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Total Petroleum Hydrocarbon and Heavy Metal Reduction: A Case Study of Enhanced Degradation Potential of Animal Waste by Fungal Isolates

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Abstract

This study is conducted to determine the role of poultry litter and cow dung in enhancing the degradation of diesel in contaminated soils by fungal isolates. The treatment sets were used for soils with three levels of diesel pollution (50 ml, 100 ml and 150 ml). The microbiological properties, total petroleum hydrocarbon content and heavy metals were analyzed for six months using standard analytical procedures. The highest and lowest levels of total petroleum hydrocarbon utilization percentages were observed for C_1 as (98.5 %), soil with 50 ml diesel, amended with poultry and cow dung, and also for Control 1 as (31.6 %). The results of heavy metal analysis revealed Pb having the highest value of 3.20 mg kg-^{1} recorded for C_3, soil with 150 ml diesel amended with poultry litter and cow dung in the month of July, 2016; Cr had its highest value of 2.8 mgkg-^{1} recorded for B_3, soil with 150 ml diesel amended with cow dung in July, 2016, and Fe had its highest value of 182 mg kg-^{1}for C_3 also in the month of July, 2016. The results of the total heterotrophic and hydrocarbon-utilizing fungal counts ranged from 22.0±2.0 to 42.5±2.5 x10^4 cfu/g for C_1 and B_3 and 24.0±2.0 to 51.0± 2.0 x10^4 cfu/g for C_3 and B_1, soils with 50 ml diesel amended with cow dung respectively. Seventeen fungal species were isolated. *Aspergillus* spp. had the highest frequency of occurrence, 31.69 % and the least percentage of frequency of occurrence was recorded for *Candida albicans* (0.7 %) and *Botrydiplodia* sp. (0.7 %). This study stresses the enhanced potential of fungal population in the reclamation of diesel-contaminated soils.

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Keywords: Diesel oil pollution, animal waste, hydrocarbon-utilizing fungi, Total Petroleum Hydrocarbon, Heavy metals.

1. Introduction

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 pollution is on the rise in Nigeria as well as in other developing countries in the world (Stephen et al., 2010). Contaminations occurs through leakage from diesel-powered vehicles, generators, through wrecks of oil tankers carrying diesel oil, cleaning of diesel tanks by merchants, war ships carrying diesel oil and motor mechanics (Hill and Moxey, 1980). Poultry litter is a mixture of feces, wasted feeds, bedding materials, and feathers (Wilkinson et al., 2011, Kim et al., 2012). It contains significant amounts of nitrogen because of the presence of high levels of protein and amino acids. Owing to its high nutrient content, poultry litters has been considered one of the most valuable animal waste to be used as organic fertilizer (Wilkinson, 1979). Cow dung, on the other hand, is the waste product of bovine animal species. It contains a vast reservoir of the following nutrients (Akinde and Obire, 2008): 3 % nitrogen, 2 % phosphorus, and 1 % potassium (3-2-1 NPK) essential for microbial growth and metabolism, and hence has a wide array of microorganisms with a potential hydrocarbon-degrading capacity.

Bioremediation is a waste management technique that involves the use of organisms to remove or neutralize pollutants from a contaminated site (Omotayo et al., 2012). It can also be defined as the treatment that uses naturally occurring organisms to break-down hazardous substances into less toxic or non-toxic substances. This process is an efficient remediation method of petroleum by-products, pesticides, and other harmful chemical (Castro-Gutierrez et al., 2012). It is an environmentally friendly technique, cost effective and efficient process (Gadd, 2000).

