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Biodegradation Potentials of Contaminated Soil around Ministry of Works Workshop Aba, Abia State, Nigeria

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Abstract: Global industrialization over the past centuries has resulted in widespread contamination of the environment with organic and inorganic wastes and their pattern of disposal. The study aimed at isolating fungi from spent diesel contaminated soil around Ministry of Works in Aba, Abia State for biodegradation potentials on the soil properties. About four (4) fungal species were isolated from the five (5) sites using cultural and biochemical characteristics. The isolate was screened, and optical density measured using spectrophotometer. A total of 5 soil samples from each location (0-15 cm and 15-30 cm) were collected and homogenized to have composite sample. Samples were taken to the laboratory for analysis of soil physiochemical parameters, fungi count and biodegradable potential of the fungi using standard methods. Data obtained revealed that, physical property of the soil such as sand (85.20% ±0.01%), silk (6.4% ± 0.01%) were lower than the control location except clay (17.39% ± 0.01%). Chemical properties revealed highest concentration of element such as pH (4.76 ±0.01), total nitrogen (0.18% ±0.011%), total organic carbon (3.41 ±0.01), sodium (0.21 ± 0.01), potassium (0.24 ± 0.001), magnesium (4.41 ± 0.015), calcium (5.21 ± 0.015), organic matter (6.18 ± 0.011), and available phosphorus (30.99 ± 0.01). All elements in the study site were higher than the control site with an exception to sodium (Na), which was lower. Fungi isolate identified were *Aspergillus niger*, *Trichoderma virdae*, *Aspergillus flavus*, and *Pencillum corylophlum*. The degradation potential of fungi identified shows that consortium degraded 29% of diesel oil from the soil followed by *A. flavus*, *T. virdae*, *A. niger* and the least was *T. corylophlum*. The study concludes that despite indiscriminate disposal of spent diesel oil, the nutrient content was still higher than control and consortium performed well in degradation.

Key words: Contaminated soil, heavy metals, fungal, waste, Aba, biodegradation.

1. Introduction

Global industrialization over the past centuries has resulted in widespread contamination of the environment with organic and inorganic wastes. Contaminated land has generally resulted from industrial activities connected with the production, use, and disposal of substances potentially hazardous to the environment. The problem is worldwide, and the estimated number of contaminated sites is significant and increasing [1, 2]. Soil contaminants include heavy metals, mineral pollutants, monocyclic aromatic hydrocarbons, phenolic compounds, polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons, pesticides and other pollutants such as mineral oils and gasoline [3]. Release of hydrocarbons into the environment whether accidentally or due to human activities is one of the main causes of water and soil pollution. Soil contamination with hydrocarbons causes extensive damage of body system since accumulation of pollutants in animals and plant tissue may cause death or mutations [4]. These oil spills can even cause damage to the sea and shoreline organisms [5]. Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon as energy for metabolic activities and these microorganisms are ubiquitous and widely distributed in nature [6]. Soil contaminated with petroleum is hazardous to human health, pollutes ground water and decreases the agricultural productivity of soils [7]. The health concern stems

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primarily from direct contact with the contaminated soil, vapours from the contaminants, and from secondary contamination of water supplies within the soil. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion and washing [8-12]. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. The use of bioremediation process to detoxify or remove pollutants is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry. Bioremediation technology is believed to be non-invasive and relatively cost-effective [13]. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [14] and is cheaper than other remediation technologies [15]. Contamination of existing and potential agricultural lands is a major problem associated with the processing and distribution of crude and refined petroleum products in many oil producing countries like Nigeria. It is against this challenge that the study aimed at assessing the contaminated soil by spent diesel oil in Ministry of Works workshop, Aba Abia State. The objectives are to determine the physio-chemical properties of the contaminated soil, isolate, characterize and identify diesel degrading microorganism from diesel contaminated soil and assess the biodegradable potential of isolated microorganism.

2. Materials and Methods

2.1 Study Area

The study area is the State Ministry of Works workshop space located in the city of Aba in Abia State. Aba lies in the tropical rain forest zone of West Africa, in the south-eastern region of Nigeria. Aba's metropolitan region is divided into two local governments: Aba-North and Aba-South, as well as a portion of Ugwunagbo, Osisioma-Ngwa, and Obi Ngwa. Geographically, Aba is roughly located between latitudes 5°05′ N and 5°08′ N, and longitudes 7°20′ E and 7°28′ E, with a total area of 26.7 km^2 [16]. According to Ogbonna et al. [16] Aba is a large commercial metropolis in Nigeria, with economic ramifications extending throughout West and Central Africa and beyond. The informal sector accounts for the majority of commercial and manufacturing operations in the city, hence the prevalence of street vending. The dominant occupation of the city is trading, with some light manufacturing activities on leather works, wood and furniture, shoes, bags, and related goods. According to Godswill et al. [17] and Ijioma [18], Aba city has extended to Ugwunabo, Osisioma Ngwa, and Obi Ngwa local government areas due to its cosmopolitan nature. Aba is the most populous city in Abia State and one of the most populated cities in Nigeria's Southeast region. According to Nkwocha et al. [19], the city is located in West Africa's tropical rain forest zone. Aba's infrastructure is well-developed, with multiple main roads and highways. Economically, Aba is well-known for its bustling markets and manufacturing industries. The city is well-known for the manufacture of leather goods, textiles, and plastics. It also houses various large-scale manufacturing businesses, including as breweries, oil mills, and soap factories. Every year, the city experiences two weather conditions: dry and rainy seasons. Seasonal variations affect both the dry season (October-March) and the wet season (April-September). The Harmattan season, which is characterized by dusty winds and dry conditions, typically lasts from December to February, however this might vary. The average temperature in Aba is between 24-34 ℃.

