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Comparative Effects of Bone Char and NPK Agricultural Fertilizers on Hydrocarbon Utilizing Bacteria and Fungi in Crude Oil Polluted Soil

Ikiogha DB[a]*, Otaraku IJ[b], Momoh OLY[c], Welcome MO[d]

[a,c]Environmental Engineering Technology, Faculty of Engineering, University of Port Harcourt, Choba, Port-Harcourt, Rivers State, Nigeria

[b]Department of Chemical Engineering, Faculty of Engineering, University of Port Harcourt, Choba, Port-Harcourt, Rivers State, Nigeria

[d]Department of Physiology, College of Health Sciences, Nile University of Nigeria, Abuja, Nigeria

[a]Email: bovieikiogha@gmail.com

[c]Email: yusuf.momoh@uniport.edu.ng

[d]Email: welcome.menizibeya@nileuniversity.edu.ng

Abstract

Bone char and NPK fertilizers are stimulants for bioremediation of crude oil polluted soil were investigated. Cells were constructed in-situ with dimensions of 1.5m by 1.5m. Crude oil samples were applied to cells with crude penetration depths of 30 cm. The hydrocarbon content (THC), total organic carbon (TOC), bacterial and fungal contents of the soils of the cells were investigated before and 8 weeks after addition of 0.5kg, 2kg and 3.5kg of bone char and NPK fertilizer. The control cells had no bone char or NPK fertilizer. The results showed that bone char and NPK fertilizer significantly reduced THC and TOC when compared with the control. Furthermore, both bone char and NPK fertilizers significantly increased the number of hydrocarbon utilizing bacteria and fungi as well as total heterotrophic bacteria population. Consequently, THC removal efficiency ranged from 62.24 to 87.74% and TOC removal efficiency ranged from 62.93 to 77.37% for NPK fertilizer and bone char amended cells, respectively. The stimulatory efficiency for THC ranged from 82.00 to 87.23% and stimulatory efficiency for TOC ranged from 72.40 to 77.55% for NPK and bone char respectively. In conclusion, our results suggest that the stimulatory effects of bone char for bioremediation of crude oil contaminated soil are comparative with those of NPK fertilizer.

Keywords: Bone char; biochar; NPK fertilizer; crude oil pollution; soil contamination; bioremediation.

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* Corresponding author.

1. Introduction

Over the past decades, anthropogenic activities such as oil exploration and exploitation contributed substantial harm to the environment due to oil spills originating from leaking pipelines, wellheads, and flow stations [1, 2]. Crude oil spillage results to contamination of surface water, ground water, seafood, crops, ambient air, thereby endangering wildlife, seabirds, valuable fish and humans [1-3].

Oil exploration and exploitation is a frequent cause of oil spills, especially in Nigeria, where statutory bodies saddled with the responsibilities of regulating the activities of oil companies and securing pipelines and oil stations are inadequately equipped to carry out their functions or are adamantly not keeping up to their task [1-5]. Despite impact assessment reports, environmental degradation and health impacts are likely to continue because the technical teams responsible for the impact assessments do not usually consist of all the needed personnel [4]. Nigeria is the largest producer of oil in Africa, and has one of the top ten largest reserves in the world. Statistics indicate that crude oil sales account for more than 90% of foreign exchange earnings for Nigeria [2]. The Niger Delta region, which constitutes 7.5% of Nigeria's land mass and an area of 70,000 km^2 is where the largest reserves of oil are found. Oil exploration in this region began since the 1950s, exporting about 15 million tons of oil every day. The region is defined by the delta of the Niger River on the Gulf of Guinea [5]. The Niger River has a total length of about 4100 km and a drainage area of 2.3 million km^2, about 7.5 % of the African continental landmass. The mangrove and freshwater swamp forest of the Niger Delta is the largest in Africa, and the third largest in the world, covering some 70 000 km^2. Farming strives well; however, recent investigations have shown huge reduction in agricultural productions, not only in regions affected by oil spills, but also, in oil-bearing communities [1].

Apparently, spills from oil exploration has made the land desolate, contaminating and degrading the soil nutrients and water bodies with significant reduction in agricultural production and acquisition of seafood for human consumption [1, 2]. For example, Ordinioha and Brisibe (2013) reported a 60% reduction in household food security caused by oil spillage in Nigeria [4]. The authors also noted 36% reduction of ascorbic acid content of vegetables and 40% reduction of crude protein content of cassava, and consequently, resulting to a 24% increase in the prevalence of malnutrition among children [4]. It is therefore not a surprise that Nigeria has one of the highest prevalence of malnutrition in the world since cassava is the major food in the country [6-8].

Contamination of the environment by crude oil spillage not only requires several millions of dollars and decades to clean up, but also, poses huge threat to human wellbeing. Evidences indicate that some components of crude oil can cause acute and chronic effects on human health – infertility, blood and liver cancer [4]. Indeed radiotoxic substances such as Ra-226, Pb-210, and Po-210 as well as carcinogens like polycyclic aromatic hydrocarbons and benxopyrene, found in crude oil pose a huge health risk to humans [2, 4].

