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Bioremediation of Spent Engine Oil Contaminated Soils Using Indigenous Fungi Species

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ABSTRACT

Spent engine oil is derived from lubricating oil which has been used to lubricate various internal combustion engines and it is drained out for disposal during servicing of the engine. Spent engine oil causes great damage to soil and soil microflora when disposed indiscriminately. Thus, the bioremediation of spent engine oil contaminated soil was studied using indigenous degrading fungi isolated from hydrocarbon contaminated soils obtained from automobile mechanic workshops located at both Okpe and Uvwie Local Government Areas of Delta State, in the Niger Delta region of Nigeria. Three (3) fungi isolates with high engine oil biodegradability potential were used for the spent engine oil (SEO) bioremediation study. The fungi isolates used for the test were identified as, *Aspergillus glaucus*, *Trichoderma polysporum* and *Talaromyces flavus*using the API 20C method. The test microcosms were incubated for four weeks at 28 ± 2°C. Physicochemical parameters such as, Sulphate concentrations, Total petroleum hydrocarbon, Nitrate concentrations, Phosphate concentrations, Total organic carbon content, pH and Hydrocarbon utilizing fungi counts were monitored weekly using standard ASTM methods to assess the biodegradation of the spent engine oil. At the end of the test duration, *Talaromyces flavus* recorded the highest percentage spent engine oil biodegradation (69.66%) for the 5% SEO experimental set up. Similarly, *Aspergillus glaucus* recorded the highest percentage SEO biodegradation (66.16%) for the 10% experimental set up. Thus, *Talaromyces flavus* and *Aspergillus glaucus* could be used to effectively bioaugment the bioremediation process of spent engine oil contaminated soils to restore the soil to its original state within a short period of time.

Keywords : *Spent engine oil contaminated soils, bioremediation and indigenous fungi species.*

I. INTRODUCTION

In Nigeria, it is common among motor mechanics not to have a specified and appropriate disposal method for spent engine oil used in their various workshop, which leads to disposal into gutters, water drains and soil (Okonokhua, *et al.*, 2007). Spent engine oil is defined as used lubricating oils obtained after servicing and subsequently drained from automobile and generator engines. Spent oils contain high percentage of aromatic and aliphatic hydrocarbons, nitrogen and sulphur compounds and metals such as Manganese, Calcium, Zinc, Lead, than fresh oils. These metals are introduced into the fresh oil as a result of wears and tears of the engine (Mohd, *et al.*, 2011).

Engine oil is described by Klamann (1984) as the oil that principally functions as cleaning the motor engines, lubricating the moving parts of motor

engines, inhibiting corrosion of motor engines, improving sealing and cooling the engine by transporting heat away from the moving parts. The present day engine oils are derivative of petroleum-based and non-petroleum produced chemical compounds. Engine oils are, therefore, mainly blended by employing base oils composed of hydrocarbons (organic compounds containing carbon and hydrogen exclusively), for instance mineral oil (Corsico, *etal.*, 1999).

Spent engine oil causes great damage to soil and soil microflora. It creates unsatisfactory condition for life in the soil due to poor aeration, immobilization of soil nutrients and lowering of soil pH (Ugoh and Moneke, 2011). It has been shown that marked changes in properties occur in soil contaminated with hydrocarbon, this affects the physical, chemical and microbiological properties of the soil (Okonokhua, *et al.*, 2007). At low concentrations, some of these heavy metals are essential micronutrients for plants, but they can cause metabolic disorders and growth inhibition when the concentration is at high levels. The key components typical of spent engine oil consist of aliphatic and aromatic hydrocarbons such as phenol, naphthalene, benzo (a) anthracene, benzo (a) pyrene, and fluoranthene (Irwin, *et al.*, 1997). After any oil spillage, Polycyclic Aromatic Hydrocarbons (PAHs) which are component of spent oil, are important contaminant which are retained in the environment. PAHs could disrupt the endocrine system of animals affect (Kathi and Anisa, 2012). Spent Oil has contaminated soils used for agricultural lands and has not spared the aquatic and marine plants and animals in Nigeria. Ground water has also been contaminated hence polluting the crops and farm animals (Eneh, 2011). Therefore, there is the need for bioremediation of spent engine oil (hydrocarbon) contaminated.

