THE APPLICATION OF SPENT MUSHROOM COMPOSITE TEA (SMCT) FOR THE DECONTAMINATION OF PAHs IN NIGER DELTA'S CRUDE OIL POLLUTED SOIL

Woyengibunugha Ere[1] and **Balogun Timothy, Ayodeji**[2]**.**

[1]Department of Agricultural and Environmental Engineering, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.
[2]Department of Chemical and Petrochemical Engineering Rivers State University, P.M.B 5080, Port Harcourt, Rivers State, Nigeria.

Correspondence e-mail: woyinse1234@gmail.com

Abstract

In the present study, spent mushroom compost tea (SMCT) was utilized for the removal of PAHs in petroleum polluted soil. The characteristics of the uncontaminated soil showed a pH of 5, and phosphorus content 28 mg/kg. After a 30days remediation period, the residual amount of PAH constituents was found to be 0.0033, 0.0367, 0.01, 0.026, 0.0833, 0.02, 0.0067, 0.04 and 0.0033 mg/kg for Naphthalene, Acenaphthylene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene and Dibenzo(a,h)anthracene respectively while others are 0.000 mg/kg. These values were compared with the control which shows a significant difference in constituent concentration. Also, the percentage reduction in total PAH was determined to be 81.7%. Hence, there is a significant reduction in the amount of PAHs.

Keywords: SMCT; decontamination; PAHs; crude oil polluted soil.

1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) has been seen as one of the most widespread carcinogenic substance in the environment (Harms et al., 2003) and are commonly linked with combustion of materials or petroleum residues (Suess, 1976). PAHs are grouped into ubiquitous hydrocarbon compounds that affect both terrestrial and aquatic ecosystems. They exist as two or more fused benzene rings and have low solubility in water which results in high octanol-water partition coefficients. This property accounts for their preferential partitioning to natural organic matter, limited availability to microbial interaction, and long environmental persistence (Mackay et al., 1999). PAHs are highly toxic and pose considerable human health risks, thus have generated significant interest worldwide and they are lipophilic, meaning they mix more easily with oil than water. The United States Environmental Protection Agency (USEPA) has listed sixteen PAHs as priority pollutants that are carcinogenic (Keith and Telliard, 1979).

These substances are common site pollutants but they are not regarded as hazardous wastes. Hence as pollutants, they are of concern because some compounds have been identified as carcinogenic, mutagenic, and teratogenic because of their properties. It is estimated that 1.7 to 8.8 million metric tons of petroleum hydrocarbons are released annually into the global environment through spills, leaks, natural seeps, offshore production, transportation, industrial wastewater, and urban runoff (Leahy and Colwell, 1990). Oil pollution has a damaging effect on the entire ecosystem and makes the soil condition unfavorable for plant growth due to a reduction in the level of available plant nutrient or rise in the toxic levels of certain elements. The effect on plants ranges from chlorosis, bleaching, spotting of leaves, necrosis, malformations to epidermal cells and mesophyll layers, yield reduction and impaired fecundity, reduced leaf growth through stomata conductance caused by root stress, etc. However, a number of approved technologies are currently used to remediate PAHs at sites. Although, biological technologies have shown to be effective and less costly for in-situ remediation of crude oil pollution in comparison to conventional remediation methods such as excavation, incineration, thermal desorption, soil vapor extraction, and chemical oxidation etc. Additionally, some of the conventional treatment technologies may result in the release of high concentrations of hydrocarbons into the atmosphere, further compounding the risk to human health if inhaled. For example, incineration used for the treatment of crude oil contaminated soils, not only causes soils to become sterile due to loss of organic matter but also results in the release of toxic contaminants into the atmosphere. Although soil vapor extraction and chemical oxidation have received increased interest, the total remedial cost of applying these methods is still high.

The purpose of the present study is to demonstrate that low cost and widely available agricultural products are effective in rapidly decreasing the toxicity of highly PAHs contaminated soils. To implement the treatment process, spent mushroom compost was applied as the remediating agent.

2. Materials and Method

2.1. Description of the study area

The study was carried out at the Rivers Institute of Agriculture and Research Training (RIART) farmland located in the Rivers State University, Port Harcourt, Nigeria. Port Harcourt which is a popular city in the Niger Delta Region of Nigeria is the capital of Rivers State. Rivers state is known for its high rate of oil and gas activities within the Niger Delta Region (Ayotammuno et al., 2006). The Niger Delta Region of Nigeria is known to produce over 98% of Nigerian's economic resource material which is crude oil. It lies between 5.5325° N and 5.8987° E. Port Harcourt lies within the tropical rainforest vegetation belt of the country and receives an annual rainfall of about 2700mm and the average temperature of the experimental area is about 27^0C between 4.8156° N and 7.0498° E (Ayotamuno et al., 2006) and as a result of these characteristics, the soil in the city is usually moist all year round due to excessive rainfall. Figure 1 shows the map of the study location.