Bioremediation has emerged as viable and efficient approach in management of oil contamination of land. The approach involves the use of microorganisms such as bacteria, yeast, or fungi or application of artificial nutrients to stimulate the growth of the microorganisms that breakdown hazardous substances into minimal toxic or nontoxic substances [9-13]. Accumulating research data have shown the importance of using inorganic

(e.g. NPK) fertilizer to reduce the negative impact of crude oil contaminated soil on the environment and to increase agricultural production [14, 15]. Ubochi and his colleagues (2006) reported significant increase in hydrocarbon utilizing bacterial count and about 30-51% decrease in hydrocarbon content upon addition of 60g of NPK agricultural fertilizer to 100g crude oil contaminated soil [14]. Similar findings about biodegradation of hydrocarbons by N-P-K fertilizer in crude oil contaminated soil have been reported elsewhere [15]. NPK is most widely used source of nutrients in bioremediation of soil. However, this inorganic fertilizer can potentially lead to increase in soil acidity, leaching of minerals, and produce by-products that are environmentally unfriendly. Furthermore, the NPK fertilizers are relatively expensive [14, 15]. Emerging evidences indicate that animal waste such as cow dung, goat manure, and poultry droppings enhance the rate of biodegradation of crude oil polluted soil through significant increase in total heterotrophic bacteria and bacterial utilizing hydrocarbon [9]. Available evidences also indicate that biochar, a solid carbon-rich material obtained by pyrolysis of different biomasses, can significantly enhance soil quality in crude-oil pollution of the environment [16, 17]. However, it is not clear how these results compare significantly to the use of inorganic fertilizer such as NPK agricultural fertilizer. Furthermore, there is scanty information on the effects of bone char on the microbial utilization of hydrocarbon in crude oil contaminated soil. Therefore, the aim of this study was to investigate the effects of bone char on degradation of hydrocarbon by soil microorganisms in crude oil contaminated soil, and to compare the outcome with NPK agricultural fertilizer stimulated bioremediation. We also determine the optimal nutrient required for biodegradation of petroleum hydrocarbon by using different concentrations of NPK and bone char fertilizers.

2. Materials and Methods

2.1. Sampling site

The experimental site was located at the chemical Engineering Department, University of Port Harcourt Choba, Rivers State, Nigeria. The inland part of Rivers State consists of tropical rainforest. Rainy season is from April to October, while dry season is from November to March. The soil is usually a mixture of sand, clay and silt but is sandy loam and fertile. The daily minimum temperature is 23^0C (average of 31.5^0C). The average monthly relative humidity is 85% and the average monthly rainfall is 200mL (average annual rainfall – 2,400mL. The present study was carried out in uncontrolled environment during the dry season.

2.2. Sample collection and preparation

2.2.1. NPK agricultural fertilizer

The inorganic fertilizer (20:10:10 NPK) was bought from fertilizers’ retail outlet at Obio/Akpo Local Government Area, ADP (Agricultural Development Programme) office, Port Harcourt, Nigeria.

2.2.2. Bone char

The bone char organic fertilizer was produced by pyrolosis of cow bone at the General Science Laboratory, University of Port Harcourt, Abuja Campus, Abuja, Nigeria.

2.2.3. Crude oil

Crude oil was collected from Adibawa North East oil spill site at Biseni, Bayelsa State of Nigeria. The oil was collected using plastic bale into glass bottles at the recovery tanks positioned by the clean-up contractor for disposal. The samples crude oil were assessed for quality assurance criteria according to the Department of Petroleum Resource (of the Nigerian Federal Ministry of Petroleum Resources) environmental regulations and standard for Petroleum industry. Other chemicals used in this experiment were purchased from Austino Laboratory Services Ltd, Choba, Port Harcourt (Nigeria) and were of analytical grade.

2.3. Soil treatment

Land portion without any previous history of crude oil contamination was marked and divided into cells (Table 1). Each cell had a dimension of 1.5 x 1.5 meters. The cells were separated from each other by a cell-to-cell gap of 10 cm with wooden structures. Each cell was polluted with 20L of crude oil and allowed for 2 weeks until the soil was contaminated to 30cm depth. At this period, the vegetations on the study site were all dead (Fig. 1). Then different concentrations of NPK and bone char fertilizers were added to the cells and tilled to ensure adequate effects of the amendments for biostimulation (Table 1 & Fig. 2). Appropriate quantity of water was also added to the cells taking into consideration the optimal soil moisture conditions.

Table 1: Summary of treatments conducted in the study

Cells	**Description of treatment**
C	20 L of crude oil + soil only (control experiment)
X_1	20 L of crude oil + soil + 0.5kilogram of 20:10:10 NPK fertilizer + tilling
X_2	20 L of crude oil + soil + 0.5 of bone char fertilizer + tilling
Y_1	20 L of crude oil + soil + 2.0kg of 20:10:10 NPK fertilizer + tilling
Y_2	20 L of crude oil + soil + 2.0kg bone char fertilizer + tilling
Ƶ$_1$	20 L of crude oil + soil + 3.5kg of 20:10:10 NPK fertilizer + tilling
Ƶ$_2$	20 L of crude oil + soil + 3.5kg bone char + tilling

Figure 1: Pictorial view showing the design of each cell in the crude oil contaminated soil

Figure 2: Pictorial view of cells with different concentrations of NPK and bone char

2.4. Laboratory analysis

2.4.1. Physiochemical analysis

Soil physiochemical parameters (pH, total hydrocarbon content, organic carbon, phosphorus, and total nitrogen) were determined and used as indicators of soil contamination. Soil samples were collected using spade at depths of 6 cm, 18 cm and 27 cm from different experimental sites in each cell before and after crude oil contamination. Samples were air dried and homogenized by passing them through a 2 mL mesh sieve. Samples from same cell were mixed into composite samples which were labeled and sealed in small polyethylene bags for further analysis. Analysis of physiochemical parameters was conducted according Walkley and Black (1934) [18]; Bray and Kurtz (1945) [19] and Odu and his colleagues (1985) [20].

