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DEGRADATION OF SPENT OIL CONTAMINATED SOIL USING FUNGI FROM ORGANIC MANURE

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ABSTRACT

Soil is the key component of natural ecosystem because environmental sustainability depends largely on a sustainable soil ecosystem. Microbial breakdown of hydrocarbon pollutants is generally a very slow proceed, but it could be optimum biodegradation can only occur if the right environmental condition such as pH, temperature, nutrients and relevant microbial consortia are present, as well as the pollution of petroleum hydrocarbons caused a major changes in the physical and chemical properties of the soil. The aim of this study is to determine the total petroleum Hydrocarbon degradative potentials of the intrinsic microbes. Two kilograms (2kg) of soil was thoroughly mixed with 200ml and 400ml of spent oil to give 5% and 10% contamination levels and a set of control was kept at 0%, 10%(w/w) each of the organic manure from poultry litter (PL), Cow dung (CD), and the mixed poultry litter and cow dung (MPLCD) was individually introduced into each spent oil contaminated soil and the rate of biodegradation was monitored for a period of 12 weeks. The percentage of total Petroleum Hydrocarbon (TPH) loss was significantly higher in the soil contaminated with MPLCD (40.46%) followed by PL (35.53%) and CD (27.70%) while 32.42% loss only was recorded in the soil contaminated with 10% spent oil and amended with MPLCD while PL was 30.04% and 25.60% for CD. The hydrocarbon-initializing fungi isolated and identified include Aspergillus spp. and Penicillium spp. The amendment of spent oil contaminated soil with organic manure can significantly enhance the rate of biodegradation of petroleum hydrocarbon. These activities can be used to remove or neutralize the contaminants of the soil, by petroleum hydrocarbon.

KEYWORDS: *Total Petroleum Hydrocarbon (TPH), Fungi, Organic Manure, contaminated soil,spent Oil.*

1. INTRODUCTION

Contaminated lands abound throughout the world but are mostly rampant in developing countries where environmental laws are at best rudimentary (Adams *et al.*,2014)

The soil is the key component of natural ecosystem because environmental sustainability depends largely on a sustainable soil ecosystem.(Adetokun and Ataga, 2007; Adenipekun 2008).

Soil contamination is the presence in soil of unwanted impure materials from human activities. It can also be the distortion of the soil environment by human activities. Soil is the habitat for variety of organisms, including fungi, bacteria, protozoa, insects, nematodes, worms, and many other animals. Viruses are also present in soils. This complex biological community contributes to the formation, maintenance, and in some situations, the degradation and disappearance of soils (Prescott *et al.*,2005)

Hydrocarbon component have been known to belong to the family of carcinogens and neurotoxic organic pollutants (Das and Chandran,2010). Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations (Alvarez and Vogel,1991). Many techniques of remediation of contaminated soils have been developed, such as physical, chemical, degradation, photo degradation. However, most of these methods have some drawbacks in completely remediating hydrocarbon contaminated soil.Biological treatment offers the best environmental friendly method for remediating hydrocarbon and heavy metals contaminated soil because it utilized the capability of the indigenous micro-organisms in the soil environment to break down the hydrocarbons and heavy metals into the innocuous substances.

This process relied upon the microbial enzymatic activities to transform or degrade the contaminants from the environments (Philip *et al.*,2005).

Organisms such as fungi are also capable of degrading the hydrocarbons in engine oil to a certain extent but they take longer periods of time to grow when compared to their bacterial counterpart(Lee *et al.*,2007). It was observed that the addition of spent mushroom compost to the concentrated medium reduced the toxicity added enzymes, micro-organisms and nutrients involved the degradations of PAHS (Lau *et al.*,2003). Organic waste like banana skin, spent mushroom compost and brewery spent grain were found to enhance the biodegradation of used lubricating oil up to 90% within the period of 3 months (Abioye *et al.*,2009b;2010).

The main aim of the study is to determine the total petroleum hydrocarbon degradative potentials of the intrinsic microbes

2. METHODOLOGY

2.1 STUDY AREA

The experiment was carried out at the experimental house (screened house) of the Department of Crop ProtectionAnd Environmental Biology, University of Ibadan, Nigeria.

