INTRINSIC BIOREMEDIATION OF HYDROCARBON POLLUTED MANGROVE SOIL IN NIGER DELTA NIGERIA USING SELECTED ORGANIC AMENDMENTS

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Abstract— Crude oil contaminated mangrove swamp soil from Tai local government area of Rivers State was amended with two different organic waste (poultry droppings and cow dung). The contaminated soil taken at depth of 0-30cm was treated for a period of 21 days. pH, Moisture content, Total Nitrogen, Available Phosphorus, Total organic Carbon (TOC), Total Hydrocarbon Content (THC), Total Petroleum Hydrocarbon (TPH), Total Heterotrophic Bacteria Count (THBC), Total Heterotrophic Fungi Count (THFC), Total Hydrocarbon Utilizing Bacteria Count (THUBC) and Total Hydrocarbon Utilizing Fungi Count (THUFC) as well as the physico-chemical and microbial parameters were measured. The treatment samples were tilled twice a week and watered with 50ml of distilled water weekly. There was a general consistent increase in microbial counts for all amended samples in all the days except for poultry waste (PW) and mixture of poultry/cow dung (PC). These reductions were from day 7 to day 21; $2.0x10^5$cfu/g - $1.4x10^5$cfu/g for THFC count and $3.5x10^5$ - $2.6x10^5$cfu/g for HUFC count respectively. The THBC for control, poultry waste, poultry/cow dung and cow dung treatment options increased from $1.55x10^8$ -$2.6x10^8$cfu/g, $1.62x10^8$ - $2.50x10^8$cfu/g, $2.26x10^8$ - $2.68x10^8$cfu/g and $1.25x10^8$ - $2.75x10^8$cfu/g respectively. The THUBC for control, poultry waste, poultry/cow dung and cow dung treatment options increased from $9x10^4$ - $3.4x10^5$cfu/g, $1.2x10^5$ - $4.4x10^6$cfu/g, $8.3x10^5$ - $4.4x10^6$cfu/g and $7.8x10^5$ - $4.4x10^6$cfu/g respectively. Cow dung showed the highest number of THFC with $8.3x10^5$cfu/g. The THUFC for control, poultry waste, poultry/cow dung and cow dung treatment options increased from $1.6x10^4$ - $1.6x10^5$cfu/g, $6x10^3$ - $2.0x10^5$cfu/g, $1.9x10^4$ - $2.6x10^5$cfu/g and $2.3x10^4$ - $4.3x10^5$cfu/g respectively. The results showed that at day 21, the percentage loss of total petroleum hydrocarbon for poultry/cow dung, poultry waste and cow dung were 78.15%, 77.24%, and 74.78% respectively. The percentage loss of total petroleum hydrocarbon for control was 16%. Eight hydrocarbon utilizing bacterial isolates obtained were *Pseudomonas* sp. *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp. *Alcaligenes* sp. *Arthrobacter* sp. *Aeromonas* sp. *klebsiella* sp. Also, the hydrocarbon utilizing fungi isolated included *Aspergillus* sp., *Candida* sp., *Rhizopus* sp., *Fusarium* sp., *Penicillium* sp. *Saccharomyces* sp. and *Acremonium* sp. The Results of this research indicated that nutrient amendment enhanced the rate of biodegradation and the significant reduction in TPH was achieved using selected organic waste which can release limiting nutrients such as Nitrogen and Phosphorus. The TPH biodegradation process was further simulated using a regression model, degradation trend was established with time leading to significant TPH reduction.

Index Terms— Bioremediation, Cow dung, poultry waste, swamp soil.

I. INTRODUCTION

The quest for crude oil in Nigeria started in the early 1937 [1]. Nigeria is among the top oil producers of the world, having crude oil and Natural gas are the main sources of revenue. It is also the major source of foreign exchange in Nigeria, accounting for 95% of its internal revenue and foreign exchange earnings [2]. Natural gas and crude oil are found in storage zones in the geological structures underlying the earth that includes the mangrove and associated ecosystems of the Niger Delta region. This makes the Niger Delta the core of active exploration and production activities.

Petroleum exploration leads to petroleum pollution of the environment leaving adverse effects on the farmland, fisheries, vegetation, wildlife and potable water [3]. Since the people of the Niger Delta depends on fishing and farming as a source of livelihood and survival, the rate of hunger, epidemics and disease outbreak is rapidly increasing so as the pollution of the mangrove and it's a major concern.

Mangrove forests are tropical or subtropical intertidal forests composed of halotolerant plant species; species that survives

in hypersaline lakes, coastal dunes, saline deserts, salt marshes and inland sea salts. Mangrove forest are often located in the muddy, no oxygen soils of estuaries, lagoons and river deltas [4]. Mangroves support the preservation of biological diversity by providing habitats for finfish, crabs, shrimps etc, spawning grounds, nurseries and nutrients for a number of organisms including many commercial species [5] and also protect coastal communities from rises in sea-level, storm surges and tsunamis [6][7].

Mangrove forests are known to be highly susceptible to oil spills. Floating oil settles with the tide and smothers both feeder and breathing roots, and innumerable associated resident fauna [8], mangrove sediments act like a sink, absorbing the toxicity of pollutants making them one of the most threatened habitats in the world [9].

Bioremediation can be explained as a technology that utilizes naturally occurring organisms such as bacteria, fungi and yeast to degrade hazardous substances into non-toxic or less-toxic substances by chemically changing and breaking down of organic molecules into other substances producing carbon dioxide, fatty acids and water utilized as energy and nutrients.

Bioremediation is defined by the American Academy of Microbiology (AAM) as the use of living organisms to reduce or eliminate environmental hazards resulting from accumulation of toxic chemicals and other hazardous substances. Bioremediation involves the use of microorganisms and their products to remove contaminants from the environment [10].

Studies have shown that poultry droppings and cow dung may be suitable as growth substrate for remediation of hydrocarbon polluted soil [11]. Nitrogen, Phosphorus and Potassium are present in high quantities in poultry droppings and cow dump and NPK (inorganic fertilizer).

II. THE PROBLEM

In the Niger Delta, mangrove remains very important to the indigenous people and covers approximately 5000 - 8580 km^2 of land [12] fishing is one of the major occupations of the Niger delta people and makes up part of Nigeria's sustainability because it provides much needed protein and nutrients. People living around mangrove swamp rear fishes in mangroves. Sadly, crude oil pollution of the swamp causes reduction of dissolved oxygen thereby killing fish, threatening the food security and the geopolitical economy of the Niger Delta. Toxins from the oil pollution are stored up in the organism's tissues which upon ingestion leads to adverse health effects in humans. Some of the adverse health effects may be cancer causing.

Oil deposits enters mangrove swamp through high tide conditions, they rest on the aerial roots which are the mangrove tree breathing pores and on the muddy sediment surface. Mangroves are killed by this event as heavy and viscous oil covers the trees breathing pores leading to a state of anoxia. Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbons (PAHs) can knock of mangrove. However, there is need to determine the quality and shelf life of major brands of bottled water marketed in Port Harcourt.

Petroleum hydrocarbon exploration and production in Nigeria has left hash negative impacts, damaging the environment in the Niger Delta. This has led to the use of several remediation techniques to combat the hazard of petroleum hydrocarbon pollution with various degrees of accomplishments and high cost which prevents many individuals from doing acceptable and effective remediation. Crude oil distorts proper soil aeration by forming a blanket over the surface of the soil preventing proper penetration of air into the soil. It also affects the Physiochemical (Moisture, Temperature, Oxygen content, Nitrogen content, pH, Conductivity etc) properties of the soil, introduces toxic heavy metals into the soil that affects agricultural yield and production [1].

III. STUDY OBJECTIVES

a) Evaluate the physicochemical properties and microbiological characteristics of the selected organic amendments.
b) Evaluate the physicochemical properties of polluted and unpolluted soil
c) Determine, isolate and identify microbial population present in crude oil polluted soil that is capable of degrading hydrocarbon.

d) Monitor remediation trend of soil amended with organic amendments (poultry droppings and cow dung) in various concentrations.