Physical, chemical and mechanical processes are traditional methods used in remediation of contaminated areas. Physical remediation method includes incineration, brick making and skimmers etc. This method cannot biodegrade more than 10-15% of spilled oil (Thavasi, *et al.,* 2011). Use of chemical surfactants as remediating agent on the other hand is not favourable due to their toxic effects on flora and fauna (Thavasi, *et al.,* 2011). However, this type of treatment system requires heavy machinery and the environmental consequences of this pollutant removal may result in massive air pollution (Bhupathiraju, *et al.,* 2002).

Fungi and bacteria are being used for biodegradation of hydrocarbons (Snape, *et al.,* 2001). The filamentous fungi possess some attributes that enable them to be good potential agents of degradation. A fungus attaches itself quickly on the substratum then digests the substratum through the secretions of extracellular enzymes (Okerentugba and Ezeronye, 2003). Fungi are capable of growing under environmental stress including, low pH, poor nutrients and low water activity. Fungal bioremediation is an attractive approach over other techniques like physical-chemical techniques, for it is simple, easy to maintain, cost effective and can be produced in mass (Achal, *et al.,* 2011). It also requires little energy input and preserves the soil structure. Studies by Smita, *et al.,* (2012) shows that, *Aspergillus, Penicillium, Fusarium,Rhizopus, Alternaria* and *Cladosporium* species have been identified as effective hydrocarbon biodegraders.

II. MATERIALS AND METHODS

A. Collection of Spent Engine Oil

One (1) litre of spent engine oil was bought from an automobile mechanic workshop located at Okuokoko in Okpe LGA, Delta State (latitude 5.578557,

longitude 5.830875). It was collected in 1L glass bottle and preserved in the refrigerator until required for use. Spent engine oil obtained was filter sterilize with WHATMAN NO 42 filter paper. Its physicochemical properties were analyzed with the following parameters; pH and total petroleum hydrocarbon (TPH)/total hydrocarbon content (THC) using standard methods of ASTM and APHA.

B. Collection of Hydrocarbon contaminated Soils

Samples of hydrocarbon contaminated soils were collected from automobiles mechanic workshops located in Uvwie and Okpe Local Government Areas (LGA) of Delta state. Sample A was collected from a workshop at Okuokoko (latitude 5.578557, longitude 5.830875) in Okpe LGA, while sample B was taken from a workshop at Ugbomro (latitude 5.563975, longitude 5.819289) in Uvwie LGA, Delta state.

C. Collection of Hydrocarbon Uncontaminated (Native) Soil

Nine (9) kilogrammes of pristine sandy loam was collected from the Federal University of Petroleum Resources, Effurun farm at Ugbomro in Uvwie LGA, Delta state at latitude 5.569708 and longitude 5.844322 and air dried. The dried soil was sieved with a 2mm sieve and stored at ambient temperature ready for use. Its physicochemical properties were analyzed with the following parameters; pH, total organic content (TOC), total petroleum hydrocarbon (TPH)/total hydrocarbon content (THC), soil texture, nitrate concentration(NO_3),phosphate concentration(PO_4^{3-}), sulphate concentration (SO_4^{2-}) and heterotrophic fungi counts.

D. Collection of Compost

Five (5) kilogrammes of chicken droppings was collected from a poultry at Agbarho in Ughelli North LGA, Delta state at latitude 5.590188 and longitude 5.851524 and air dried. The dried droppings were crushed to tiny particles before use. It was stored at ambient temperature ready for use. The chicken droppings collected was air dried and crumbled into smaller particles. Its physicochemical properties were analyzed with following parameters; pH, total organic content (TOC), total hydrocarbon content (THC), nitrate concentration (NO_3), phosphate concentration (PO_4^{3-}), Sulphate concentration(SO_4^{2-}) and heterotrophic fungi counts according .