- **Total hydrocarbon content (THC):** Toluene (10 mL) of was added to 10 g of the sample, and vortexed. THC was determined by absorbance at 420nm wavelength using the Bausch & Lomb spectronic-70 spectrophotometer (Bamko-Surplus, Texas, United States). Calculation of substance concentration in soil sample was done according to the manufacturer specification and with reference to Odu and his colleagues (1985) [20].
- **Total nitrogen:** Two grams of the sample was weighed into designated flasks and catalyst mixture of selenium, copper sulfate ($CuSO_4$) and sodium sulfate (Na_2SO_4) was added followed by 10 milliliters of concentrated analytical sulphuric acid. The contents of the flask were mixed by gentle swirling and then digested in a Foss Tecator digestor (Kjeldahl™ Model 2300, Pittsburgh, USA) until a high green or gray color was formed, indicating the digesta has been cleared. Heating was continued for another one hour before the digesta was allowed to cool. The digesta was then transferred into a 250 mL comical flask and made up to the mark with distilled water [20]. Aliquots of the solution were used to determine nitrogen using an autoanalyzer.
- **pH:** The pH of soil samples collected was determined using corning pH meter model 7 (Corning

Scientific, NY, USA). The pH determination was performed according to the manufacturer specification.

- **Phosphorous:** The phosphorus content of the soil samples was determined by the Bray and Kurtz method [19]. Briefly, 2.85g of the sample was weighed. 20 mL of Bray No.1 extractant was added (0.025N + 0.03N NH_4) and shaken for 1 min. Thereafter, 10 mL of filtrate were pipetted into a 50 mL volumetric flask and diluted to about 20 mL with distilled water, then ascorbic acid solution (4 mL) was added. This was allowed for at least 30 minutes for full color development before reading with the spectronic-20 spectrophotometer at 660nm wavelength. Phosphorous content of the soil sample was measured according to the manufacturer specification.
- **Total organic carbon (TOC)**: TOC was determined by wet combustion method of Walkey and Black (1934) [18]. Briefly, 1g of finely grinded soil sample was weighed in duplicates into beakers. Then potassium dichromate solution (10 mm) was pipetted into each beaker and rotated gently with wet soil sample, followed by addition of H_2SO_4 (20 mL). The beaker was rotated again to ensure complete oxidation and allowed to stand for 10 min before dilution with distilled water to about 200-250 mL. Finally, 10.5N ferrous ammonium sulfate (25 mL) was added and treated with 0.4N potassium permanganate under strong light.
- **Determination of soil texture**: The particle size distributions were determined using Bouyoucous hydrometer [21, 22] with modification according to Day (1965) [23].

2.4.2. Microbiological analysis

Microbiological analysis was conducted according to the procedure described by Buchanan and Gibbons (1974) [24] and Cowan (1974) [25]. Media and all diluents were sterilized in an autoclave at 121^0C for 15 min. Glass waves were sterilized in dry hot air oven at 160^0C. In preparing the normal saline, the diluent used, 0.85g of NaCl was weighed and transferred into 100 mL of distilled water and mixed thoroughly. The resulting solution was then dispensed in 9 mL into a test tube, followed by sterilization at 121^0C for 15 min. The final solution was used for serial dilution of the soil samples.

- **Cultivation and enumeration of soil bacteria:** 1g of previously air-dried fine soil obtained from the rhizosphere was mixed with 1.0 mL of sterile distilled water and shaken until homogenous. 1mL of the 10^{-1} diluted solution was transferred into test tube containing 9.0 mL normal saline (diluent) and diluted serially in one tenth step wise up to 10^{-3} dilution [26]. A 0.1 mL aliquot of the solution was transferred into freshly prepared nutrient agar plates and spread with a bent glass rod. The inoculated plates were incubated at 37^0c for 24 hours after which the plates were examined for bacterial growth. The total viable heterotrophic bacteria was counted by the number of Colony Forming Units (CFU) using the colony counting technique to measure cells capable of dividing [27]. CFU was calculated as CFU/g = number of colonies x dilution factor / volume of culture plate.
- **Isolation and identification of soil bacteria:** Cultures of bacteria were obtained aseptically by streaking colonies of different cultures which appeared in the plate on to freshly prepared nutrient agar plate and incubated at 28^0C for 24 hours for the bacteria to grow. After this period, colonies began to appear. Isolates were selected based on colony morphology and those exhibiting zones of inhibition on the primary culture. Purification of isolates was done with dilute nutrient broth plus agar media and stocked at –80ºC. Standard characterization was done using gram stain (methyl and violet test) to confirm the bacterial growth and then

viable count was done manually and isolates were observed under inverted microscope at × 100 oil immersion [27]. The CFU was calculated.

