonial and microscopicmorphology of the fungal

isolates are displayed in Table 5. Result showed that the identified isolates were members of the genera *Mucor, Rhizopus, Aspergillus, Fusarium, Penicillium.*

The bioutilization of the used engine oil was assessed using Spectrophotometer for a 5 day period, with their turbidity examined at intervals of 0 hour, 48 hours, 96 hours and 144 hours. Result showed that the turbidity of all the samples increased with time (Table 6).

Table 1:Total Heterotrophic Bacterial (THB) counts obtained from the samples

Sample	Total Heterotrophic Bacterial (THB) counts (CFU/ml)	Control (CFU/ml)
1	$3.40x10^5$	$4.45x10^8$
2	$3.20x10^5$	$4.35x10^8$
3	$3.00x10^4$	$4.25x10^8$
4	$2.60x10^5$	$3.50x10^7$
5	$2.00x10^5$	$3.00x10^8$
6	$5.20x10^4$	$2.20x10^7$

Table 2: Total Fungal (TF) counts obtained from the samples

Sample	Total Fungi (CFU/g)	Control (CFU/g)
1	$8.70x10^4$	$9.69x10^6$
2	$2.08x10^4$	$3.68x10^6$
3	$9.74x10^3$	$1.66x10^5$
4	$1.40x10^4$	$2.64x10^6$
5	$1.32x10^4$	$2.44x10^5$
6	$4.20x10^3$	$1.10x10^5$

Table 3:Microscopic and Biochemical characteristics of bacterial isolates

Isolates	Gram reaction	Morphology	Indole red reaction	Methyl red reaction	Vogesproskauer reaction	Catalase reaction	Citrate reaction	Triple sugar iron butt/deep	Slant	Most Probable Bacteria
1	+ve	Rod in chain	-	-	+	-	-	G production	Alkaline	*Bacillus* spp
2	-ve	Rod in chain	+	+	-	-	-	G.P/Alkaline	Alkaline	*Pseudomonas* spp
3	+ve	Cocci in cluster	-	-	-	+	-	AG production	Alkaline	*Staphylococcus*
4	+ve	Rods in chain	-	-	+	-	-	AG/Alkaline	Alkaline	*Bacillus subtilus*
5	+ve	Large rods chain	+	+	+	-	+	G production	G.P	*Enterobacteria*spp
6	-ve	Large rods in chain	+	-	-	+	+	AG/Alkaline	Alkaline	*Bacillus* spp
7	+ve	Cocci in cluster	-	-	-	-	-	AG production	G production	*Micrococcus* spp
8	-ve	Rod in chain	-	-	+	-	+	G production	Alkaline	*Bacillus* spp
9	-ve	Cocci in cluster	+	+	-	+	+	G.P/Alkaline	G.P/Alkaline	*Staphylococcus* spp
10	+ve	Tiny rods in chain	-	-	-	-	+	G production	G. production	*Corynebacterium*spp
11	+ve	Rod in chain	-	+	-	-	+	G.P/Alkaline	GP/Alkaline	*Lactobacillus*spp

Key: + = Positive, - = Negative, G.P = Gas Production

Table 4: Percentage occurrence

SN	Most Probable Bacteria	Prevalence (%)
1	*Bacillus*spp	36.40
2	*Pseudomonas*spp	9.10
3	*Staphylococcus*spp	18.18
4	*Enterococcus*spp	9.10
5	*Micrococcus*spp	9.10
6	*Corynebacterium*spp	9.10
7	*Lactobacillus*spp	9.10

Table 5: Cultural and microscopic characterization of fungal isolates

Isolates	Colonial morphology	Microscopic morphology	Fungi
FSI	Young colonies are white and Cottony, later becomes brown	Brown aerial branched sporangiophores. Sporangium at the tip. No rhizoid is produced.	*Mucor*sp
FS2	Young colonies are white and Cottony later becomes gray	Largeprophilesporangi. Thick walled non septate hyphae	*Rhizopus*sp
FS3	Reddish colonies	Micoconidia on elongated Conidiophores and macroconidia on short conidiophores	*Fusarium*sp
FS4	Colonies are flat and wrinkled Yellow-green to dark green	conidial heads consist of spherical vesicle, without metulae and one in colour row of phialides producing chains of conidia. Hyphae septate	*Aspergillusflavus*
FS5	Young colonies are white and Cottony, later becomes brown	Brown aerial branched sporangiophores. Sporangium at the tip. No rhizoid is produced.	*Mucor*sp
FS6	Dark green dense colonies	Spores in unbranched chains, borne from clusters of cylindrical colony to bottle shape phialides	*Penicillium*spp

Key: FS – Fungal sample

Table 6: Turbidity of the sample during a 5 days analysis

Sample	0hour	48hours	96hours	144hours
1	0.066	0.956	1.411	1.467
2	0.066	0.906	1.616	1.672
3	0.066	1.268	1.342	1.616
4	0.066	1.035	1.255	1.351
5	0.066	1.305	1.490	1.790
6	0.066	1.113	1.550	1.178
Control	0.066	0.022	0.022	0.105

DISCUSSION

Result showed that the total heterotrophic bacterial and fungal counts of the impacted soils decreased with time, and there are higher THB and TF in the un-impacted soils than the impacted ones. This corroborates the findings of Samuel*et al.*[21] that the total heterotrophic bacterial counts of contaminated soil sites are lower when compared to the control (uncontaminated). The decrease in counts could be attributed to the toxic persistent effect of engine oil in the soil [22].