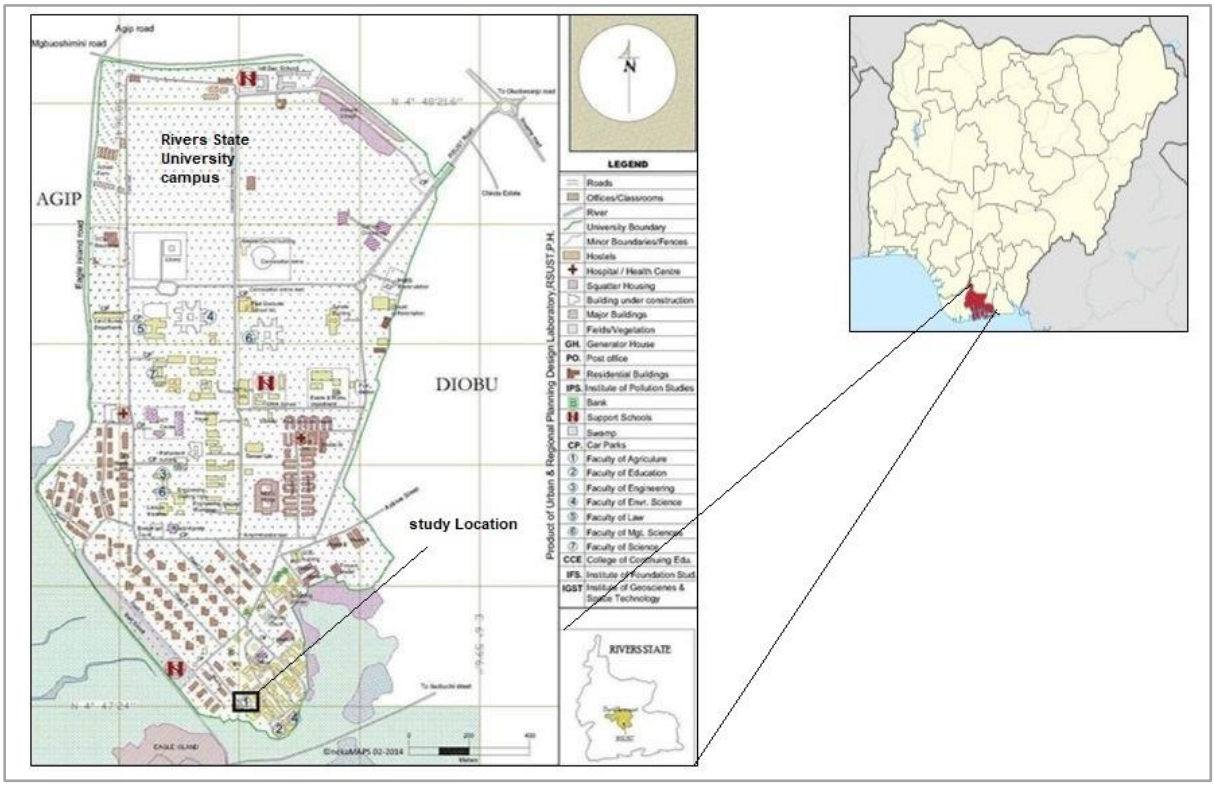


Figure 1.Map of the study area.

2.2. Experimental design and procedure

The soil samples were augered at different random spots with a 9 (inch) hand dug soil auger at about a depth of 30cm and the augered samples were homogenized to form a composite sample

before putting into test tubes. The test tubes would be divided into 9 treatment options and replicated twice having one replicate as the control. 36ml of Bonny light crude oil was sprinkled on each treatment test tubes until it completely covers the surface of the sample with a thin layer of oil. The objective of this sprinkling is to stimulate the condition of a major oil spill condition. After this was done, the contaminated soil would be turned with the aid of a spatula so as to enable a good mix of the soil and crude oil. After collecting the uncontaminated soil from our site which had no record of oil pollution before, it was put into test tubes that were labeled and taken periodically to the laboratory for the analysis for both microbiological and physiochemical characteristics. Another round of sampling would be done after the contamination of the soil has taken place. The remediation procedure now takes place and soil sampling would now be carried out every 30days till the end of the 60days period of treatment.

2.2.1. Nutrient application

Organic Spent Mushroom Compost Tea was applied using a sprinkler system for each test tube of the E_2 section as this was done for comparison. This application was done till the soil was at a 60% moisture content and it was regularly checked every 3days to maintain this level of moisture content.

(A) **(B)**

Figure 2. (A) Spent Mushroom Compost (SMC) produced from sawdust and (B) in a mushroom house in Nigeria (Okerentugba et al., 2015).

Treatment cells were stuck with a stick at different spots in order to create holes for a proper condition for aeration and adequate mixing of nutrients and microbes with the contaminated soil to take place. After this form of tilling was carried out, the tubes would be covered with perforated aluminum foils as this also would ensure good aeration. 0.4 (Liter) of water was added once in every 3days for a period of 30-days.

2.3. Laboratory analysis and procedure

The soil samples would be collected and taken to the Laboratory for analysis. The analysis that would be carried out is to be done before contamination, after contamination, and after treatment and this analysis can be grouped into 3 main categories: (1) physiochemical analysis (soil moisture content, soil pH, total nitrogen, total organic carbon, particle size distribution) (2) geochemical analysis (TPH) and (3) microbial analysis (bacterial counts of the soil). All parameters were analyzed using standard methods derived from (Ayotamuno et al., 2007).

2.3.1. Soil pH

The pH values of the soil samples were analyzed with the aid of a corning pH meter model (Jackson, 1964). This procedure of determination was carried out by dipping the electrode into a 1:2:5 soil, water suspension that had been stirred and allowed to equilibrate for about an hour.

2.3.2. Particle size distribution

The particle size was obtained by making use of the Bouyoucous hydrometer method. It was carried out by first dispersing the soil with the addition of sodium hexametaphsophate (Calgon 44g/L) and sodium carbonate (8g/L) solutions. After which, a textural triangular chart was used to ascertain the textural class of the soil sample.

2.3.3. Soil moisture content

A weighed wet sample of soil was placed in an oven at a temperature of 20^0C for 24hours. After this period, the sample was taken, removed and reweighed in order to determine the actual water content of the soil. This calculation would be done based on the ratio of the mass of water driven off to the mass of the dry solid (Smith and Smith, 1998). This dry weight of soil serves as an index in the determination of the gravimetric water content/moisture content and can be deduced using the formula:

$$\text{Gravimetric water content} = \frac{\text{Mass of water}}{\text{mass of oven dry soil}} \times 100\% \qquad [1]$$

2.3.4. Total Organic Carbon (TOC)

The total Organic Carbon Content of the soil sample was determined using the method derived from Walkey and Black (1934). 1g of a finely ground sample of soil would be weighed in duplicates and put into beakers where there would be an addition of 10ml of potassium dichromate solution which would be pipette adequately into each beaker and gently swirled in order to dissolve the sample completely by wetting. This process was followed up by the addition of 20ml concentrated sulphuric acid (H_2SO_4) using a graduated cylinder. After this, the mixture would be allowed to settle for about 20minutes before diluting it with distilled water to

about 250ml. After, 5 – 6 drops of an indicator (potassium permanganate) would be added before titration with 0.5N of ferrous ammonium sulfate (F_eSO_4) would be carried out under strong light.