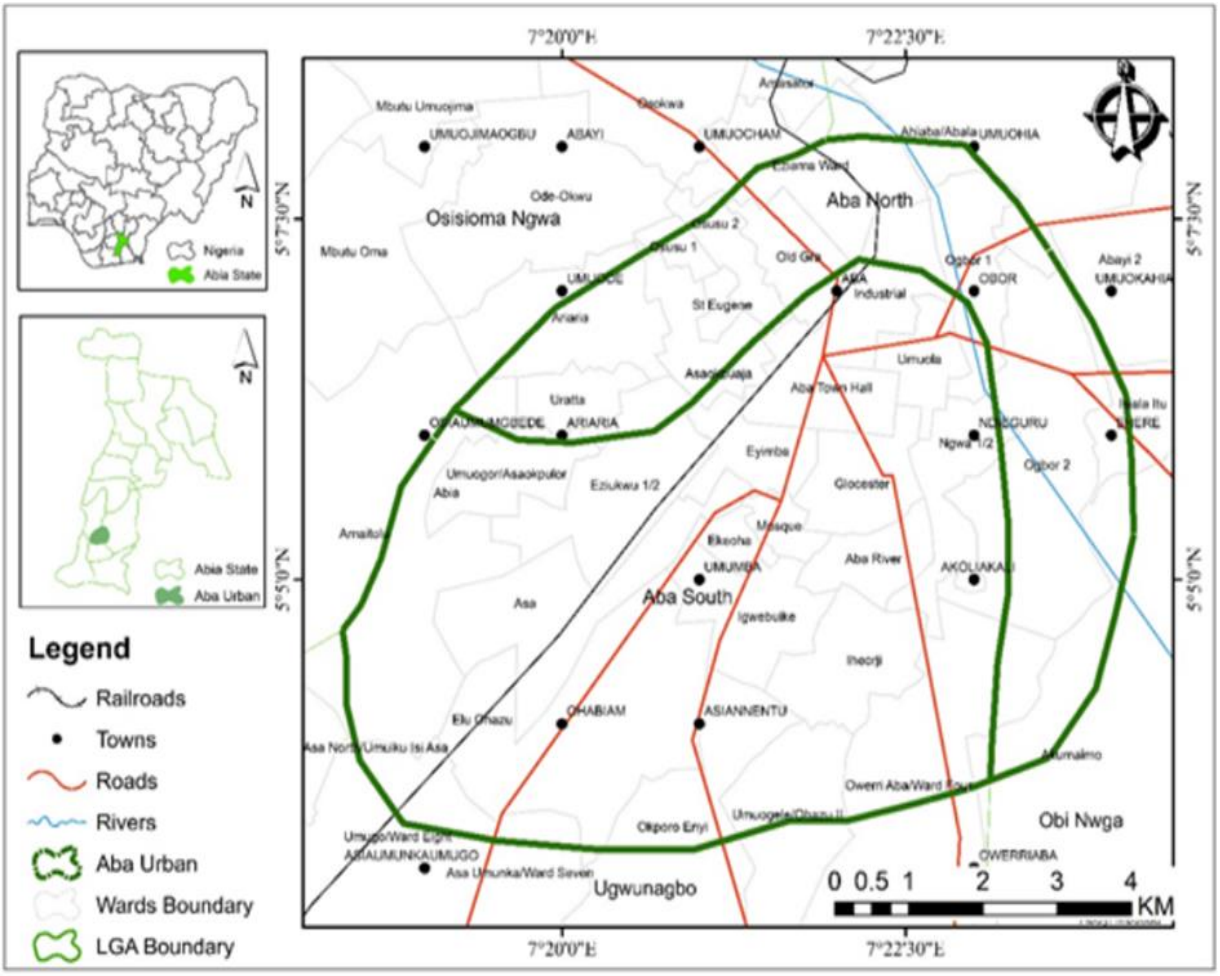

Fig. 1 Geographical map of Aba urban.

2.2 Sample Collection and Preparation

Spent engine contaminated soil samples were collected from Ministry of Works workshops as shown in figure 1. The sampling site and locations were frequently receiving spent engine oil and at each of these sampling locations, soil auger-boring instruments was used to collect soil from depths 0-15 cm (top soil) and subsoil 15-30 cm. The top soils (0-15 cm) collected from the five workshop points were homogenized in a clean bucket and a composite sample was drawn. Same process was repeated for the sub-soil (15-30 cm) and a control sample was collected 2 km away from the study area free from contamination. The samples were poured into polythene bags, labeled adequately and transported to the laboratory immediately for analyses.

2.3 Determination of Soil Physicochemical Parameters

2.3.1 pH Determination

Ten grams (10 g) of soil sample was weighed into a 50 mL beaker, and 20 mL of distilled water was added and the suspension was stirred for 30 min. The suspension was allowed to stand for 30 min undisturbed and the electrode was immersed about half way into the mixture, the pH meter reading taken after 30 s. This was repeated using 0.01 M $CaCl_2$ $2H_2O$ solution. The pH meter was standardized before the readings of the soil samples were taken.

2.3.2 Determination of Total Nitrogen in Soil

Nitrogen concentration was determined according to Kjeldahl digestion method. The sample was digested in a mixture of H_2SO_4, K_2SO_4 and selenium which converted all nitrogen into ammonium sulphate. The

distillation of ammonia (liberated after sodium hydroxide was added to ammonium sulphate), over boric acid was condycted and titrated against standard acid to determine the nitrogen content. Total nitrogen in soil (mg/kg) = $((S\text{-}B) \times N \times 14) \times (1{,}000)$/sample weight (g). where: S = Volume of acid used against sample; B = Volume of acid used against blank; N = Normality of the acid.