E. Isolation of Spent Engine Oil degrading Fungi

The isolation of spent engine oil degrading fungi was done according to the method ofBhattacharya, *et al.*, (2015). Bushnell-Haas (BH) media with the following composition (g/L): K_2HPO_4 (0.1g), KH_2PO_4 (0.1g), NH_4NO_3 (0.1g), $MgSO_4 \cdot 7H_2O$ (0.02g), $FeCl_3 \cdot 6H_2O$ (0.005g), $CaCl_2 \cdot 2H_2O$ (0.002g), was used as enrichment medium with filter sterilize spent engine oil 1% (v/v) added as the sole carbon source. The BH media was autoclave at 120^0C for fifteen (15) minutes and allowed to cool before the soil samples and carbon source were added. Soil samples (10g) each, from the two hydrocarbon contaminated sites sampled, were enriched by adding 100 ml BH media in two 250 mL Erlenmeyer culture flasks each. It was then stirred and incubated for seven (7) days at 30^0C. The mixture was stirred daily for effective aeration. After seven (7) days incubation, serial dilution of the enriched soil was done using normal saline prepared from 0.85g of sodium chloride (NaCl) in 100ml of distilled water. One (1) ml each was collected from the supernatant of the two (2) Erlenmeyer culture flasks containing the enriched soil for the serial dilution. Pour plating technique was used for plating inoculum, using $10^{-4.}$ And 10^{-5}. Potato dextrose agar (PDA) was used for the cultivation of fungi. Three (3) drops of acetic acid was added to the PDA to inhibit bacteria growth. The culture was incubated for 3 - 6 days at 28 ± 2^0C.

Pure fungi cultures was obtained by streaking a single colony on solidified PDA plates. It was allowed to grow for four to six days at 30^0C. Discrete colonies were subculture on PDA slant, allowed to grow for four to six days and then stored in the refrigerator until required for use.

F. Screening and selection of Spent Engine Oil Degrading Fungi

For the selection of spent engine oil degrading fungi, the isolated fungi pure cultures were screened for effective spent engine oil degradation. The isolates were aseptically put into 100ml BH broth in culture flasks with 1% (v/v) filter sterilized spent engine oil as carbon source. The flasks were then incubated for 7 days. Turbidity of the culture was read at the end of the incubation period (Mounteer, 2006). Isolates with high turbidity values were selected for the biodegradation tests.

G. Identification of Selected Fungi Isolates

Screened and selected spent engine oil degrading fungi were identified using API test kit (API 20C method).

H. Spent engine oil Biodegradation Studies

Three isolates (*Aspergillus glaucus*,*Trichoderma polysporum* and *Talaromyces flavus*) with the highest turbidity values were used for the biodegradation test. The spent engine oil contaminated soil was treated with variable culture conditions which included; incubation period (0, 7, 14, 21 and 28 days) and spent engine oil concentration (5 and 10 % v/v). 500g of the native soil and compost was mixed in a ratio of 3:1. The mixture of native soil and compost was contaminated with 5% v/v (50,000mg/kg) and 10% v/v (100,000mg/kg) spent engine oil. 20ml of the activated isolates in BH medium was added to the soil, compost and spent oil mixture. 50ml of distilled water was added at four (4) days intervals days to maintain the moisture content of the soil. A control containing only the soil and spent oil, devoid of the isolates and compost was prepared along with the test. The degradation microcosm was prepared in duplicates. Biodegradation of the spent engine oil in the test microcosms was assessed and monitored weekly(0, 7, 14, 21 and 28days) for four (4) weeks. The biodegradation of the spent oil was monitored by analyzing the following physicochemical and microbiological parameters in the test soils; pH, total organic content (TOC), total petroleum hydrocarbon(TPH)/total hydrocarbon content(THC), Nitrate concentration(NO_3^-), Phosphate concentration(PO_4^{3-}), Sulphate concentration(SO_4^{2-}) and hydrocarbon utilizing fungi counts (Bhattacharya, *et al.*, (2015).Standard ASTM methods were used for the physicochemical parameters analysis.