Fungi have been shown to play a major role in the bioremediation of polluted environments. Amongst their features which enable them to play a great role in bioremediation are: the secretion of extracellular enzymes, the ability to grow under stressed environmental conditions (low nutrient, pH, and water activity), extension in biomass location through hyphal growth, easy and rapid growth on agricultural or forest waste, and other enzyme systems (Obire and Putheti, 2008; George-Okafor et al., 2009). Fungi are known to secrete extracellular enzymes during biodegradation. These inherent capabilities make fungi initiate primary attack of more complex and recalcitrant pollutants thereby facilitating secondary attacks by bacteria. Fungal genera (*Amorphoteca, Neosartorya, Talomyces*, and *Graphium*), yeast such as *Candida*, *Yarrowia,* and *Pichia* and terrestrial fungi; *Aspergillus, Cephalosporium*, and *Penicillium* have been

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implicated in hydrocarbon degradation (Chaillan et al., 2004; Singh, 2006; Das and Chandran, 2011).

The presence of heavy metals in the soil has been attributed to pedogenetic processes of weathering of parent materials and anthropogenic sources (Kabata-Pendias and Pendias, 2001). The presence of heavy metals in the soil has been attributed to petroleum prospecting and mining as well as oil spills, (Osuji and Onojake, 2004). The most significant natural sources are weathering of minerals, erosion and volcanic activity, while the anthropogenic sources depend upon human activities such as mining, smelting, electroplating, use of pesticides and phosphate fertilizer discharge, as well biosolids (e.g., livestock manures, composts, and municipal sewage sludge), atmospheric deposition, *etc*. (Summer, 2000). Bioremediation is based on their catalyzed redox conversion to insoluble forms. These reduction or oxidation reactions take place due to enzymatic activity and the biomass concentration of microbes (Khan et al., 2005). More work is being done on the use of organic waste to remediate oil-polluted sites. The remediation of crude oil-polluted soil using cow dung manure in relations to the growth of maize (*Zea mays l.*) has also been demonstrated (Oyedele and Amoo, 2014). Obasi et al., (2013) conducted a comparative study using horse manure, cow dung and poultry litter to remediate polluted soil, and discovered that poultry litter remediated most contaminated soils compared with the other organic manures. The aim of this study is to determine the role of poultry litter and cow dung in enhancing degradation of diesel- contaminated soils by fungal isolates.

2. Materials and Methods

2.1. Soil and Animal Waste Collection

The soil samples were collected from the Animal and Environmental Biology (AEB) Experimental Garden, Faculty of Life Sciences, University of Benin, Benin City at a depth of 15 cm. The animal waste samples including cow dung and poultry litter were collected from the University of Benin Agricultural Farm and the Cattle Ranch at Benin Technical College Road, Benin City respectively. The samples of petroleum products (diesel) were collected from a Commercial Petroleum Products Station in Benin, Benin storage facility, Edo State.

2.2. Soil and Animal Waste Collection

A total of eleven treatments were set up in triplicate for the assessment of enhanced biodegradation. The experiments were monitored for a period of six months between July, 2016 and December, 2016. The experimental designs are as follows:

Table 1. TREATMENT SET-UP

	Treatments	Soil (g)	Diesel (ml)	PL (g)	CD (g)	PL+ CD (g)	Total
	Control 1	2000	–	–	–	–	2000
	Control 2	2000	50	–	–	–	2050
1	A_1	2000	50	100	–	–	2150
	A_2	2000	100	100	–	–	2200
	A_3	2000	150	100	–	–	2250
2	B_1	2000	50	–	100	–	2150
	B_2	2000	100	–	100	–	2200
	B_3	2000	150	–	100	–	2250
3	C_1	2000	50	–	–	100	2150
	C_2	2000	100	–	–	100	2200
	C_3	2000	150	–	–	100	2250

KEYS: *CD = Cow Dung; PL = Poultry Litter*
Control 1: soil only, control 2: soil + 50 ml diesel, A_1: soil + 100 g poultry litter + 50 ml diesel, A_2: soil + 100 g poultry litter + 100 ml diesel, A_3: soil + 100 g poultry litter + 150 ml diesel, B_1: soil + 100 g cow dung + 50 ml diesel, B_2: soil + 100 g cow dung + 100 ml diesel, B_3: soil + 100 g cow dung + 150 ml diesel, C_1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C_2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C_3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel

The experiments were conducted over a period of six months (three months of rain and three months of dry weather). The perforated buckets containing the soil samples were kept in the open, but they were protected from the direct effect of rain. During this period, the soil samples were stirred, and the temperature of the soils were taken at regular intervals. The soil samples were bi-monthly collected for analysis.