2.3.3 Phosphorus Determination

Phosphorus was determined by the method of Lim [20]. Ten (10) mL of the acid digest of the soil sample was placed in a 50 mL volumetric flask; 10 mL of vanadate-molybdate reagent was added and diluted to 50 mL with distilled water. The contents were mixed thoroughly and the absorbance was read after 10 min in a spectrophotometer at 420 nm wavelength as a function of phosphorous concentration. Zero, 1, 2, 3, 4 and 5 mL of the 10.0 mg/L phosphorus solutions were taken in 50 mL volumetric flask and colour developed in identical manner. The spectrophotometer was calibrated with known phosphorus concentration and the individual sample concentrations read with the standard.

2.3.4 Determination of Exchangeable Bases (Mg, Ca, K and Na)

Ten grams (10 g) of soil was weighed into a funnel fitted over 100 mL volumetric flasks. The soil in the funnel was leached with 1 M ammonium acetate solution into a volume of 100 mL (leaching was done for about 2 h with about 10 mL of the acetate at each washing) the funnel was removed and the flask corked and shaken thoroughly. Na and K were determined by flame emission while Ca and Mg were determined by atomic absorption spectrometry using the leachate.

2.3.5 Determination of Total Organic Carbon and Organic Matter

The soil sample was sieved through 2 mm sieve and 1 g of sieved soil sample was put in a 100 mL flask. Ten milliliters (10 mL) potassium dichromate and 20 mL sulphuric acid were added to the sample and shake very well. The mixture is allowed to cool on asbestos sheet and the volume is made up of 100 mL with distilled water and kept overnight. The optical density was measured at 660 nm wavelength using a spectrophotometer.

Organic carbon (%) = optical density × Factor F

Organic matter (%) is determined by: Organic Carbon × 1.724.

2.4 Heavy Metal Analysis

The heavy metal analysis was carried by Atomic Absorption Spectrophotometry (AAS). For each of the metal: iron (Fe), zinc (Zn), lead (Pb), cadmium (Cd), copper (Cu), AAS is calibrated using a standard solution of the metal. Five grams (5 g) of soil sample was digested in 20 mL of hydrogen chloride on a heating mantle to dryness. The extract was aspirated directly into the AAS and reagent blank was used to estimate the amount of each metal.

2.5 Determination of Total Fungal Count

The total fungal counts of the soil samples were estimated using potato dextrose agar (PDA). One gram (1 g) of the soil and 1 mL of the water sample was mixed with 9 mL of normal saline. One milliliter (1 mL) of the soil and water each was diluted from 10^{-1} to 10^{-10} mL. An aliquot of 1 mL dilution 10^{3} mL was seeded into PDA. The inoculated plates were incubated at 28 ℃ for 72 h. The fungal colony forming units (CFU/g) were then counted. Repeated sub-culturing was done for each isolate to get pure culture of the fungi for easy identification.

2.6 Screening of Diesel Hydrocarbon Degrading Fungi

For preliminary screening of diesel hydrocarbon degrading fungi oil agar, the oil agar medium was prepared by adding 1% crude oil to mineral salt medium (MSM) (*v*/*v*) of Mills et al. [21] as modified by Okpokwasili and Okorie [22]. The composition of MSM was NaCl (10.0 g), $MgSO_4 \cdot 7H_2O$ (0.42 g), KCl (0.29 g), KH_2PO_4 (0.83 g), Na_2HPO_4 (1.25 g), $NaNO_3$ (0.42 g), agar (20 g), distilled water (1 L) and pH 7.2. Plate was inoculated with spore suspension of each

isolate, incubated at 26 ℃, and the appearance of substantial growth was monitored daily up to 7 days [23].

2.7 Characterization and Identification of Fungal Isolate

Pure fungal cultures were observed while still on plates and the isolates were characterized using cultural characteristics such as the colour of aerial and substrate hyphae, type of hyphae, shape and kind of asexual spore, presence of foot cell, sporangiophore or conidiophores and the characteristics of spore head using microscopic and macroscopic methods. The identities of the organisms were determined by comparing their characteristics with those of known taxa as described by Domsch and Gams [24].

2.8 Estimation of Diesel Hydrocarbon Degradation

The efficiency of the isolates in degrading diesel hydrocarbon was evaluated for some common petroleum fuels, kerosene, diesel and octane, where the rate of degradation is expressed as percent degradation as described by Juwarkar and Khirsagar [25]. Briefly, the isolates were cultured in MSM supplemented with 1% each of diesel. Following 7 days incubation at 26 ℃ with constant shaking, the petroleum fraction of cell free culture broth was extracted with three volumes of toluene. The optical density (OD) was measured at 420 nm by Visible-UV spectrophotometer (UV-VIS RS Spectrophotometer, LaboMed). The extent of degradation was calculated using the following equation:

$$\text{Degradation (\%)} = (C_0\text{-}C)/C_0 \times 100$$

where C_0 and C refer to initial and final concentration, respectively.

The assay was carried out in triplicate.

2.9 Statistical Analysis

Data collected from the laboratory analysis were subjected to descriptive statistical analysis using Statistical Package for the Social Sciences (SPSS) version 20. Results were presented in tables (mean, and standard deviation) and charts.

