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Physiochemical Properties and Biodegradation Potentials of Soil Microbes Isolated from Mechanic Workshops in Benin Metropolis

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ABSTRACT

The release of used engine oil into the environment is gradually becoming a challenge to our environment. This research was carried out to determine the physiochemical properties and biodegradation potentials of some soil microbes isolated from mechanic workshops in Benin metropolis. The physiochemical properties of the soil samples were investigated according to the methods of Association of Official Analytical Chemists. The microbial analysis was carried out according to standard microbiological and biochemical methods. Results revealed bacterial count ranged from $1.6\times10^4 - 3.8\times10^4$ cfu/g, Hydrocarbon utilizing bacterial counts ranging from $0.4\times10^4 - 1.0\times10^4$ cfu/g. Fungal counts ranging from $0.1\times10^4 - 0.7\times10^4$ cfu/g. The bacteria isolated were *Pseudomonas aeruginosa, Micrococcus luteus, Bacillus subtilis, Acinetobacter* spp., *Corynebacterium* spp. and *Klebsiella* spp. The fungi isolates identified were *Trichoderma* spp., *Penicillium* spp., *Mucor* spp., *Aspergillus niger* and *Fusarium* spp. The screening test revealed *Bacillus subtilis, Pseudomonas aeruginosa*, *Trichoderma* spp. and *Aspergillus niger* to have biodegradative potentials. Shake flask experiment revealed increased in the turbidity of the mineral salt medium amended with engine oil. The consortium of bacterial and fungal showed better degradation potentials especially with the gradual increase turbidity and reduction in pH. The results suggest that the consortium of the isolates could be used in remediating contaminated soils.

Keywords: Bacteria, Fungi, Optical density, Consortium, Microbes

INTRODUCTION

Environmental pollution and the presence of complex mixtures of hydrocarbons and other organic compounds, including some organo-metabolic constituents used to lubricate the metallic parts of an automobile engine, have become major challenges today in the environment (Udeani et al., 2009). When spent engine oil enters the environment, it causes alterations in some soil properties, microbial activities, and influences the concentration of organic matter, nitrogen, magnesium, and heavy metals in the soil (Okonokhua et al., 2007). Prolonged exposure to high oil concentrations may cause the development of liver or kidney disease, possible damage to the bone marrow, and an increased risk of cancer (Mishra et al., 2001). Used engine oil pollutes the environment and constitutes a potential threat to humans, animals, and vegetation (Edewor et al., 2004; Adelowo et al., 2006). Used motor oil can cause great damage to sensitive environments and soil microorganisms. Substantial volumes of soil have been contaminated by used oil in many countries, especially industrialized nations. Large amounts of used engine oil are released into the environment when the oil from motor cars, motorbikes, and generators is changed and disposed of into gutters, water drains, open vacant plots, and farmlands—a common practice by motor and generator mechanics (Odjegba and Sadiq, 2002). The presence of spent engine oil in the soil creates a chemical impurity which contributes to chronic hazards resulting in mutagenicity and environmental risk (Blodgette, 2001). Various contaminants, such as used engine oil and heavy metals, have been found to alter soil biochemistry, including changes in soil microbial properties such as pH, O_2, and nutrient availability (Odjegba and Sadiq, 2002). Spent engine oil contains metals and heavy polycyclic aromatic hydrocarbons (PAHs), which could contribute to chronic hazards, including mutagenicity and carcinogenicity (Boonchan et al., 2000). As it is inevitable for the efficient and effective functioning of automobile engines, soil contamination with used engine oil is becoming one of the major environmental problems (Mandri and Lin, 2006), mainly due to uncontrolled disposal, particularly in developing economies. The widespread ability of microorganisms to assimilate these hydrocarbons is of great significance, and when it occurs in the natural environment, the process is known as biodegradation. Hydrocarbons, including PAHs, have long been recognized as substrates supporting microbial growth. A wide range of hydrocarbon utilizers (HCUs) found to be useful in the soil includes species such as *Pseudomonas*, *Rhodococcus*, *Mycobacterium*, *Bacillus*, *Acinetobacter*, *Providecin*, *Flavobacter*, *Corynebacterium*, and *Streptococcus* (Bhattacharya et al., 2002). Other organisms, such as fungi, are also capable of degrading hydrocarbons in engine oil to a certain extent, but they take longer periods to grow compared to their bacterial counterparts (Prenafeta-Boldu et al., 2001). The aim of this study was to determine the physiochemical properties and biodegradation potentials of soil microbes isolated from mechanic workshops in Benin metropolis, Nigeria.

MATERIAL AND METHODS

Study Sites

The five locations in Benin City metropolis used in this study were Uwelu around the mechanic workshops, Evbareke around the mechanic workshops, Igun around the mechanic workshops, Ugbowo around the mechanic workshops opposite University of Benin, and Oluku around the mechanic workshop close to the Bypass.