IV. STUDY AREA

The study area is Gio mangrove swamp, in Tai Local Government Area of Rivers State. The geographical coordinate of the study area is 4º42'58.52232" N and 7º14'51.67248" E.

Tai LGA has a tropical climate and rainfall is significant for most month of the year with a short dry season that has little effect. Rainy season last from March to October and dry season from November to February. The average yearly temperature is around 25^0c, average humidity level of 73% and precipitation has an average of 2,708 mm. Tai covers an area of 159km^2 with a population of 117,797 as recorded by the 2006 cencus [13]. The intended study area is approximately 3500m^2 of secondary forest in the rainforest belt. The swamp is extensive; it covers a very large area of table land and top layer of mud slurry that covers a hard substratum. This area has the characteristics of mangrove swamps, dominated with mangrove vegetations.

This site was carefully selected because of illegal oil bunkering activities, vandalization and oil spillage from a pipeline owned by an upstream industry in Nigeria. The predominant mangrove plants in this area are *Nypa fruticans, Rhizophora racemose, Paspalum vaginatum and Avicennia Africana.* The major occupation of the Gio people is mainly fishing and agricultural/ land farming.

Figure 1: Map of Rivers State showing Gio, Tai L.G.A. the study location

V. BRIEF REVIEW OF RELATED LITERATURE

The Niger Delta is known to be the delta of the Niger River that directly sits on the Gulf of Guinea on the Atlantic Ocean in Nigeria [14]. The scientific assessment, carried out by the United Nations Environment Program (UNEP), showed that the accumulative pollution from over 50 years of oil exploration and exploitation in the region has eaten into the core of the region, [15]. [16], stated that microorganisms have the ability to breakdown petroleum hydrocarbon into simple substances used as energy and carbon, producing carbon dioxide, water and biomass as end products. [17] Stated that there is an increase in numbers of HUB in oil polluted environment undergoing bioremediation by natural attenuation. They also stated that hydrocarbon must be applied to growth medium as the sole source of carbon and energy and no other preferable source.

[18] reported Bacteria, Fungi and Yeast as the primary hydrocarbon degrading agents in the environment. They reported efficiency ranges of these organisms as 6% to 82% for soil fungi, 0.13% to 50% for soil bacteria. [19] listed 22genera of bacteria and 31 genera of hydrocarbon degrading fungi which were isolated from marine environment. Similarly, [20] listed 25 genera of bacteria ang 25 general of hydrocarbon degrading fungi.

[21] reported in a marine oilspill study that Carbon-Nitrogen ratio is distorted, this causes deficiency of nitrogen, other nutrients may also be lacking, this causes limitations in bioremediation processes. [22] reported that nutrient enhancement increases bacterial count which impacts significantly with hydrocarbon attenuation after examining bacteria dynamics and crude oil degration. [23] Concluded that presence of indigenous microorganisms in the treatments triggered high fungal count of total heterotrophic hydrocarbon utilizing fungal count and the oil polluted soil with the various treatments stimulated higher microbial proliferation in soil. [24] [25] reported cow dung and goat manure can be effective

organic amendments for biodegradation of diesel contaminated soil as they enhance the multiplication of indigenous microbes and local materials such as cow dung and poultry dropping can be used for bioremediation of lands polluted with crude oil.

VI. MATERIALS AND METHODOLOGY

A. *Sample Type and Collection*

The hydrocarbon polluted soil samples were collected using a hand auger at a sample depth of 0-15cm (top and bottom soil) from four different points at the oily contaminated mangrove swamp and same was done in an uncontaminated mangrove swamp. The samples were mixed in a polythene bags and transported to Environmental Microbiology and Mycology Research Laboratory of the University of Port-Harcourt for bioremediation studies.

B. *Source of Material*

2000grams of cow dung obtained from Trans Amadi cattle abattoir was sun dried for 5 days to remove moisture and then stored in a clean environment for usage. 2000grams of poultry waste obtained from a poultry farm in Umueblulu 2, Etche L.G.A, was sun dried for 5 days to remove moisture and then stored in a clean environment for usage. Both samples were mashed into power for easy mixing with soil samples. Crude oil referred to as bonny light was obtained from NNPC, Eleme, Port Harcourt, Nigeria.

C. *Preparation and Treatment of Soil Sample*

The contaminated swamp soil samples were mixed in one polythene bag to get a composite mixture. 1000 grams of swamp polluted soil sample was put in each of the four containers labelled A, B and C. D serves as a control. Treatment cells A, B & C was amended with 100g of poultry waste, 100g of cow dung and mixture of 50g each of poultry waste and cow dung respectively.

The different treatment options were watered with 50ml sterile water weekly after treatment to moisten the soil and they were mixed twice a week for aeration individually. While treatment cell D was just mixed twice a week and watered with 50ml of sterile water weekly, that was to check the ability of naturally existing microorganisms to degrade hydrocarbon without amendment. The samples analysis was taken every other week for a period of 21days; 0day, 7day, and 21day.

Table 1. Bioremediation design of the study

Experimental set	Test Experiment
A	1000g of contaminated soil+100g of poultry waste
B	1000g of contaminated soil+100g of cow dung
C	1000g of contaminated soil+50g each of poultry waste and cow dung
D	1000g of contaminated soil. Tilling and watering

D. *Physicochemical Parameters*

Physico-chemical analysis was conducted on parameters such as conductivity, pH, Nitrogen content, Phosphorous content, moisture, total organic carbon (TOC), total hydrocarbon content, heavy metal (Fe, Zn and Mn) content using Absorption Spectrophotometry, TPH was analyzed using Gas Chromatography with a Mass Spectrophotometer, this was carried out on both the treatment and the control at the day 0, 7 and 21 of the experiment.

E. *Enumeration of Total Culturable Heterotrophic Bacteria*

The spread plate method using NURIENT AGAR in a petri dish (plate) was employed to enumerate heterotrophic bacteria.

Swamp soil suspension of 10-fold serial dilution was prepared using 1g of soil sample put into a sterile test tube containing 9ml of sterile saline as diluents. The 10-fold serial dilution was done to a dilution of 10^{-5} i.e (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}).

An aliquot of 0.1ml from each dilution was inoculated on the nutrient agar and was replicated in three nutrient agar plates. The plates were incubated at 28^0C room temperature in an incubator for 24hours. Colonies counts were calculated using the formula below;

$$\frac{\text{No of colonies x dilution factor}}{\text{Amount used}}$$

Expressed as colony forming units per gram (cfu/g). The above method was used and reported by some authors [26] [27].

F. *Enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (TCHUB)*

The vapour phase method was used for enumeration of total culturable hydrocarbon utilizing bacteria. Appropriate dilutions of swamp soil from the various treatment conditions at specified days were inoculated into modified mineral salts medium. The medium contained; 0.29g of KCl, 1.27g of K_2HPO_4, 0.42g of $MgSO_47H_2O$, 0.424g of $NaNO_3$, 0.85g of KH_2P0_4, 20g of Agar powder, and 250g

of Amphotericin B known as Fungizone.

These compounds were weighed to their appropriate requirements, hydrated into a 1000ml of sterile water in an Erlenmeyer flask. Appropriate dilution of swamp soil was inoculated into the gelled mineral salt agar (MSA). Hydrocarbon in this case, bonny light crude oil was introduced into the medium by saturating filter paper (Whatman No 1) with it. The saturated filter paper was placed aseptically onto the covers of the pri dished and placed downside up. The saturated filter paper supplied hydrocarbon through vapour phase transfer to the inocula [28]. The inverted plates were incubated in an incubator at a temperature of 28^0c for a period of 7 days. The colonies were counted from the triplicates, mean values were calculated in colony forming units per gram (cfu/g).