3. Results and Discussions

3.1 Effect of Diesel Oil on Soil Physicochemical Properties

The results in Table 1 show the physical and chemical properties of the diesel oil contaminated soil obtained at different depth and the control. Physico-chemical analysis of the soil samples (Table 1) shows a significant difference between the control sample and diesel oil contaminated soil obtained at different soil depth. From the result obtained it is observed that the control sample soil has a textural class of loamy sands, with sand fraction having 86.0% ± 0.00%, silt fraction 7.4% ± 0.01% and clay fraction 11.8% ± 0.001% when compared to the diesel oil contaminated soil having sand fraction having 86.0% ± 0.00%, silt fraction 7.4% ± 0.01% and clay fraction 11.8% ± 0.001% for the top soil (0-15 cm) and the sub-soil (15-30 cm) having sand fraction having 86.0% ± 0.00%, silt fraction 7.4% ± 0.01% and clay fraction 11.8% ± 0.001%.

The result shows a soil reaction of very strongly acidic property for the diesel oil contaminated samples with the pH 4.76 ± 0.001 at depth 0-15 cm and 4.74 ± 0.001 at a depth 15-30 cm when compared to the control with the pH 6.20 ± 0.001. A pH value between 6.5 and 7.5 is considered optimum for the growth of many plants. pH affects plant growth primarily through its effects on nutrient availability. High or low pH causes deficiencies in essential nutrients that plants need to grow. The low pH value of the polluted soils may be attributed to higher concentration of particulates from spent engine oil. These particulates would normally settle on the top soil, and hence, affects its acidity. It could also be as a result of the leaching away by rain water of the basic cation (Ca^{2+}, Mg^{2+}, K^+, Na^+) and replacement of many of these basic cations by hydrogen ion (H^+) from carbonic acid (H_2CO_3) formed from water and dissolved CO_2. The low pH may also be due to increased degradation of diesel oil by the organisms in the soil, resulting in accumulation of acidic metabolites [26]. Acidic soil often

Table 1 Summary of physical and chemical properties of soil samples (mg/kg).

Sample	Sand (%)	Silt (%)	Clay (%)	pH	Total N (%)	Total org. C	Na	K	Mg	Ca	OM	EA	ECEC	BS	Available P
Control	86.20 ± 0.01	7.4 ± 0.01	11.8 ± 0.001	6.20 ± 0.01	0.10 ± 0.011	1.08 ± 0.01	0.30 ± 0.01	0.14 ± 0.001	1.22 ± 0.017	2.01 ± 0.015	1.85 ± 0.011	0.86 ± 0.015	4.38 ± 0.00015	62.8 ± 0.01	25.69 ± 0.01
0-15 cm	85.20 ± 0.01	6.4 ± 0.01	13.8 ± 0.001	4.76 ± 0.01	0.18 ± 0.011	3.41 ± 0.01	0.21 ± 0.01	0.24 ± 0.001	4.41 ± 0.015	5.21 ± 0.015	5.91 ± 0.011	1.37 ± 0.015	11.50 ± 0.002	76.30 ± 0.01	30.99 ± 0.01
15-30 cm	79.23 ± 0.01	6.2 ± 0.1	17.39 ± 0.01	4.74 ± 0.01	0.16 ± 0.011	3.19 ± 0.01	0.11 ± 0.01	0.19 ± 0.0001	4.22 ± 0.015	5.13 ± 0.015	6.18 ± 0.011	3.14 ± 0.015	12.94 ± 0.039	92.51 ± 0.001	23.20 ± 0.01

Source: Field data (2024).

causes the stunting and yellowing of leaves, resulting in the decrease in growth and yield of crops as the pH levels falls. Additionally, plants grown in adverse pH conditions may be more prone to disease and fungal attack. The availability of plant nutrients is considerably affected by soil pH. Calcium, potassium, magnesium and sodium are alkaline elements, which are lost with increasing acidity whereas phosphorous is more available in acidic soil conditions. There were varying degrees of increase in organic carbon and organic matter in each of the contaminated samples compared to the control with the least organic carbon content of 3.19% ±0.01% and the highest being 3.41% ± 0.01% and the control being 1.08% ± 0.01% The increase in organic carbon could be attributed to the spent engine oil contamination in the soil samples. Diesel oil contains principal elements such as oxygen, nitrogen and sulphur other than hydrogen and carbon. This is in line with the findings of Lovely and Cackette [27] that significant increases in total nitrogen and organic carbon of soil have been observed as a result of oil contamination. There were also varying increases in total nitrogen in each of the contaminated samples compared to the control with the least total nitrogen content of 0.16% ±0.01% (sub soil) and the highest being 0.18% ±0.01% (top soil) and the control being 0.10% ± 0.01%. The reason for the increase in total nitrogen content may be due to mineralization of N content in the diesel oil or dead degrader may also be contributed to the significant increase in the total N. The increase in nitrogen content of the diesel oil contaminated soil was in line with earlier reports by [28, 29]. Odu [28] reported that the increase in total N may be due to increase in non-symbiotic fixer attracted to the contaminated soils. There is increase in Ca in the contaminated samples compared to the control with the least calcium content of 5.13% ± 0.015% (15-30 cm) and the highest being 5.21% ± 0.01% (0-15 cm) and the control being 2.01% ±0.01%. The increase in calcium content of the soil may be due to the release of calcium from the diesel oil as a result of microbe's activities on diesel. The result shows decrease in K content of the contaminated soil samples with the least potassium content of 5.13% ±0.015% (15-30 cm) and the highest being 5.21% ± 0.01% (0-15 cm) and the control being 2.01% ± 0.01%. The reasons for the decrease may be due to immobilization and leaching of the nutrient. The result similarly showed decrease in Na content of the contaminated soil sample when compared to the control. The reason for the decrease in Na content may be due to leaching of nutrient. The result shows increase in exchange acidity (EA) in the contaminated samples as compared to the control. The reason for increase in EA may be due to leaching of basic cations leading to increase in Al^{3+} and H^{+} content of the soil effective cations exchange capacity (ECEC). The result shows increase in ECEC content in all the treatments compared to the control. The reason for the increase may be due to the increase observed in EA.