2.2 COLLECTION AND PROCESSING OF SAMPLES

2.2.1 Soil sampling: The soil sample was collected randomly at a depth of 0-30cm from the fallow field in the University of Ibadan, Nigeria.. They were bulked to form a composite sample and transported in a polythene bags to the laboratory, air dried and sieved through a 2mm mesh.

2.2.2 Collection ofSpent Motor Oil: The spent motor oil used for the experiment was collected from a freshly drained motor car engine.

2.2.3 Poultry Litter and Cow Dung: The poultry litter (PL) and cow dung (CD) was obtained from the animal farm in the University of Ibadan, Nigeria. They were sun dried for 72 hours to allow moisture removal and accelerate the distribution of nutrients to the microbes.

2.2.4 Preparation of soil for Bioremediation. 2 Kg of sieved (2mm) soil was contaminated with 5% and 10% of spent lubricating oil and thoroughly mixed and left for 24 hours for homogenization. 10% (w/w) of each organic manure, poultry litter (PL), cow dung (CD), mixed poultry litter and cow dung (MPLCD) was individually introduced into each spent oil contaminated soil and thoroughly mixed.

The experimental pots were filled with the soil-oil – organic manure mixture. The control pots consist of soil-oil mixture without organic manure was also set up. The experiment was set up in four replicates. Periodic sampling from each experimental pot was carried out on day 0 and subsequently 4 weeks interval for 12 weeks of post contamination. Composite samples were obtained by mixing 5g of soil collected from four different areas of the pots for isolation and enumeration of bacteria and fungi and also the determination of Total Petroleum Hydrocarbon (TPH).

TABLE 1: EXPERIMENTAL DESIGN

Treatment	Details of Treatment
1	2kg soil + 5% spent oil.
2	2kg soil + 10% spent oil.
3	2kg soil + 5% spent oil + 10% PL
4	2kg soil + 5% spent oil + 10% CD
5	2kg soil + 5% spent oil + 10% MPLCD
6	2kg soil + 10% spent oil + 10% PL
7	2kg soil + 10% spent oil + 10% CD
8	2kg soil + 10% spent oil + 10%MPLCD

Key:

PL = Poultry litter
CD = Cow dung
MPLCD = Mixed poultry litter and cow dung

2.3 ENUMERATION AND IDENTIFICATION OF MICROBIAL POPULATION

Four replicate samples from each oil-contaminated soil were withdrawn in every four weeks for the enumeration and identification of fungi. 1g of oil-contaminated samples were weighed and poured into 9ml of sterile distilled water and mixed thoroughly. Concentration of dilutions were made at $10^1$to 10^5 for fungi. 0.1ml of dilution levels of 10^{-2}, 10^{-3}, and 10^{-5} for fungi was cultured using pour plate method on Potato Dextrose Agar (PDA) to determine the loads of Total Heterotrophic Fungi (THF). All media, sterile distilled water were sterilized by autoclaving at 121^{0}C for 15minutes.

The PDA plates for the enumeration of Total Heterotrophic Fungi were incubated at 28± 2^{0}C for 7daysThe PDA was fortified with lactic acid for fungi after sterilization to avoid bacteria contamination. The isolates from different plates were purified by repeated streaking on fresh agar medium.

Cultural features microscopic characteristics described (Singh *et al.*,1991) were used for the identification of fungi.

2.4 LABORATORY ANALYSES

Sample analyses were carried out at the Multidisciplinary Central Laboratory (MCL), Nigeria Institute of Science Laboratory Technology (NIST), Microbiology Laboratory of University of Ibadan, Toxicology and Pathology Laboratory of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria.

2.5 PHYSICO – CHEMICAL PARAMETERS OF THE SOIL.

The physical and chemical characteristics of the contaminated soil and the organic manure were determined.

2.5.1 Soil pH determination

The pH was measured using Jenway 3510 pH meter (Hendershot *et al.*,1993).

2.5.2 Determination of Organic Carbon (Walkley – Black Method)

The method of the Association of official Analytical Chemists (AOAC,2003) was used

2.5.3 Determination of Total Nitrogen (KjeldahlMethod)

This was determined by the method of the Association of Official Analytical Chemists (AOAC,2003).