I. Statistical Analysis

Analysis of variance (ANOVA) was used to determine if there is any significant difference between the sample treatments and between the control and treatments. It was calculated using libre calc version 6.0.6.2 on linux OS 4.15.

III. RESULTS AND DISCUSSION

A. Mean Concentration of Physicochemical Parameters of Native Soil

This result showed that the concentration of total petroleum hydrocarbon in the native soil was below the detection limit of the measuring equipment. This result shows that the concentration of total petroleum hydrocarbon in the native soil is low/negligible. The native soil have a neutral pH (7.09 ±0.21).The nutrient content in the native soil reveals a high concentration of phosphate(7.66± 0.04%) , a lower concentration of nitrate (0.029± 0.14ppm/kg) and a low concentration of sulphate (0.974 ± 0.40ppm/kg) .The soil texture was sandy loam, which

is suitable for the bioremediation process (ref). The hydrocarbon utilizing fungi counts werelow (1.00 x 10^3 ± 0.15CFU/g).This counts in the native soil, is corroborated by findings ofRitz, *et al.*, 2003, who stated that bacteria and fungi counts in a given soil could range from 10^3 to 10^4/g.

B. Mean Concentration of Total Petroleum Hydrocarbon of Spent Engine Oil

TPH value obtained for the spent engine oil was 2953 ± 2.24mg/l. The result shows a very high concentration of total petroleum hydrocarbon in the spent oil used for the biodegradation test.

According to Leahy and Colwell (1990), the rates of uptake and mineralization of many organic compounds by microbial populations present in the environment are largely proportional to the concentration of the compound itself. High concentrations of hydrocarbons can inhibit biodegradation by nutrient or oxygen limitation or through toxic effects exerted by volatile hydrocarbons. There is the possibility that if the concentration of oil is high, negative effects on biodegradation rates following oil spills in quiescent, low-energy environments such as beaches, harbours, and small lakes or ponds, in which the oil is relatively protected from dispersion by wind and wave action would be experienced (Leahy and Colwell, 1990).

C. Mean Concentration of Physicochemical Parameters of Compost

The total petroleum hydrocarbon in the compost used for the experimental design was below the detection limit of the test equipment, indicating the absence of hydrocarbon contamination. 9.08 ± 0.22 was recorded for pH. This indicates that the compost is basic with a moderate hydrocarbon utilizing fungi count of 1.20 x 10^3CFU/g. The nutrients concentration reveals a high nutrient content for sulphate with a value of 81.45ppm. Nitrate and phosphate recorded 2.25ppm and 5.39ppm respectively. The high nutrient content of the compost will biostimulate the growth of the hydrocarbon degrading fungi in the test microcosms ((Nduka, 2012).

D. Results obtained for Identification of selected Fungi Isolates

The three selected fungi isolates with high spent engine oil biodegradative ability isolated from hydrocarbon contaminated soils were identified using the characteristics shown in the table 2 below.Asrecorded using the API 20C method, the fungi wereidentified as*Aspergillus glaucus, Trichoderma polysporum and Talaromyces flavus.* These fungi have been known todegrade hydrocarbons and organic compounds (Obire, *et al.,* 2008).