- **Isolation and identification of soil fungi:** Isolation of fungi using dilution plate method on potato dextrose agar. Isolates of soil fungi that were successfully identified macroscopically and microscopically and identified by matching the characteristics of the fungus obtained from observations, according to the method described by Umboh and his colleagues (2016) [28].

The analysis of bioremediation of crude oil polluted soil was observed for a period of 8 weeks.

2.5. Statistical analysis

The experimental data were statically analyzed by Microsoft Excel and SPSS for Windows version 18. Paired t-test was performed to test the difference between the NPK and bone char treatments.

3. Results and Discussion

The particles size analysis of top 30 cm of the soil before treatment indicated that soil texture is sandy loamy: sand – 75%; silt – 10%; clay – 15%. The soil texture triangle was used to confirm the soil texture [29] and the findings were similar to that reported by Odokuma and Dickson [30, 31] for tropical rain forest top soils.

The physiochemical parameters of the non-contaminated and crude oil contamination soil are shown in Table 1.

Table 1: Physicochemical properties of non-contaminated soil and crude oil contaminated soil just before amendments

Sample	Non contaminated soil	Contaminated soil before amendments
pH	5.86	6.74
EC (µS/cm)	30	79
Soil Porosity (%)	85	52
TP (mg/kg)	18.24	6.41
TN (%)	0.85	0.27
Nitrate (mg/kg)	8.93	1.42
Phosphate (mg/kg)	55.92	84.28
Na (mg/kg)	10.28	4.17
K (mg/kg)	163.74	42.79
Ca (mg/kg)	1305.82	153.80
Sulphate (mg/kg)	5.86	13.85
SAR	2.05	11.47
CEC (meq/100g)	13.94	74
Oil & Grease (mg/L)	305.86	14720.73
TOC (%)	3.28	8.52
THC (mg/kg)	136.74	19849.47
TBC (cfu/mL)	3.64×10^6	1.31×10^2
HUB (cfu/mL)	2.23×10^2	1.64×10^2
HUF (cfu/mL)	1.05×10^2	1.01×10^2
MC (%)	8.46	6.24

Note: EC – electrical conductivity; TP – total petroleum hydrocarbon; TN – total nitrogen; SAR – sodium adsorption ratio; CEC – cation exchange capacity ; TOC – total organic carbon; THC – total hydrocarbon

content; TBC – total bacteria count; HUB – hydrocarbon utilizing bacteria; HUF – hydrocarbon utilizing fungi; MC – moisture content.

The data in Table 1 (TBC, HUB, THC and TOC) show that crude oil contamination resulted to heavy contamination of soil. Interestingly, concentration of ions in the contaminated soil was substantially lower than similar indices in non-contaminated soil i.e. before addition of crude oil.

The results of physiochemical analysis of bone char are showed in Table 2. The high level of essential ions required for plant growth in bone char suggests that remediation will probably lead to degradation of petroleum hydrocarbon.

Table 2: Results of physiochemical analysis of bone char

Parameter	MC (%)	pH	Density (g/mL)	Porosity (%)	Ca^{2+} (mg/kg)	TN (%)	K (%)
Bone char	1.86	7.18	3.06	5.80	3740.36	3.05	98.20

Note: All abbreviations are similar to those in Table 1.

Indeed the predominant bacteria identified upon contamination of soil with crude oil were Bacillus and Pseudomonas species. However, there was significant increase in total heterotrophic bacteria in all cells following addition of bone char. Two weeks after addition to NPK and bone char to the crude oil contaminated soil, the heterotrophic bacteria count increased to 2.42×10^5cfu/g and 1.26×10^6cfu/g for NPK (Z_1 cell) and bone char (Z_2 cell) amended cells, respectively. The total bacterial count also increased significantly beginning from the 4th week of the experiment (Fig. 3).