2.3. Enumeration of Total Heterotrophic Fungal Counts

Total fungal analysis was carried out on treatments by weighing 10 g of the soil samples into 90 ml of distilled water and was serially diluted to obtain a ten-fold diluent. Aliquot (1 ml) of 10^{-4} and 10^{-6} dilutions were dispensed unto Potato Dextrose Agar (PDA), amended with chloramphenicol (0.02-1 µg/ml) and used for the isolation of fungi. The plates were prepared and inoculated in duplicates, and were incubated at room temperature for five days. After incubation, the colonies of the isolates were counted and expressed in cfu/g; the isolated colonies were further purified by sub-culturing and were identified by comparing their growth with the standard manual and microscopy (Barnett and Hunter, 1998).

2.4. Screening for Hydrocarbon Utilizing Fungal Populations

The vapor phase transfer technique was employed for the screening of hydrocarbon- utilizing fungi. Sterile Whatman filter papers soaked in diesel were aseptically placed into the lids of each inoculated Bushnell-Haas Agar plates. Aliquots (1 ml) of 10^{-4} and 10^{-6} dilutions of the crude oil soil suspension were seeded onto Bushnell-Haas Agar and incubated at room temperature for six days (Chikere and Azubuike, 2014). Average colony counts were recorded and used for hydrocarbon utilizers within the fungal population. The isolated colonies were further purified by sub-culturing onto Potato Dextrose Agar (PDA) medium to obtain a pure culture. They were examined both macroscopically and microscopically for the identification of the fungi (Barnett and Hunter, 1998).

2.5. Heavy Metal Analysis

The soil samples were initially digested using concentrated nitric acid (HNO_3) before being analyzed for heavy metals (chromium, lead and iron). The digested samples were analyzed for heavy metals using Atomic Absorbance Spectrophotometer (AAS) (Onyeonwu, 2000).

2.6. Determination of Total Petroleum Hydrocarbon (TPH)

TPH was analyzed using organic solvent extraction procedures (Onyeonwu, 2000). Ten grams (10 g) of the soil were weighed and 5 g of anhydrous sodium sulfate ($NaSO_4$) were added and stirred with a stirring rod. Thereafter, 100 ml of HPLC n-Hexane were then added into the sample and stirred with a magnetic stirrer for thirty minutes. The extract was then cleaned up and fractionated using silica gel Solid Phase Extraction (SPE). The TPH was analyzed using Gas Chromatograph Agilent 6890 Series, with an Agilent FID detector under the following conditions. Column Temperature Program 60 °C to 280 °C 10°C/min to 280 °C at 10 °C/min for 8 mins, Injector Temperature 200°C, Detector Temperature 300 °C, Carrier gas Nitrogen gas, Pressure Program (Setpoint) 14.5 psi, injected volume 1 µl and Column Dimensions was Capillary 30.0 m x 320 µm x 0.00 µm. The total cycle of time run was forty-two minutes for each sample injected. External calibration was carried out using TPH's Standards. The retention times of the standards were used for the identification and quantization of the individual TPH. All solvents used were of high-purity analytical grade. The percentage of crude oil degraded after six months was determined using the equation:

% Crude oil degraded = x 100

Weight of crude oil degraded = Original weight of crude oil – Weight of residual crude oil.

2.7. Statistical Analysis

Results obtained were subjected to test using Two Way Analysis of Variance (ANOVA) without replication to test the level of significance between the groups of means for the different treatment samples. Microsoft excel package was used.