E.Hydrocarbon Utilizing Fungi counts inspent engine oil degradation microcosms

As shown in figure 1 below, there was anincrease in the fungi counts from Day 0 to Day 28 in both spent engine oil concentrations (5% and 10%) for the three fungi isolates used, in relation to the counts in the control. On day 0, for the 5% SEO test microcosms, counts ranged from 5.80 x 10^5FU/g (*Aspergillus glaucus*) to8.0 x 10^8 CFU/g (*Talaromyces flavus),* while on day 28, fungi counts ranged from 5.0x10^7CFU/g *Trichoderma polysporum*) to 5.80 x 10^9 CFU/g (*Talaromyces flavus).* The control recorded a slight increase in count from 3.62 x 10^3 CFU on day 0 to 4.80 x 10^5CFU/g on day 28. The counts recorded on day O for the 10% SEO microcosms, ranged from 5.40 x 10^5 CFU/g (*Trichoderma polysporum)* to 6.80 x 10^5(*Aspergillus glaucus),*whilethecounts for day28, ranged from 4.50 x 10^6CFU/g (*Trichoderma polysporum)* to 6.40x 10^7CFU/g (*Aspergillus glaucus).*The control recorded a decline in fungi growthfrom 4.0 x 10^3 CFU/g to 2.80 x 10^3CFU/g at the end of 28 days test period. Thus, *Talaromyces flavus* recorded the highest count for the 5% test microcosms, while *Aspergillus glaucus* recorded the

highest count for the 10% microcosms. The control recorded the lowest count in both test concentrations. Theenhanced counts of the fungi in the SEO test soils, could be attributed to the effects of bioaugmentation with the fungi isolates and biostimulation with compost, which must have enhanced the biodegradative potentials of the microorganisms, as well as the improved soil texture by the compost (Smita, *et al.,* 2012).Adding compost to contaminated soil have been shown to enhance bioremediation because of the structure of the organic compost matrix (Kastner and Mahro, 1996). Compost has also be shown to enhance the oxidation of aromatic contaminants in soil to ketones and quinones, which eventually disappear (Wischmann and Steinhart, 1997).

F. Concentration of % Total Petroleum Hydrocarbon degraded in the SEO contaminated soils.

From figure 2 below, it was discovered that at 5%v/v spent engine oil contamination, the sequence of % degradation of the spent engine oil at Day 28 was; *Talaromyces flavus* (69.66%) >*Aspergillus glaucus* (52.76%) >*Trichoderma polysporum* (36.88%).The control microcosm recorded 20.50% SEO degradation. At 10%v/v spent engine oil contamination, spent engine oil % degradation sequence at Day 28 was; *Aspergillus glaucus* (66.16%) >*Talaromyces flavus* (50.61%) >*Trichoderma polysporum*(40%), with Control showing the least percentage degradation (15.33%). This indicates that Talaromyces *flavus* degraded the 5%v/v spent engine oil contamination the most, while *Aspergillus glaucus*degraded the 10%v/v spent engine oil contamination the most. These findings coroborates with the fungi counts recordedin subsection G above. In previous studies similar results were obatained by Husaini *et al.* (2008).

G. Mean Concentration of pH of the SEO biodegradation soils studies.

For the 5%v/v SEObiodegradation soils studies, the highest pH value was recorded for Isolate J (*Aspergillus glaucus*) at Day 21 (7.76) and the lowest is Isolate G (*Talaromyces f,lavus*) at Day 14 (5.93). The Control had the highest value on Day 28 (6.18), showing that the biodegradation has a neutral pH value. At 10%v/v spent engine oil contamination, Isolate G (*Talaromyces flavus*) at Day 28 (8.82) and Isolate J (*Aspergillus glaucus*) at Day 14 (6.51) has the highest and lowest pH value respectively, with the Control highest value at Day 28 (7.17) (Figure 3). This shows that the biodegradation of the spent oil in the control has a neutral pH value, while the test set up with the 5% and 10% spent oil concentration affected the biodegradation process tended towards alkaline and acidic. The values were however within the recommended pH range of 6.5- 8.0. The results of the present study are partly consistent with those obtained by Atlas and Bragg (2009).