2.5.4 Determination of Available Phosphorus (Bray11 method)

Available Phosphorus was measured colorimetric ally by Bray II method (Olsen *et al.*,1954)

2.5.5 Hydrometer method of Soil Mechanical Analysis ;

This was determined using hydrometer method.

2.6 Total Petroleum Hydrocarbon (TPH) Analysis

This was carried out using the method of Adesodun and Mbagwa(2008). Ten grammes (10g) of soil samples were weighed into 50ml flask and 20ml Toluene (Analar Grade) was added. After shaking for 30 minutes on an orbital shaker ,the liquid phase of the extract was measured at 420nm (nanometer) absorbance using DR/4000 Spectrophotometer. The Total Petroleum Hydrocarbon in the soil was estimated with the reference to a standard curve derived from fresh used engine oil diluted with Toluene.

2.6.1 Procedure for Preparing Standard Curve for TPH Analysis

Preparation of the standard solution was by diluting 0.2ml of fresh spent oil in 100mls of Toluene to give 2000ppm. It was then filled into the 100ml flask to meet the required mark using Toluene.

Preparation of 100ppm, 200ppm, 300ppm, 400ppm, 500ppm and 600ppm from the stock solution using the formula $C1V1 = C2V2$,

Where, C_1 = Concentration of stock solution
V_1 = Unknown volume
C_2 = Concentration of desired solution e.g. 100ppm
V_2 = Desired volume e.g. 10ml

The concentration of each volume was put in a 10ml flask and add more Toluene till it reaches the desired

mark. The absorbance for each concentration was taken using the spectrophotometer and plotting the calibration curve of Absorbance value against concentration.

3.RESULTS

Table 2: Physicochemical Parameters of Contaminated soil and Organic manure.

Properties	Contaminated soil	PL	CD	MPLCD
Ph	6.31±0.55	8.60±0.25	8.20±0.28	9.60±0.36
Total Nitrogen (%)	3.65±0.51	6.00±0.95	5.20±0.64	6.50±0.64
Available phosphorus (%)	18.34±0.36	25.00±0.24	22.00±0.25	24.00±0.12
Total Organic carbon (%)	10.35±0.28	17.17±0.16	16.92±0.67	17.20±0.35
Total organic matter (%)	17.89±0.38	29.69±0.28	29.25±0.46	29.74±0.16
Moisture content (%)	39.00±0.20	17.20±0.06	16.81±0.68	11.35±0.24
Sand (%)	87.50±0.70			
Silt (%)	5.15±0.64			
Clay (%)	7.35±0.07			

Means of triplicate ± standard deviation
PL: Poultry litter
CD: Cow Dung
MPLCD: Mixed Poultry litter and cow dung.

Table 3: Baseline Total Heterotrophic microbial population counts in the contaminated soil and organic manure.

Samples	Total heterotrophic fungi
Contaminated soil with 5% spent oil	$0.32\pm0.64 \times 10^3$
Contaminated soil with 10% spent oil	$0.005\pm0.68 \times 10^3$
Poultry Litter (PL)	$4.80\pm0.11 \times 10^5$
Cow dung (CD)	$3.30 \pm 0.68 \times 10^5$
Mixed Poultry Litter and Cow Dung	$36.00\pm0.10 \times 10^5$

Table 4: Morphological characteristics of fungi isolated from degraded contaminated soil.

Macroscopy features	Microscopic features	Organisms
Raised; Black colonies; creamy bottom	Long erect septateconidor shore; sterigmata;	*Aspergillus sp.*
Raised; green colonies; creamy bottom	Septate hyphae; phialides vesicles; metula; conidia	*Penicillium sp.*

Table 5: Mean concentration of total petroleum hydrocarbon in the Biodegradable soil (mg/kg).

Treatments	Weeks		
	4	8	12
PL + 5% spent oil	189l	2643i	3253f
CD + 5% Spent oil	1314n	2368j	2918g
MPLCD + 5% Spent oil	2195k	3433e	4545c
PL + 10% Spent oil	1606m	2218k	4728b
CD + 10% Spent Oil	1260n	2840gh	3810d
MPLCD + 10% Spent oil	2815h	3228f	5803a

Means with the same letter on the same column not significantly different at $P < 0.05$.