Sample Collection

Composite soil samples from designated mechanic workshops in the five areas of study were collected in a sterile polyethylene bag using a sterile spatula from the edge of a motor oil-stained patch in the workshop by scooping to about 5 cm. The samples were immediately transported to the laboratory for analyses.

Physiochemical Properties of the Soil Samples

The physiochemical properties of the soil samples were investigated according to the methods of A.O.A.C. (2000).

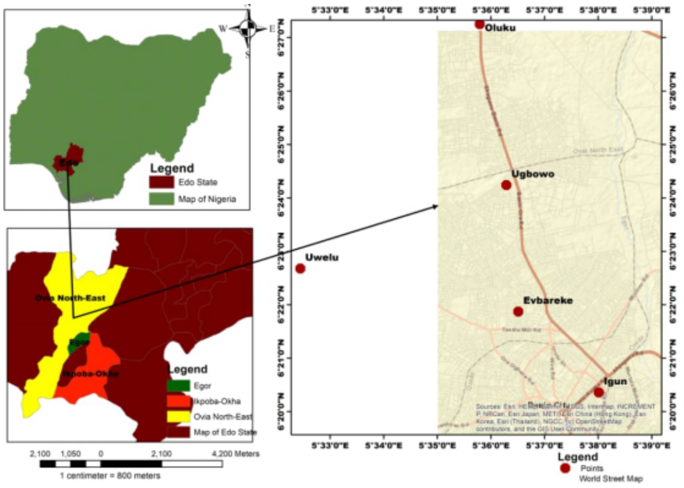

Figure 1: Map showing GPS coordinates of soil samples collected around the study sites.

Bacterial Enumeration

One gram (1 g) of the contaminated soil was weighed using an analytical balance (Model no AX423/E, USA Ohaus Cooperation) into test tubes containing 9 ml distilled water, and 10-fold dilution was carried out to 10^{-3} dilution. One milliliter (1 ml) of each dilution was inoculated into nutrient agar and potato dextrose agar for bacteria and fungi using the pour plate method. The plates for bacteria were incubated at 37°C for 48 hrs, while the plates for fungi were incubated at 30°C for 48 hrs. Hydrocarbon utilizing bacteria counts (HUB) were enumerated by inoculating 1 ml of aliquot of 10^{-1}, 10^{-2}, 10^{-3} into mineral salt agar plates prepared according to Chessbrough (2000). The plates were incubated for 7 days, and the discrete colonies were counted.

Characterization and Identification of Bacterial and Fungal Isolates

The bacteria and fungi isolates were characterized based on preliminary cultural and biochemical characteristics. Identification of the isolates was performed according to the method of Chessbrough (2000). The pure cultures were maintained in slants in McCartney bottles containing tryptic soy broth-glycerol (TSB-glycerol). They were stored at 4°C until required for analysis.

Screening Test for Biodegradation Potential of Engine Oil Contaminated Soil

The isolates were screened for the ability to utilize spent engine oil using mineral salt medium. Nine milliliters (9 ml) of mineral salt medium were dispensed into six test tubes for bacteria and five test tubes for fungi. In each of the test tubes, one milliliter of spent engine oil was added as the main source of carbon. Thereafter, all the test tubes were inoculated with 0.5 MacFarland (10^8 cfu/ml) of an isolate previously grown. All the test tubes were incubated at 30°C for 7 days, after which the turbidity of the solution was checked to determine the potentials of the isolates. Those with high degradation potentials were utilized, while those with poor degradation potential were eliminated from the experiment (Somiari et al., 2022).

Determination of the Ability of the Bacterial and Fungal Isolates to Utilize Spent Engine Oil from Contaminated Soil Using Shake Flask Method

A known volume of 98 ml of the mineral salt medium was transferred into 250 ml conical flasks followed by 1 ml spent engine oil before sterilization. Thereafter, all the conical flasks except for the control sample were inoculated with 0.5 MacFarland (10^8 cfu/ml) of a twenty-four (24 h) culture. The utilization of the engine oil was monitored at three-day intervals for a total of twelve days. The optical density and pH readings were monitored at 3-day intervals for 12 days. The optical density was measured with a M501 UV-Vis Camspec Spectrophotometer using 600 nm, while the pH was measured with a pH meter (Onyeiwu et al., 2022; Veerapagu et al., 2019).

Statistics

The data were subjected to One-way ANOVA using SPSS for various parameters. Further tests, such as Duncan's multiple range test, were carried out to ascertain significance among the parameters.