The turbidity of the engine oil sample over a 5 day period at intervals of 0 hour, 48 hours, 96 hours and 144 hours revealed that the turbidity value increased with time. This increase showed that there was an increase in concentration of engine oil utilizing microorganisms, which indicates that the engine oil was been bioutilized by the organisms. It could be deduced thatthe engine oil selected the existence of engine oil utilizing bacteria and fungi from the existing heterotrophic bacterial and total fungi. In a related study,Okpokwasili and Nwosu, [23] reported that pesticides in any environment selects for the existence of pesticide utilizing microorganisms within the heterotrophic population. This is also in tandem with the findings of Enabulele and Obayagbona[24], which deposited that the preliminary fungal counts isolated from contaminated soils from mechanic workshop could be due to the adaptive abilities of the fungi to survive even in hydrocarbon contaminated soil overtime. Oluwafemi*et al.*[25] also reported similar findings in earlier studies, that microorganisms used engine oil contaminated soil, overtime evolved and developed the ability to withstand and survive in harsh conditions, and also leading to the displacement of less adaptable microbial population.

The longer the mineral salt medium stays, the more turbid it becomes and as such increases the rate of utilization of used engine oil in the soil. Result showed that *Bacillus* species has the capacity to utilize used engine oil. Hence could be applied for bioremediation of engine oil polluted sites. The relative absence of utilization in the control could be attributed to absence of inoculum and the high total heterotrophic bacterial and fungal counts could be due to the soil normal flora.

Isolates identification and determination of percentage occurrence revealed that most of the used engine oil utilizing bacteria was gram positive (+) rods. *Bacillus* spp, *Pseudomonas* spp, *Staphylococcus*spp, *Escherichia* coli, *Micrococcus* spp, *Saphylococcus*spp, *Corynebacter*spp, *Lactobacillus*sppand *Bacillus* sppwere the probable organisms found. *Bacillus* species has the highest prevalence (36.40%) while *Pseudomonas*spp, *Enterobacteriaspp*, *Micrococcus*spp, *Corynebacter*spp and *Lactobacillus*spp had the lowest prevalence of 9.10%. This corroborates the findings of Asikong and Ebana[26] that among the hydrocarbon degraders isolated from petrol station in Calabar Metropolis, *Bacillus* species has the highest percentage degradation rate. The enumeration of fungi isolates showed that *Mucor, Rhizopus, Penicillium, Fusarium and Aspergillus*were among engine oil utilizing fungi.

This result agrees with the findings of Samuel *et al*. [21] which observed the presence of the following bacterial species; *Klebsiellapneumoniae, Plesiomonasshigelloide, Acinetobacter*species, *Bacillus licheniformis, Pseudomonas aeroginosa*, and *fungal species; Aspergillusalabamensis, Rhodotorulamucilaginosa, Aspergellusterreus, Aspergillusflavus, Sarocladiumhominis, Trichodermacapillare, Aspergillusaculeatus* from engine oil impacted soils.

This result also agrees with the result of Ugoh and Moneke[13] which reported the presence of *Pseudomonas* spp., *Micrococcus*spp, *Serratia*spp and *Bacillus*spp, with *Bacillus*spp been the most dominant (100% occurrence) followed by *Micrococcus* and *Pseudomonas*sppwith 80% occurrence each, and lastly *Serratia*spp with the least of 40% occurrence. Relatedly, Olannye*et al.*[27] in a study of the potential of soil mycoflora isolated from four mechanic workshops, in remediating used engine oil contaminated soil identified the presence of *Trichoderma* spp., *Fusarium* spp., *Aspergillusniger*, *Aspergillusflavus*, *Mucor* spp., *Rhizopusstolonifer*, *Penicilliumchrysogenum*, *Penicillium* spp., *Aspergillus* spp., *Saccharomyces* spp., *Geotrichum* spp., *Rhizopusstolonifer*, *Cladosporium* spp. and *Rhizopusoryzae*, with *Aspergillusflavus* occurring most frequently.

The Spectrophotometric result corroborates the findings of Olannye*et al*. [27] which reported an increase in turbidity (0.08-0.90) during a 25 day incubation period of engine oil impacted soil, inoculated with *Aspergillusflavus*. Amongst the test organisms used as treatments for hydrocarbon utilization, *Aspergillusflavus*, consortium of *Aspergillusflavus*+ *Trichoderma*spp and consortium of *Aspergillusflavus* + *Trichoderma*spp + Fusariumspp caused the highest percentage reduction of total hydrocarbon content of used engine oil contaminated soil after eight weeks of hydrocarbon utilization. Soils from mechanic workshops are good sources of hydrocarbon utilizers.

It has been shown that marked changes in properties occur in soils contaminated with hydrocarbon; this affects the physical, chemical and microbiological properties of the soil ([28], [29]).Wyszkowski and Ziolkowska [30] reported that petrol and diesel oil affected the organic carbon and mineral components in soils at different rates. This means that the growth and development of organisms

that depends on such soils can be affected at different rates by petroleum products. Nkwoada*et al.* [31] deposited that the detrimental effects of auto-mechanic village activities were on humans and also disrupted growth and flowering of arable plants and advocated that Nigeria should provide standard repairs and services to automobiles in-line with emerging technology and best environmental practices.

CONCLUSION

The discharge of used engine oil in farms around a mechanic workshop distorts the microbial equilibrium within such soil environment. Hydrocarbon utilizing organisms were isolated from engine oil impacted soil samples, leading to reduction in total heterotrophic bacterial counts.This could affect soil fertility and crop production. Also, the bacterial and fungi isolates obtained from this study could be explored for remediation of oil spill clean-up in similar environments. However, environmental consciousness should be installed into auton 8 mechanics to avoid indiscriminate disposal of engine oil.

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