Table 6: Net Percentage loss of total Petroleum Hydrocarbon (TPH) on the Biodegradable soil.

Treatments	Weeks		
	4	8	12
PL + 5% spent oil	19.55e	20.22e	15.38d
CD + 5% Spent oil	29.90c	23.28d	18.21c
MPLCD + 5% Spent oil	35.24b	27.06b	29.14a
PL + 10% Spent oil	18.01f	25.12c	7.65f
CD + 10% Spent Oil	23.87d	18.12f	13.09e
MPLCD + 10% Spent oil	44.42a	29.48a	19.47b

Means with the same letter on the same column are not significantly different at $P < 0.05$.
Net % loss = Percentage loss in TPH of soil contaminated soil amended with organic manure - % lossin TPH of unamended contaminated soil.

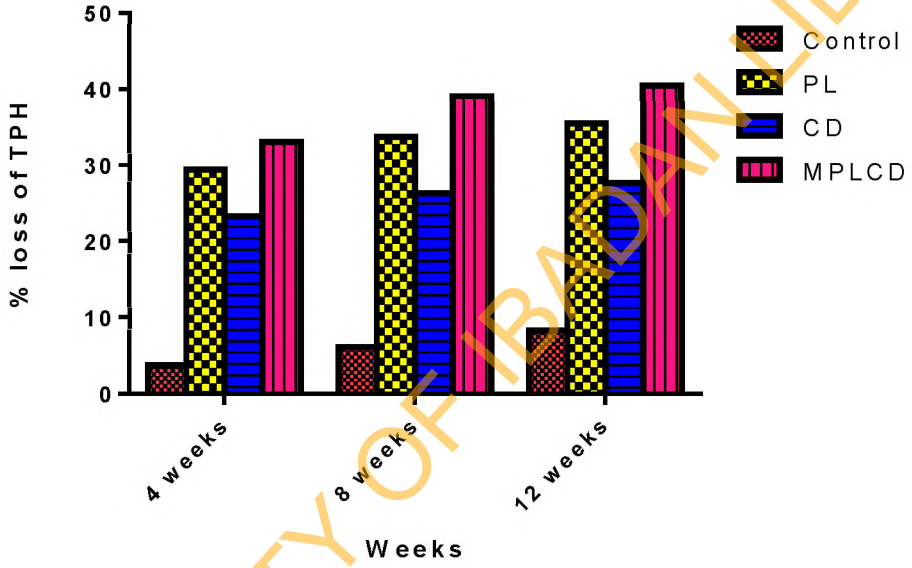

Fig 1 - Percentage loss of Total Petroleum Hydrocarbon in a contaminated soil with 5% spent oil.