H. Mean Concentrations of Nutrients in SEO biodegradation soils studies.

The mean concentration recorded for phosphate at the end of the test duration in the 5% v/v SEO biodegradation test soils, ranged from 3.88% (*Talaromyces f,lavus)*to 5.38%*)Trichoderma polysporum)* while in the test microcosms with 10% SEO concentrations, values obtained ranged from 3.80% (*Aspergillus glaucus*) to 4.47%*(Trichoderma polysporum).*

The mean concentrations recorded for sulphate in the 5% SEO biodegradation test soils ranged from 1.125ppm in the test microcosm with *Aspergillus glaucus*to 1.562ppm in the test microcosm with*Talaromyces flavus,* at the end of 28 days.In the 10% SEO biodegradation test soils,concentrations of sulphate rangedfrom 7.47ppm in the test microcosm with*Trichoderma polysporum*to 12.15ppm in the test

microcosm with *Talaromyces* flavus at the end of the test period.The sulphate nutrient was more readily available at the 5% concentration than in the 10% test microcosms.

Concentrations recorded for nitrate in the 5% SEO test microcosms at the end of the test period, ranged from 0.139ppm (*Trichoderma polysporum)* to 0.345ppm *(Aspergillus glaucus).* In the 10% SEO test soils, concentrations ranged from0.161ppm in the microcosms with *Trichoderma polysporum* to 0.973ppm in the microcosm with *Aspergillus glaucus.* The control test soils recorded 0.029ppm and 0.068ppm for the 5% and 10% at the end of the test period. In the three nutrients monitored it was observed that the there was a higher availability in the 5% SEO biodegradation test soils than the 10% SEO test soils.Alexander, *et al*. (1982) reported that a C: N: P ratio of 100:10:2 was enough to ensure optimal growth of microorganisms.However, some of these nutrients in surplus or limited amounts could become limiting factors, therefore, affecting the process of biodegradation (Zhu *etal*., 2001; Nilanjana and Preethy, 2010). Hence at higher SEO concentration(10%), availability of nutrients could have been impaired.

IV. STATISTICAL ANALYSIS RESULTS

The values computed showed the ANOVA for the percentage TPH degradation of the Isolates in relation to the control At alpha level of 0.05, the F value (1.310) is lesser than the F critical (2.423), which indicate that there is no significance difference in TPH concentrations among the group means of the two SEO concentrations tested.

The ANOVA for the pH of the Isolates with their Control, at alpha level of 0.05, the F value (5.566) is greater than the F critical(2.313) which indicate that there is a significance difference among the group(treatment) means.The ANOVA for the hydrocarbon utilizing fungi Isolates in relation to the Control, at alpha level of 0.05, the F value(0.758) is lesser than the F critical(2.313) which indicate that there is no significance difference among the group means.

Table 1 : Colonial and morphological of identification of fungi isolates

Isolates	Colonial Appearance	Morphology	Identity
J	Felt-like green with brownish top. Reverse was slightly yellowish.	Conidiophores were of different sizes in length and were smooth. Sterigmata are single, radiate columnar.	*Aspergillus glaucus*
B	Slightly raised aerial mycelium with whitish colonies and whitish border.	Oblong and smooth conidia, with long straight phialides, typically flask-shaped and enlarged in the middle.	*Trichoderma polysporum*
G	Fast growing colonies of higher aerial mycelia, having beautiful mat-like appearance with creamish colour. Reverse side was tan.	Conidiophores were borne from aerial mycelium with bi-verticillate appearance. The metulae were in verticils having collula which lappers gradually with conidia.	*Talaromyces flavus*