2.3.5. Total nitrogen

2g of a representative air-dried soil was taken to a weighing scale. It was weighed into tecator digestion flasks and a catalyst constituting selenium, Copper Sulphate (C_uSO_4) and Sodium Sulphate (Na_2SO_4) would be added into the flask followed by 10ml of concentrated sulphuric acid (H_2SO_4). The contents of the flask would then be mixed by gently swirling the flask before it is then digested on a tecator block until the moment the digest is cleared (light green or grey color). Heating would be continued for about an hour or less before being allowed to cool. The digest would then be transferred quantitatively with distilled water into a 300ml conical flask and made u mark with the distilled water (Odu et al.,1985). Aliquots of this mix was taken and used in the determination of ammonium nitrogen with the aid of an auto-analyzer. The percentage of nitrogen contents of the soil would then be calculated after considering various dilution factors.

2.3.6. Total petroleum hydrocarbon (TPH)

The crude oil content of the soil was determined by shaking 10g of a representative soil sample with 10ml of toluene and the oil extracted is then determined by the absorbance extract at a 240mm wavelength in a spectronic photometer. Then after, a standard curve of the absorbance of the different known concentrations of oil in the extract would be drawn after takings the readings from the spectrophotometer. With reference to the standard curve and the multiplication by the corresponding dilution factors, the oil concentration would then be determined (Odu et al.,1985).

2.3.7. Microbial analysis

The soil aerobic heterotrophic bacteria and hydrocarbon utilizing bacteria were estimated using the soil dilution plate count method (IPS, 1990) using nutrient agar medium respectively. However, the bioremediation process is described below in Figure 2.

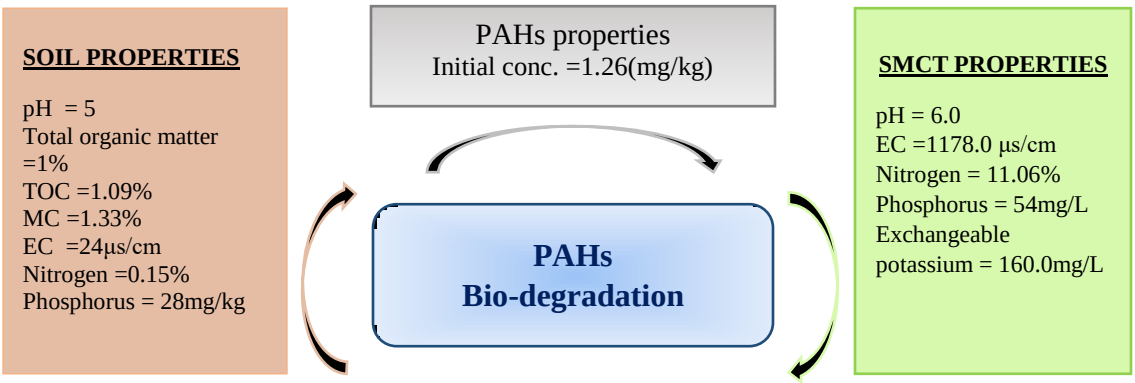


Figure 2. Bioremediation process

3. Result and Discussion

3.1. Experimental result

The uncontaminated soil sample had a moderately low organic carbon and nitrogen content of 1.09% and 0.15% respectively, moisture content of 1.33% on a dry basis, organic matter content of 1.88% as seen in table 1.

Table 1. Physical characteristics of soil sample

Particle size distribution	Value (%)
Sand	77.8
Silt	2.0
Clay	20.2
Texture	Sandy-clay-loam

Table 2. Physico-chemical characteristics of uncontaminated soil

Parameter	Value
Soil pH	5.00
Total Organic Matter (%)	1.00
Total Organic Carbon (%)	1.09
Moisture Content (%)	1.33
Electrical conductivity (μs/cm)	24.6
Total Nitrogen (%)	0.15
Available phosphorus (mg/kg)	28.0

Table 3. Properties of spent mushroom compost tea (SMCT)

Parameter	Value
pH	6.00
Electrical conductivity (μs/cm)	1178.00
Total Nitrogen (%)	11.06
Available phosphorus (mg/L)	54.00
Exchangeable Potassium (mg/L)	160.00

In Table 2 and 3 which shows the results of the analysis that was carried out on the uncontaminated soil and spent mushroom compost tea (SMCT) respectively, we can observe that the pH of both analyses showed that their acidic nature is low with the SMCT having the lowest characteristic. Also, we can observe that the SMCT has high electrical conductivity (1178μS/cm) while the uncontaminated soil has a lower electrical conductivity (24.6μS/cm) which indicates that the SMCT possesses high electrical charges. The study revealed that the spent mushroom

compost tea has a high availability of supplement nutrients as it is very important to supplement contaminated soil with nutrients generally nitrogen and phosphorus to complement carbon utilization by the microorganisms (Okerentugba et al., 2015).