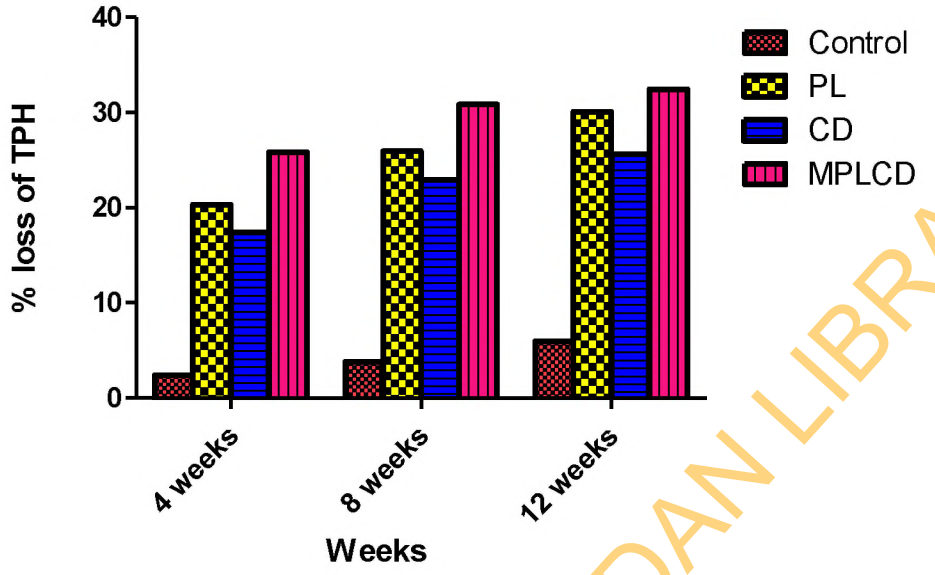

Fig 2 – Percentage loss of Total Petroleum Hydrocarbon in a contaminated Soil with 10% Spent Oil

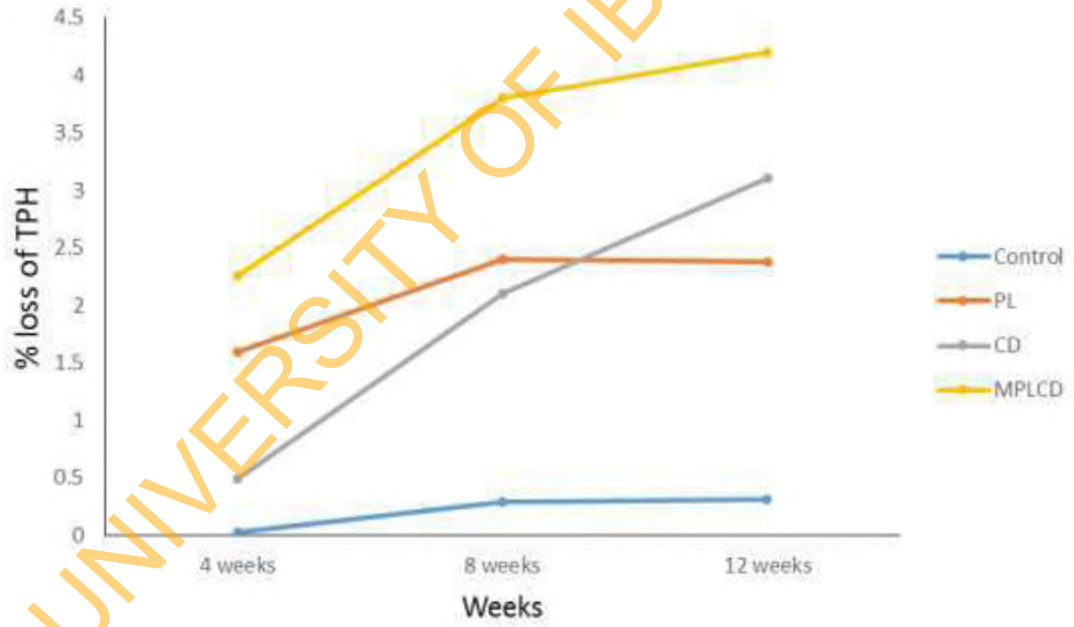

Fig .3:- Counts of Hydrocarbon – utilizing fungi in the soil contaminated with 5% spent oil (cfu/g).