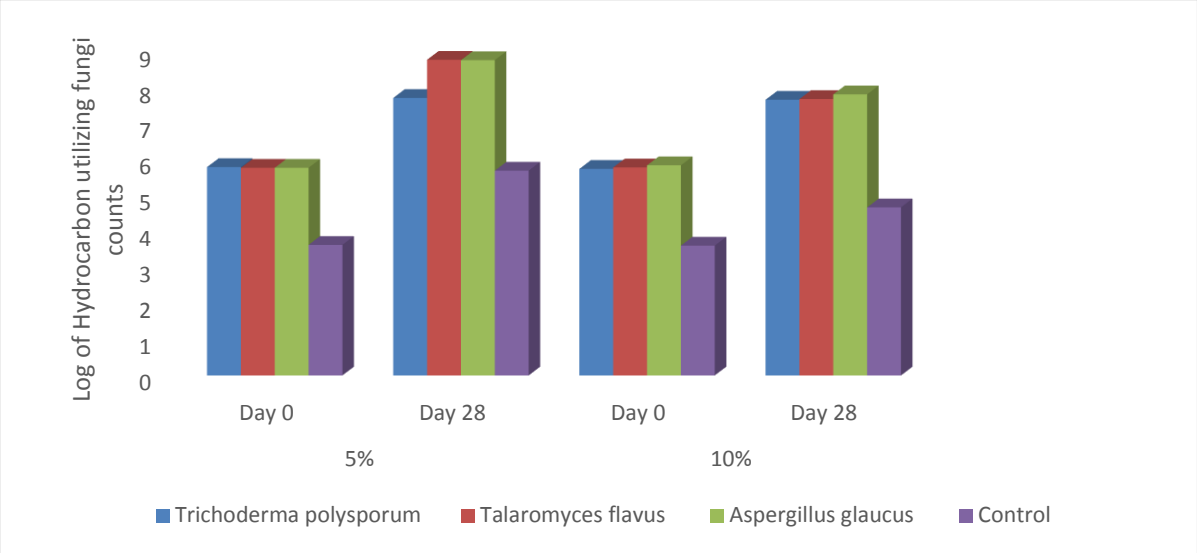

Fig 1: Counts of Hydrocarbon utilizing fungi species in spent engine oil degradation microcosms

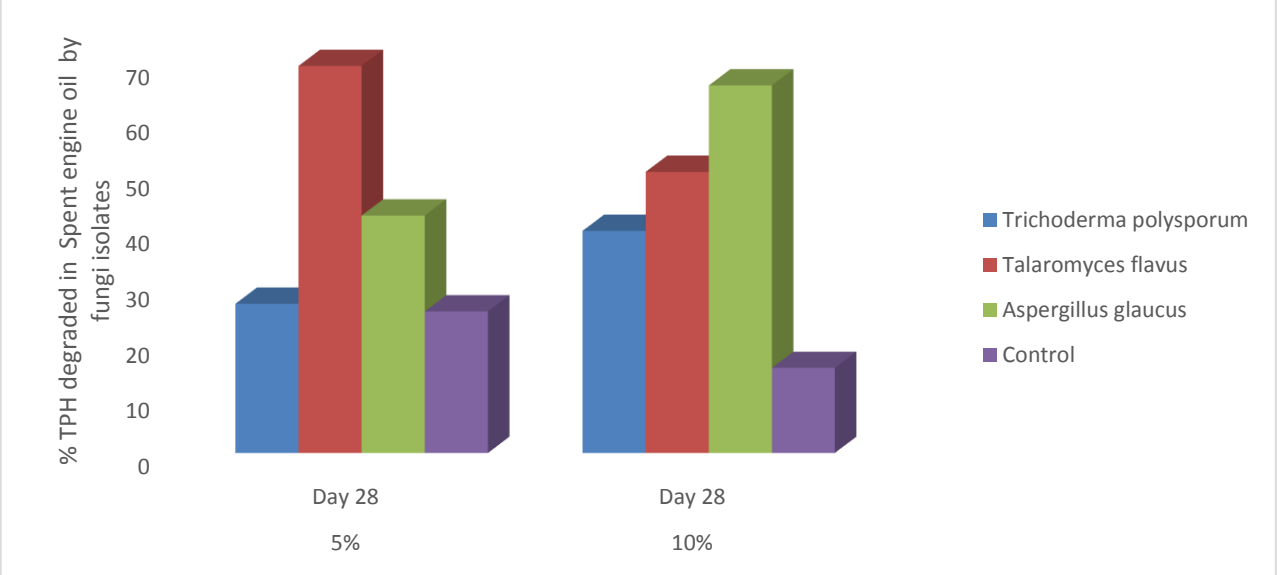

Fig 2: % TPH degraded in spent engine oil contaminated soils by fungi species.