The results of the heavy metal analysis presented in Table 2 show that diesel contaminated soil with significantly high concentrations of heavy metals (Cd, Cr, Cu, Fe, Zn, Pb) compared to the control sample. The mean concentration of Cd (10.00 mg/kg) in the spent gasoline-contaminated soil was above the permissible limit (0.003 mg/kg) and control sample (0.58 mg/kg).

Table 2 Summary of heavy metal concentration in soil samples of spent engine oil (mg/kg).

Sample	Cd	Cr	Cu	Fe	Zn	Pb
0-15 cm	5.3	6.2	9.6	6.0	6.5	15.1
15-30 cm	9.4	11.5	7.0	5.8	6.4	18.2
Mean	10.00	11.95	13.1	5.90	6.45	16.65
Control	0.58	1.43	1.07	0.6	2.43	3.0
FAO/WHO limit	0.003	100.0	36-75	50,000	20	2-300
USEPA limit	0.48					200

FAO/WHO 2002, 2011 Report.

The mean concentration of Cr (11.95 mg/kg) in soil samples was found under the standard limit by FAO/WHO range (36-75 mg/kg) while its concentration in the soil samples was higher than the control (1.07 mg/kg). Cu and Fe concentrations among the trace metals were found higher in samples around the workshop sites because both the metals are used in the manufacturing of various items including wires, steel equipment, and alloys that find their way into the soil. Their high concentration could be an indication of the migration of leachate rich in Cu and Fe or movement by runoff water in the surrounding soils. The concentration of Cu and Fe in soil was found within the reference range recommended by WHO (FAO/WHO: 36-37 and 50,000 mg/kg respectively) whereas their concentration was found higher than the control sample (1.07 and 0.6 mg/kg respectively). Moreover, Zn and Pd concentrations in the uncontaminated garden soil were within the permissible limits of WHO/FAO limit for soil. Although the concentrations were higher than the control, this shows that there is anthropogenic impact on the soil [30-32].

3.2 Isolation of Total Heterotrophic Fungi and Hydrocarbon Utilizing Fungi Counts from Diesel Oil Contaminated Soil

Four fungal strains were isolated from the diesel contaminated soil samples in the present investigation (see table 3). *Aspergillus niger*, *Trichoderma virdae*, *Aspergillus flavus*, and *Pencillum corylophlum* were isolated. These fungi have been reported by other authors' studies in the degradation of hydrocarbon [22].

Total heterotrophic fungi count expressed as the number of colony forming units was 45.2×10^3 CFU/g in the top soil (0-15 cm) diesel oil contaminated sample and 3.5×10^6 CFU/g in sub soil (15-30 cm) diesel-contaminated soil and the uncontaminated soil sample (control) was 50.67×10^3 CFU/g (Table 4). The lower microbial population in the contaminated soil could be a direct or indirect effect of the diesel oil. Considering direct effect, it was demonstrated that the presence of C_5-C_{10} homologues in the petroleum fraction is inhibitory to the majority of the hydrocarbon-degrading microorganisms [33]. These solvents tend to disrupt

Table 3 Colony characteristics and microscopic observation of fungi isolates from diesel oil contaminated soil.

Isolates	Cultural appearance	Microscopic features	Possible organism
1	Black mycelia growth and fully extended in growth medium	An upright conidiophore that terminates in a devate swelling bearing philalides at the apex or radiating from the entire surface, conida are I-celled and globule.	*Aspergillus niger*
2	Green mycelia growth	A branched conidiophore hyaline, not verticillate; phialides single, conida hyaline, I-celled, avoid, borne in small terminal clusters.	*Trichoderma virdae*
3	Yellow mycelia growth	Upright conidiophores that terminate in a devate swelling phalides at the apex or radiating from the entire surface, conida are I-celled and globule.	*Aspergillus flavus*
4	Greenish white colony	Long, smooth, erect. Conidiophores were long, smooth, erect, hyaline, barded, bwertrallati, frequency brunded below the level of mutulae, philaides borne in one series bearing chain of corrida forming brush like cluster	*Pencillum corylophlum*

Table 4 Summary of total heterotrophic fungi and hydrocarbon utilizing fungi counts.

Sample	Total heterotrophic fungi count (CFU/g)	Total diesel utilizing fungi count (CFU/g)
Top soil (0-15 cm)	45.20×10^3	22.50×10^3
Sub soil (15-30 cm)	38.30×10^3	18.30×10^3
Control	50.67×10^3	14.20×10^3

membrane lipid structures of microorganisms. In addition, polycyclic aromatic hydrocarbons are highly toxic to microbial cell membranes, having both carcinogenic and mutagenic activity [34]. Indirectly, an extremely high level of TPH in contaminated soil can result in impairment of gaseous exchange and retention of soil carbon dioxide [35]. These conditions in the present study might have resulted in increased acidity and decreased porosity of the contaminated soil.

3.3 Biodegradation Potential of Isolated Fungi Strain and a Mixed Fungi Consortium

Results (Fig. 2) showed that the mycoflora of the study area possess the ability to utilize diesel oil. All the fungal isolates showed varying degree of biodegradation of crude oil in MSM indicating that they all utilize crude oil as their main source of carbon.