3. Results

3.1. Total Heterotrophic and Hydrocarbon Utilizing Fungal Counts

Figure 1 shows the total heterotrophic fungal counts, Control 1: 21.0±1.0 to 28.0±14.5 x10^4 cfu/g, Control 2: 24.5±0.5 to 39.0±6.0 x10^4 cfu/g, Treatments A_1, A_2 and A_3 counts range between 25.0±1.0 to 41.5±4.5 x10^4 cfu/g, 26.5±9.5 to 39.0±3.0 x10^4 cfu/g, 23.5±0.5 to 36.0±1.0 x10^4 cfu/g respectively. Treatments B_1, B_2, B_3 counts range between 24.0±2.0 to 32.5±3.5 x10^4 cfu/g, 23.5±0.5 to 24.5±6.5 x10^4 cfu/g, and 22.0±2.0 to 28.0±7.0 x10^4 cfu/g respectively and for treatments C_1, C_2 and C_3, they ranged between 22.5± 0.5 to 42.5± 5.5 x10^4 cfu/g, 27.5±5.0 to 37.5±8.5 x10^4 cfu/g, 27.0±3.0 to 38.5±6.5 x10^4 cfu/g respectively. Higher fungal counts were observed between July to September, 2016 and decreased slightly between October to December, 2016 for total heterotrophic fungal counts. Figure 2 shows the mean total hydrocarbon utilizing fungal counts, Control 1: 23.0±1.0 to 34.0±4.0 x10^4 cfu/g, Control 2: 28.5±0.5 to 39.5±2.5 x10^4 cfu/g, Treatments A_1, A_2 and A_3 counts range between 28.5±1.5 to 38.5±1.5 x10^4 cfu/g, 35.0±3.0 to 41.5±3.5 x10^4 cfu/g, 29.5±5.5 to 40.5±0.5 x10^4 cfu/g respectively. Treatments B_1, B_2, B_3 counts range between 24.0±2.0 to 38.0±0.0 x10^4 cfu/g, 30.5±0.5 to 38.5±0.5 x10^4 cfu/g, and 33.0±2.0 to 41.0±2.0 x10^4 cfu/g respectively and for treatments C_1, C_2 and C_3, they ranged between 38.0± 2.0 to 43.5± 4.5 x10^4 cfu/g, 36.0±1.0 to 47.5±1.0x10^4 cfu/g, 44.5±2.5 to 51.0±2.0 x10^4 cfu/g respectively. Also, for total hydrocarbon-utilizing fungal counts, higher fungal counts were observed between July to September, 2016 and decreased slightly between October to December, 2016.

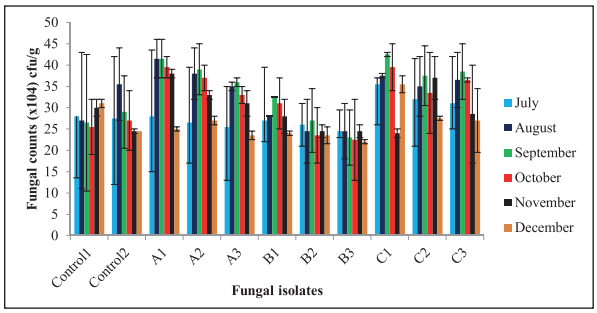

Figure 1. Total heterotrophic fungal counts (July- December 2016)
Keys: control 1: soil only, control 2: soil + 50ml diesel, A_1: soil + 100 g poultry litter + 50 ml diesel, A_2: soil + 100 g poultry litter + 100 ml diesel, A_3: soil + 100 g poultry litter + 150 ml diesel, B_1: soil + 100 g cow dung + 50 ml diesel, B_2: soil + 100 g cow dung + 100 ml diesel, B_3: soil + 100 g cow dung + 150 ml diesel, C_1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C_2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C_3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel.