3.2. Physico-chemical characteristics of soil

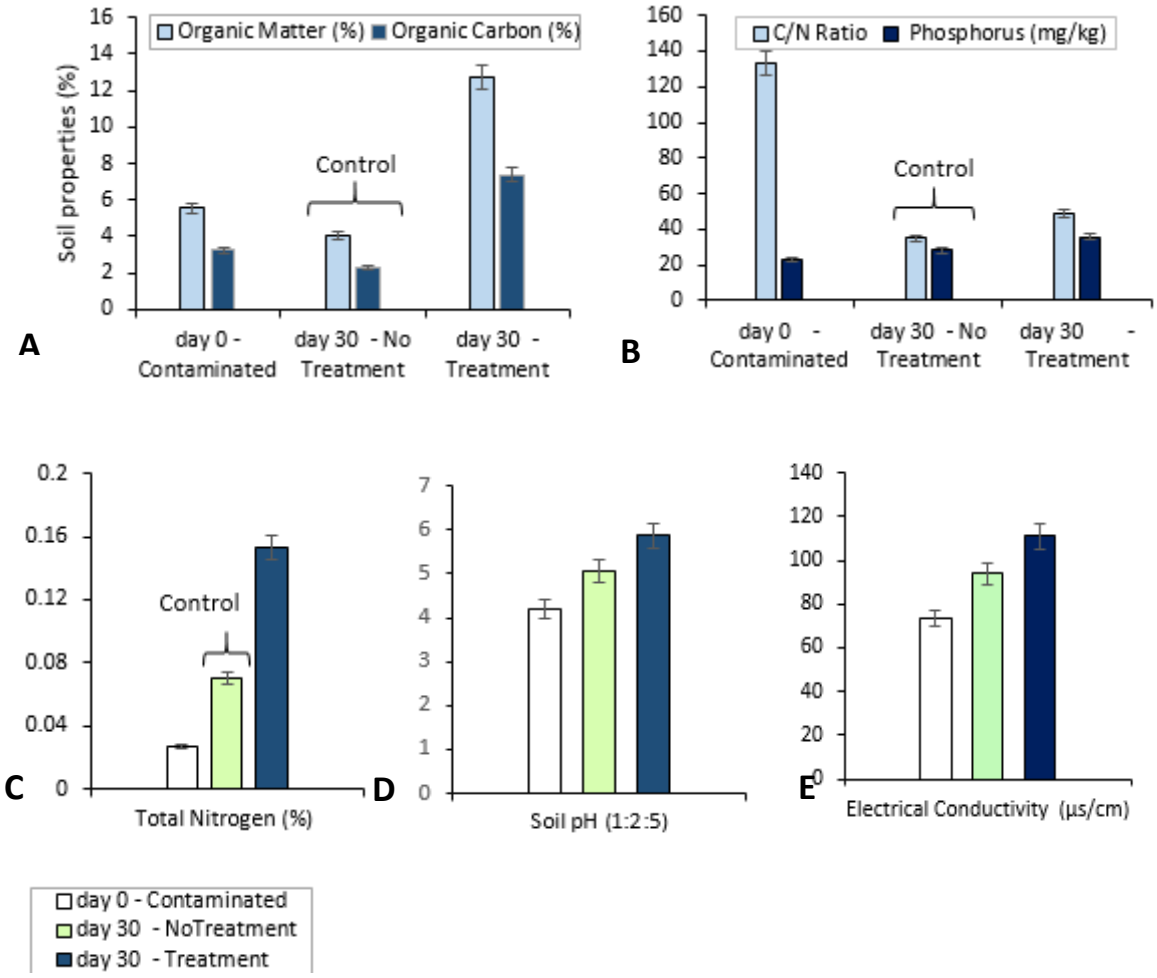


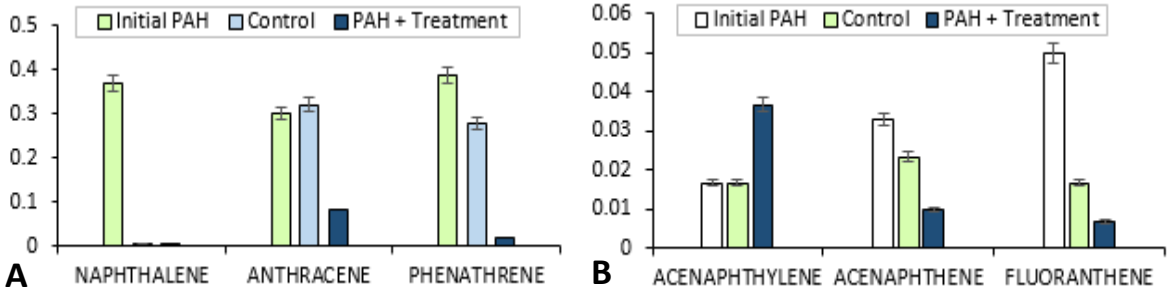
Figure 3. Soil physico-chemical characteristics

The pH of the soil samples at the various stages respectively as their pH values were found to vary between the ranges 3.90 - 4.5, 4.9 - 5.2 and 5.7 - 6.0 for the contaminated soil (day 0), control (day 30) and treated soil (day 30) respectively (see fig. 3C). It has been reported that high pH values impede the ability of microbial populations to degrade hydrocarbons, as heterotrophic bacteria and fungi are most active near pH of neutrality (Leahy and Colwell, 1990; Davis, 2016; Sims *et al*., 1990). This increase in the nitrogen content of the treated sample is in line with the observation made by Morgan (1991) and he postulated that the essence of an increase in nitrogen content of a soil is to enhance the build-up of microbial population since it has been reported as one of the limiting nutrients in any hydrocarbon contaminated soil. Similarly, the available

phosphorus present in the uncontaminated soil was higher than that present in the contaminated soil (day 0). The plausible explanation for this observation might be attributed to metabolism, immobilization in biomass, immobilization on soil colloids and washing out (Margesin and Schinner, 2001). At the end of the remediation period, it was observed that the available phosphorus level increased in the control and treated soil samples with the treated soil sample having the highest increase. This is supported with the report by the Canola Council of Canada (2017) where it was stated that at a lower pH, when the soil is very acidic, more iron and aluminium cations are available to form insoluble phosphate compounds and therefore, less phosphate is available while in contrast, at very high pH, phosphorus can react with excess calcium and magnesium cations to also form unavailable phosphorus compounds in the soil. Also, electrical conductivity (EC) of the contaminated soil increased compared to the uncontaminated soil. This observed increase indicates that the application of crude oil affected the ionic stability of the soil, hence, causing the soil to have high ionic strength (Agbogidi *et al*., 2007). Also, there was a general increase in the EC of the control and treated soil samples after the treatment period compared to that of the initial contaminated soil where the EC of the treated soil possesses the highest EC value. According to Ayotamuno *et al* (2006), Odu *et al* (1985) and Gallardo-Lara and Nogales (1987), the high increase in EC of the treated soil samples is as a result of the availability of soluble salt content induced by the introduction of the SMCT especially in high doses which favoured the degradation of the contaminants.