In this study, the percentage removal of hydrocarbon components by the fungal isolates is shown in Fig. 2. At 7th day, mixed consortium had the highest percentage (45.37%) removal of spent engine oil analysed followed by *Aspergillus niger* (42.52%), *Aspergillus flavu* (40.32%), *Trichoderma viride* (40.1%) and *Pencillum corylophlum* (35.39%). At 14th day, mixed consortium had the highest percentage (53.23%) removal of spent engine oil analysed followed by *Aspergillus niger* (78.3%), *Aspergillus flavu* (72.28%), *Trichoderma viride* (72.32%) and *Pencillum corylophlum* (65.22%). At 21 day, mixed consortium had the highest percentage (64.72%) removal of spent engine oil analysed followed by *Aspergillus flavu* (53.69%), *Aspergillus niger* (53.26%), *Trichoderma viride* (51.34%) and *Pencillum corylophlum* (48.35%). After 28 days, the mixed consortium had the highest percentage (83.3%) removal of spent engine oil analysed followed by *Aspergillus niger* (78.3%), *Aspergillus flavu* (72.28%), *Trichoderma viride* (72.32%) and *Pencillum corylophlum* (65.22%). This could be an indicator of a synergistic relationship among individual fungi of the consortium that might have invariably boosted the degradative potential of the fungi consortium. The result also agrees with the theory that individual organisms in a consortium of microorganism perform substantial roles and also depend on the presence of different species and strains to effectively degrade crude oil in the environment [36].

An interesting demonstration generated in this work shows an increase in rates of fungal growth in the media containing diesel oil which might be due to the fact that the fungi used diesel oil as a substrate for their growth using extra cellular enzymes to break down the recalcitrant hydrocarbon molecules, by dismantling the long chains of hydrogen and carbon, thereby, converting crude oil into simpler forms or products that can be absorbed for the growth and nutrition of the fungi as shown by Adekunle and Adebambo [37]. From the biodegradation study, it was observed that *Trichoderma viride, Aspergillus flavus*, and *Aspergillus niger* had the best growth in the oil broth. This is in line with the work of other researchers such as Vidali [15] and Bhattacharya et al. [38], who reported similar findings which made their application for bioremediation efforts suitable. The efficient crude oil degrading ability of the organisms may be due to the competent degradative enzyme system of the organisms. The high degradation ability of these organisms especially *Aspergillus flavus* and *Trichoderma viride* may be due to the higher production of extracellular enzymes and organic acids that enable the organisms to utilize the hydrocarbon faster [39]. This agrees with the findings of Stamets [40] that mycelial mats are used for bioremediation because they produce extracellular enzymes and acids that break and dismantle the long chains of hydrocarbon, the base structure common to oils, petroleum products and many other pollutants. The fact that the species of *Aspergillus*, *Trichoderma* and *Pencillum*, could degrade crude oil efficiently points to their potential usefulness in cleaning oil spills in tropical soils. This study is in line with the findings of April et al. [41] that reported 22 species of *Penicillium* and 5 species of *Aspergillus*

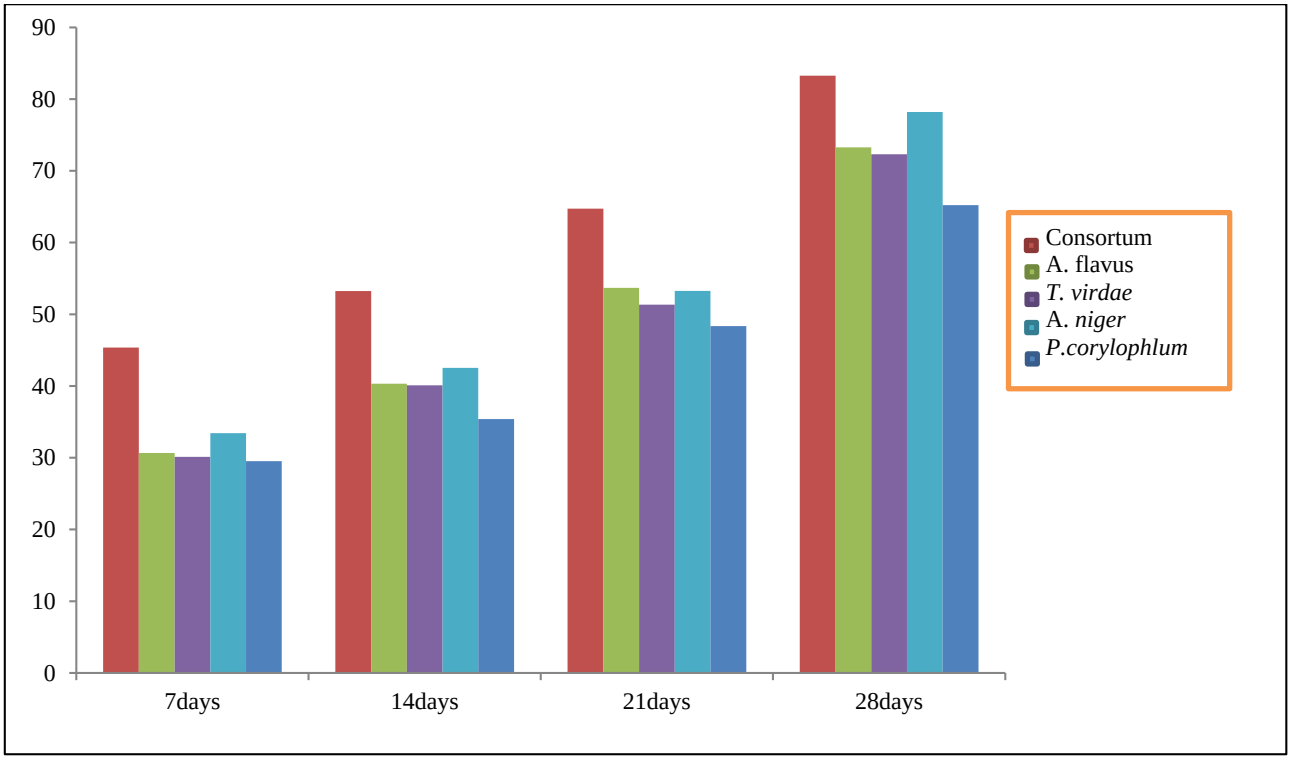

Fig. 2 Biodegradation potentials of isolated fungi strain and a mixed fungi consortium.

isolated from the flare pit soils in Northern and Southern Canada. These isolates showed the ability to degrade hydrocarbons on solid medium amended with crude oil.