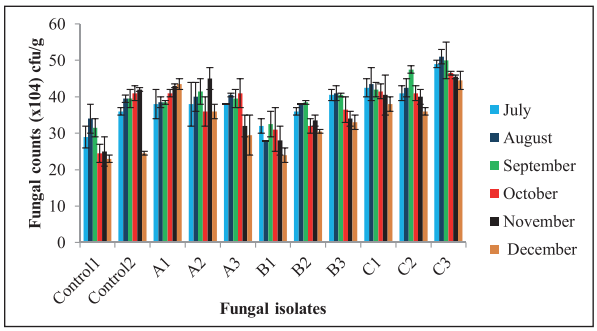

Figure 2. Total petroleum hydrocarbon utilizing fungal counts (July- December 2016)
Keys: control 1: soil only, control 2: soil + 50ml diesel, A1: soil + 100 g poultry litter + 50 ml diesel, A2: soil + 100 g poultry litter + 100 ml diesel, A3: soil + 100 g poultry litter + 150 ml diesel, B1: soil + 100 g cow dung + 50 ml diesel, B2: soil + 100 g cow dung + 100 ml diesel, B3: soil + 100 g cow dung + 150 ml diesel, C1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel.

Seventeen heterotrophic and Five petroleum hydrocarbon utilizing fungal species were isolated. Among the fungal isolates, *Aspergillus* spp. (31.6 9%) had the highest percentage of frequency of occurrence and the least percentages were recorded for *Botryodiplodia* sp. (0.7 %) and *Candida* sp. (0.7 %).

Table 2. Percentage frequency of occurrence of the fungal isolates in the various treatments

Isolates	Control 1	Control 2	A (A_1,A_2,A_3)	B(B_1,B_2,B_3)	C(C_1,C_2,C_3)	Total
**Aspergillus* spp.	8	6	11	8	12	45(31.69 %)
**Penicillium* spp.	7	6	4	1	4	22 (15.49 %)
Helmintosporium sp.	1	1	3	2	2	9(6.34 %)
Rhizopus sp.	-	-	2	1	-	3(2.11 %)
**Mucor* sp.	1	1	3	2	3	10(7.04 %)
**Trichorderma* spp.	2	2	3	4	6	17(11.97 %)
Fusarium sp	-	-	1	-	3	4(2.82 %)
Candida albican	1	-	-	-	-	1(0.70 %)
**Cladosporium* sp	1	2	3	3	3	12(8.45 %)
Neurospora crassa	-	-	2	1	1	4(2.82 %)
Curvularia sp.	-	-	-	1	1	2(1.41 %)
Sclerotium sp	-	-	1	1	1	3(2.11 %)
Rhodoturula sp	-	-	1	-	1	2(1.41 %)
Saccharomyces sp.	1	1	-	1	-	3(2.11 %)
Geotrichium sp	-	1	1	1	-	3(2.11 %)
Botrydiplodia sp	-	-	-	-	1	1(0.70 %)
Botrytis sp	-	-	-	1	1	2(1.41 %)
Total	**22(15.38%)**	**20(13.99)**	**35(24.48%)**	**27(18.88%)**	**39(27.27%)**	**143(100 %)**

** Total petroleum hydrocarbon degrading fungi species*
Keys: control 1: soil only, control 2: soil + 50ml diesel, A_1: soil + 100 g poultry litter + 50 ml diesel, A_2: soil + 100 g poultry litter + 100 ml diesel, A_3: soil + 100 g poultry litter + 150 ml diesel, B_1: soil + 100 g cow dung + 50 ml diesel, B_2: soil + 100 g cow dung + 100 ml diesel, B_3: soil + 100 g cow dung + 150 ml diesel, C_1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C_2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C_3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel.

