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APPRAISAL ON BACTERIOLOGICAL QUALITY AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF MICROORGANISMS IN HYDROCARBON-CONTAMINATED SOILS

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Abstract
Spent engine oil in various mechanical workshops is inadequately disposed into gutters, water drains and soil in Nigeria, and this could have overall environmental advert effects on human and aquatic lives. Thus, investigation on bacteriological value and antibiotics susceptibility profile of isolates from contaminated hydrocarbon soil were carried out using pour plate and disc diffusion techniques respectively. The findings revealed that the sample from site A had the highest mean bacterial count (274.67 x 10^6 CFU/g). The isolates presumptively obtained from the hydrocarbon-polluted soil samples included: *Bacillus* sp. *Pseudomonas* sp., *Streptococcus* sp. and *Staphylococcus epidermidis.* Antibiotic susceptibility test revealed ofloxacin the most potent antibiotic among the selected antibiotics. Findings from this study confirmed hydrocarbon hydrocarbon-contaminated soils are a potential source of pathogenic microbes thus, proper hygiene should be observed by people working in hydrocarbon-contaminated areas.

Keywords: Bacteria, antibiotics, hydrocarbon, ofloxacin.

Introduction

The majority of motor mechanics in Nigeria, do not have a precise and suitable disposal technique for spent engine oil in their various factory sites, which leads to disposal and direct seepage into the soil, municipal gully and draining system (Nwite & Alu, 2015). Lubricating oils obtained or drained after servicing vehicle and generator engine are referred to as spent oil and have a mixture of aromatic and aliphatic hydrocarbons, nitrogen and sulphur compounds and some metals such as manganese, calcium, zinc, and lead, due to wear and tear of engine Kiyohara *et al.* (2014). According to Blodgette (2017), the main function of engine oil in automobile and mechanical engines is cleaning, lubricating and inhibiting corrosion of the engines. It also enhances the sealing and cooling of the engine by discharging heat away from the moving parts. The commercially available engine oils in the market in recent times are byproducts of petroleum- and non-petroleum chemical compounds. Hence engine oils are, often produced from a combination of base oils composed of hydrocarbons (Griffiths *et al.*, 2018).

The introduction of spent oil into the soil environment has a negative effect on the microflora and fauna of the soil by creating unfavourable environmental conditions for life in the soil due to the viscous nature of the oil leading to deprived aeration, restriction of mobility of soil nutrients and declination of soil pH (Griffiths *et al.*, 2018). Hydrocarbon contamination has been implicated in the degradation of soil, which in turn affects the physical, chemical, flora and fauna properties of the soil (Ohenhen *et al.*, 2016). Large-scale accidental spills have

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been linked with environmental pollution with petroleum and petroleum products. If this happens, hydrocarbons may percolate the water table before becoming immobilized in the soil. They spread horizontally on the groundwater surface and continue to partition into groundwater, soil pore space, air and the surface of soil particles. The diverse substrates and metabolites present in hydrocarbon-impacted soils surely provide an atmosphere for the growth of a quite complex microbial community (Butier & Mason, 2017). These microorganisms can hydrolyze a wide range of target constituents present in oil sludge (Barathi and Vasudevan, 2011; Mishra *et al.*, 2011). A large number of *Pseudomonas* strains with polycyclic aromatic hydrocarbon-degrading capability have been isolated from soil (Johnson *et al.*, 2017; Kiyohara *et al.*, 2014).

Other microbes with hydrocarbons degrading capability include *Yokenella* spp., *Alcaligenes* spp., *Roseomanas* spp., *Sreanotrophomanas* spp., *Acinetobacter* spp., *Flavobacter* spp., *cyanobacterium* spp., *Capnocytophage* spp., *Moraxella* spp. and *Bacillus* spp. (Antai, 2010).
Petroleum products such as engine oil, petrol, diesel and kerosene are used daily in various forms in mechanic workshops. This study aimed at surveying the bacteria present in used engine oil-contaminated soil environment and their antibiotic susceptibility pattern to some readily available antibiotics to enlighten the populace of the inherent danger associated with hydrocarbon-contaminated soil.