3.3. The effect of treatment on PAH constituents

Analysis of the contaminated soil showed that there was a total petroleum hydrocarbon (TPH) content of 26777 mg/kg in the soil and the identification and quantification of the concentrations of crude oil PAHs in both the contaminated soil and remediated soil at day 0 and 30 was done based on highest relative retention times compared with standards and mass spectral library searches. A total of 16 PAHs (including 15 of the 16 USEPA priority PAHs) were identified and measured in the agricultural soil sample with a total concentration of 1.26 mg/kg as shown in table 4.



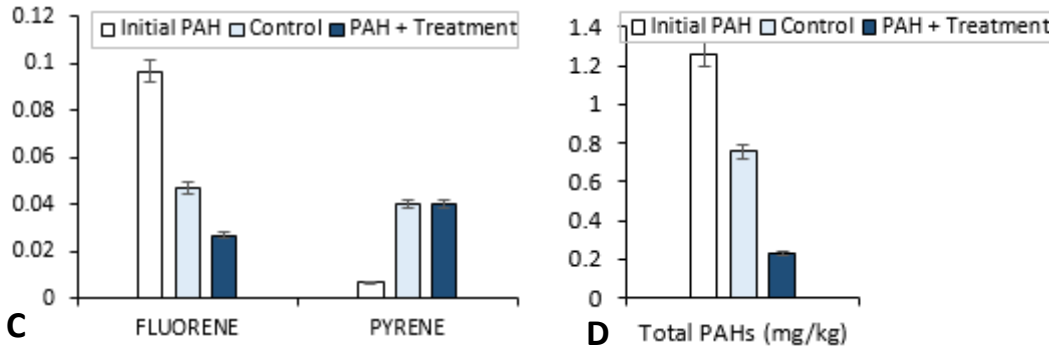


Figure 4. The impact of treatment on PAHs constituents

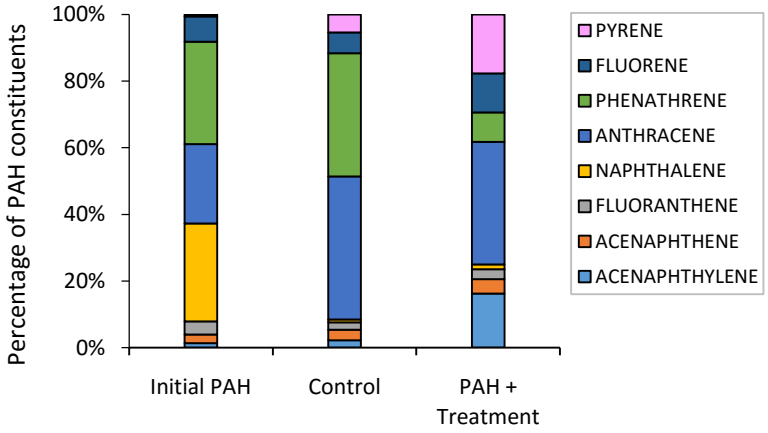


Figure 5. showing the percentage of PAH constituents in the treated soil

3.4. Statistical evaluation

Analysis of variance (ANOVA) was carried out using a statistical software (Minitab 17) to compare the untreated, control and the treated samples of residual PAHs. Fisher's test was employed at 95% confidence interval. The result shows significant difference in the residual concentration of PAHs in the different samples. According to Sadiq et al. (2018), SMC is rich in residual nutrients and enzymes therefore it could be used advantageously as low-cost bioremediation tool to degrade many pollutants. This accounts for the significant reduction in the concentration total PAHs of the treated soil when compared with the control sample and the untreated (see figure 6).

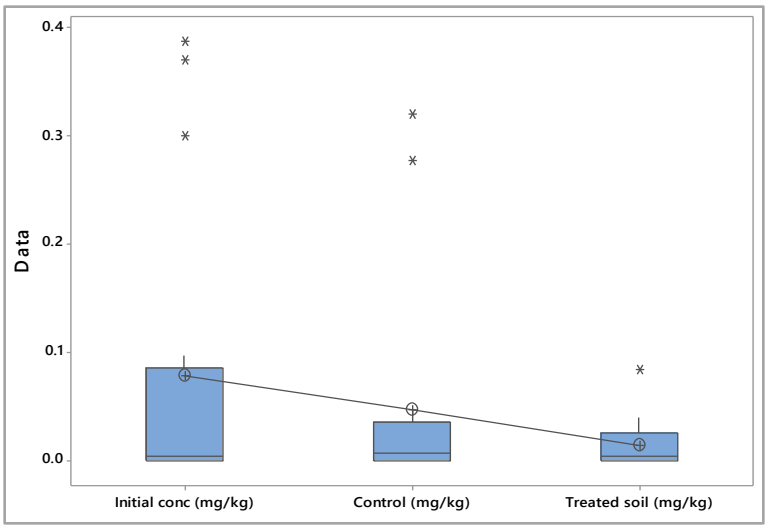


Figure 6. Box plot of analysis of variance

Table 4. InitialPAHs Concentration in crude oil impacted soil

PAHs	Molar mass, g/mol	Ring group	Concentration, mg/kg
Naphthalene	128	2-ring	0.3700 ± 0.33181
Acenaphthylene	152	3-ring	0.0167 ± 0.02082
Acenaphthene	153	3-ring	0.0333 ± 0.03512
Fluorene	166	3-ring	0.0967 ± 0.08083
Anthracene	178	3-ring	0.3000 ± 0.32187
Phenanthrene	178	3-ring	0.3867 ± 0.35076
Fluoranthene	202	4-ring	0.0500 ± 0.05568
Pyrene	202	4-ring	0.0067 ± 0.00577
Benz(a)anthracene	228	4-ring	0.0000 ± 0.00000
Chrysene	228	4-ring	0.0000 ± 0.00000
Benzo(b)fluoranthene	252	5-ring	0.0000 ± 0.00000
Benzo(k)fluoranthene	252	5-ring	0.0000 ± 0.00000
Benzo(e)pyrene	252	5-ring	0.0000 ± 0.00000
Dibenzo(a,h)anthracene	276	5-ring	0.0000 ± 0.00000
Indeno(1,2,3-cd)pyrene	278	6-ring	0.0000 ± 0.00000
Benzo(ghi)perylene	276	6-ring	0.0000 ± 0.00000
Total PAHs	-	-	1.2600 ± 0.00000

The PAHs concentration of both the control soil samples and treated soil samples at the end of the 30-day compost treatment and the percentage reduction at the beginning and ending for the samples are shown in Tables 5 and 6 respectively.

