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Effect of Oil Palm Bunch Ash (*Elaeis Guineensis*) on the Bioremediation of Diesel Oil Polluted Soil

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Abstract
The physicochemical and microbiological characteristics of unpolluted soils, soils simulated with conditions of a major spill (diesel oil pollution) and soils treated with different weights of an organic supplement (oil palm bunch ash, OPBA) after simulation were investigated. Physicochemical parameters such as pH, water holding capacity and moisture contents decreased in both the unpolluted soils (control 1) and polluted soil without OPBA (control 2) throughout the study periods (Day 1 to Day 42). Addition of different weights of OPBA to the polluted soils caused an initial increase in pH, water holding capacity and moisture contents and decreased afterwards. Exchangeable cations also decreased in both the unpolluted soil and polluted soil without OPBA treatment. Treatment with OPBA increased nitrogen, phosphorus, calcium, magnesium, potassium and sodium contents. Both heterotrophic and diesel utilizing bacteria and fungi were isolated from unpolluted soil, polluted soil and OPBA treated soils. There was significant difference among controls and diesel oil polluted soils treated with different weights of OPBA for heterotrophic and diesel utilizing bacteria and fungi. Application of diesel oil and OPBA according to weight applied stimulated both bacteria and fungi which gradually declined with time as higher fractions of diesel oil were degraded. TPH ranges and percentage reduction observed throughout the experiment for all the treatments are as follows: US 16.96±1.27 to 11.21±2.47 (33.9%), PS 131.31±11.96 to 125.50±11.25 (4.4%), PS+50gOPBA 127.89±12.86 to 18.29±0.96 (85.7%), PS+100gOPBA 125.54±2.28 to 20.68±0.51 (83.5%), PS+200gOPBA 124.71±11.50 to 28.21±0.49 (77.38%) and PS+500gOPBA 124.04±5.02 to 36.25±0.38 (70.78%). Means of microbial counts are as follows: THB ($\times 10^5$ cfu/g) US 1.22±0.08, PS 0.9±0.06, PS+50gOPBA 16.99±0.0, PS+100gOPBA 18.79±0.0, PS+200gOPBA 21.70±0.0 and PS+500gOPBA 27.15±0.0. THF ($\times 10^5$ cfu/g) US 0.88±0.08, PS 0.33±0.06, PS+50gOPBA 15.70±0.0, PS+100gOPBA 17.32±0.0, PS+200gOPBA 20.38±0.0 and PS+500gOPBA 24.23±0.0. HUB ($\times 10^5$ cfu/g) US 0.93±0.08, PS 0.84±0.06, PS+50gOPBA 3.51±0.0, PS+100gOPBA 5.20±0.0, PS+200gOPBA 5.54±0.0 and PS+500gOPBA 6.58±0.0. HUF ($\times 10^5$ cfu/g) US 0.73±0.08, PS 0.48±0.06, PS+50gOPBA 2.81±0.0, PS+100gOPBA 3.70±0.0, PS+200gOPBA 4.02±0.0 and PS+500gOPBA 4.43±0.0. Microbial counts increased at the beginning of the experiment upon addition of OPBA and decreased with time. Bacterial and fungal species isolated were: *Klebsiella sp, Acinetobacter sp, Streptomyces sp, Flavobacterium sp, Pseudomonas sp, Nocardia sp, Bacillus sp, Aerococcus sp, Alcaligenes sp* and *Micrococcus sp., Rhizopus sp, Aspergillus sp, Penicillium sp, Trichodema sp, Mucor sp, Fusarium sp* and *Cladosporium sp.* OPBA enhanced oil degradation with %TPH reduction highest at PS+50gOPBA which is the optimal level likely to impact maximum economic gain.

1. Introduction

Soil is the key component of natural ecosystem because environmental sustainability depends largely on a sustainable soil ecosystem (Adedokun and Ataga, 2007; Adenipekun, 2008). Soil is a primary recipient by design or accident of a myriad of waste products and chemicals used in modern society. Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. Since commercial exploration of petroleum started in Nigeria in 1958 (Okoh, 2003), petroleum has continuously grown to be mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and water ways (Eneje *et al.,* 2012). The increasing use of diesel oil in diesel engines of cars, industrial trucks and generators has led to an increased demand for diesel oil (Ogbo, 2009) and this has resulted in accidental spillage of diesel oil along Nigerian high ways, and in turn pollution of agricultural lands (Ekpo and Nya, 2012). Diesel oil is one of the major products of crude oil and it constitutes a major source of pollution to the environment (Nwaogu *et al.,* 2008). Diesel oil can enter into the environment through leakage from storage containers, refueling of vehicles, wrecks of oil tankers and through improper disposal by mechanics when cleaning diesel tankers (Ekpo and Nya, 2012).

The addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Margesin *et al.,* 2007; Abioye *et al.,* 2009). Positive effects of nitrogen amendment using nitrogenous fertilizer on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated (Akinde and Obire, 2008, Agarry *et al.,* 2010a, 2010b).

One of the agricultural wastes commonly generated in Nigeria is the Oil Palm Bunch Refuse. Nigeria is still the world's third largest producer and clearly the largest producer in Africa (Oviasogie *et al.,* 2010). The oil palm waste, which has been estimated from this industry at about seven million metric tonnes annually, is yet to be harnessed for the production of organic manures and agricultural development generally (Oviasogie *et al.,* 2010).

Soils in the Niger Delta region are usually acidic due to soil pollution like oil spillage and deficient in essential plant nutrients due to frequent rain fall associated with erosion and leaching. Contamination of soil with diesel oil and its effect on the soil environment and human health require highly efficient and cost effective means of restoration or treatment. Several methods including the use of chemical fertilizers to augment for mineral element limitations during soil biodegradation has been conflicting in terms of its effectiveness and cost. In developing countries like Nigeria, fertilizers are not sufficient for agriculture, let alone for cleaning oil spills. The need to search for cheaper, locally available and environmentally friendly options like OPBA for enhancing petroleum hydrocarbon degradation is very important. More so, the involvement of microorganisms in the degradation of petroleum hydrocarbons in the environment has been established as an economic, efficient, versatile, and environmentally friendly treatment method. There are no adequate literatures on the potential use of this un-exploited product (OPBA) as biostimulating agent for soil biodegradation.

The main objective of this research is to determine the effect of Oil palm bunch ash on the bioremediation of diesel oil contaminated soil.

1.1. Characteristics of Oil Palm Bunch Ash

Palm bunch ash is an effective fertilizer for mature oil palms on peat soils (Othman *et al.,* 2005). It is used as a substitute for NPK fertilizer (Awodun *et al.,* 2007). It has also shown to be an effective adsorbent in flue gas desulfurization (Mohamed *et al.,* 2005). It is a by-product or residue that is burnt. It is also a fine powdery substance gotten after burning of the palm bunch that bears the palm fruits (Devendra *et al.,* 1981). It constitutes varying amounts of calcium, phosphorus, potassium and magnesium which affect the yield of crops (Aya and Lucas, 1979). It is hygroscopic and highly basic (pH 12.0). It contains potassium (30%), calcium (5%), phosphorus (4%) and magnesium (6%) according to study carried out by Hasnol *et al.,* (2005). The ash, with a potassium content of about 30%, (Lim, 2000) was used as fertilizer. Its pH and calcium content improves the soil by reducing its acidic content (liming effect). Calcium is important both as a soil conditioner and as a plant nutrient which greatly increases the symbiotic and non-symbiotic bacteria which helps in adding more available nutrients to the soil (Adeyemo, 1986).

1.2. Sources of Diesel Oil Pollution

Diesel oil is produced from petroleum and from various other sources. Petroleum diesel, also called petro-diesel, or fossil diesel is produced from the fractional distillation of crude oil between 200 °C (392 °F) and 350 °C (662 °F) at atmospheric pressure, resulting in a mixture of carbon chains that typically contain between 8 and 21 carbon atoms per molecule (Collins, 2007).