G. ***Enumeration of Total Culturable Hydrocarbon Utilizing Fungi***

Mineral salt agar (MSA) that contained 25g of agar powder, 0.42g of KCl, 0.45g of $MgSO_47H_2O$, 0.43g of $NaNO_3$, 0.29g of KH_2P0_4, 10g of NaCl, and 0.86g of $NaHPO_4.H_2O$ was used. Bonny light crude oil was introduced into the medium by saturation of Whatman No 1 filter paper with the crude. The crude served as the sole source of carbon, the saturated filter paper was placed on the cover of the petri dish and was inverted. Appropriate dilution of swamp soil was inoculated into the MSA plates by spread plate method. The MSA medium was amended with 250miligrams of chloramphenicol and tetracycline [29]. The inoculated plates were incubated at room temperature (26^oc-30^0c) for a period of 7days in an incubator.

H. ***Enumeration of Total Culturable Heterotrophic Fungi***

Enumeration of total culturable heterotrophic fungi was done using potato dextrose agar (PDA). The medium was prepared based on the manufacture's instruction (Oxo id Ltd). 0.1ml of each dilution was inoculated into the PDA plates that are replicated in 3s. The inoculated plates were incubated for 7days at 28^0c followed by colony count in cfu/g.

I. ***Statistical Analysis***

All data were keyed in and subsequently analysed using the SPSS for Windows Version 21 and MATLAB (MathWorks, USA). Variations in effect of amendment materials across treatment and duration were investigated using a two-way analysis of variance (ANOVA) technique. Prior to ANOVA, all data were first checked for normality by conducting homogeneity of variance test or F ratio test. Where results of ANOVA are found to be significant, mean separation were further carried out using the Tukey test. All statistical analysis was conducted at the 95% significance level.

VII. RESULTS AND DISCUSSION

Bioremediation of crude oil polluted swamp soil was investigated using animal waste (cow dung and poultry droppings) to similate the indigenous microbial population.

The microbial and physicochemical parameters of the study are represented in Table 2. The bacteria count for hydrocarbon utilizing bacteria was in the range of 10^8cfu/g and heterotrophic bacteria count was in range of 10^5cfu/g which indicates that most of the bacteria communities making up the total heterotrophic bacteria could utilize petroleum hydrocarbon. This phenomenon occurs in environments that has been chronically exposed to hydrocarbon due to anthropogenic activities. The pH of the hydrocarbon polluted soil was 6.6±0.14 which is slightly acidic but could be considered favourable for bioremediation.

Table 2: Physico-chemical and Microbiological properties

Parameters	Baseline	Polluted Soil
pH	4.69±0.58	6.6±0.14
Moisture (%)	22.3±0.06	16.6±0.14
Phosphorous (mg/kg)	31.6±0.58	0.04±0.00
Nitrogen (%)	1.18±0.58	0.08±0.01
Total Hydrocarbon(mg/kg)	49.5±0.26	98881.51±1.41
Total organic carbon (%)	52.46±1,49	82.55±0.35
TPH (mg/kg)	49.5±0.08	7056.83±0.92
THBC ($Log_{10cfu/ml}$)	5.36±0.67	1.56±0.01
THUBC ($Log_{10cfu/ml}$)	5.18±0.67	0.91±0.01
THFC ($Log_{10cfu/ml}$)	4.57±0.50	0.61±0.01
THUFC ($Log_{10cfu/ml}$)	2.57±0.50	0.17±0.01

A. ***Bacterial and Fungal Populations***

Changes in total heterotrophic bacteria count and total heterotrophic fungi count during the 21 days bioremediation study are

represented in Figure 1 and Figure 2 respectively. The population count of heterotrophic bacteria in all treatment levels was higher than that of heterotrophic fungi throughout the experimental days. The same goes to the population count of hydrocarbon utilizing bacteria and fungi.

The heterotrophic bacteria count for control sample on day 7 decreased to 1.26±0.01 from the initial day 0 of 1.56±0.01. Same was observed for the heterotrophic fungi count of day 7 as it decreases from 3.05±0.07 to 2.1±0.14 of day 21, with samples amended with cow dung (CD) showing the highest population growth from the initial count of 1.26±0.01 to 2.77±0.03 cfu/g and 3.05±0.07 to 8.4±0.14cfu/g for heterotrophic bacteria count and heterotrphic fungi counts respectively.There were significant differences in the total heterotrophic bacterial and fungi counts for PW, PC, CD ($p<0.05$).

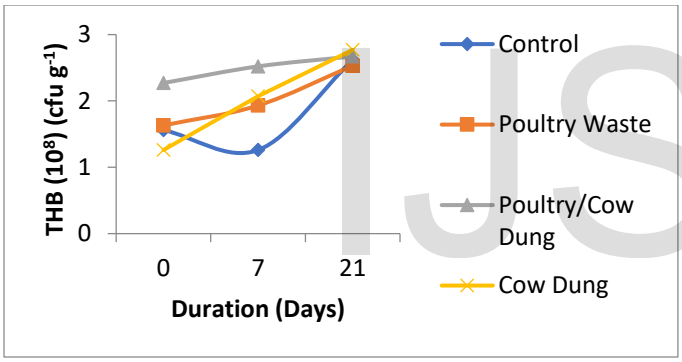

Figure 1: Changes in Total Culturable Heterotrophic Bacterial Count (THBC) Of Hydrocarbon Polluted Soil After 21 Days Bioremediation

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung **CTRL;** control

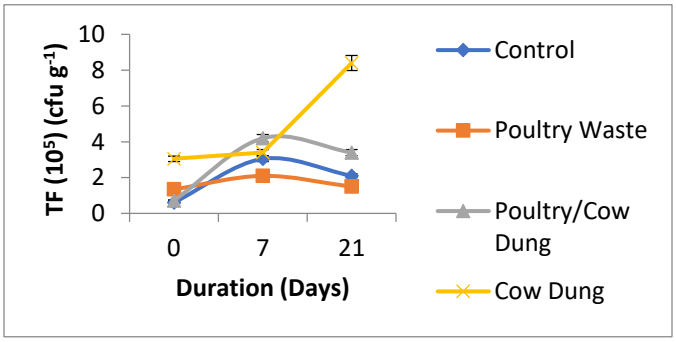

Figure 2: Changes in Total Culturable Heterotrophic Fungi Count (THFC) Of Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung **CTRL;** control

The hydrocarbon utilizing bacteria and fungi responded to the nutrient amendment with poultry waste represented in Figures 3 and 4. The logarithmic population of hydrocarbon utilizing bacteria and fungi in all soil samples amended with various organic waste were higher when compared with to counts from control unamended soil samples. poultry waste (PW) increased from 1.25±0.07 to 44.05±0.07 from day 0 to day 21 day of the study that of cow dung (CD) was recorded to have increased from 7.85±0.07 to 44.1±0.14 from day 0 to day 21. Similarly, the logarithmic total culturable hydrocarbon utilizing bacteria in poultry waste and cow dung (PC) increased from 8.4±0.14 to 44.3±0.42. From the control sample, the logarithmic THUB count increased from 0.91±0.01 to 75.25±0.35 on day 7 but then decreased to 34.5±0.71 on day 21. There was no significant difference between PW, CD and PC treatment options ($p>0.05$).

The hydrocarbon utilizing bacteria isolated in this study were: *Pseudomonas* species, *Staphylococus* species, *Bacillus* species, *Aeromonas* species, *Alcaligenes* species, *Klebsiella* species, *Micrococus* species and *Arthrobacter* species. Amongst which *Bacillus* species were dominate. The hydrocarbon utilizing fungi isolated in this study were: *Saccharomyces* species, *Fusarium* species, *Rhizopus* species, Aspergillus species, *Microsporum* species, *Penicillium* species, *Candida* species, *Acremonium* species and *Geotrichum* species. Amongst these, *Aspergillus* species were dominate.