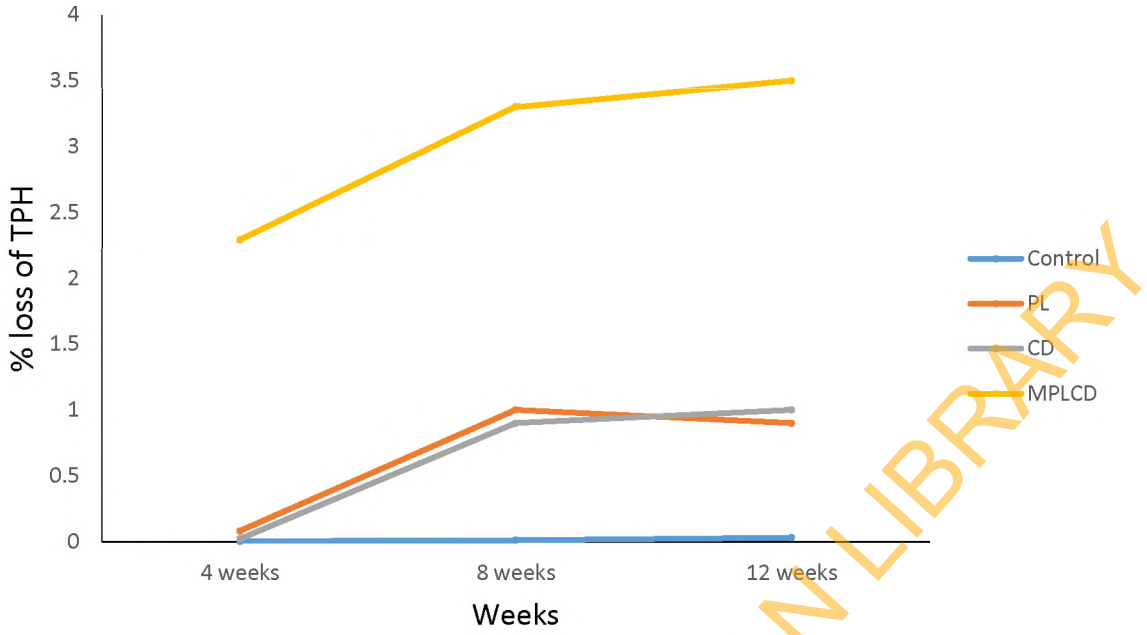

Fig 4:- Counts of Hydrocarbon – utilizing fungi in the soil contaminated with 10% spent oil (cfu/g).