2. Materials and Methods

The study area was a fallow patch of land in ADP, Rumuodomaya, Port Harcourt, Rivers State, Nigeria. Sampling period was one season during rainy season. The sampling intervals were recorded in days as D_1, D_{14}, D_{28}, and D_{42}. All glassware and media were sterilized before use. Ten kilogram of surface soil (0 – 15cm depth) was collected with a sterile auger borer from ten (10) random plots and bulked to form composite sample. The soil samples were homogenized, dried, sieved through a 2-mm mesh sieve and stored at room temperature. 1kg of the soil each was transferred into sterile bucket for treatment. Six treatments (Unpolluted soil (US), Polluted soil (PS), PS + 50g OPBA, PS + 100g OPBA, PS + 200g OPBA, and PS + 500g OPBA), replicated three times, were used. 1kg each of the soil was properly mixed with 100ml of Diesel oil to simulate condition of a major spill. Each sample was tilled for 2min twice a week and analyzed every two weeks for six weeks for even mixing of the OPBA. Physicochemical parameters analyzed on US, PS and PS+OPBA samples include pH, TN, Av. P., exchangeable cations, WHC and MC (Jamie and Richard, 1996). The determination of total organic carbon (%) was done by titration method using potassium permanganate as oxidant whereas the moisture content was determined using the method previously described by Stewart *et al.,* (1974), The extraction of petroleum hydrocarbon was done with dichloromethane (DCM) using cold extraction method with ASTM D-3694 heavy machine for 1 hour and procedure as reported by Saari *et al*., (2007). while microbiological parameters include THB, THF, HUB and HUF. Ten-fold serial dilution procedure was used to get appropriate dilutions of the samples. Aliquots (0.1ml) of the appropriate dilutions of samples were spread plated onto petri dishes of nutrient agar and potato dextrose agar for enumeration of THB and THF respectively (Chikere *et al.,* 2009; Orji, 2011). Inoculated plates were incubated at 28°C for 24hrs. HUB and HUF of samples were enumerated on modified mineral salt agar (MSA) using vapor phase transfer method and incubated at 28°C for 3 - 5days. 1ml of lactic acid was added to the fungal media to inhibit the growth of bacteria (Obire *et. al.,* 2008). Discrete bacterial and fungal colonies were further purified by sub-culturing on nutrient agar and malt extract agar respectively. Pure isolates were identified morphologically, biochemically, macroscopically and microscopically.

3. Results and Discussion

The data tabulated in the result sections are the means of three replicates.

Physicochemical Parameters of the Unpolluted soil, Diesel Oil Polluted and Oil Palm Bunch Ash.

The results of the physicochemical composition (mean±SD) of the unpolluted soil (Control), polluted soil and Oil palm bunch ash sample used in the experiment are shown in Table 1.1.

Table 1.1. *Physicochemical Characteristics (mean±SD) of unpolluted soil, polluted soil and Oil Palm Bunch Ash used in the experiment*

Parameters	Unpolluted Soil	Polluted Soil	OPBA
pH	5.98±0.04	6.12±0.03	10.62±0.06
Water holding capacity (%)	32.7±0.58	31.33±0.58	ND
Moisture content (%)	85.8±0.1	83.10±0.06	ND
Available phosphorus (%)	8.34±0.05	8.57±0.03	4.17±0.01
Total nitrogen (%)	0.05±0.005	0.07±0.01	0.15±0.03
Ca^{2+}(mg/kg)	7.13±0.03	7.03±0.02	5.28±0.07
Mg^{2+} (mg/kg)	1.91±0.01	1.89±0.01	1.31±0.02
K^{+}(mg/kg)	0.78±0.01	0.54±0.02	0.03±0.01
Na^{+} (mg/kg)	1.05±0.01	1.03±0.01	0.53±0.02
TOC (%)	1.54±0.02	2.02±0.01	0.34±0.02
TPH (mg/kg)	16.96±1.27	151.68±13.21	ND