Table 5. Variation in PAHs concentration in crude oil impacted soil after remediation

PAHs	Control, mg/kg	Treated soil, mg/kg
Naphthalene	0.0067 ± 0.00577	0.0033 ± 0.00577
Acenaphthylene	0.0167 ± 0.00577	0.0367 ± 0.05508
Acenaphthene	0.0233 ± 0.01155	0.0100 ± 0.01000
Fluorene	0.0467 ± 0.02082	0.0267 ± 0.01528
Anthracene	0.3200 ± 0.09849	0.0833 ± 0.04041
Phenanthrene	0.2767 ± 0.22234	0.0200 ± 0.00000
Fluoranthene	0.0167 ± 0.01155	0.0067 ± 0.00577
Pyrene	0.0400 ± 0.03606	0.0400 ± 0.04000
Benz(a)anthracene	0.0000 ± 0.00000	0.0000 ± 0.00000
Chrysene	0.0067 ± 0.00577	0.0000 ± 0.00000
Benzo(b)fluoranthene	0.0000 ± 0.00000	0.0000 ± 0.00000
Benzo(k)fluoranthene	0.0033 ± 0.00577	0.0000 ± 0.00000
Benzo(e)pyrene	0.0000 ± 0.00000	0.0000 ± 0.00000
Dibenzo(a,h)anthracene	0.0000 ± 0.00000	0.0033 ± 0.00577
Indeno(1,2,3-cd)pyrene	0.0000 ± 0.00000	0.0000 ± 0.00000
Benzo(ghi)perylene	0.0000 ± 0.00000	0.0000 ± 0.00000
Total PAHs	0.7568± 0.00000	0.2300 ± 0.00000

Table 6. Percentage reduction in PAHs concentration after treatment

PAH constituents	% PAHs Reduction			
	E_1,30 days	E_2, 30 days	E_1, 0 w.r.t E_1, 30	E_1, 0 w.r.t E_2, 30
Naphthalene	0.0067	0.0033	98.2	99.1
Acenaphthylene	0.0167	0.0367	0.00	-119.1
Acenaphthene	0.0233	0.0100	30.0	70.00
Fluorene	0.0467	0.0267	51.7	72.4
Anthracene	0.3200	0.0833	-6.7	72.2
Phenanthrene	0.2767	0.0200	28.4	94.8
Fluoranthene	0.0167	0.0067	66.6	86.6
Pyrene	0.0400	0.0400	-497	-497
Benz(a)anthracene	0.0000	0.0000	0.00	0.00
Chrysene	0.0067	0.0000	0.00	0.00
Benzo(b)fluoranthene	0.0000	0.0000	0.00	0.00
Benzo(k)fluoranthene	0.0033	0.0000	0.00	0.00
Benzo(e)pyrene	0.0000	0.0000	0.00	0.00
Dibenzo(a,h)anthracene	0.0000	0.0033	0.00	0.00
Indeno(1,2,3-cd)pyrene	0.0000	0.0000	0.00	0.00
Benzo(ghi)perylene	0.0000	0.0000	0.00	0.00
Total PAHs	0.7568	0.2300	39.9	81.7

E_1, 0 = contaminated soil at day 0; E_1, 30 = untreated soil at day 30; E_2, 30 = treated soil at day 30; "wrt" = with respect to; negative (-ve) means that there was an increase

3.4.1. Degradation of PAHs constituent fraction by SMCT

From table 6, it was observed that PAHs under investigation could be classified into the 2 and 3-ring PAHs, 4-ring PAHs and 5 and 6-ring PAHs and hence, they are generally defined as small, medium and large molecular weight PAHs (Davis, 2016). Naphthalene (2-ring and molar mass, 128 g/mol) had the highest degradation as decrease of 98.2% and 99.1% were achieved in the control and treated soil respectively at the end of the remediation period. This is in agreement with the findings of Okparanma et al. (2011) where they stated that the high rate in reduction of naphthalene is as a result of its physical properties such as high volatility, slight solubility in water (31 mg/l), high vapor pressure, presence of two number of rings and low molecular mass of 128 g/mol. It also argues the works of Yunker and MacDonald (1995) and Johnsen et al. (2005) where they postulated that the low molecular mass PAHs are less likely sorbed onto soil matter hence they are made unavailable for degradation by PAH-degrading organisms. Similarly, in fluorene (3-ring and molar mass, 166 g/mol) where its degradation was 51.7% in the control and 72.4% in the treated soil after the remediation period. Again, acenaphthene (3-ring and

molar mass, 153 g/mol) showed a minimal rate of degradation with a 30% decrease in the control and 70% decrease in the SMCT treated soil at the end of the remediation period. Also, phenanthrene (3-ring and molar mass, 178 g/mol) had a poor degradation in the control and a high degradation in the SMCT treated soil both having a percentage reduction of 28.4% and 94.8% respectively. Table 5 shows that acenaphthylene did not degrade (remained constant) in the control soil at the end of the research while it increased in the SMCT treated soil. The reasons for this are uncertain, but maybe it is due to changes in the characteristics of the amendment option (SMCT) due to microbial activities during the process, peradventure resulting in the high availability of the PAH for extraction during analyses or its low laboratory detection limit. Anthracene (3-ring and molar mass, 178 g/mol) showed a 6.7% increase in the control and 72.2% decrease in the SMCT treated sample. This anomalous degradation pattern of the 2 and 3-ring PAHs by SMCT was fair complete biodegradation to harmless end-products. On the other hand, the 4-ring PAHs excluding pyrene, fluorathene and including benz(A)anthracene and chrysene showed no signs of reduction as they have very low detection limits. Fluoranthene (4-ring and molar mass, 202 g/mol) had a 66.6% reduction in the control and 86.6% reduction in the SMCT treated the soil. Pyrene (4-ring and molar mass, 202 g/mol) generally had a high increase in both the control and treated soil after the remediation period. All the resolved 5 and 6-ring PAH compounds were all below the laboratory detecting limit (LDL) in the initial analysis done for the contaminated soil and hence, had no percentage increase or decrease in the control and treated soil after the treatment period from the analysis done.