Results of heavy metals analyses conducted on chromium, lead and iron (fig. 3-8) revealed that Pb had its lowest value as 0.00 mg kg^{-1} in control 2 in the month of September, 2016 (fig. 5) and its highest value of 3.20 mg kg^{-1} in C_3 in the month of July, 2016 (fig. 3). Chromium (Cr) had its lowest value of 0.10 mg kg^{-1} in control 1 in the month of December, 2016 (fig.8) and its highest value of 2.80 mg kg^{-1} in B_2 in the month of July, 2016 (fig. 3). The lowest and highest values for Iron (Fe) were 71.0 and 182.0 mg kg^{-1} recorded for Control 1 and C_3 in the months of December and July, 2016 respectively. In all the three metals analyzed (lead (Pb), chromium (Cr) and iron (Fe)), there was a reduction in the concentration of heavy metals in both control and the amended soils throughout the months (July- December, 2016) of analysis. Also, there was an increase in the concentrations of heavy metals in the various treatments as the volume of diesel pollution increased. Table 3 shows the initial and the final TPH utilization with treated soil with $P < 0.05$, and also the percentages of degradation which were 31.6 %, 39.3 %, 94.8 %, 93.0 %, 89.8 %, 93.2 %, 92.2 %, 85.9 %, 98.5 %, 97.7 %, 96.2 % for control 1, control 2, A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2 and C_3 treatments respectively. Diesel-oil degradation was highest in C_1 (98.5 %) and lowest in control 1 (31.6 %).

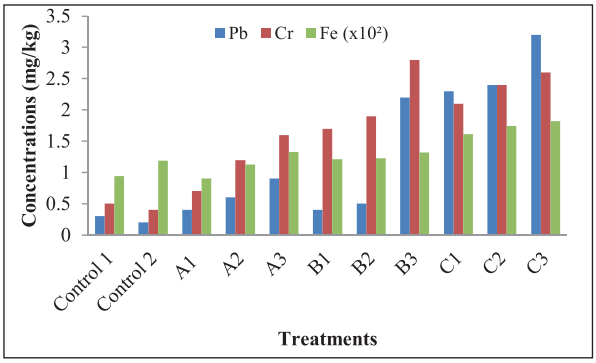

Figure 3. Heavy metal concentrations (July, 2016)

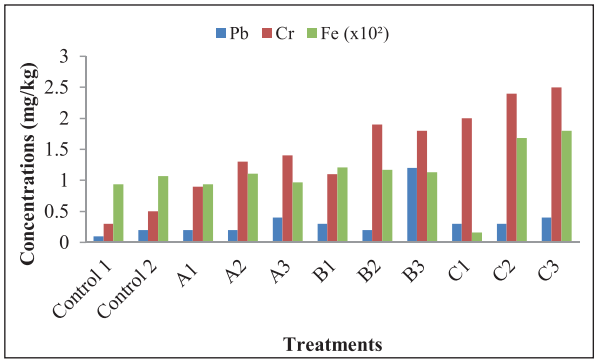

Figure 4. Heavy metal concentrations (August, 2016)

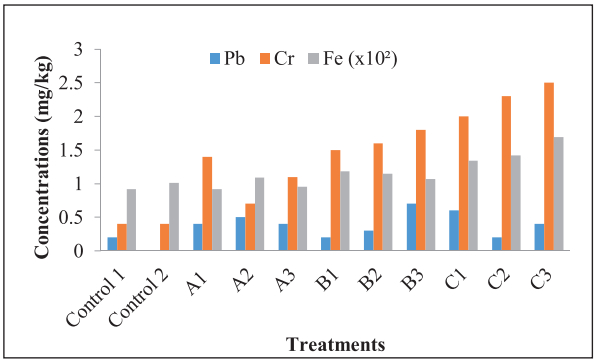

Figure 5. Heavy metal concentrations (September, 2016)

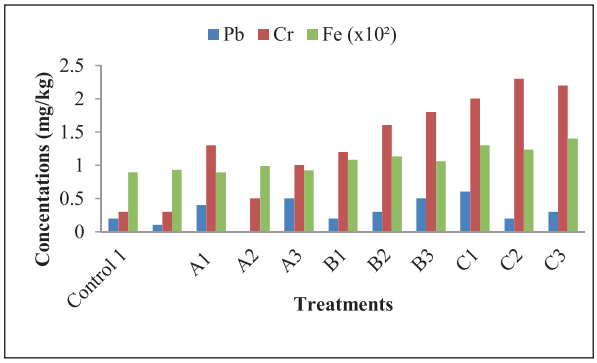

Figure 6. Heavy metal concentrations (October, 2016)