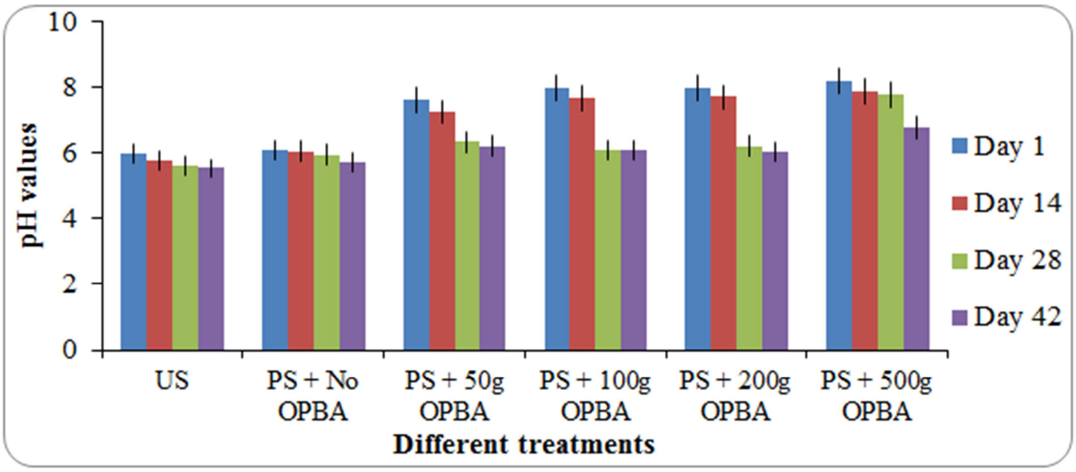

Figure 1.1. *pH values of US, PS without and with different weights of OPBA at different days*

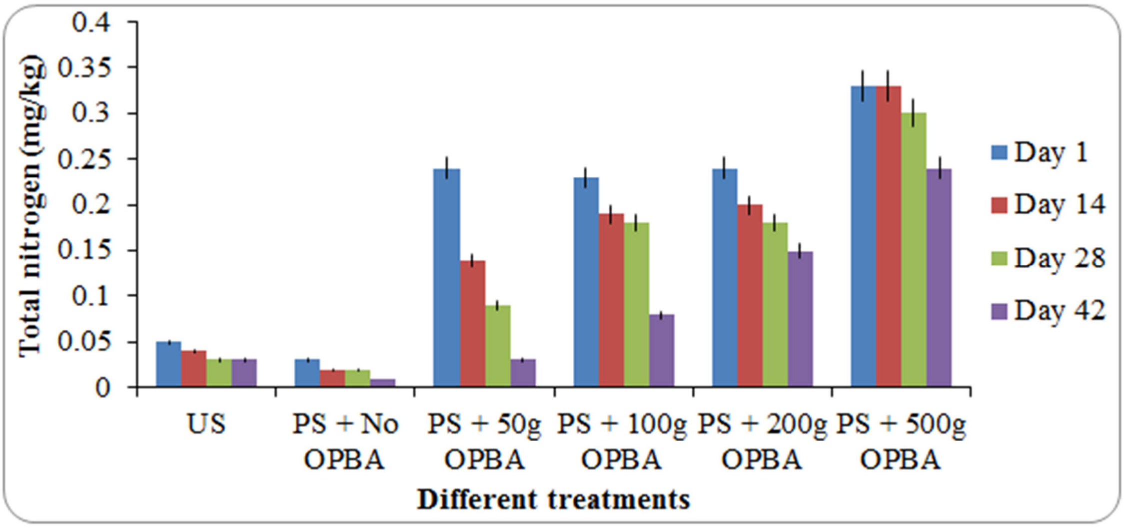

Figure 1.2. *Total nitrogen values of US, PS without and with different weights of OPBA at different days*

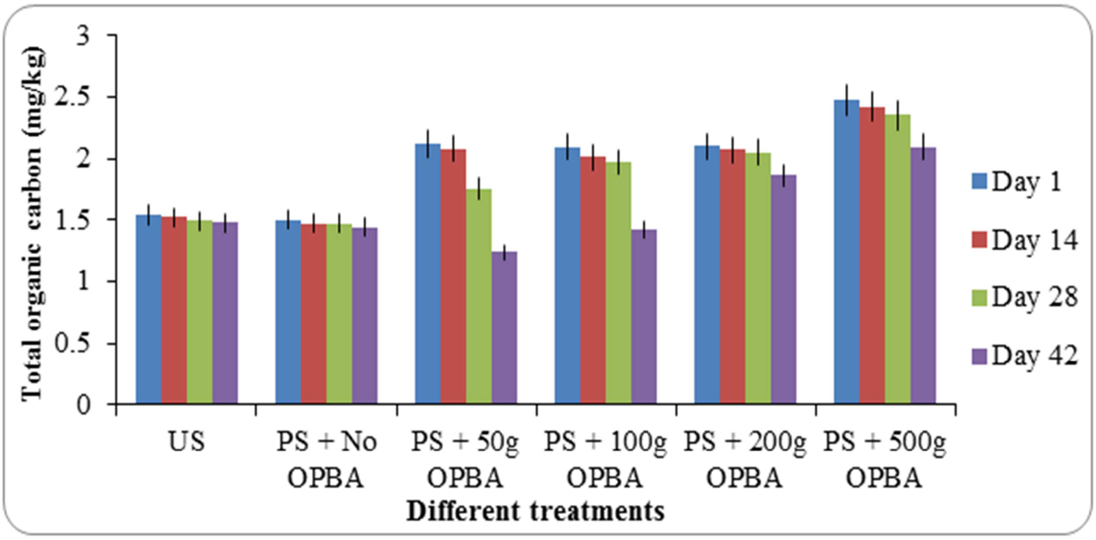

Figure 1.3. *Total organic carbon values of US, PS without and with different weights of OPBA at different days*