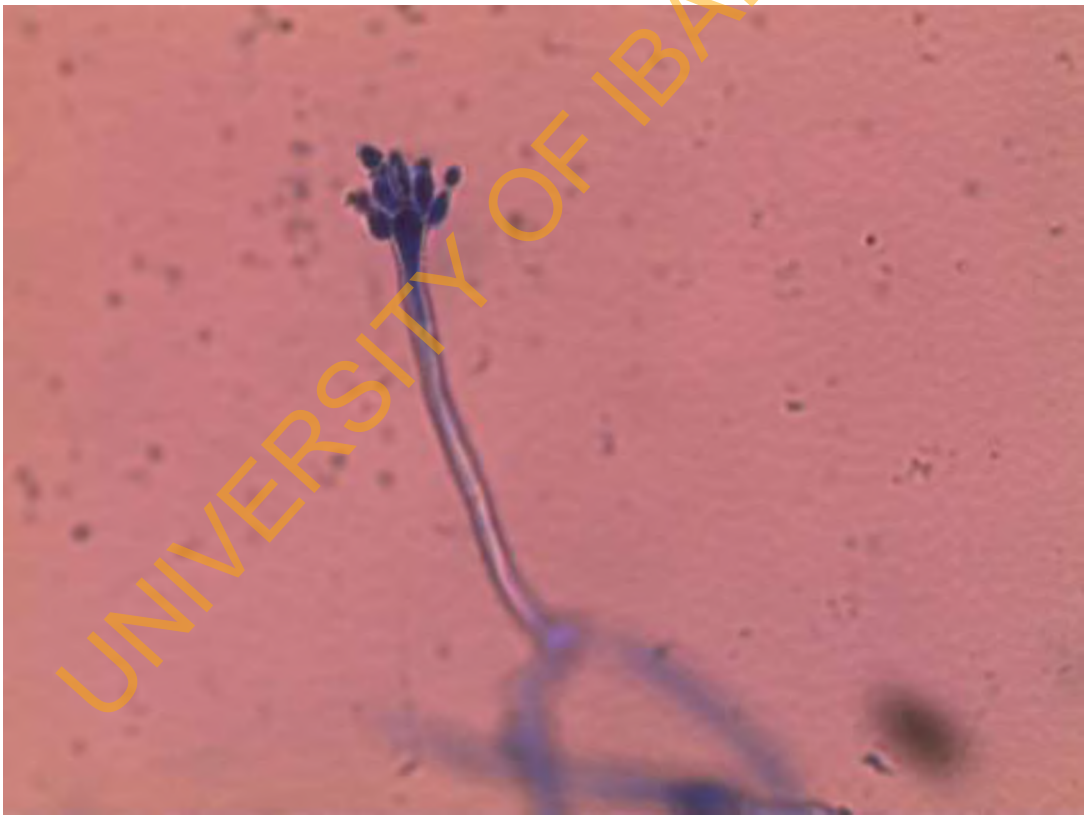

Plate 1: Isolated *Penicillium species*

4. DISCUSSION

The physicochemical properties of the contaminated soil and the organic manure. These were shown in table 2. The soil had a pH of 6.31±0.55 and a low concentration of total nitrogen, organic carbon, organic matter and available phosphorus as 3.65±0.51%, 10.35±0.28%; 17.89±0.38% and 18.34±0.36mg/kg respectively. The organic manure used comprised of Poultry litter, cow dung and the mixed Poultry litter and cow dung which had a pH of 8.60±0.25; 8.20±0.28 and 9.60±0.36 respectively. The mixed Poultry litter and cow dung had a higher percentage of total nitrogen of 6.50±0.64% followed by Poultry litter of 6.00±0.95% and cow dung 5.20±0.64%. Whereas, the available phosphorus of Poultry litter is s25.00±0.24 mg/kg; cow dung is 22.00±0.25mg/kg while the phosphorus of mixed poultry litter and cow dung was 24.00±0.12mg/kg. The poultry litter, cow dung and the mixed poultry litter and cow dung had an organic carbon (%) of 17.17±0.16; 16.92±0.67 and 17.20±0.35 respectively while the total organic matter (%) was higher 29.74±0.16% in the mixed poultry litter and cow dung when compared with the low percentage recorded in poultry litter 29.69±0.28% and cow dung litter 29.69±0.28% and cow dung 29.25±0.46%. The moisture content was higher in contaminated soil (39.00±0.20%) compared to the organic manure of poultry litter of 17.20±0.06%; cow dung 16.81±0.68% and mixed poultry litter and cow dung of 11.35±0.24%.

The result in table 3 shows the total heterotrophic fungi in the contaminated soil and the organic manure. The total heterotrophic counts of fungiwas 0.32±0.64 x 10^3sfu/g and 0.005±0.68 x 10^3sfu/g. While, the total heterotrophic fungi counts in poultry litter, cow dung and the mixed poultry litter and cow dung and the mixed poultry litter and cow dung was 4.80±0.11 10^5; 3.30±0.68 x 10^5 and 36.00±0.10 x 10^5sfu/g respectively.

Figure 1 and 2 shows marked percentage loss of total petroleum Hydrocarbon content during the period of study with the addition of different organic manures (Poultry litter, cow dung and mixed poultry litter and cow dung). At the end of the first 4 weeks of the study, the contaminated soil with 5% spent oil showed a significant in total petroleum hydrocarbon of 29.38%; 23.32% and 33.07% in the soil amended with PL, CD and MPLCD respectively compared with the unamended control contaminated soil of 3.83% while there was low degradation of hydrocarbon in the soil contaminated with 5%. The contaminated soil with 10% spent oil showed a significant loss in total petroleum hydrocarbon content of 20.27%; 17.41% and 25.83% in to contaminated soil amended with poultry litter, cow dung and mixed poultry litter and cow dung whereas 2.4% reduction from the control contaminated soil.

AT 8 weeks of the study, there was a reduction in the degradation of contaminated soil with 5% spent oil amended with poultry litter was 33.73%, cow dung was 26.32% while mixed poultry litter and cow dung loss 39.07% and the unamended control soil loss 6.10%. Also, the contaminated soil with 10% spent oil, loss of total petroleum hydrocarbon content in the soil amended with poultry litter was 25.93%, cow dung 22.93% and for mixed poultry litter and cow dung was 30.83% while the unamended control soil loss 3.80%.

AT the end of 12 weeks, there was a significant loss of total petroleum hydrocarbon content in the amended contaminated soil with 5% and 10%.

The level of total petroleum hydrocarbon loss in the amended contaminated soil with 5% spent oil was for poultry litter 35.53%,cow dung 27.70% and mixed poultry litter and cow dung was 40.46% while unamended control soil was 8.32%. However, for contaminated soil with 10% of spent oil, a significant reduction of 30.04%, 25.60% and 32.42% respectively for poultry litter, cow dung and mixed poultry litter and cow dung whereas the total petroleum hydrocarbon content loss in the unamended control soil was 5.95%.