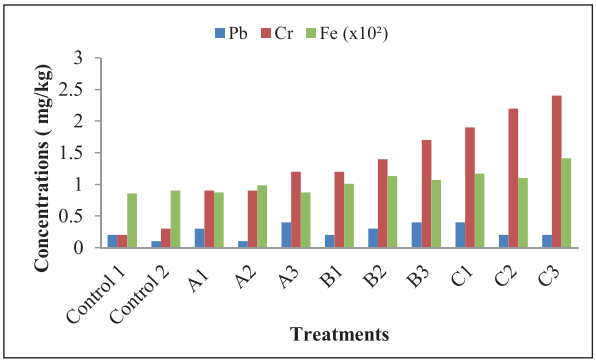

Figure 7. Heavy metal concentrations (November, 2016)

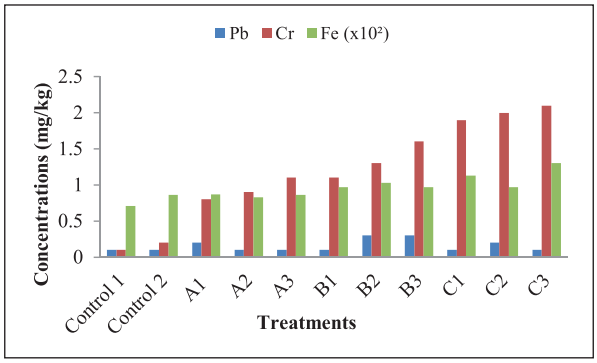

Figure 8. Heavy metal concentrations (December, 2016)

Keys: control 1: soil only, control 2: soil + 50ml diesel, A_1: soil + 100 g poultry litter + 50 ml diesel, A_2: soil + 100 g poultry litter + 100 ml diesel, A_3: soil + 100 g poultry litter + 150 ml diesel, B_1: soil + 100 g cow dung + 50 ml diesel, B_2: soil + 100 g cow dung + 100 ml diesel, B_3: soil + 100 g cow dung + 150 ml diesel, C_1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C_2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C_3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel.

Table 5. Percentage (%) total petroleum hydrocarbon (TPH) utilization with treated soil (July 2016 – December 2016)

Treatments	Control 1	Control 2	A_1	A_2	A_3	B_1	B_2	B_3	C_1	C_2	C_3	P-value
Initial TPH (mg/kg)	3.8917	13292.4	11362.3	12140.5	13595.4	11656.7	12193.7	12576.5	11447.1	11558.2	11622.3	P<0.05
Final TPH (mg/kg)	2.6618	8056.41	592.25	848.766	1392.57	796.067	957.015	1774.65	169.69	270.164	446.83	P< 0.05
% Degradation	31.6	39.3	94.8	93.0	89.8	93.2	92.2	85.9	98.5	97.7	96.2	

Keys: control 1: soil only, control 2: soil + 50ml diesel, A_1: soil + 100 g poultry litter + 50 ml diesel, A_2: soil + 100 g poultry litter + 100 ml diesel, A_3: soil + 100 g poultry litter + 150 ml diesel, B_1: soil + 100 g cow dung + 50 ml diesel, B_2: soil + 100 g cow dung + 100 ml diesel, B_3: soil + 100 g cow dung + 150 ml diesel, C_1: soil + 50 g cow dung + 50 g poultry litter + 50 ml diesel, C_2: soil + 50 g cow dung + 50 g poultry litter + 100 ml diesel, C_3: soil + 50 g cow dung + 50 g poultry litter + 150 ml diesel..