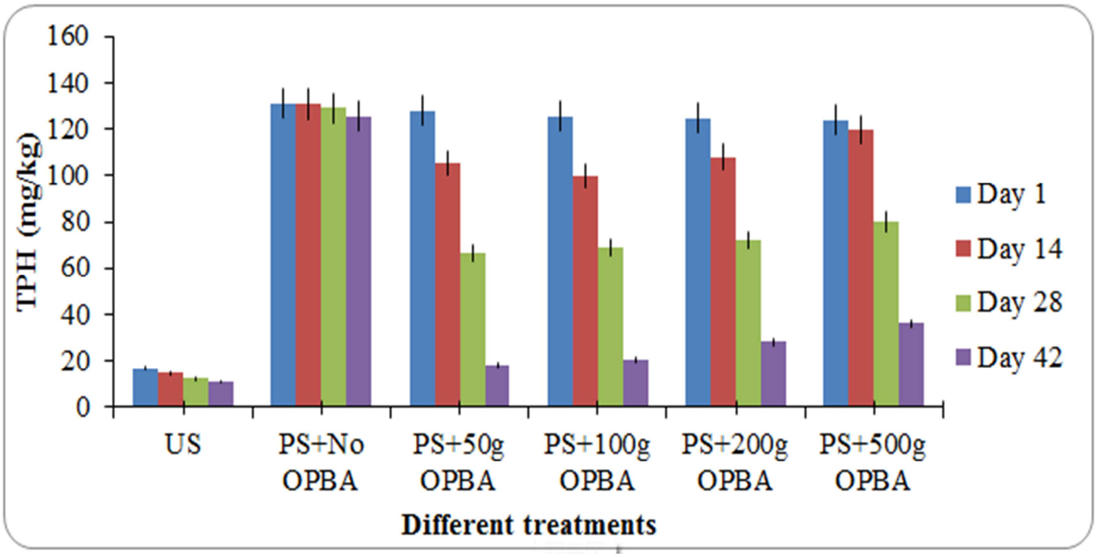

Figure 1.4. *TPH values of US, PS without and with different soil treatments at different days*

4. Discussion

pH values of both polluted soil with diesel oil and oil palm bunch ash were higher than that of unpolluted soil. Increase in pH of soil after pollution with diesel oil is in line with the work of Njoku *et al* (2008) who reported that diesel oil pollution increased the pH of the soil. pH value of oil palm bunch ash was highest compared to both the unpolluted soil and the polluted soil. This has been shown in the work of Hasnol *et al* (2005). pH values of polluted soil treated with different weights of OPBA appeared slightly basic at the on-set of the investigation but gradually reduced from near neutrality to moderately acidic during the investigation. This agrees with the work of Toogood (1977a) who reported that addition of hydrocarbon appeared to have raised the pH of the soil. Low pH value observed in both US and PS + no OPBA is in line with the findings of Osuji and Nwoye (2007) who reported that soil pH was reduced due to the presence of hydrocarbon that produce organic acids when acted upon by micro-organisms. Also, reduction in pH value of PS with organic nutrient treatment has been observed by Akpan and Ekpo (2006; 2013). There was progressive decrease in pH values for all the treatments during the investigation. This implies increased acidity which could be due to the fact that hydrocarbons contain many free cations causing them to have properties of a weak acid (Akpoveta *et al.,* 2011). There was increase in pH values with the addition of different weights of OPBA between Day 1 and Day 14 which shows that organic nutrient supplement used (OPBA) contain both micro- and macro-nutrients capable of improving soil fertility (Cooke, 1975). Generally, there is significant difference at $p \leq 0.05$ for all the treated plots with different weights of OPBA at different times whereas there is no significant difference at $p \leq 0.05$ for both the US and PS without OPBA throughout the investigation (Figure 1.1).