Materials and Methods

Collection of sample materials

Four different hydrocarbon-polluted soil samples were obtained from four different mechanical factory sites in four different locations in Offa (Taiwo Road, Tipper garage, Amuyo area, and Ijagbo. The soil samples were collected into sterile containers with screw caps and brought to the laboratory for analysis. The analyses were performed at the Department of Science Laboratory Technology, Federal Polytechnic, Offa, Kwara State.

Sterilization of materials and culture media

This was carried out to ensure that materials and media use were free from microorganisms which could be a source of contamination in this work. All the glass wares were washed with soap and water, and sterilized in an oven at 160 ^{0}C for 60 minutes. Seventy per cent (70 %) of alcohol was used to disinfect (swabbing) the bench work surface. All culture media were prepared according to the manufacturers' descriptions and were further decontaminated in an autoclave at 121 ^{0}C for 15 minutes at 15 Pascal.

Determination of microbial load of hydrocarbon-polluted soil samples

Sixfold serial dilutions were set up for the sampled soil. An aliquot (1 ml) of the diluent was siphoned and dispensed into Petri-dished. An aliquot of 20 ml of molten nutrient agar was poured plated at 45 ^{0}C and allowed to gel. The emergent colonies were counted using a colony counter, and values were recorded after 24 hours of incubation.

Identification of bacterial isolates in soil samples

The standard procedure of Cheesbrough (2010) was used in the identification of bacterial isolates. Morphological qualities and cellular and biochemical reactions were used in the identification procedures for the bacteria isolates. Each bacterial colony was observed and reported after 24 hours old growth. The colony appearance documented comprises the cell shape, optics, elevation, surface, edge,

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consistency and pigmentation. Biochemical tests also included the Gram staining, coagulase, starch hydrolysis, oxidase, urease, catalase test, production of hydrogen sulphide (H_2S) indole, methyl red, motility, methyl red, vogesproskauer and various selected sugar fermentation. The result was compared with Bergey's Manual of Determinative Bacteriology (Fawole & Oso, 2007).

Standardization of inoculums for antibiotic susceptibility test (0.5 McFarland standard)

A 0.1 ml of 1% Barium chloride and sulphuric acid was mixed in a ratio of 0.1 to 9.9 ml. This was further added to 10 ml of autoclaved distilled water to make 0.5 ml McFarland standard solution. The turbidity of the 24 hours broth culture of the test organism was further compared to 0.5% McFarland, an equivalent to 10^5cfu/ml. A loopful of the standardized culture was used for the antibiotic susceptibility assay (Usman *et al.*, 2021).

Antibiotics susceptibility test

The disk diffusion method as described by the National Committee for Clinical Laboratory Standard Institute (CLSI, 2017) was used to determine the antibiotic susceptibility of the bacterial isolates. A 0.1 ml of each bacterial isolate was seeded into each of the Petri dishes containing Mueller-Hinton agar and allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs contain the following antibiotics: ceftazidine (CAZ, 30µg), cefuroxime (CRX, 30µg), gentamicin (GEN, 10µg), ceftriaxone (CTR, 30µg), erythromycin (ERY, 5µg) cloxacillin (CXC, 5µg), ofloxacin (OFL, 5µg), augmentin (AUG, 30µg), cefixime (CXM, 5µg), nitrofurantoin (NIT, 300µg), ciprofloxacin (CPR, 5µg) (Liverpool L9 7AR, UK) were aseptically placed on the surfaces of the susceptibility agar plates with a sterile forceps and incubated at 37 ^oC overnight. Zones of inhibition after incubation were observed and the interpretation was made using susceptible breakpoints of CLSI (2017). The diameter of zone inhibition around the disc was measured to the nearest millimetre using a metal caliper

Statistical Analysis of Data

Descriptive statistics such as mean, frequency, standard deviation, percentage and graph were used in the discussion of the results, to give a lucid representation of the data analyzed; Statistical Package (IBM-SPSS) version 20 was used to test for the level of significance of the results obtained and the differences were considered significant at $p < 0.05$.