4. Discussion

The total heterotrophic fungal counts were in this order; $C_1 > C_2 > C_3 > A_1 > A_2 > A_3 > B_1 > B_2 > B_3 >$ Control 1 > Control 2. The greater fungal counts observed in the combined amendment could be attributed to the synergism of the fungal population in the organic waste. It was observed that fungal counts were higher in poultry-amended soils than in soils amended with cow dung; this agrees with the report of Obasi *et al.* (2013), which showed a higher microbial count in soils amended with poultry litter than soils amended with cow dung, sawdust and horse manure. Control 1 had a more fungal population than Control 2, although both decreased with time. The reason for this difference could be attributed to the toxicity of the pollutant (diesel) that was applied. Within each treatment, with increasing levels of diesel pollution, there was slight reduction in fungal counts in this order: ($A_1 < A_2 < A_3$, $B_1 < B_2 < B_3$, $C_1 < C_2 < C_3$). For Total Petroleum Hydrocarbon Utilizing Fungal (TPHUB) counts, the reverse was the case as the fungi were able to utilize the hydrocarbon as sole source of carbon and energy, and thus increased in number as the volume of hydrocarbon increased. This finding corroborates with the results of Adebusoye et al. (2007), who reported an increase in TPHUC. Findings also revealed an observable decrease from July-December, 2016, this agrees with the work done by Stephen et al. (2015), who observed that the microbial count reduces with time. This could be attributed to the exhaustion of nutrients with time.

This observation is in consonance with the findings of Stephen et al, (2015), who observed higher microbial counts in oil-free soils than in oil-polluted soils. There was a significant difference ($P < 0.05$) in fungal counts isolated from individual amendment (poultry litters, cow dung) compared to that of the combined amended soil.

Further observations showed a significant difference ($P<0.05$) in the percentage of degradation between the amended and non-amended soils, and also between the initial and the final percentages of the total petroleum hydrocarbon values. The percentage of degradation in soils amended with poultry litter was greater than that in the soils amended with cow dung, but a greater percentage of degradation was observed in the soils amended with poultry litter and cow dung.

Findings from this experiment showed that there was a reduction in the concentration of lead (Pb) and chromium (Cr) with time (July- December, 2016). This observation supports the fact that fungi are able to accumulate these metals, and convert them to insoluble forms where some are reduced, volatilized or precipitated (Gadd, 1990). The lead (Pb) concentration as well as Cr and Fe in the amended soil were slightly higher than the control soil which confirms the results of Essien et al. (2015), who reported that lead (Pb) content in soils amended with cow dung were higher than those in oil-free soils. Most striking was the fact that the concentrations of all the metals increased with increasing levels of diesel pollution indicating the presence of these heavy metals in the diesel oil applied. This is in agreement with earlier reports by Tanee and Kinako (2008) and Obasi et al. (2013) who reported a marked decrease in the total hydrocarbon content of amended soils polluted with crude oil relative to the control soils. Atuanya and Ibeh, (2004) and Chijioke-Osuji et al. (2014) reported that, the treatments amended with poultry litter showed enhanced utilization of petroleum product. Umar et al. (2012) also reported that bioremediated soils using cow dung and chicken droppings have a high removal rate of TPH compared to control soil. A higher loss of TPH was evident in the combined compost amendment followed by poultry litter-amended soil and then by cow dung-amended soil. It was observed that the percentage of degradation reduced slightly within each set of treatment with increasing the levels of pollution. The control soils percentage of degradation was slow when compared to the amended soils. This shows that organic waste is a good source of nutrients that stimulate the resident microorganisms to degrade the pollutants.

5. Conclusions

The results of the current study confirm that diesel oil impacts the soil ecology negatively, but with the application of organic waste as amendments (in the form of poultry litter and cow dung), the resident fungal population was stimulated bringing about an enhanced degradation. The combined compost amendment had a better percentage of degradation, and is recommended for bioremediation processes. However, this study has shown that poultry litter offered better degradation potentials than the cow dung, and is therefore preferred when recommending organic waste for bioremediative purposes.

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