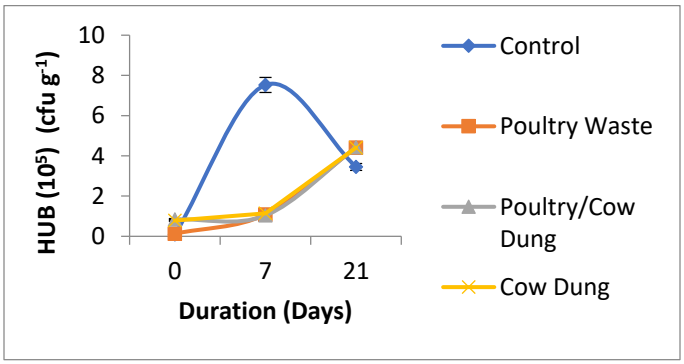

Figure 3: Changes in Total Culturable Hydrocarbon Utilizing Bacteria Count After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung **CTRL;** control

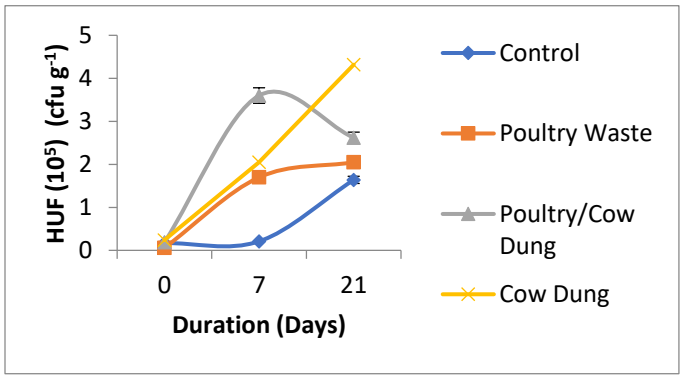

Figure 4: Changes in The Total Culturable Hydrocarbon Utilizing Fungi Count After 21 Days Bioremediation.

PW; poluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

The increase in microbial count in amended soil samples as compared to the unamended soil could be ascribed to the presence of appreciable quantities of nitrogen and phosphorous in animal waste. The same trend was observed by [24], [23] and [25]. [30] examined bacterial dynamics and crude oil degradation after biostimulation and found that nutrient enhancement boosts bacterial counts which correlate significantly with hydrocarbon attenuation. Increase in total heterotrophic bacterial and fungi counts as well as the total hydrocarbon utilizing bacteria and fungi counts in all amended samples (PW, PC and CD) were reported by other researchers such as [30], [34] and [31]. This could be attributed to the presence of appreciable quantities of nitrogen and phosphorous in animal waste which are essential nutrients for microbial degradation of hydrocarbon, [32], [33]. It could be said that the presence of indigenous microorganisms in the organic waste could be responsible for the higher counts. [23] clearly stated the ratio of biodegradation depends majorly on soil nutrient availability. The bacteria count for all amended samples were higher than fungal counts of similar treatments.

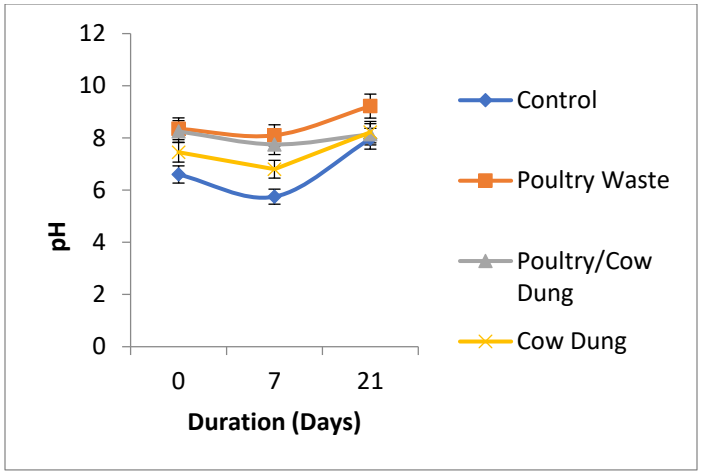

Figure 5: Changes in pH of Hydrocarbon Polluted Soil After 21st Day Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

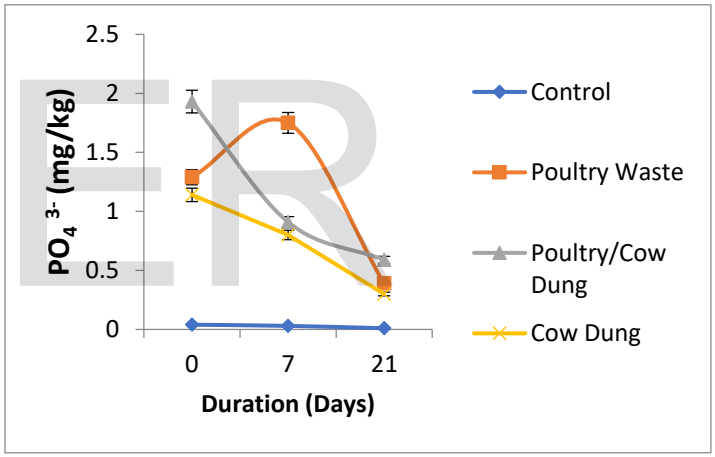

Figure 6: Changes in Phosphorous of Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

B. *Physico-chemical Analysis*

The pH of the mangrove soil was acidic 6.6±0.14. The pH values for the various amended soil ranged from 6-9.24 as shown I Figure 5. The poultry waste treatment option had pH at days 0, 7, and 21 as 8.35±0.07, 8.1±0.14 and 9.22±0.02 respectively. The poultry waste and cow dung treatment option had pH values for days 0, 7 and 21 as 8.25±0.07, 7.75±0.07 and 8.14±0.02 respectively. While in the cow dung treatment option, pH values for days 0, 7 and 21

was 7.45±0.07, 6.8±0.14 and 8.22±0.02 respectively. They maintained an alkaline state except for day 7 of cow dung treatment option which was slightly acidic. The control sample had pH values for days 0, 7 and 21 as 6.6±0.14, 5.75±0.07 and 7.97±0.01 respectively within treatment options. The control sample was acidic for days 0 and 7 but became alkaline on day 21 (Figure 5). Statistical analyses showed that there was a significant difference for the various treatment options ($p<0.05$). In the study, pH was recorded to fluctuate with time, the fluctuation may be as a result of metabolites produced by the microorganisms during the remediation period. The environmental factors (pH, Nitrogen, phosphate) recorded in this study are among are among those recorded to affect bacterial growth. [32], [33].

The available Phosphate in poultry waste, poultry waste/cow dung and cow dung amendments were 1.14±0.62mg/kg, 1.14±0.63mg/kg and 0.75±0.38mg/kg respectively within treatment options. The concentration of phosphate in polluted soil immediately after amendment increased from 0.04±0.00mg/kg to 1.29±0.01mg/kg, 1.93±0.01mg/kg and 1.14±0.01mg/kg in poultry waste, poultry waste/cow dung and cow dung samples respectively (Fig. 6). The amount of phosphate in the poultry waste treatment option increased from 1.29±0.01mg/kg at day 0 to 1.75±0.01mg/kg at day 7 but decreased to 0.39±0.00mg/kg at day 21. Whereas phosphate decreased in poultry waste/cow dung and cow dung treatment options in days 7- 21 from 1.93±0.01mg/kg to 0.59±0.00mg/kg and 1.14±0.01mg/kg to 0.3±0.00mg/kg respectively. Phosphate concentration in the control sample decreased from 0.04±0.00mg/kg to 0.01±0.00mg/kg on day 21. There was a significant difference for treatment options ($p<0.05$)

The nitrogen level in all treatment options increased from 0.08±0.01% to 0.12±0.00%, 0.13±0.00% and 0.11±0.00% in poultry waste, poultry waste/cow dung and cow dung respectively. They all increased at day 7 and finally decreased to 0.08±0.01%, 0.14±0.00%and 0.12±0.01% at day 21 respectively (Figure 7). The control sample decreased from 0.08±0.01% to 0.08±0.01% at day 21. In between treatment options, Poultry waste amendments had the highest percentage of nitrate at 0.17±0.11% followed by the combination of poultry waste/cow dung at 0.15±0.02% and cow dung at 0.13±0.02%. There was a significant difference in all the experimental conditions at $p<0.05$ level.