3.5. Microbial analysis

This analysis was carried out for the spent mushroom compost tea (SMCT), the contaminated soil sample at day 0, the untreated soil and treated soil at day 30 respectively in order to identify the microbial organisms present and their population.

The microbes present in the SMCT, the contaminated soil sample at day 0, the untreated soil and treated soil at day 30 are shown in figure 4. Hydrocarbons in the environment are biodegraded mainly by bacteria and fungi and the fraction of the total heterotrophic community represented by the hydrocarbon utilizing bacteria and fungi is highly variable (Leahy and Colwell, 1990). Microbial population revealed that the total heterotrophic bacteria (THB) count and the total heterotrophic fungal (THB) count of the organic amendment were 8.4×10^3 CFU/ml and 2.0×10^2 CFU/ml respectively. This result shows that the bioavailability of microbes in the SMCT was high and hence, it may have the ability to degrade the pollutants in the soil.

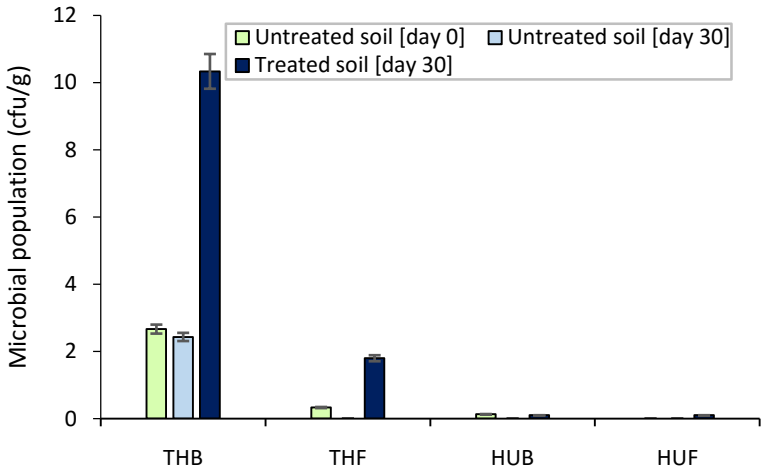


Figure 4. Distribution of the microbial population in the untreated and treated soil at the end of the compost-bioremediation period

Nevertheless, the total heterotrophic bacteria (THB) count in the freshly contaminated soil reduced in the control from 2.7×10^4 CFU/g to 2.4×10^4 CFU/g after the remediation period whereas it increased in the SMCT treated soil from 2.7×10^4 CFU/g to 10.3×10^4 CFU/g. Hence, the microbial activities increased during the remediation and it is supported by the theory postulated by Ausma et al. (2002) where it was stated that the addition of organic amendments containing nutrients stimulates the degradative capabilities of the indigenous microorganisms thus allowing the micro-organisms to break down the organic pollutants at a faster rate. The result is in agreement with the finding of Abioye et al. (2010) where they recorded higher counts of both heterotrophic bacteria and hydrocarbon utilizing microbes in used lubricating oil contaminated soil amended with brewery spent grain, banana skin and spent mushroom compost (SMC). Therefore, the higher microbial population counts (see figure 4) in oil-contaminated soil amended with SMCT is accompanied by significant oil biodegradation. In addition, the high counts in hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) is as a result of the fact that the SMCT (organic amendment) was able to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physico-chemical properties (Floch et al, 2011 and Basharudin, 2008) as well as improved the soil aeration and hence providing sufficient oxygen required by the microbial community which in turn favored the growth of indigenous microbes in the soil.

4. Conclusion

In this study, bioremediation was carried out within a period of 30-days using SMCT as remediating agent. It was revealed that significant reduction occurred in the total PAHs content

of the treated soil. This was due to the rich residual nutrients and enzymes in SMCT. Although, the statistical evaluation showed significant variation among the untreated, control and treated soil samples when compared using ANOVA. Nevertheless, the total heterotrophic bacteria (THB) count in the contaminated soil reduced in the control sample after the remediation period whereas it increased in the SMCT treated soil. Hence, the microbial activities increased during the remediation. Therefore, the higher microbial population counts in oil-contaminated soil amended with SMCT is accompanied by significant oil biodegradation. In addition, the high counts in hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) is as a result of the fact that the SMCT was able to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physico-chemical properties.

References

Abioye, O.P., Abdul, A.A. and Agamuthu, P. (2010) 'Enhanced Biodegradation of Used Engine Oil in Soil Amended with Organic Wastes', *Water Air and Soil Pollution*, 209: 173 – 179.

Agbogidi, O.M., Eruotor, P.G., Akparobi, S.O. and Nnaji, G.U. (2007) 'Evaluation of Crude Oil Contaminated Soil on the Mineral Nutrient Elements of Maize (Zea mays L.)', *Journal of Agronomy,* 6: 188-193.

Ausma, S., Edwards, G.C., Fitzgerald-Hubble, C.R., Halfpenny-Mitchell, L., Gillespie, T.J., and Mortimer, W.P. (2002) 'Volatile Hydrocarbon Emissions from a Diesel-Fuel Contaminated Soil Bioremediation Facility', *Air Waste Manage. Assoc*,52: 769-780.