4. Conclusion

The study concludes that diesel-contaminated soil has extremely high concentration of petroleum hydrocarbons, which affected soil physicochemical properties. High organic carbon and total nitrogen concentration, low soil fertility index, low pH and low moisture probably could decrease fungal growth in the contaminated soil. Growth and activity of microorganisms in such sites could be enhanced by increasing moisture content and substrate which may further increase bioavailability of petroleum hydrocarbons for microbial degradation in the soil. Based on the findings, the study recommend that spent diesel oil should not be disposed indiscriminately on the soil and strong enforcement should be put in place to control soil contamination.

References

[1] Mougin, C. 2002. "Bioremediation and Phytoremediation of Industrial PAH Polluted Soils." *Polycyclic Aromatic Hydrocarbons* 22: 1011-43.

[2] Kaimi, E., Mukaidani, T., Miyoshi, S., and Tamaki, M. 2006. "Ryegrass Enhancement of Biodegradation in Diesel-Contaminated Soil." *Environmental and Experimental Botany* 55: 110-9.

[3] Bamforth, S. M., and Singleton, I. (2005). Bioremediation of polycyclic aromatic hydrocarbons: Current knowledge and future directions**.** *Journal of Chemical Technology and Biotechnology* 80(7):723 – 736.

[4] Sheetal, S. 2012. "Bioremediation: A Boon to Hydrocarbon Degradation." *International Journal of Environmental Sciences* 2 (4): 2408.

[5] Rodr ́guez-Mart ́nez, E. 2006. "Microbial Diversity and Bioremediation of a Hydrocarbon Contaminated Aquifer." *International Journal of Environmental Research and Public Health* 3 (3): 292-300.

[6] Jyothi, K. K., Surendra, B., Nancy, C. K., and Amita, K. 2012. "Identification and Isolation of Hydrocarbon Degrading Bacteria by Molecular Characterization." *BioAxis DNA Research Centre (P) Ltd, Hyderabad, Helix* 2: 105-11.

[7] Wang, J. Z. Z., Zhang, Y. M., Su, W., He, F., and Song, H. G. 2008. "Phytoremediation of Petroleum Polluted Soil."

Petroleum Science 5 (2): 167.

[8] Antai, S. P., and Mgomo, E. 1989. "Distribution of Hydrocarbon Utilizing Bacteria in Oil-Spill Areas." *Microbiology Letters* 40: 137-43.

[9] Das, K., and Mukherjee, A. K. 2011. "Crude Petroleum-Oil Biodegradation Efficiency of *Bacillus subtitles* and *Pseudomonas aerations* Strains Isolated from a Petroleum-Oil Contaminated Soil from North-East India." *Bioresearch Technology* 98 (7): 1339-45.

[10] Johnsen, A. R., Wick, L. Y., and Harms, H. 2005. "Principle of Microbial PAH-Degradation in Soil." *Environmental Pollution* 133: 71-84.

[11] Colwell, R. R., Walker, J. D., and Nelson, J. D. 1993. "Microbial Ecology and the Problem of Petroleum Degradation in Chesapeake Bay." In *The Microbial Degradation of Oil Pollutants*, edited by Ahearn, D. G., and Myers, S. P. Baton Rouge: Louisiana State University, pp. 185-97.

[12] Leahy, J. G., and Colwell, R. R. 1990. "Microbial Degradation of Hydrocarbons in the Environment." *Microbial Reviews* 53 (3): 305-15.

[13] Millioli, V. S., Servulo, E. L. C., Sobral, L. G. S., Declor, W., and Iho, D. E. 2009. "Bioremedation of Crude Bearing Soil: Evaluating the Effect of Rhamnnolipid Addition to Soil Toxicity and to Crude Oil Biodegration Efficiency." *Global Nest Journal* 11 (2): 181-8.

[14] Rosenberg, E., and. Ron, E.Z (1996). Bioremediation of petroleum contamination In: Crawford RL and Crawford DL (eds.) Cambridge: Cambridge University Press. Bioremediation: Principles and Application pp.100-124.

[15] Vidali, M. 2001. "Bioremediation: An Overview." *Journal of Applied Chemistry* 73 (7): 1163-72.

[16] Ogbonna, C. G., Nwaeze, C., Okoye, V. U., and Eleazu, E. I. 2016. "The Resilience of Street Vendors and Urban Public Space Management in Aba, Nigeria." *Developing Country Studies* 6 (11): 11.

[17] Godswill, O. C., Chinweoke, N., Ugonma, O. V., and Ijeoma, E. E. 2016. "The Resilience of Street Vendors and Urban Public Space Management in Aba Nigeria." *Developing Country Studies* 6 (11): 83-93.

[18] Ijioma, U. D. 2021. "Evaluation of Water Situation and Development of Drinking Water Management Plan for Aba City Southeast Nigeria." Doctoral dissertation, BTU Cottbus-Senftenberg.