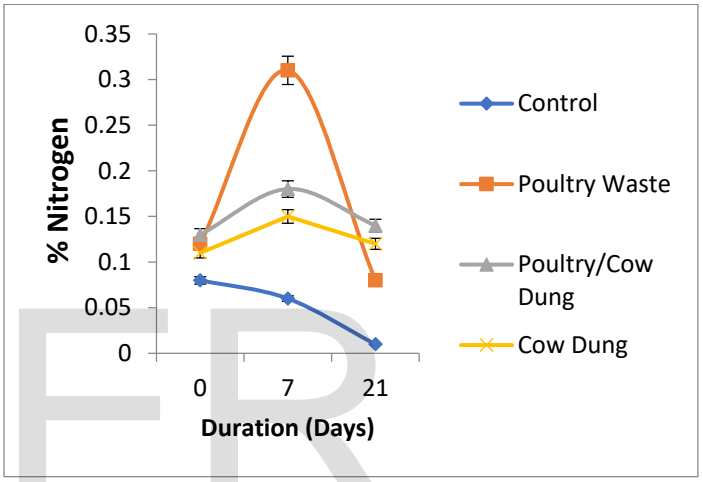

Figure 7: Changes in Nitrogen of Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

The Changes in phosphate and nitrogen content were represented in Figures 6 and 7 respectively. There was an apreciatable decrease in phosphate and nitrogen levels in all amended options as compared to the control, indicting that the phosphate and nitrogen were used by microorganisms during bioremediation. [23] clearly stated the ratio of biodegradation depends majorly on soil nutrient availability. The same trend was observed by [24], [23] and [25]. [30] examined bacterial dynamics and crude oil degradation after biostimulation and found that nutrient enhancement boosts bacterial counts which correlate significantly with hydrocarbon attenuation. Similar observations have been reported by other researchers [35], [36] and [31].

The recorded changes in total petroleum hydrocarbon (TPH) concentration of the amended soil samples are shown in

Figure 8. In the poultry waste, poultry waste/cow dung and cow dung treatment options, the petroleum hydrocarbon decreased from 5871.32±1.63mg/kg, 6256.79±2.47mg/kg and 5365.39±4.45mg/kg to 1335.31±1.63mg/kg, 1366.29±1.7mg/kg and 1353.74±0.57mg/kg respectively at day 21. The control experiment had a reduction in total hydrocarbon content form 7056.83±0.92mg/kg to 5927.77±1.7mg/kg at day 21, showing the effectiveness of natural attenuation. There was a significant mean difference between treatment options and control ($p<0.05$). The percentage loss of total petroleum hydrocarbon (TPH) was 77.24±0.01%, 78.15±0.01% and 74.78±0.01% for PW, PC and CD respectively on day 21 (Figure 9). The percentage loss in TPH on the day 21 for the control sample was 16±0.01%. (Figure 9). There was a significant mean difference between the treatment options ($p<0.05$).

At the end of day 21, polluted mangrove swamp soil amended with PC had the highest percentage of degradation with 78.15% followed by PW at 77.24% and CD at 74.78% compared to the un-amended control sample which had 16% degradation rate. [23] reported similar percentages of TPH loss in their study on bioremediation of crude oil polluted soil using animal waste. The treatment options had greater oil degradation capacity when compared to the control sample in this study. The addition of limiting nutrients stimulated the degradative capability, allowing the indigenous microorganisms to break down the organic pollutants at a faster rate [37], [24].

Poultry waste/Cow dung (PC) recorded the highest hydrocarbon degradation of 78.15% compared to poultry waste (PW) and cow dung (CD). [23] noted the potency of poultry waste as compared to cow dung. This might be as a result of the differences in nutrient contents in this case, Nitrogen and Phosphorous in the various organic amendments in stimulating the indigenous microorganisms. Same results were recorded by [24]. It is therefore established that Nitrogen and Phosphorous are the most important nutrients needed in biostimulation for hydrocarbon utilizing bacteria and fungi to carry out effective and efficient degradation of xenobiotics in the soil environment.

There was a 16% degradation in the control un-amended sample. This may be due to non-biological factors like evaporation or photo-degradation and of course the potency of existing hydrocarbon degrader available in the soil. Hence, its biodegradation capacity was low when compared to treatment options.

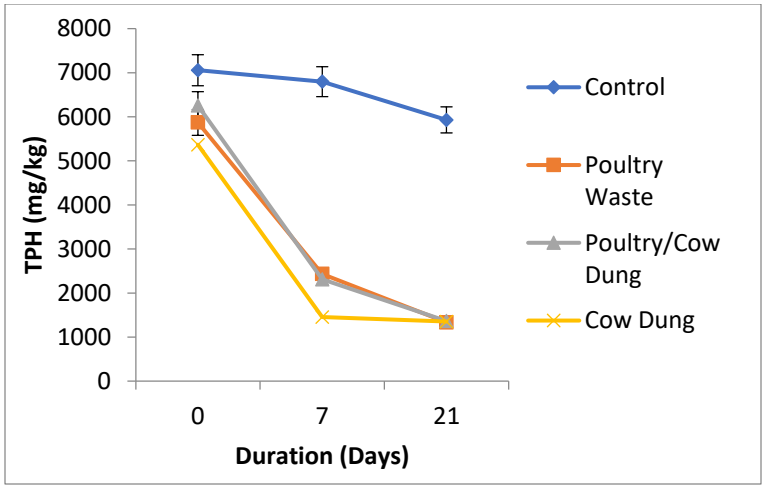

Figure 8: Changes in Total Petroleum Hydrocarbon (TPH) Of Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

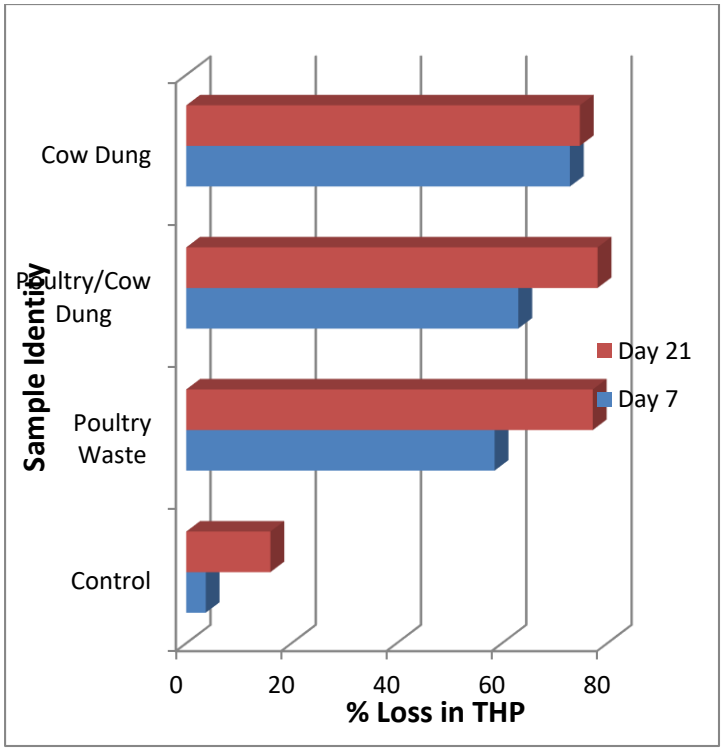

Figure 9: Changes in %Loss of TPH in Samples in treatments at Varying Times (Days)

C. *HeavyMetals*

The amount of manganese (Mn) recorded in the polluted soil sample was 12.15±0.07mg/kg. after amendment, the poultry waste, poultry waste/cow dung and cow dung treatment samples had 110.12±0.03mg/kg, 85.62±0.04mg/kg and 50.07±0.02mg/kg respectively on day 0 and 25.82±0.73mg/kg, 15.87±0.74kg and 6.87±0.12mg/kg on day 21 respectively (Figure 10). Statistical record between treatment options showed that poultry waste, poultry waste/cow dung and cow dung as 64.53±38.08mg/kg, 39.44±35.77mg/kg and 22.74±21.26mg/kg respectively. There was significant reduction in concentration between day 0 and 21 of the experiment. There was a significant mean difference between poultry waste, poultry waste/cow dung and cow dung treatment options (p,0.05).