Ayotamuno, M.J., Kogbara, R.B., and AgunWamba, S.C. (2007) 'Comparative Analysis of some techniques in Biological Reclamation of Crude oil polluted Agricultural soils in Nigeria', *Nigerian Journal of Tech.*, 25.

Ayotamuno, M.J., Kogbara, R.B., Ogaji, S.O.T. and Probert, S.D. (2006) 'Bioremediation of a crude-oil polluted agricultural-soil at Port Harcourt, *Nigeria',Applied Energy*, 83(11), pp. 1249-1257.

Basharudin, H. (2008) 'Bioremediation of Oil Contaminated Wastewater using Mixed Culture', Master Dissertation, Universiti Malaysia Pahang. Retrieved on December 12, 2017from http://umpir.ump.edu.my/507/1/bioremediation_of_oil_contaminated_wastewater_using_mixed_culture_thesis.pdf

Canola Council of Canada (2017) 'Canola Encyclopedia; Field characteristics/Effects of soil characteristics', Available online: http://www.canolacouncil.org/canola-encyclopedia/field-characteristics/effects-of-soil-characteristics/ [Accessed 03/03/2018].

Davis, D.D. (2016) '*Bioremediation of Hydrocarbon Contaminated Soils and Drill Cuttings Using Composting with Agricultural Wastes*', PhD. Thesis, School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne, UK.

Floch, C., Chevremont, A.C., Joanico, K., Capowiez, K. and Criquet, S. (2011) 'Indicators of Pesticide Contamination: Soil enzyme compared to Functional Diversity of Bacterial Communities via Biolog® Ecoplates', *European Journal of Soil Biology,* 47(4): 256-263.

Gallardo-Lara, F. and Nogales, R. (1987) 'Effect of the Application of Town Refuse Compost on the Soil-Plant System: a review', *Biological Wastes,* 19:35-62.

Harms, H., Bokern, M., Kolb, M. and Bock, C. (2003) 'Transformation of Organic Contaminants by different plant system in Phytoremediation; Transformation and control of contaminants', S. C. McCutcheon, J. L. Schnoor, eds., John Wiley & Sons, Inc., Hoboken, New Jersey.

Institute of pollution Studies (IPS), (1990). Ecological Impact Assessment of EbubuOchani, Shell Petroleum Development Company, Institute of Pollution Studies, Rivers State University of Science & Technology Port Harcourt, 236pp.

Jackson, M.L. (1964) 'Soil Chemical Analysis'', *Engle Wood Cliffs, New York, Prentice Hall*, pp. 498.

Johnson, A.R., Wick, L.Y. and Harms, H. (2005) 'Principles of microbial PAH degradation in soil', *Environment Pollution,* 133, pp. 71-84.

Keith, L. and Telliard, W. (1979) 'ES & T Special Report: Priority Pollutants: 1-a Perspective view', Vol. 13: Issue 4: pp. 416-423.

Leahy, J.G. and Colwell, R.R. (1990) 'Microbial Degradation of Hydrocarbons in the Environment', *Microbiological Reviews*, 54(3), pp. 305–315.

Mackay, D.M., Finarson, M.D., Wilson, R.D., Fowler, B., Scow, K., Hyman, M., Naas, C., Schirmer, M. and Durrant, G.D. (1999) 'Field studies of In-situ Remediation of an MTBE Plume at Site 60, Vandenberg Airforce Base, California', *In Proceedings of the 2000 Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation, National Ground Water Association, Westerville, OH*. Pp. 145-157.

Margesin, R. and Schinner, F. (2001) 'Biodegradation and Bioremediation of Hydrocarbons in Extreme Environments', *Applied Microbiology Biotechnology*, 56: 650–663.

Morgan, P. (1991) 'Biotechnology and Oil Spills', *An invited paper at the Society of Chemical Industry. Biotech. UK Conference Leeds* September 1991, pp. 7.

Odu, C.T.I., Esuruoso, O.F., Nwoboshi, L.C. and Ogunwale, J. (1985) 'Environmental Study of the Nigerian Agip Oil Company Operational Areas, Soils and Fresh Water Vegetation', Milan, Italy.

Okerentugba, P.O., Orji, F.A., Ibiene, A.A. and Elemo, G.N. (2015) 'Spent Mushroom Compost for Bioremediation of Petroleum Hydrocarbon Polluted Soil: A Review', *Global Advanced Research Journal of Environmental Science and Toxicology* (ISSN: 2315-5140) Vol. 4(1) pp. 001-007.

Okparanma, R.N., Ayotamuno, J.M., Davis, D.D. and Allagoa, M. (2011) 'Mycoremediation of Polycyclic Aromatic Hydrocarbons (PAH)-Contaminated Oil-Based Drill-Cuttings', *African Journal of Biotechnology*, 10(26), pp. 5149-5156.

Sadiq, S. Mahmood-ul-Hassan, M., Ahad, K. and Nazir, S. (2018). Bioremediation of hexachlorocyclohexane (HCH) in soil using spent mushroom compost of Pleurotusostreatus, Bioremediation Journal, 2018, Vol. 22, No. 3-4, 126–135 https://doi.org/10.1080/10889868.2018.1516615.

Sims, J.L., Sims, R.C. and Matthews, J.E. (1990) 'Approach to Bioremediation of Contaminated Soil', *Hazardous Waste and Hazardous Materials*, 7, 17-49.

Smith, G.N. and Smith, I.G.N. (1998) 'Elements of Soil Mechanics (7th Ed.)', *Blackwell Science, Oxford,* pp. 9-10.

Suess, M.J. (1976) 'The Environmental load and cycle of polycyclic aromatic hydrocarbons',*Sci. Tot. Env*. 6, 239-250.

Walkey, A. and Black, I.A. (1934) 'Determination of organic Carbon in soil Science', 37: 29 – 38.

Yunker, M.B., MacDonald R.W. (1995) 'Composition and Origins of Polycyclic Aromatic Hydrocarbons in the Mackenzie River and on the Beaufort Sea Shelf', *J. Arctic. Inst. North Am*. 48(2): 118-129.