[19] Nkwocha, K. F., Phil Eze, P. O., Iheukwumere, S. O., and Shettima, M. K. 2023. "Municipal Solid Waste Disposal in the City of Aba: Challenges and Solutions." *Journal of Waste Manage Xenobio* 6 (1): 183.

[20] Awual, M. R., Jyo, A., Ihara, T., Seko, N., Tamada, M., and Lim, K. T. (2011). Enhanced trace phosphate removal from water by zirconium (IV) loaded fibrous adsorbent. *Water research*, *45*(15), 4592-4600.

[21] Mills, A. L., Breuil, C., and Colwell, R.R. (1978). Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. *Can J Microbiol.* 24(5):522-7. PMID: 350362.

[22] Eze, V. C., and Okpokwasili, G. C. 2010. "Microbial and Other Related Changes in a Niger Delta River Sediment Receiving Industrial Effluents." *Continental Journal of Microbiology* 4: 15-21.

[23] Desai, A., Jitendra, J., Desai, D., and Hanson, K. G. 1993. "A Rapid and Simple Screening Technique for Potential Crude Oil Degrading Microorganisms." *Biotechnology Techniques* 7 (10): 745-8.

[24] Domsch and Gams, W. (1970). Przipisy- Phialophora foetens In: Nova Hedwigia 18:17.

[25] Juwarkar, A., and Khirsagar, D. G. 1991. "The Utilization of Certain Hydrocarbon by Microorganisms." *Journal of Bacteriology* 41: 653-73.

[26] Ijah, J. J., and Abioye, O. P. 2003. "Assessment of Physicochemical and Microbiological Properties of soil, 30 Months after Kerosene Spill." *Journal of Research and Science Management* 1: 24-30.

[27] Lovely, C. A., and Cackette, T. A. 2001. "Diesel Engine: Environmental Impact and Control." *Air and Waste Management Association* 57: 805-47.

[28] Odu, C. T. I. 1972. "Microbiology of Soil Contaminated with Petroleum Hydrocarbons." *Journal of the Institute of Petroleum* 58: 201-8.

[29] Udo, E. J., and Fayemi, A. A. 1975. "The Effect of Oil Pollution of Soil on Germination, Growth and Nutrient Uptake of Corn (*Zea mays* L.)." *Journal of Environmental Quality* 1: 540-7.

[30] Awashthi, S. K. 2000. *Prevention of Food Adulteration Act No. 37 of 1954, Central and State Rules as Amended for 1999*. New Delhi: Ashoka Law House.

[31] Sharma, R. K., Agrawal, M., and Marshall, F. M. 2006, "Heavy Metals Contamination in Vegetables Grown in Waste Water Irrigated Areas of Varanasi, India." *Bulletin of Environmental Contamination and Toxicology* 77: 311-8.

[32] Gupta, S., Nayek, S., Saha, R. N., and Satpati, S. 2008. "Assessment of Heavy Metal Accumulation in Macrophyte, Agricultural Soil and Crop Plants Adjacent to Discharge Zone of Sponge Iron Factor." *Environmental Geology* 55: 731-9.

[33] Okoh, A. I. 2006. "Biodegradation Alternative in the Clean-Up of Petroleum Hydrocarbon Pollutants." *Biotechnology and Molecular Biology Review* 1: 38-50.

[34] Amellal, N., Portal, J. M., Vogel, T. (2001). Distribution and location of polycyclic aromatic hydrocarbons (PAHs) and PAH-degrading bacteria within polluted soil aggregates. *Biodegradation* 12, 49–57. https://doi.org/10.1023/A:1011909107858

[35] Ujowundu, C. O., Kalu, F. N., Nwaoguikpe, R. N., Kalu,

O. I., Ihejirika, C. E., Nwosunjoku, E. C., and Okechukwu, R. I. 2011. "Biochemical and Physical Characterization of Diesel Petroleum Contaminated Soil in Southeastern Nigeria." *Research Journal of Chemical Sciences* 1: 57-62.

[36] Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A. B., and Basri, M. 2004. "Biodegradation of Hydrocarbons in Soil by Microbial Consortium." *International Biodeterioration & Biodegradation* 54: 61-7.

[37] Adekunle, A. A., and Adebambo, O .A. (2007). Petroleum hydrocarbon utilization by fungi isolated from *Detarium senegalense* (j. f gmelin) seeds. Journal of American Science. 2007; 3(1): 69-76.

[38] Bhattacharya, D., Sarma, P. M., Krishnan, S., Mishra, S., and Lal, B. 2003. "Evaluation of Genetic Diversity among *Pseudomonas citronellolis* Strains Isolated from Oily Sludge Contaminated Sites." *Applied and Environmental Microbiology* 69 (3): 1435-41.

[39] Damisa, D., Oyegoke, T. S., Ijah, U. J. J., Adabara, N. U., Bala, J. D., and Abdulsalam, R. 2013. "Biodegradation of Petroleum by Fungi Isolated from Unpolluted Tropical Soil." *International Journal of Applied Biology and Pharmaceutical Technology* 4 (2): 136-40.

[40] Stamets, P. (1999). Helping the ecosystem through mushroom cultivation In: Growing gourmet and medicinal mushroom. Batellet, A. (edition). Ten speed press, Berkeley, California. p. 452.

[41] April, T. M., Foght, J. M., and Currah, R. S. 2000. "Hydrocarbon-Degrading Filamentous Fungi Isolated from Flare Pit Soil in Northern and Western Canada." *Canadian Journal of Microbiology* 46 (1): 38-49.