Total nitrogen content of unpolluted soil decreased with time throughout the investigation. Total nitrogen in the unpolluted soil was very low. This indicates that the soil was low in plant nutrients and amendment with the OPBA was ideal to boost the soil nutrient. Pollution of soil with diesel oil led to decrease in total nitrogen content compared to the unpolluted soil. This is because petroleum-products such as diesel oil are known to reduce nitrogen availability as observed by Agbogidi *et al.,* (2007). Addition of 50g to 500g OPBA to diesel oil polluted soils led to an increase in total nitrogen on Day 1 compared to both the unpolluted soil and the polluted soil without OPBA. There was significant reduction of total nitrogen in soils treated with 50g to 200g OPBA from Day 1 to Day 42 whereas soils treated with 500g OPBA remained fairly constant indicating that aerobic conditions were achieved or rather the use of high value of OPBA resulted in high microbial activity leading to equilibrium in the mineralization of this nutrient. This is in line with the work of Ling and Isa, 2006 who reported that initial drop in the concentration of nitrogen could be attributed to microbial use of nitrate as a nutrient or as an electron acceptor. Increase in total nitrogen on Day 1 may be due to mineralization of nitrogen content in diesel oil or dead degrader may also contribute to significant increase in total nitrogen as reported by Agarry and Jimoda (2013). More so, increase in total nitrogen on Day 1 is in line with earlier reports by Odu (1972), Udo and Fayemi (1975). Odu (1975) reported that increase in total nitrogen may be due to increase in non-symbiotic fixer attracted to the contaminated soils.

There is significant difference at $p \leq 0.05$ for PS + 50g to 200g OPBA. Significant reduction in total nitrogen was high in PS + 50g OPBA and least in PS + 500g OPBA. Addition of OPBA also led to increase in total nitrogen on Day 1 for PS + 50g to 500g OPBA. The highest value was observed in PS + 500g OPBA. The values of total nitrogen were low in both unpolluted soil (control 1) and polluted soil + No OPBA (control 2). Reduction of total nitrogen in PS + 500g OPBA was slow which could be an indication that aerobic conditions and microbial utilization of total nitrogen as nutrient reached its saturation with time. Generally, there was no significant difference at $p \leq 0.05$ for the US, and PS + No OPBA.

Total organic carbon of unpolluted soil (US) and PS + No OPBA decreased slightly throughout the experiment. Pollution of soil with diesel oil led to an increase in the value of total organic carbon. Higher increase in total organic carbon was observed in all the soils treated with OPBA at different weights on Day 1. Increase in organic carbon in all the treated soils on Day 1 may be attributed to the hydrocarbon used in polluting the soil. This is in line with the work of Godwin (2013). Osuji and Onojake (2006) also noted that there were significant increases at $p \leq 0.05$ in organic carbon and attributed it to the metabolic processes following oil pollution which facilitated agronomical addition of organic carbon from petroleum hydrocarbon by reducing carbon mineralizing capacity of the micro flora. Initial drop in the concentration of total organic carbon was observed in all the polluted soil treated with different weights of OPBA on Day 14, which continued till Day 42 of the investigation. Wyszkowski and Ziolkowska (2008) also reported that addition of diesel oil to soil led to a significant reduction of organic carbon content of the soil with time. It could be deduced that total organic carbon of both unpolluted soil and PS + No OPBA remain fairly constant throughout the investigation while an increase in organic carbon content was observed for all the treated soils on Day 1 of experiment. This marked increase declined from Day 14 to Day 42 for all the soils. This revealed that there was significant difference at $p \leq 0.05$ for PS + 50g to 500g OPBA whereas there was no significant difference for both unpolluted soil and PS + No OPBA.

Results showed that the unpolluted soil had low values of total petroleum hydrocarbon (TPH) throughout the investigation. The reduction of TPH from Day 1 to Day 42 was very slow. This reduction observed could be attributed to natural attenuation where indigenous microorganisms use available nutrients as both carbon and nitrogen sources to degrade hydrocarbon compounds. There was no significant reduction in TPH values at $p \leq 0.05$ (Figure 1.4). There was