Results

Mean counts of bacteria isolated from hydrocarbon-polluted soil samples

The mean count of bacterial isolates from hydrocarbon-polluted soil samples obtained in Offa Local Government Area, Kwara State, Nigeria is shown in Table 1. The findings revealed that the sample collected from Taiwo Road recorded the highest means bacterial count (274.67 x10^6 CFU/g) followed by the sample from Ijagbo which recorded a means count of 120.00 x10^6 CFU/g whereas, the lowest means count was recorded from sample Amuyo which recorded 93.33 x 10^6 CFU/g and there were no significant differences among the values obtained at $p \leq 0.05$.

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Table 1: Mean counts of bacteria isolated from hydrocarbon-polluted soil samples

S/N	Samples	Samples' sites	Means Population x 10^6 CFU/ml
1	Soil	Taiwo road	274.67 ±69.895[a]
2	Soil	Tipper garage	213.33 ±165.125[a]
3	Soil	Amuyo	93.33 ±41.633[a]
4	Soil	Ijagbo	120.00 ±40.000[a]

Values are mean ±SD of replicates (n=3). Values with the same alphabets in the same column are not significantly different while values with different alphabets are significantly different ($\alpha < 0.05$)
Key: S/N = serial number,

Bacterial isolates from hydrocarbon-polluted soil samples

Using standard conventional methods, the bacteria suspected to be present in samples from Taiwo road, Tipper garage, and Ijagbo analyzed included *Bacillus* sp. *Pseudomonas* sp. and *Staphylococcus epidermidis* while in sample from Amuyo *Streptococcus* spp. were presumptively confirmed present (Tables 2 and 3).

Table 2: Bacterial isolates from hydrocarbon-polluted soil samples

S/N	Samples	Samples' sites	Bacteria isolates
1	Soil	Taiwo road	*Bacillus* sp. *Pseudomonas* sp. and *Staphylococcus epidermidis*
2	Soil	Tipper garage	*Bacillus* sp. *Pseudomonas* sp. and *Staphylococcus epidermidis*
3	Soil	Amuyo	*Streptococcus* sp.
4	Soil	Ijagbo	*Bacillus* sp.*Pseudomonas* sp. and *Staphylococcus epidermidis*

Key: S/N = serial number

Table 3: Conventional characterization of bacterial isolates from hydrocarbon-polluted soil samples
(+) = positive, (G) = Gas, (-) = negative, (Nd) = Not determined, (Na) = Not aplicable, (Ab) = Absent,(P) = Present (AG) =Acid and Gas

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Serial number	Colonial morphology	Gram reaction	Shape	Structural arrangement	Catalase reaction	Indole test	Methyl red test	Simmon citrate test	Oxidase test	Vegetative hyphae	spore	Nitrate reduction	Coagulase test	hydrolysis	Motility	Glucose fermentation	Mointol fermentation	Hydrogen sulfide	Inositol fermentation	Microorganisms
1	Circular, opaque, flat, rough	+	cocci	chain	+	-	Nd	Nd	Nd	p	+	+	Na	Starch, gelatin, adenine	-	Na	-	Na	Na	*Streptococcu*
2	Circular, opaque, convex yellowish, smooth colony	+	cocci	Clustered	+	-	-	Na	-	Ab	-	+	-	Na	-	Na	G	Nd	Na	*Staphylococcus*
3	Cream, circular, smooth, entire	+	bacilli	Singly	Na	Na	-	-	Na	Ab	-	Na	-	urea	Na	Na	Na	Na	Na	*Bacillussp*
4	Cream, circular raised and smooth	-	bacilli	Singly	+	+	+	+	-	Ab	-	+	-	urea	+	G	-	-	AG	*Pseudomona*

Antibiotic sensitivity pattern of bacteria isolated from hydrocarbon-polluted soil samples

The findings from antibiotic sensitivity pattern revealed that the three isolates from sample sites (A, B, C and D) were susceptible to ofloxacin while only *Pseudomonas* sp. was susceptible to gentamicin among the challenged bacteria (Table 4)

Table 4: Antibiotic susceptibility pattern of isolates from hydrocarbon polluted soil from Offa Local Government Area, Kwara State.