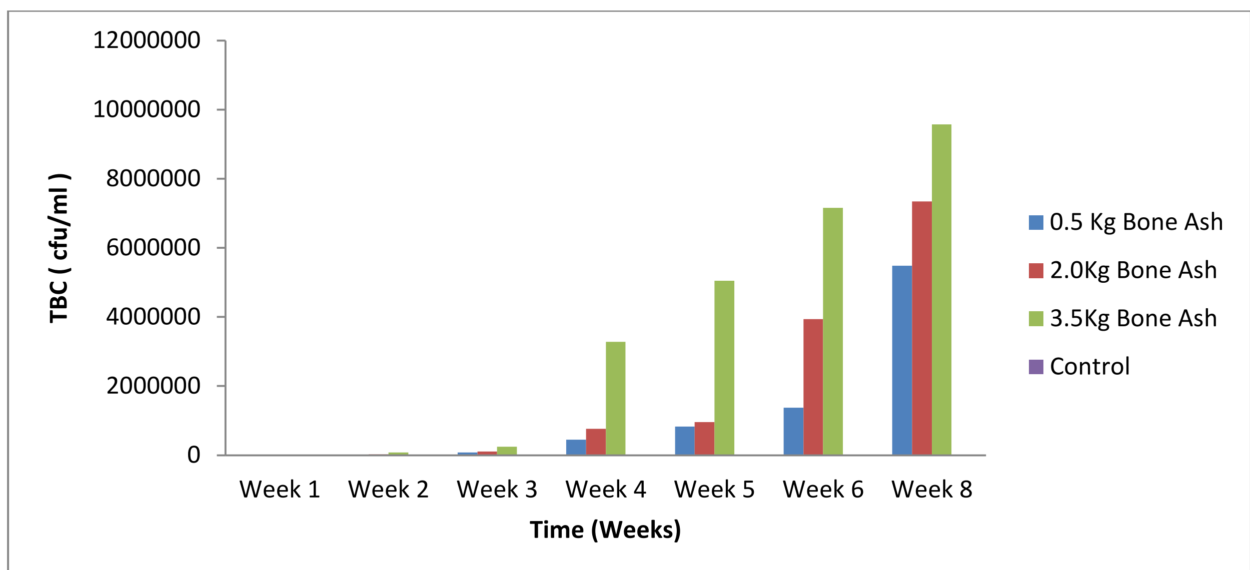

Figure 3: Total bacteria count (TBC) using different quantities of bone char.

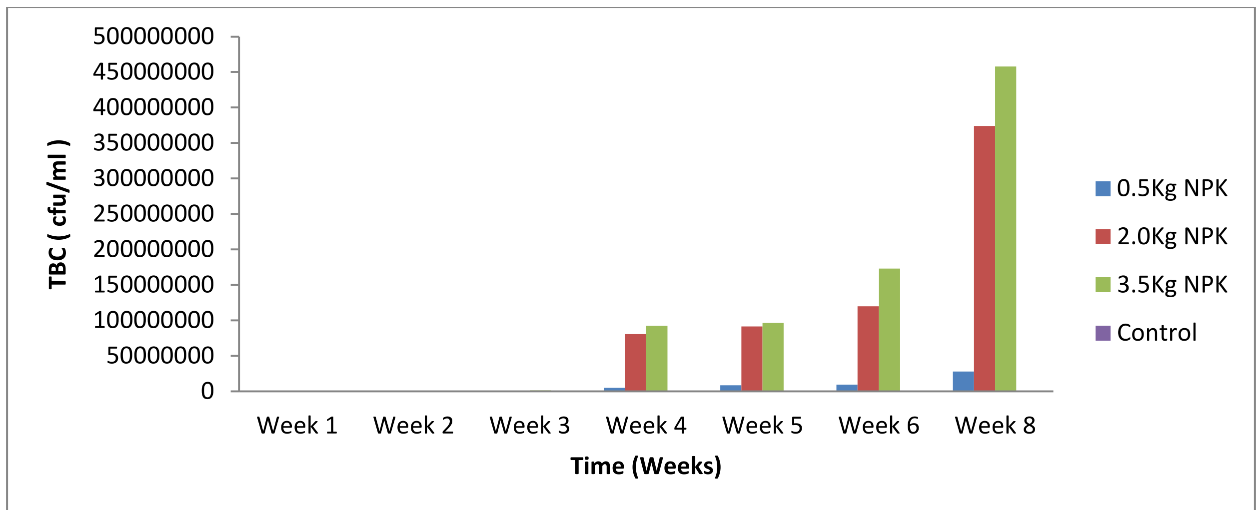

Figure 4: Hydrocarbon utilizing bacteria (HUB) growth rate using various proportions of NPK

There was a significant growth in hydrocarbon utilizing bacteria in the contaminated soil upon addition of NPK (Fig. 4) and bone char (Fig. 5) fertilizers. For both fertilizers, HUB growth increased with greater quantity of the amendments. However, it appears that 3.5 g bone char had a greater stimulatory effect on HUB than the same quantity of NPK fertilizer. HUB such as *alpha-Proteobacteria sp., Actinobacterium sp., Alcanivorax borkumensis, Micrococcus luteus, Rhodococcus erythropolis, Rhodococcus opacus* [32], *Aspergillus Niger, Pseudomonas Aeruginosa* [9, 13], *Alicagenes* sp., *Arthrobacter* sp., *Aeromonas* sp., *Bacillus* sp., *Citrobacter* sp., *Corynebacterium* sp., *Escherichia coli*, *Flavobacterium* sp., *Micrococcus* sp. [9] degrade aliphatic alkanes and some aromatic hydrocarbon through a series of metabolic reactions, required for acquisition of nutrients and energy for their survival [32-35]. Their activities are dependent on sufficient supply of nutrients (e.g. nitrogen and phosphorous), oxygen supply, pH, among others [36, 37].