33.9% loss in TPH for the unpolluted soil throughout the experiment. Results of polluted soil without OPBA showed increase in TPH value on Day 1 compared to the value obtained for the unpolluted soil, followed by minimal reduction from Day 14 to Day 42 of the experiment. The reason for high concentrations of TPH observed on the Day 1 is due to pollution of soil with diesel oil in such a quantity as to simulate pollution. Slow reduction in the concentration of TPH could probably be due to lack of nutrients in the soil which led to inability of indigenous microorganisms to utilize the hydrocarbon compounds. There was no significant reduction in TPH values at $p \leq 0.05$. There was 4.4% loss in TPH for the polluted soil throughout the experiment. Results of Polluted soil treated with 50g OPBA showed significant reduction in TPH concentrations from Day 1 to Day 42. A marked reduction observed during the first two weeks of treatment with OPBA could be due to the ability of microorganisms to use the OPBA as both carbon and nitrogen sources to degrade the hydrocarbon compounds. Ibekwe *et al.,* (2006) has reported that amendment of 100g contaminated soil with 30g organic nutrient led to loss of 40% TPH. There was significant reduction in TPH values at $p \leq 0.05$ throughout the investigation, which also resulted in 85.7% loss of TPH values. From this, it could be deducted that the use of OPBA effectively stimulated organisms into utilization of diesel oil. All the polluted soil treated with different weights (that is, 100g to 500g) of OPBA followed the same trend with that of polluted soil treated with 50g OPBA. There was decrease in TPH values throughout the experiment for all the soils with different weights of OPBA. From the observation, PS + 50g OPBA showed the highest percentage reduction in TPH value (85.7%), followed by PS + 100g OPBA (83.5%), then PS + 200g and 500g which had percentage reduction in TPH value 77.4% and 70.8% respectively. Ameh *et al.,* (2011) had reported that the quantity of manure is important for bioremediation (in supplying nitrogen). Higher weights of OPBA as treatment recipe for diesel oil degradation may probably not be ideal as a biostimulant

Results of both total heterotrophic fungi and hydrocarbon utilizing fungi followed the same trend in all treatments used in this study throughout the experiment. However, total heterotrophic fungi had higher load compared to hydrocarbon-utilizing fungi. Results of heterotrophic fungi and hydrocarbon utilizing fungal counts showed gradual decrease from Day 1 to Day 42 for the unpolluted soil (Control 1). The decrease in unpolluted soil throughout the experiment could be due to lack of nutrient which may have influenced fungal growth. However, the fungal counts observed in unpolluted soil could probably be attributed to the fact that most fungi are aerobic which is in line with the report by Nester *et al.,* (2004). More so, presence of fungi in unpolluted soil depicts that microorganisms are widely distributed in nature and have been found in areas not directly contaminated with hydrocarbons as reported by Atlas (1981). Results showed that both heterotrophic fungal and hydrocarbon-utilizing fungal counts of unpolluted soil from Day 1 to Day 42 did not differ significantly when subjected to statistical analysis at $p \leq 0.05$. Results of heterotrophic fungal and hydrocarbon-utilizing fungal counts for the polluted soil (Control 2) from Day 1 showed decrease in fungal counts which later increased on Day 14. Results obtained in Day 1 could depict that the fungi had not started breaking down the hydrocarbon into simpler molecules as reported by Battelle (2002). Immediate effect of diesel oil contamination in the soil is the depression of the fungal population due to presence of additives in diesel oil as reported by Ujowundu *et al.,* (2011). It was evident from the study that the proportion of fungal population increased on Day 14 as a result of diesel contamination. Increase in fungal counts on Day 14 could probably be attributed to utilization of diesel oil as carbon source. Low *et al.,* (2008) had reported that many native strains including ligninolytic fungi have great potential for remediation of pentachlorophenol (PCP) and polycyclic aromatic hydrocarbon from diesel-contaminated soils in an oil refinery sites. Fungal population decreased gradually from Day 28 to Day 42 which could be a reflection of nutrient depletion. Generally, it could be deducted that diesel oil contamination enhanced growth of diesel-degrading fungi. There was no significant difference in fungal counts at $p \leq 0.05$. Results of polluted soil treated with different weights of OPBA showed the same trend of fungal activities in terms of its population. Day 1 of PS + 50g OPBA showed that fungal counts of both the total heterotrophic fungi and hydrocarbon utilizing fungi were higher than that observed in both unpolluted soil and polluted soil without OPBA. Higher counts of both THF and HUF were observed on Day 14 compared to that observed on Day 1. Battelle (2000) reported that growth medium helped in creating a favorable environment for rapid development of fungi especially at the time when the fungi had not started breaking down the hydrocarbons into simpler molecules. The contribution of fungi in PS + 50g OPBA treatment could be evidenced in TPH reduction which was observed during the first two weeks of treatment. Day 28 and Day 42 revealed gradual decrease in fungal load compared to Day 14 which could be due to nutrient depletion. This is in agreement with Dua *et al.,* (2002) who reported that microorganisms are capable of using organic substances as sources of nutrients and energy hence, exhibiting remarkable range of degradative capabilities. The study showed that the fungi were able to feed on the oil (substrate), using it as food in order to build up their cells and hence get energy for growth, development and reproduction. This was indicated by the concomitant increase in fungal counts especially on Day 14 and reduction in TPH concentration from Day1 to Day 42 for all the treatments. This is in line with the work by Eneja (2007). All the polluted soil treated with different weights of OPBA followed the same trend as observed for PS + 50g OPBA treatment from Day 1 to Day 42 for both the heterotrophic fungi and hydrocarbon utilizing fungi. It was observed that increase in weight of OPBA from 50g to 500g resulted in a corresponding increase in fungal counts throughout the experiment. There was significant difference at $p \leq 0.05$ for all the treated soil with different weights of OPBA. Results obtained in this study showed that many of the fungal species isolated from diesel oil polluted soil were capable of degrading diesel. Increase in fungal counts at a stage in the study indicated that they were able to degrade and utilize the oils for their growth and development.