S/N	Isolates	Antibiotic used (mm zone of inhibition)									
		CXC	OFL	AUG	CAZ	CRX	GEN	CTR	ERY	MAR	MARI
1	*Bacillus* spp.	R	16	R	R	R	R	R	R	7	0.88
2	*Pseudomonas* sp.	R	20	R	R	R	19	R	R	6	0.75
3	*Staphylococcus epidermidis*	R	22	R	R	R	R	R	R	7	0.88
4	*Streptococcus* spp.	R	20	R	R	R	R	R	R	7	0.88

Key:
S/N = serial number, CXC = cloxacillin, OFL = of laxacin, AUG = augumentin, CAZ = ceftazidine, CRX = cefuroxime, GEN = gentamincin, CTR= ceftriazone, MAR= Multiple Antibiotic Resistance, MARI = Multiple Antibiotic Resistance Index

DISCUSSION

There were no significant differences in the microbial means population of this study which somewhat disagreed with the findings of Ekhaise and Nkwelle (2011) who confirmed a significant difference ($p \leq 0.05$) in the means population of

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microbes isolated from oil-contaminated soil from mechanic workshops in Benin city metropolis, Edo State, Nigeria. More so the findings of this present study disagreed with the findings of Ekhaise and Nkwelle (2011) whose total heterotrophic bacterial counts ranged between 1.98 x 10^7 and 2.95 x 10^7cfu/g. However, findings from this study agreed with Jain *et al.* (2011) findings who indicated that there is an increase in heterotrophic bacteria population in the presence of dispersant agents and that the relationship between the petroleum contamination gradient and bacterial diversity is still not clear (Xuneke *et al*., 2019).

Findings from the microbiological study observed in this study disagreed with the findings of Igborgbor *et al.* (2022) who confirmed the presence of *Serratia*, *Pseudomonas*, *Enterococcus* and *Providencia* sp. in the samples obtained from an auto-mechanic workshop, Orubor-Agbo, Delta State, Nigeria. The disparities observed may be due to variations in the level of hydrocarbon composition and level of contamination. Nevertheless, the findings of Xuenke *et al.* (2019) on soil

contaminated with petroleum from the Dagang Oilfield, China, somehow agreed with the findings of this study where the presence of *Pseudomonas* was confirmed. The results of this study also conformed to the finding of Sabina *et al.* (2010) which also confirmed the presence of *Bacillus* and *Pseudomonas* in hydrocarbon-polluted soil. The availability of the microbes in the soil confirmed their potential ability to biodegrade hydrocarbon compounds which has major benefits in eliminating contaminants and is considered efficient, environmentally friendly, and cost-effective (Xuneke *et al.*, 2019).

Findings from antibiotic susceptibility tests on bacteria isolated from hydrocarbon-contaminated soil agreed with the finding of Usman *et al.* (2021), who confirmed the activity of oflaxacin as the most potent against bacteria isolated from untreated wastewater from Offa Local government Area, Kwara State. The activity of the antibiotic (ofloxacin) could be a result of the non-readily availability of ofloxacin which makes it irresistible to microorganisms due to lack of abuse of the drug. Finally, hydrocarbon-contaminated soil should be avoided since some microorganisms are capable of dwelling in hydrocarbon-contaminated areas.

Conclusion

Findings from this study presumptively confirmed the presence of *Bacillus* sp, *Pseudomonas* sp., *Staphylococcus epidermidis*, and *Streptococcus* sp. in hydrocarbon-contaminated soil analyzed. The findings also confirmed ofloxacin as the most effective antibiotic among the selected antibiotics used

Recommendations

Thus, further studies need to be done to identify and characterize these organisms down to the species level and more effective strains could also be developed to aid the bioremediation of oil-contaminated soil. More so proper hygiene should be obeyed while working in hydrocarbon-contaminated areas.

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