RESULTS AND DISCUSSION

Table 1 reveals the physicochemical properties of the crude oil-contaminated soil samples. The research by Mahmood et al. (2020) shows that the physicochemical properties of soil can be affected by hydrocarbon contamination. These properties have been found to aid in the proliferation of bacteria in the soil. The interaction between living organisms and petroleum contaminants is highly dependent on the type of soil (Onyeiwu et al., 2022). Mahmood et al. (2020) further show that soil pH plays a critical role in the absorption of nutrients by plants and has a significant effect on the interaction of microbes, which can affect the potential of microbes to carry out bioremediation. In this study, the pH ranged from 6.31 ± 0.07 to 6.79 ± 0.10, a comfortable range that could facilitate the bioremediation activities of the bacteria isolated from the soil. Similar pH ranges have been reported by Udinyiwe and Aghedo (2023), and Udinyiwe et al. (2022), which supports better comparison and understanding. Soil pH is a key factor influencing the uptake of available nutrients for plant utilization (Udinyiwe and Aghedo, 2023; Mahmood et al., 2020). Bada et al. (2018) reported a similar pH range of 4.48 ± 0.48 to 6.72 ± 0.72, which favors microbial metabolic activities. Okoh (2006) noted that low or acidic pH can affect the rate of oil biodegradation by bacteria in oil-contaminated soil. Acidic soil releases heavy metals such as Cd, Ni, Pb, Cu, Hg, and Zn, which creates problems for crop production (Bada et al., 2014).

The significantly higher values of electrical conductivity obtained from various sampling sites in this study could have resulted from the high concentration of charged ions in the impacted sites. Oyem and Oyem (2013) revealed electrical conductivity values higher than those reported in this study. The electrical conductivity in this study ranged from 306.30 ± 05.40 to 497.50 ± 0.90, which could be attributed to the presence of high Na^+ and Mg^+ salts in the environment. This range is consistent with the values reported by Abosede (2013), Sari et al. (2018), and Oyem and Oyem (2013). High electrical conductivity can negatively impact soil (Abdulfattah et al., 2016; Precti and Shah, 2015).

Table 2 reveals bacterial counts ranging from 1.6×10^4 to 3.8×10^4 cfu/g, while Table 3 shows hydrocarbon-utilizing bacterial counts ranging from 0.4×10^4 to 1.0×10^4 cfu/g. This indicates that bacteria can survive and thrive in this environment. Udinyiwe et al. (2022) reported total heterotrophic bacteria counts and hydrocarbon-utilizing bacterial counts similar to those reported in this study. Ogbonna et al. (2020) reported total heterotrophic bacterial counts (THBC) ranging from $2.58 \times 10^8 \pm 0.07$ cfu/g to $2.10 \times 10^7 \pm 0.50$ cfu/g, and total hydrocarbon-utilizing bacterial counts (THUBC) ranging from $8.1 \times 10^3 \pm 0.50$ cfu/g to $5.0 \times 10^4 \pm 0.50$ cfu/g, which agree with the findings of this study, confirming that bacteria can thrive in hydrocarbon-polluted sites.

Table 1: Physicochemical properties of hydrocarbon-contaminated soil samples

Parameters	Sample A	Sample B	Sample C	Sample D	Sample E
pH	6.79 ± 0.10	6.47 ± 0.01	6.31 ± 0.07	6.41 ± 0.08	6.56 ± 0.12
EC (mS/m)	456.45 ± 1.55	306.30 ± 5.40^{ab}	396.00 ± 0.90	391.90 ± 4.60^{a}	497.50 ± 0.90
Carbon (%)	3.31 ± 0.05	3.23 ± 0.04	3.44 ± 0.05	3.19 ± 0.03	3.05 ± 0.23^{a}
Nitrogen (%)	0.09 ± 0.03^{a}	1.04 ± 0.22^{a}	0.74 ± 0.06^{a}	0.62 ± 0.05^{ab}	0.79 ± 0.06
Phosphorus (%)	1.61 ± 0.03	1.43 ± 0.04	1.68 ± 0.09	1.66 ± 0.01	1.57 ± 0.02
Water holding capacity (%)	80.70 ± 0.70	78.50 ± 0.50	80.28 ± 0.72	75.55 ± 0.55^{a}	76.55 ± 0.30^{a}
Bulk density (g/cm^3)	2.66 ± 0.02	2.40 ± 0.01^{a}	1.90 ± 0.02^{ab}	2.70 ± 0.04	2.05 ± 0.11^{a}
Moisture (%)	12.06 ± 0.30	13.09 ± 0.24	13.40 ± 0.20	12.33 ± 0.55	12.07 ± 0.30

Table 2: Total heterotrophic bacterial counts (cfu/g) of soil from mechanic workshop

Samples	10^{-1}	10^{-2}	10^{-3}	Mean counts
Uwelu	139	74	31	3.8 × 10^4
Evwaike	110	59	24	3.4 × 10^4
Igun	88	35	19	2.3 × 10^4
Ugbowo	72	29	13	1.6 × 10^4
Oluku	94	51	24	3.0 × 10^4