5. Conclusion

Diesel oil had increasing and decreasing effects on soil physicochemical parameters. Parameters such as pH, water holding capacity and moisture content decreased in both the unpolluted soil and polluted soil without OPBA throughout the investigation. However, parameters such as pH, available phosphorus and nitrogen increased in all polluted soils treated with different weights of OPBA during the on- set of the investigation but decreased afterwards. Exchangeable cations such as calcium, magnesium, potassium and sodium also decreased in content in both the unpolluted soil and polluted soil without OPBA treatment. However, organic carbon increased in diesel oil polluted soil without OPBA compared to unpolluted soil. Treatment with organic nutrient supplement (OPBA) increased nitrogen, phosphorus, calcium, magnesium, potassium and sodium in the soil thereby improving the bioremediation potential of microorganisms isolated from the samples. pH also increased to neutral region upon addition of OPBA.

This study revealed the presence of diesel utilizing bacteria and fungi in the soil which can be exploited in oil spill cleanup. For the unpolluted soil, lag phase was observed to suggest that the organisms from the study site had not been previously exposed to diesel oil hydrocarbons. The same observation was made for the polluted soil at the on-set of the investigation. There was significant difference among controls and oil polluted soils treated with different weights of OPBA for total heterotrophic bacteria and fungi as well as hydrocarbon utilizing-bacteria and fungi. For the polluted soil treated with different weights of OPBA, the study confirmed that these organisms metabolized the organic nutrient supplement (OPBA) especially during Day 14 which resulted in higher microbial counts but gradually declined as the diesel hydrocarbon fractions are degraded. In order words, this investigation showed that microbial numbers are stimulated on application of diesel oil and organic nutrient supplement (OPBA) which depended on the weight applied and gradually declined with time as the higher fractions of hydrocarbon are exhausted.

This study also showed the reduction of total petroleum hydrocarbon in all the soils in varying degrees. There is no significant difference in both unpolluted soil (control 1) and polluted soil without OPBA (control 2) samples which showed that there was a slight decrease in their TPH concentrations compared to polluted soils treated with different weights of OPBA which showed significant difference at varying concentrations to indicate that the quantity of OPBA added had significant effect on the remediation process.

Based on the percentage reduction of TPH for the different weights of OPBA used as biostimulant for the remediation process, it was clear that there is optimal level of organic nutrient supplement (OPBA) that are likely to impact maximum economic gain. This is evidenced as PS + 50g OPBA showed highest TPH percentage reduction (85.7%), followed by PS + 100g OPBA with 83.5% TPH loss, then PS + 200g and PS + 500g OPBA with 77.4% and 70.8% TPH respectively.

There is ample evidence to show from the study that OPBA modified soil physical, chemical and biological properties in diesel oil polluted soil for enhanced oil degradation as applied at different weights. Optimal level of OPBA that are likely to impact maximum economic gain especially in the oil producing areas of Niger Delta is PS + 50g OPBA.

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