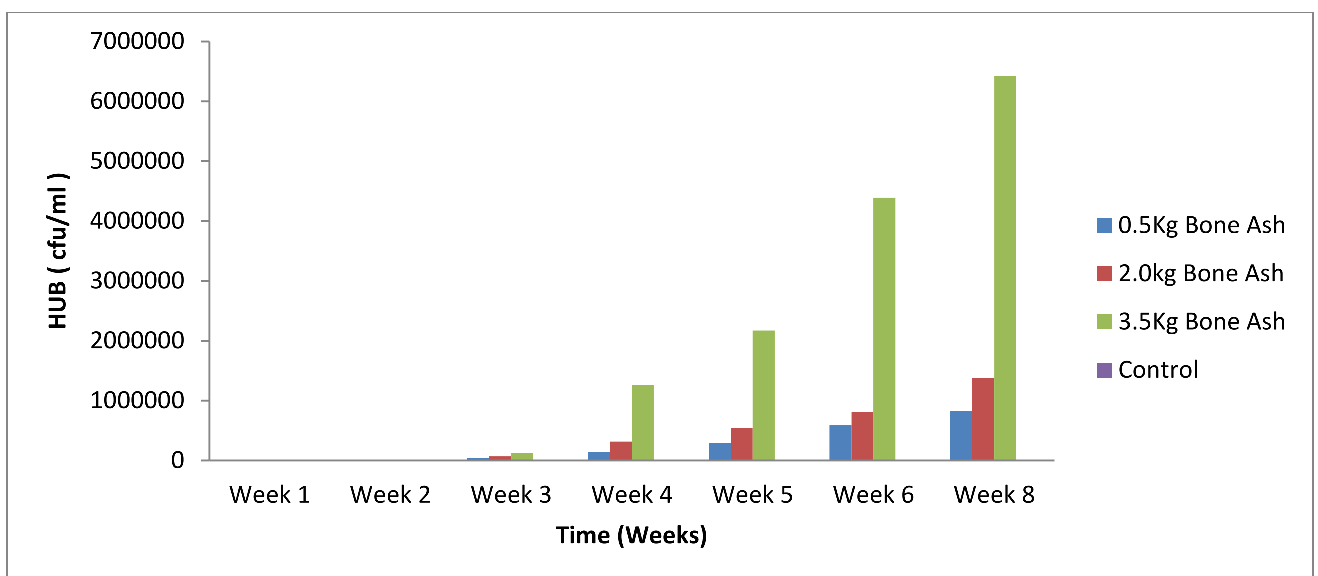

Figure 5: Hydrocarbon utilizing bacteria (HUB) growth rate following amendments of various proportions of bone char

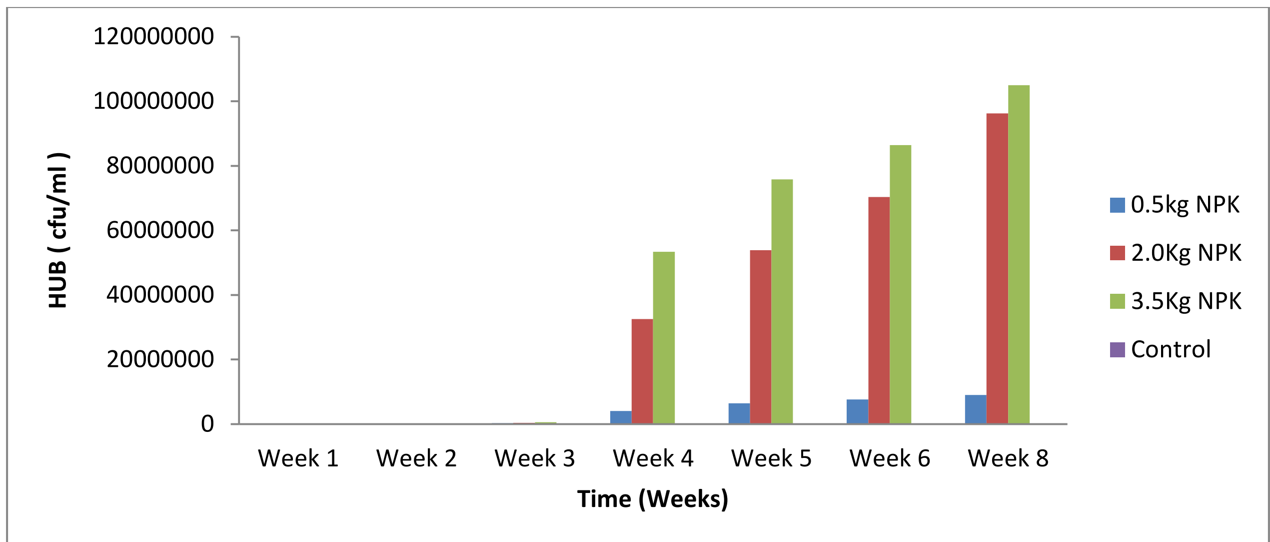

Figure 6: Hydrocarbon utilizing bacteria (HUB) growth rate following amendments of various proportions of NPK

Accumulating evidences indicate that hydrocarbon utilizing fungi are effective and environmentally efficient in degrading a wide array of toxins or contaminants. HUF are capable of degrading hydrocarbons present in soil to harmless substances [38]. Examples of HUF that have been shown to degrade crude oil polluted soil include *Aspergillus flavus*, *A. Niger*, *Mucor* sp., *Rhizopus* sp., *Talaromyces* sp., *Candida* sp., *Penicillium* sp., *Saccharomyces* sp., *Cladosporium* sp., *Fusarium* sp., and *Rhodotorula* sp. [9, 38, 39]. The hydrocarbon degrading capabilities of sawdust, one of the materials used for decontamination of oil spillage, was reportedly due to the presence of HUF. The HUF use aliphatic (C9-C40 n-alkanes) and aromatic (benzene, biphenyl, anthracene, naphthalene and phenanthrene) hydrocarbons as their main sources of energy [11, 32]. The efficiency biodegradation of crude oil of some HUF such as *Aspergillus* sp. and *Fusarium* sp. can reach 77–95% [11]. Jawhari (2014) reported a 90% efficiency of degradation of petroleum hydrocarbons with *A. Niger* and *A. Fumigatus* [12]. The results of our study revealed that growth rate of HUF during the study period of 8 weeks (Figs. 7 & 8). Similar to the results of HUB, growth rate of HUF increased with increase in the quantity of NPK or bone char fertilizers.