The concentration of Zinc (Zn) in the polluted sample was 13.52±0.03mg/kg. After amendment, Poultry waste recorded the highest value of 73.8±0.07mg/kg followed by poultry waste/cow dung mixture then cow dung at 53.12±0.02mg/kg and 35.55±0.14mg/kg respectively on day 0. They all decreased by day 21 as shown in Figure 11 with recorded values of 15.37±0.1mg/kg, 16.35±0.06mg/kg and 5.87±0.03mg/kg respectively. between treatment options, statistical values were recorded at 39.17±27mg/kg, 30.45±17.73mg/kg and 17.23±14.33mg/kg for poultry waste, poultry waste/cow dung and cow dung respectively. There was no significant difference (p>0.05).

The polluted soil sample had iron (Fe) content of 2344.32±0.02mg/kg (Figure 12). Cow dung treatment option had the highest with 2982.27±0.09mg/kg at day 0, there was a rapid drop in conc on day 7 to 633.22±0.73mg/kg and 408.37±0.68mg/kg on day 21. Same was noted about poultry waste and poultry waste/cow dung treatment options which had 2562.2±0.07mg/kg to 529.24±0.06mg/kg and 1990.37±0.02mg/kg to 264.31±0.69mg/kg on day 21 respectively. between various treatment options of poultry waste, poultry waste/cow dung and cow dung, 1276.67±1000.16mg/kg, 927.15±831.89mg/kg and 1341.28±1275.07mg/kg was recorded. There was no significant difference at p>0.05.

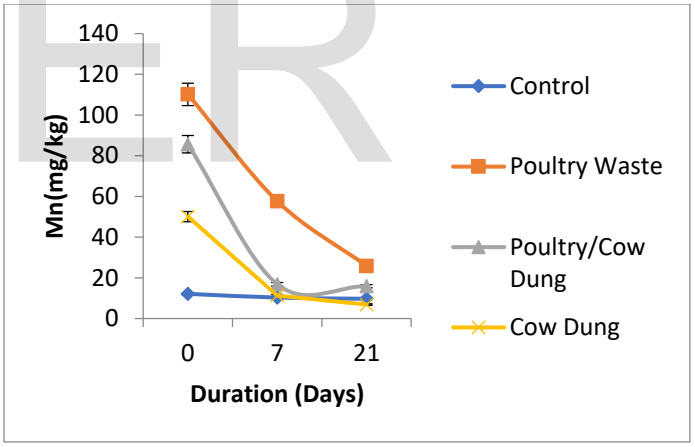

Figure 10: Changes in Manganese in Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

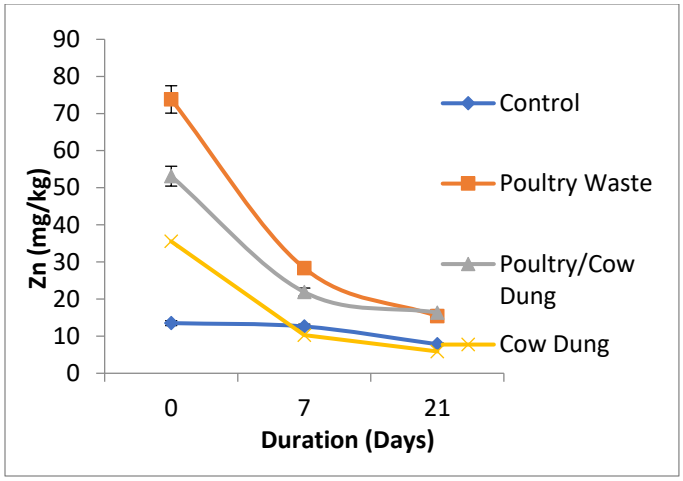

Figure 12: Changes in Zinc in Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

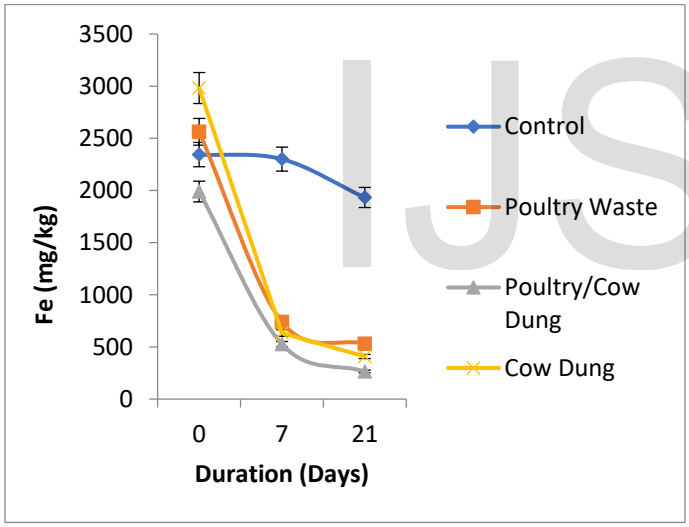

Figure 12: Changes in Iron in Hydrocarbon Polluted Soil After 21 Days Bioremediation.

PW; polluted soil + poultry waste, **CD;** polluted soil + cow dung, **PC;** polluted soil + poultry waste and cow dung, **CTRL;** control

Table 4.3: Microscopic and Biochemical Characteristics of HUB isolated during the Study.

Isolate	Shape	Gram Rxn	Spore	Catalase	Oxidase	Indole	Citrate	Motility	MR	VP	H_2S	Gas	Slant	Glucose	Lactose	Sucrose	Mannitol	Probable Organism
HUB1	Rod	-	-	+	+	-	-	+	-	+	-	-	B	-	A	A	A	*Pseudomonas* Sp.
HUB2	Cocci	+	-	+	-	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB3	Rod	+	+	+	-	-	+	+	-	+	-	-	-	A	-	-	-	*Bacillus* Sp.
HUB4	Rod	-	-	+	+	+	-	+	-	+	-	-	B	A	A	A	-	*Aeromonas* Sp.
HUB5	Rod	+	-	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB6	Rod	-	-	-	-	-	+	-	-	+	-	-	B	-	A	A	A	*Alcaligenes* Sp.
HUB7	Cocci	+	-	+	+	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB8	Rod	-	-	-	-	-	+	-	-	+	-	+	A	A/G	A/G	A	-	*Klebsiella* Sp.
HUB9	Rod	-	-	+	+	-	-	+	-	+	-	-	B	-	A	A	A	*Pseudomonas*
HUB10	Cocci	+	-	-	-	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB11	Rod	-	-	+	+	-	-	+	-	+	-	-	B	-	A	A	A	*Pseudomonas* Sp.
HUB12	Rod	+	+	+	+	-	+	+	-	+	-	-	A	A	-	-	-	*Bacillus* Sp.
HUB13	Cocci	+	-	+	+	-	+	-	-	+	-	+	A	A	A	-	A	*Staphylococcus* Sp.
HUB14	Rod	-	-															*Alcaligenes* Sp.
HUB15	Rod	-	-	-	-	-	+	-	-	+	-	+	A	A/G	A/G	A	-	*Klebsiella* Sp.
HUB16	Rod	-	-	+	+	+	-	+	-	+	-	-	B	A	A	A	-	*Aeromonas* Sp.
HUB17	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB18	Cocci	+	-	-	-	-	+	-	+	+	-	-	B	A	-	-	A	*Micrococcus* Sp.
HUB19	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB20	Rod	-	-	+	+	-	-	+	-	+	-	-	B	-	A	A	A	*Pseudomonas* Sp.
HUB21	Rod	-	-	-	-	-	+	-	-	+	-	-	A	A/G	A/G	A	-	*Klebsiella* Sp.
HUB22	Cocci	+	-	+	+	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB23	Rod	-	-	-	-	-	+	-	-	+	-	-	B	-	A	A	A	*Alcaligenes* Sp.
HUB24	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB25	Rod	-	-	+	+	+	-	+	-	+	-	-	B	A	A	A	-	*Aeromonas* Sp.
HUB26	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB27	Rod	+	-	+	-	-	+	-	-	+	-	+	B	A/G	A	A/G	A/G	*Arthrobacter* Sp.
HUB28	Rod	-	-	+	+	+	-	+	-	+	-	-	B	A	A	A	-	*Aeromonas* Sp.
HUB29	Rod	+	-	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB30	Rod	-	-	+	-	-	+	+	-	+	-	-	B	A	A	A	-	*Pseudomonas* Sp.