At the end of the study, it was observed that the organic manure applied showed a tremendous degradation in the contaminated soil with spent oil. The high degradation of contaminated soil occurred in the soil amended with mixed poultry litter and cow dung. This can be due to the presence of consortium of microbes which enhances the biodegradation of spent oil from the soil.

Figure 3 and 4 shows the counts of hydrocarbon – utilizing fungi in the soil contaminated with 5% and 10% spent oil. At 4 weeks post contamination of 5% spent oil, the soil amended with poultry litter had 1.60±0.95 x 10^5 counts of hydrocarbon – utilizing fungi, cow dung was 0.51±0.20 x 10^5 and the mixed poultry litter and cow dung was 2.26±0.21 x 10^5 while that of unamended control soil was 0.021±0.10 x 10^5sfu/g. While it was 0.08±1.20 x 10^5 for poultry litter, 002±0.10 x 10^5 for cow dung and the mixed poultry litter and cow dung was 2.30±1.21 x 10^5sfu/g at the 10% spent oil soil contamination.

There was a slight increase in the microbial population at the end of 8 weeks post contamination. The counts of hydrocarbon – utilizing fungi at 5% spent oil contamination in unamended control was 0.29±1.10 x 10^5 while that of the amended with poultry litter, cow dung and the mixed poultry litter and cow dung was 2.40±1.50 x 10^5, 2.10 ± 0.26 x 10^5 and 3.80±1.21 x 10^5sfu/g. While the soil contaminated with 10% spent oil, the hydrocarbon- utilizing fungi counts in poultry litter was 1.0±0.26 x 10^5. Cow dung was 0.90±0.22 x $10^5$and the mixed poultry litter and cow dung was 3.30±1.21 x 10^5 while the unamended

control soil was 0.10 ± 0.01 x 10^5sfu/g. After 12 weeks of application, the hydrocarbon – utilizing fungi in 5% spent oil contaminated soil showed that the counts in the amended soil with poultry litter, cow dung and the mixed poultry litter and cow dung was 2.70±0.10 x 10^5, 3.10±0.22 x 10^5 and 4.20±1.20 x 10^5 while the unamended soil was 0.31±1.01 x 10^5sfu/g. an in the 10^ spent oil soil contaminated, the counts of hydrocarbon – utilizing fungi was 0.90±1.22 x 10^5 for poultry litter, 1.00±0.03 x 10^5 for cow dung and 3.50±0.74 x10^5 for Mixed poultry litter and cow dung while the uamended control soil was 0.03±0.35 x 10^5sfu/g.

It was observed that the additives had a greater microbial population in the amended contaminated soil. However towards the end of the study, there was a microbial population drop in the treatment which is also similar to the 10% spent oil contaminated soil. Microbial counts was significantly higher in soil amended with different organic manures when compared to those of the unamended control.

Contaminated soil at the 0.05% probability level, indicating the role of nutrients in the enhancement of microbial population. There was a significantly higher hydrocarbon – utilizing microbial population in the mixed poultry litter and cow dung than those amended with cow dung and poultry litter while the microbial population in the poultry litter was significantly higher ($P < 0.05$) than the cow dung. The hydrocarbon – utilizing microbial population in the contaminated soil with 10% spent oil exhibited a similar trend as observed in 5% concentration of hydrocarbon – utilizing bacteria at 0.05% probability level in the soil amended with organic manures.

5. CONCLUSION

The amendment of spent oil contaminated soil with organic manure enhances significantly the rate of biodegradation of petroleum hydrocarbon. The spent oil contaminated soil amended with mixed poultry litter and cow dung with 5% and 10% spent oil contamination exhibited highest rate of oil biodegradation and counts of hydrocarbon- utilizing fungi compared to the soil amended with poultry litter and cow dung.

The un-amended control soil also showed that the remediation of the contaminated soil can be achieved through natural processes of biodegradation, photo-oxidation, evaporation and volatilization without external interferences.

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