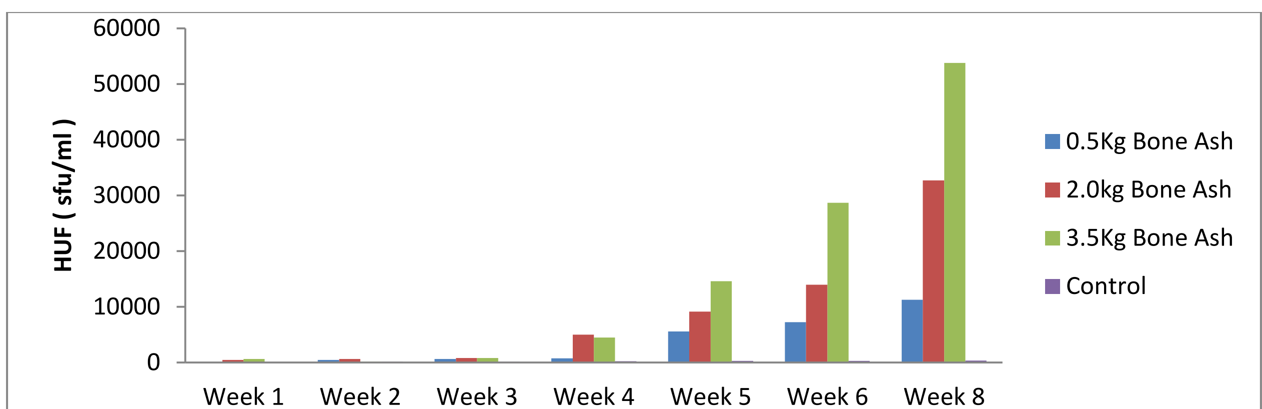

Figure 7: Hydrocarbon utilizing fungi (HUF) growth rate upon addition of different quantities of bone char

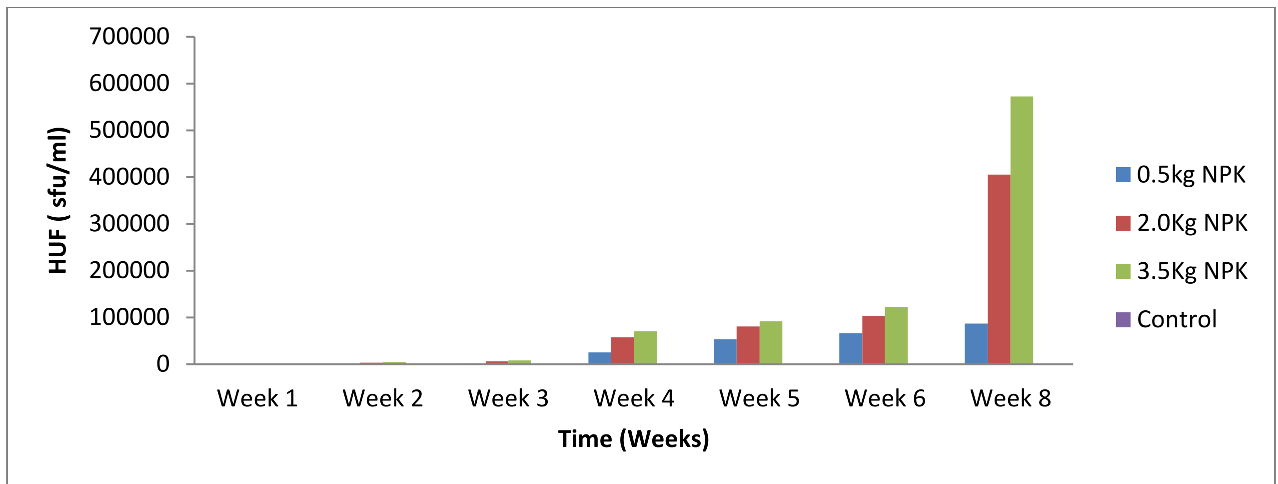

Figure 8: Hydrocarbon utilizing fungi (HUF) growth following addition of different quantities of NPK

The growth of HUB and HUF was associated with the rate of degradation of total hydrocarbon (THC). Thus, THC analysis of the soil samples of NPK and bone char treated cells showed gradual increase in the rate degradation of petroleum hydrocarbons (Table 3). By the 8th week of the experiment, there was substantial decrease in petroleum hydrocarbons. In contrast, no substantial decrease in petroleum hydrocarbons was recorded for the control cell ($P < 0.05$).