Table 3: Hydrocarbon-utilizing bacterial counts (cfu/g) of soil from mechanic workshop

Samples	10^{-1}	10^{-2}	10^{-3}	Mean counts
Uwelu	57	22	08	1.0 × 10^4
Evwaike	44	17	05	0.7 × 10^4
Igun	38	14	05	0.6 × 10^4
Ugbowo	31	11	03	0.4 × 10^4
Oluku	40	16	07	0.8 × 10^4

Table 4 shows fungal counts ranging from 0.1 × 10^4 to 0.7 × 10^4 cfu/g. The presence of fungal growth indicates that the soil has been exposed to hydrocarbon pollution, enriching it for fungal growth. Ogbonna et al. (2020) reported total fungal counts (TFC) and hydrocarbon-utilizing fungal counts (HUFC) ranging from 2.0 × 10^5 ± 0.05 cfu/g to 1.6 × 10^5 ± 0.08 cfu/g and 9.0 × 10^3 ± 0.05 cfu/g to 7.0 × 10^4 ± 0.50 cfu/g, respectively. This confirms that fungi can utilize hydrocarbons as a source of energy.

The microbes identified in this study with degradation potentials include Bacillus spp., Pseudomonas spp., Micrococcus spp., Acinetobacter spp., Fusarium spp., and Aspergillus spp. (Udinyiwe et al., 2022; Ijah and Abioye, 2003; Ajayi et al., 2008; Das and Chandran, 2011; Omotayo et al., 2012; Ogbonna et al., 2020; Bento et al., 2005). The results in Tables 5 and 6 show the growth and potential of these microbes to utilize hydrocarbons as an energy source. Bacillus subtilis, Pseudomonas

Table 4: Total heterotrophic fungal counts (cfu/g) of soil from mechanic workshop

Samples	10^{-1}	10^{-2}	10^{-3}	Mean counts
Uwelu	29	14	06	0.7×10^4
Evwaike	23	08	03	0.4×10^4
Igun	19	07	02	0.2×10^4
Ugbowo	15	04	01	0.1×10^4
Oluku	18	05	02	0.3×10^4

Table 5: Result of screening test for biodegradation of engine oil contaminated soil

Isolates	Optical Density (OD)
Acinetobacter spp	0.293
Micrococcus luteus	0.507
Bacillus subtilis	0.883
Pseudomonas aeruginosa	0.916
Corynebacterium spp.	0.128
Aspergillus niger	0.739
Penicillium	0.427
Mucor spp.	0.396
Trichoderma spp.	0.661
Fusarium spp.	0.131
Control	0.000

Keys: + = Little Growth, ++ = Moderate Growth, +++ = Heavy Growth, - = No Growth

aeruginosa, Aspergillus niger, and Trichoderma spp., selected after the screening test, demonstrated hydrocarbon degradation abilities in the 12-day shake flask experiment. The gradual increase in optical density indicates hydrocarbon utilization by these microbes. Dilmi et al. (2017) reported a similar gradual increase in optical density over fifteen days, indicating bacterial adaptation, survival, and proliferation. This study's results showed a gradual increase in turbidity of the mineral salt medium, indicating bacterial adaptation and proliferation, leading to the degradation of hydrocarbons. Udinyiwe et al. (2022) also reported increased turbidity and pH over 12 days, consistent with this study. The microbial proliferation observed using poor plate techniques in Table 6 indicates bacterial utilization and adaptation in the medium, in agreement with Udinyiwe et al. (2022) and Ogbonna et al. (2020). For instance, the microbial population of Bacillus subtilis increased from 5.9×10^6 at day 0 to 1.25×10^7 at day 15.

Conclusion

The study revealed that soil samples collected from the five mechanic workshops were contaminated with spent engine oil. Results from this study are relevant in explaining the changes in the state of the soil environment, which leads to a corresponding change in the microbial composition. Oil degraders with inherent hydrocarbon assimilatory potentials are enriched by the contaminants, while the less adapted microbial species among the total heterotrophic populations are gradually being eliminated due to changes in species composition. The results of this study showed that the microbes isolated from engine oil-contaminated soil had biodegradation potentials.

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Table 6: Optical density (600 nm) during shake flask biodegradation for 12 days

Isolates	Day 0 to Day 3	Day 3 to Day 6	Day 6 to Day 9	Day 9 to Day 12
Pseudomonas aeruginosa	0.077	0.394	0.771	0.737
Bacillus subtilis	0.086	0.525	1.103	0.919
Aspergillus niger	0.091	0.478	0.933	0.896
Trichoderma spp.	0.052	0.303	0.596	0.538
Bacterial consortium	0.103	0.768	1.394	1.361
Fungal consortium	0.119	0.694	1.286	1.209
Control	0.028	0.032	0.038	0.036

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