HUB31	Rod	-	-	-	-	-	+	-	-	+	-	+	A	A/G	A/G	A	-	*Klebsiella* Sp.
HUB32	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB33	Cocci	+	-	+	-	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB34	Rod	+	-	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB35	Rod	-	-	+	+	+	-	+	+	-	-	-	A	A	A	A	-	*Aeromonas* Sp.
HUB36	Rod	-	-	+	-	-	+	-	-	+	-	-	B	-	A	A	A	*Alcaligenes* Sp.
HUB37	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB38	Rod	+	-	+	-	-	+	-	-	+	-	+	B	A/G	A	A/G	A/G	*Arthrobacter* Sp.
HUB39	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB40	Cocci	+	-	+	-	-	+	-	-	+	-	-	A	A	A	-	A	*Staphylococcus* Sp.
HUB41	Cocci	+	-	+	-	-	+	-	+	+	-	-	A	A	A	-	A	*Micrococcus* Sp.
HUB42	Rod	+	+	+	-	-	+	+	-	+	-	-	B	A	-	-	-	*Bacillus* Sp.
HUB43	Rod	-	-	+	-	-	+	+	-	+	-	-	B	A	A	A	-	*Pseudomonas* Sp.
HUB44	Rod	-	-	-	-		+	-	-	+	-	+	A	A/G	A/G	A	-	*Klebsiella* Sp.

Table 4.4: Cultural Characteristics of Hydrocarbon Utilizing Fungi

ISO	Culture Characteristics	Microscopic Characteristics	Probable Genera.
HUF1	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cell appearing as single, budded and some clustered	*Saccharomyces* Sp.
HUF2	White fluffy colonies that is light at the reverse	The hyphae are small and separate given rise to phialides that produced single-celled microconidia	*Fusarium* Sp.
HUF3	Dense fluffy grey/brown colonies with loose dotted sporangia.	Unbranched sporangiophores with rhizoids appearing at the stolon	*Rhizopus* Sp.
HUF4	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cells appearing as single, budded and clustered	*Saccharomyces* Sp.
HUF5	Yellowish green sporing surface that is light at the reverse	Vesicles are globose and phialides produces directly from the vesicle surface	*Aspergillus* Sp.
HUF6	White fluffy colonies that is light at the reverse	The hyphae are small and separate given rise to phialides that produced single-celled microconidia	*Fusarium* Sp.
HUF7	Yellowish green sporing surface that is light at the reverse	Vesicles are globose and phialides produces directly from the vesicle surface	*Aspergillus* Sp.
HUF8	White curled surface with light reverse	Irregular branching hyphae with predominant cross walls and chlamydospores	*Microsporum* Sp.
HUF9	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cell appearing as single, budded and some clustered	*Saccharomyces* Sp.
HUF10	Black sporing/granular surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.

HUF11	White fluffy colonies that is light at the reverse	The hyphae are small and separate given rise to phialides that produced single-celled microconidia	*Fusarium* Sp.
HUF12	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cell appearing as single, budded and some clustered	*Saccharomyces* Sp.
HUF13	Blue green velvet surface with light cracked reverse	Hyphae are hyaline and septate, producing brush like conidiophores	*Penicillium* Sp.
HUF14	Yellowish green sporing surface that is light at the reverse	Vesicles are globose and phialides produces directly from the vesicle surface	*Aspergillus* Sp.
HUF15	White fluffy colonies that is light at the reverse	The hyphae are small and separate given rise to phialides that produced single-celled microconidia	*Fusarium* Sp.
HUF16	White velvet powdery colonies that is light at the reverse	Hyphae are hyaline and septate, producing brush like conidiophores	*Penicillium* Sp.
HUF17	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cell appearing as single, budded and some clustered	*Saccharomyces* Sp.
HUF18	White shinny smooth surfaced colonies that is light at the reverse	Spherical budding yeast like cells	*Candida* Sp.
HUF19	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.
HUF20	White to rose or reddish velvet surface that is pink at the reverse	Small septate hyphae that produce single unbranched tube like phialides	*Acremonium* Sp.
HUF21	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.
HUF22	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.
HUF23	Blue-green velvet surface with light cracked reverse	Hyphae are hyaline and septate, producing brush like conidiophores	*Penicillium* Sp.
HUF24	White powdery colonies that is light at the revers	Hyphae are septate and produce numerous cylindrical to barrel-shaped arthroconidia	*Geotrichum* Sp.
HUF25	Green velvet colonies and light at the reverse	Hyphae are hyaline and septate, producing brush like conidiophores	*Penicillium* Sp.
HUF26	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.
HUF27	White powdery colonies that is light at the revers	Hyphae are septate and produce numerous cylindrical to barrel-shaped arthroconidia	*Geotrichum* Sp.
HUF28	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.
HUF29	Blue-green velvet powdery colonies with light cracked reverse	Hyphae are hyaline and septate, producing brush like conidiophores	*Penicillium* Sp.
HUF30	White opaque round colonies with smooth surface and light at the reverse	Oval shaped cell appearing as single, budded and some clustered	*Saccharomyces* Sp.
HUF31	Black sporing surface with light cracked reverse	Septate hyphae with long conidiophore that support spherical vesicles	*Aspergillus* Sp.

VIII. CONCLUSION

The results of this study showed that contaminated soil amended with poultry waste/cow dung, poultry waste, cow dung and the control sample had 78.15%, 77.24%, 74.78% and 16% loss in total petroleum hydrocarbon content respectively. Hydrocarbon polluted soil amended with a mixture of poultry waste and cow dung had the highest percentage loss o f total petroleum hydrocarbon. The reports of this study showed that the rate of bioderadation depends greatly on soil nutrient availability. Hence, the low percentage loss in the control sample. This research demostated that nutrient amendment using organic waste boosted the selection of diverse bacteria and fungi population in the mangrove ecosystem. Based on the request for the Nigerian content development board, advocating for the substitution of very expensive foreign materials with locally made ones for bioremediation, this study has roven bryound reasonable doubt that Organic waste such as poultry waste and cow dung are good substitute for foreign materials as it it cost effective, efficient, environmentally friendly and sustainable. This also manages the menace of organic waste disposal experienced in poultry and cattle farms thereby, reducing environmental pollution.

REFERENCES

[1] Awobajo, S. A. (1981). An Analysis of Spill Incidents in Nigeria (1976-1980). In: Pro., Seminar on Petroleum Industry and the Nigerian Environment, NNPC/ FMOW and Housing PT, Warri.

[2] Owugah, L. (2001). Oil Transnationals, State and Development in The Oil Producing Communities of the Niger Delta. In T. W. Africa, Mining, Development and Social Conflicts in Africa. *Third World Network Africa,* 45-76.

[3] Duke, N., Meynecke, J., Dittmann, S., Ellison, A., Anger. K, U. Berger, Cannicci, S., Diele, K., Ewel, K., Field, C., Koedam, N., Lee, S., Marchand, C., Nordhaus, I. & Dahdouh-Guebas, F. (2007). A world without mangroves. *Science,* 317(5834), 41-42.

[4] Dahdouh-Guebas, F. (2002). The Use of Remote Sensing and GIS in the Sustainable Management of Tropical Coastal Ecosystems. *Environmental Development Sustainability,* 4, 93-112.

[5] Food and Agriculture Organization of the United Nations FAO. (2007). The world's mangrove 1980-2005. *Forestry Paper,* 153, 89.