Table 3: Results of total hydrocarbon (THC) degradation (mg/kg)

Amendments (kg)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Control	19849.47	19492.79	19089.62	18826.14	18436.92	18073.26	17625.84
0.5kg Bone char	18382.54	16746.19	14296.10	11463.24	10163.15	9285.374	6942.148
2.0kg Bone char	15927.63	13635.92	11538.38	9724.357	8805.293	7423.538	5196.211
3.5kg Bone char	13483.29	12025.36	10815.26	8205.693	7416.038	6518.211	3829.564
0.5kg NPK	14280.17	12864.16	10296.16	8473.216	7658.429	6810.386	3941.376
2.0kg NPK	11837.16	9694.277	8057.364	5828.194	4365.715	3587.615	2368.312
3.5kg NPK	10210.33	8425.143	7156.072	5152.302	3815.414	2918.732	1251.649

Percentage degradation (% D) of total hydrocarbon content (THC) of all cells is shown in Fig 9. The result indicates highest level of degradation for 3.5 kg NPK. Interestingly, the %D was more than 60% in all cells, suggesting that 0.5–3.5 kg of either NPK and bone char fertilizers are effective for bioremediation of crude oil polluted soil. The general trend in the THC degradation is similar to that reported by Ofoegbu and his colleagues (2004) [40], Agarry and his colleagues (2015). Consistent with the works of Ofoegbu and his colleagues (2004)

[40] and Agarry and his colleagues (2015) biodegradation with inorganic fertilizer showed a high level of effectiveness against the control. Correspondingly, 3.5 kg NPK had the highest level of degradation (87.74% for THC and 77.37% for TOC). A couple of studies have shown that NPK agricultural fertilizer is a good source for remediation of crude oil polluted sites [14], however, very little studies have been done on the use of bone char for bioremediation.

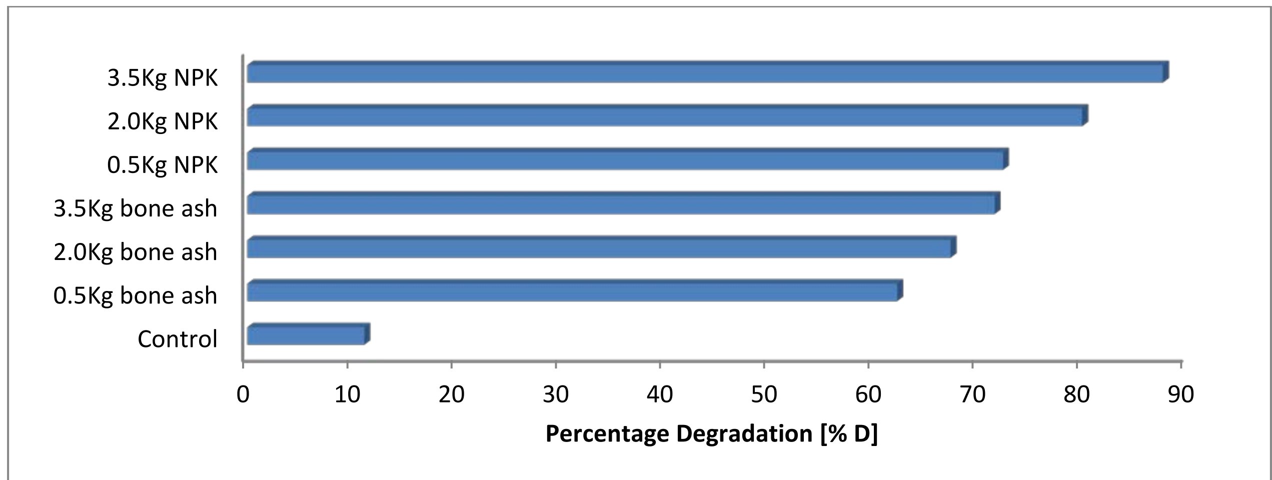

Figure 9: Percentage degradation of petroleum hydrocarbon in the cells.

Total organic carbon (TOC) is used as a rough estimate for the quality of soil contaminated with petroleum hydrocarbons. A minimum of 2% is the average value allowed for contamination. Above this value, crude oil contamination is considered significant. From Table 4, it can be deduced that bone char and NPK fertilizers substantially decreased TOC level up to normal range especially at higher quantity of the fertilizers.

Table 4: Results of total organic carbon (TOC)

Amendments (kg)	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 8
Control	8.52	8.40	8.00	7.94	7.59	7.28	7.04
0.5 bone char	7.85	7.26	6.38	4.72	4.05	3.51	2.91
2.0 bone char	7.23	6.84	6.05	4.26	3.84	3.37	2.68
3.5 bone char	6.82	6.43	5.82	3.53	3.15	2.86	2.14
0.5 NPK	7.08	6.56	5.93	3.64	3.36	3.01	2.35
2.0 NPK	6.28	6.03	5.56	3.07	2.78	2.59	1.87
3.5 NPK	6.01	5.88	5.25	2.88	2.45	2.15	1.36

4. Conclusions

The study indicated that there was substantial increase in microbe utilizing hydrocarbons and corresponding reduction in petroleum hydrocarbons in the treated cells which varied according to different quantities of bone

char and NPK fertilizers used. The use of bone char showed comparative performance with use of NPK as fertilizers for remediating crude oil contaminated soil. Similar to NPK fertilizer, increasing bone char quantity in the cell enhanced the efficiency of bioremediation.

5. Recommendations

Based on the results of this study, we recommend :

- That bone char should be used in bioremediation of crude oil polluted soil at a depth of 30cm.
- That bone char bioremediation be applied in industrial scale to help maintain a sustainable environment for living organisms.
- That the production of organic fertilizers using cow bones be encouraged for the purpose of its economic importance.
- That use of bone char should be encouraged among farmers since this organic fertilizer is environmentally friendly, and cost effective.
- That further research be conducted on the effects of bone char in remediating crude oil polluted soil using robust parameters to assess hydrocarbon degradability of specific microorganisms and factors affecting their activities.

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