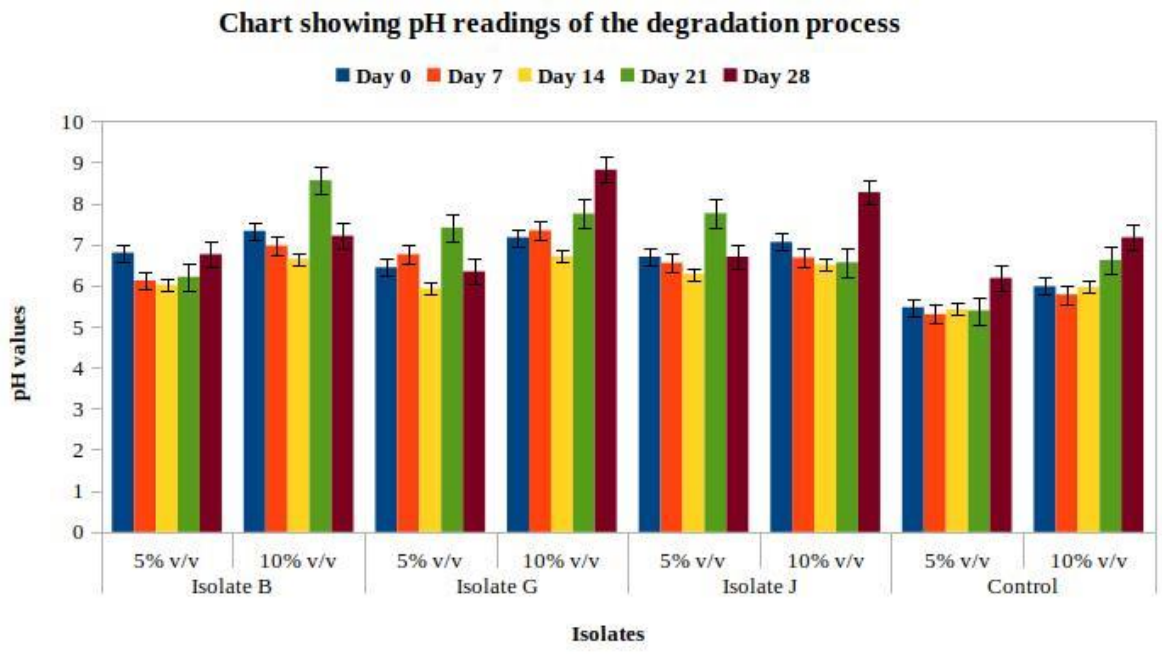

Fig 3: Mean pH values of Spent engine oil biodegradation soils.

V. CONCLUSION

The numerous reports and cases of oil spill though devastating is a major challenge which has been overcome by environmental scientists and researchers, adopting bioremediation technique which is cheap and more environmentally friendly compared to other forms of remediation. Bioaugmentation strategies, have been successfully utilized for the bioremediation of refined petroleum products by some researchers. It was successfully utilized in this research to bioremediate spent engine oil contaminated soil.

A total of three fungal isolates were isolated and characterized morphologically from hydrocarbon polluted soils from auto mechanic workshops in both Uvwie and Okpe Local Government Area of Delta state namely, Trichoderma polysporum, Aspergillus glaucus and Talaromyces flavus. The three isolates were identified using the API 20C test method. However for the percentage degradation of spent engine oil, Aspergillus glaucus degraded the the 10% treatments most with 66.16%, while Talaromyces flavus degarded the 5% treatments most with 69.66%. Further studies could be conducted to optimize the growth of these fungi on a large scale, preserved in a dehydrated form and in the event of a spill would be rehydrated to promptly remediate the polluted site in the Niger Delta.

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