[6] Dahdouh-Guebas, F., Hettiarachchi, S., Lo Seen, D., Batelaan, O., Sooriyarachchi, S., Jayatissa, L. P. & Koedam, N. (2005). Transitions in Ancient Inland Freshwater Resource Management in Sri Lanka Affect Biota and Human Populations in and Around Coastal Lagoons. *Current Biology,* 15, 579–586.

[7] Olwig, M. F., Sorensem, M. K., Rasmussen, M. S., Danielsen, F., Selvam, V, Hansen, L. B., Nyborg, L., Vestergaard, K. B., Parish, F. & Karunagaran, V. M. (2007). Using Remote Sensing to Assess the Protective Role of Coastal Woody Vegetation Against Tsunami Waves. *International Journal Remote Sensing* 28(13), 3153 -3169.

[8] Duke, N., Meynecke, J., Dittmann, S., Ellison, A., Anger. K, U. Berger, Cannicci, S., Diele, K., Ewel, K., Field, C., Koedam, N., Lee, S., Marchand, C., Nordhaus, I. & Dahdouh-Guebas, F. (2007). A world without mangroves. *Science,* 317(5834), 41-42.

[9] Brito, E. M. S., Duran, R., Guyoneaud, R., Goni-Urriza, M., Oteyza, T. G., Crapez, M. A. C., Aleluia, I. & Wasserman, J. C. A. (2009). A Case Study of In-Situ Oil Contamination in a Mangrove Swamp (Rio De Janeiro, Brazil). *March Pollution Bulletin,* 58, 418–423.

[10] United Nation Environmental Protection Agency UNEPA (2001). A Citizen's Guide to Bioremediation.

[11] Agarry, S. E., Owabor, C. N., & Yusuf, R. O. (2010) 'Bioremediation of Soil Artificially Contaminated with Petroleum Hydrocarbon Oil Mixtures: Evaluation of the Use of Animal Manure and Chemical Fertilizer'. *Bioremediation Journal,* 14(4), 189-195.

[12] Nwilo, P.C. & Badejo, O.T. (2001). Impact of oil spill along the Nigerian coast. The Association for Environmental Health and Sciences.

[13] Tambeke, N. G. & Gloria, O. A. (2016). contributions of Soil Microarthropods to Ecosystem Recovery at Tai Communities, Rivers State, Nigeria. *Resources and Environment*, 6 (6), 136-142.

[14] Michael, H. C. (2013) "Niger River", in M. McGinley (ed.), *Encyclopedia of Earth*, Washington, DC: National Council for Science and Environment.

[15] United Nation Environmental Programme, UNEP (2011). environmental assessment of Ogoniland report, Retrieved from http://www.unep/dissastersandconflicts/country-operations

[16] Artin, H. (2010). Principles of Bioremediation Processes. *Trends in bioremediation and phytoremediation*. 2-54. [16] Rosenberg, F. A. (2003). The microbiology of bottled water. *Clinical Microbiology Newsletter, 25(6),* 41-44.

[17] Chikere, B. O. & Chijioke-Osuji, O. (2006). Microbial Diversity and Physico-Chemical Properties of Crude oil Polluted Soil. *Nigeria Journal of Microbiology,* 20, 1039-1046 [18] Dawson, D. J., & Sartory, D. P. (2000). Microbiological safety of water. *British Medical Bulletin, 56(1),* 74-83.

[18] Pinholt, Y., Struwe, S., & Kjoller, A. (2006). Microbial Changes During oil decomposition in soil. *Ecography, 2,*

195-200.

[19] Bossert, I., & Bartha, R. (1984). The Fate of Petroleum in Soil Ecosystems. In. Petroleum Microbiology Atlas R.M. ed. Macmillan New York, 435-437.

[20] Floodgate, G. D. (1984). The Fate of Petroleum in Marine Ecosystems. In Atlas RM Education. *Petroleum Microbiology Macmillan Publishing Company* New York 355-398.

[21] Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol. Review,* 45*,* 180–209.

[22] Okpowasili, G. C. & Odukuma, L. O. (1994). Tolerance of *Nitrobacter* to Toxicity of Some Nigerian Crude Oils. *Bulletin of Environmental. Contamination & Toxicology,* 52*,* 388-395.

[23] Obiakalaije, U. M., Makinde, O. A. & Amakaoromo, E. R. (2015). Bioremediation of Crude oil Polluted Soil Using Animal Waste *International Journal of Environmental Bioremediation & Biodegradation,* 3(3), 79-85.

[24] Ibekwe, M, J., Ehirim, O., Emmanuel U. & Umeda, U. (2018). Bioremediation of Hydrocarbon Contaminated Soil Using Cow Dung and Poultry Droppings. *International Journal of Chemistry and Chemical processes*, 4(3), 2545-5265.

[25] Williams J. O. & Amaechi V.C. (2017). Bioremediation of Hydrocarbon Contamination Soil Using Organic Wastes as Amendment. *Current Studies in Comparative Education, Science and technology.* 4(2) 89-99.

[26] Chikere, C.B., Okpokwasili, G.C & Chikere, B.O. (2009). Bacteria Diversity in a Tropical Crude Oil Polluted Soil Undergoing Bioremediation. *African Journal of Biotechnology,* 8(11), 2535-2540

[27] Nwachukwu, M. A., Feng, H. & Alinnor. (2010). Assessment of Heavy Metals Pollution in Soil and Their Implications Within and Around Mechanic villages. *International Journal of Environmental Science & Technology,* 7(2)*,* 347-358.

[28] Abu, G. O. & Chikere, B. O. (2006). Cell surface Properties of Hydrocarbon Utilizing Bacterial Isolates from Port Harcourt marine Environment. Nigeria. *Journal of Microbiology,* 20*,* 809-816.

[29] Obire, o., Anyanwu, E.C., and Okigbo, R.N (2008). Saprophytic and Crude Oil Degrading Fungi from Cow Dung and Poultry Droppings as Bioremediating Agents. *International Journal Agricultural Science and Technology,* 4 (2), 81-89.

[30] Roling, W.F.M., Milner, M.G., Jone, D.M., Lee, K., Daniel, F. & Swannel, R.P.J. (2002). Robust Hydrocarbon Degradation and Dynamics of Bacterial Communities During Nutrient-Enhanced Oil Spill Bioremediation. *Applied and Environmental Microbiology*. 68, 5537-5548.

[31] Orji, F.A., Ibiene, A.A & Dike, E.N. (2012). Laboratory scale bioremediation of petroleum hydrocarbon-polluted mangrove swamps in the Niger Delta using cow dung. *Malaysian Journal of Microbiology*, 8(4), 219-228.

[32] Adesodun, J.K. & Mbagwu, J.S.C. (2008). Biodegradation of waste lubricating petroleum oil in a tropical soil as mediated by animal droppings. *Bioresource Technology*, 99, 5659-5665.

[33] Ijah, U.J.J. & Anita, S.P. (2003). The potential use of chicken droppings microorganisms for oil spill remediation. *The Environmentalist*, 23, 89-95.

[34] Chikere, C.B., Chikere, B.O. & Okpokwasili, G.C. (2012). Bioreactor Based Bioremediation of Hydrocarbon Polluted Niger Delta Marine Sediment. *Nigeria Biotechnology*, 2(1), 53-66.

[35] Okpowasili, G. C. & Oton, N.S. (2006). Comparative applications of bioreactor and shake flask systems in the laboratory treatment of oil sludge. *International Journal of Environmental Waste*, 1(1), 49-60.

[36] Ibiene, A. A., Orji, F. A., Ezidi, C. O., Ngwobia, C. L. (2011). Bioremediation of hydrocarbon contaminated soil in the Niger Delta using spent mushroom compost and other organic wastes. *Nigeria Journal of Agriculture, Food and Environment,* 7(3), 1-7.

[37] Ausma, S., Edwards G.C., Fitzgerald-Hubble, C.R., Halfpenny-Mitchell, L., Gilles, T.J. & Mortimer, W.P. (2002). Volatile hydrocarbon emissions from diesel fuel contaminated soil bioremediation facility. Air Waste management Association, 52, 769-780.