



Laboratory – Based Bioremediation of Hydrocarbon Polluted Mangrove Swamp Soil in the Niger Delta Using Poultry Wastes

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Authors' contributions

This work was carried out by author CCE during his research work as part of his M.Sc. The three authors designed the study and author CCE prepared the manuscript under supervision of authors ERA and AAI. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the sustainable use of poultry wastes in compost bioremediation and the effects of sterile and non – sterile poultry wastes on the bacterial degradation of petroleum in mangrove soil.

Methodology: A laboratory – based study was carried out using sterile and non – sterile poultry wastes.

Place and Duration: Department of Microbiology, Faculty of Biological Sciences, University of Port Harcourt, Choba Port Harcourt, Nigeria, between August, 2012 and June, 2013.

Results: In a 42 day study, the sterile poultry wastes treated option had an increase in total logarithmic cultural heterotrophic bacterial count from 5.18 to 7.66 while the non-sterile poultry wastes increased from 5.26 to 7.68. The untreated set up had its total logarithmic culturable heterotrophic bacterial count increased from 5.15 to 6.65. The total logarithmic culturable

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hydrocarbon utilizing bacteria in SPW and NSPW treated options increased from 3.7 to 7.11, and 3.85 to 7.20, respectively, at which time the corresponding value obtained for untreated increased from 3.60 to 5.59. Statistical analyses showed significant difference at $p < 0.05$ level for three conditions. Hydrocarbon utilizers isolated from poultry wastes were *Pseudomonas* spp., *Bacillus* spp., *Escherichia* spp., and *Salmonella* spp. At day 42, the percentage loss of total petroleum hydrocarbon (TPH) was $67.66 \pm 0.01\%$, $62.93 \pm 0.06\%$ and $29.43 \pm 0.01\%$ in SPW, NSPW and untreated, respectively.

Conclusion: These results showed that application of poultry wastes especially non – sterile poultry wastes can effectively enhance bioremediation of hydrocarbon impacted mangrove soil. This could be attributed to the presence of indigenous hydrocarbon utilizing bacteria in non sterile poultry wastes.

Keywords: Mangrove swamps; hydrocarbon pollution; poultry wastes; waste management; hydrocarbon; bacteria; Niger Delta; Nigeria.

1. INTRODUCTION

Crude oil and Natural Gas are the main sources of foreign exchange to the Nigerian economy. These sources contribute to as much as 95% to Nigeria's budgetary expenditures [1]. Oil and Natural gas are found in the geological structures underlying mangrove and associated coastal ecosystems of the Niger Delta. Therefore, the Niger Delta is the centre of intensive and extensive oil exploration and production activities. Unfortunately, these activities have inevitably resulted in several incidents of oil spillage, causing extensive deforestation and subsequent degradation of the environment [2,3,4,5]. This has become a major cause of worry and sorrow to the people of the Niger Delta region because of pollution of both water bodies and land terrestrial ecosystems. According to a Department of petroleum resources (2006) 72% of cases of oil spillage in Nigeria in 2005 were due to sabotage occasioned by bunkering and pipeline vandalisation [6].

Efforts to remediate the negative impact of hydrocarbon pollution on the water and soil has resulted in several devices such as Remediation by Enhanced Natural Attenuation (RENA) which involves many techniques including Land farming by biostimulation or bioaugmentation of soil biota with commercially available micro flora [7]. Biostimulation is the process of providing microbial communities with a favorable environment in which they can effectively degrade contaminants and in most cases involves the provision of rate – limiting resources like nitrogen, phosphorus and oxygen (usually by tilling to aerate the soil) to speed up the bioremediation process [8,9,10,11]. In cases where natural communities of degrading consortia are at low levels or not present at all,

the addition of contaminant degrading microorganisms, known as bioaugmentation, can speed the process [12,13,14]. Although research has been performed in this area, bioaugmentation is generally not practiced, since introduced microorganisms in most areas are unable to compete favorably with well-adapted autochthonous microbial communities because of the strange environmental conditions and therefore needs more time to acclimatize [15,16,17].

One of the biggest concerns associated with petroleum pollution in the environment is the damage to farmland, fisheries, and potable water supplies since most of the people's livelihood depends on farming, fishing and usage of water for domestic purposes. Pollution of mangrove swamps is also of concern. Mangrove forests are well known for their high vulnerability to oil spills since floating oil settles with the tide and smothers both breathing and feeder roots plus a myriad of associated resident fauna [18,19]. Mangrove swamps provide habitat for finfish, crabs and shrimp, among others. Presently, mangrove forests and swamps are among the most threatened habitats in the world [20,21,19]. Currently, physical and chemical methods are the most widely used procedures employed towards minimizing the effects of oil spills in a mangrove ecosystem. However, microorganisms with the ability to degrade a wide range of crude oil components are ubiquitous in marine environment [22,23,24].

The chemical, microbiological and physical characteristics of poultry wastes are suitable co-substrate and nutrient sources for potential applications in the soil bioremediation industry. Studies have shown that poultry wastes increases the rate of atrazine biodegradation [25]

and may be suitable for the remediation of gasoline - contaminated soil [26]. Poultry wastes, the combination of feces and bedding materials, has also been used as an alternative to improve soil quality for crop production. However, information regarding the utilization of poultry litter as growth substrate for bioremediation of petroleum hydrocarbon is very limited. The use of poultry wastes (PW) in bioremediation of hydrocarbon polluted mangrove swamp soil to make provision for limiting nutrient has never been published by any known scholar. Therefore, this implies that the use of sterile poultry wastes and non-sterile poultry wastes in bioremediation of hydrocarbon polluted "mangrove environment" are a pioneer laboratory scale bioremediation study.

Poultry wastes have been identified as a waste management issue and a source of potential environmental risk. Environmental problems such as eutrophication, odours and contamination of drinking waters can result from poor handling and storage of the manure. There is often too much manure to manage, and as such the manure is regarded as wastes and is often disposed of to soils, without regard to nutrient loading of soils and groundwater. There are many uses of poultry manure, for example as an organic fertilizer (the most common use), animal feeds and electricity generation, but mostly in Nigeria and most other countries the economic potential of this resource is undervalued.

Poultry wastes are organic fertilizers which contain substances of various origins that serve as soil fertilization as well as source of nutrients and energy for soil microorganism [27]. In addition to the large amount of nitrogen and considerable quantity of phosphorus found in poultry wastes, they contain useful hydrocarbon utilizing bacteria such as *Pseudomonas* spp., *Micrococcus* spp. and *Acinetobacter* spp. which further improved natural biogeochemical cycling [27,28]. Bioremediation protocols involving application of nutrients to oil polluted site to stimulate the growth of naturally occurring oil degrading microorganisms can improve the rate of recovery of environments contaminated with petroleum or its products. The presence of essential nutrients for microbial growth and appreciable population of hydrocarbon utilizers in poultry wastes probably confirmed poultry wastes as a good biostimulants as well as good source of exogenous hydrocarbon utilizers [29]. Thus the use of poultry wastes for bioremediations is

beneficial not only for oil clean – up but also for waste management as the removal and management of poultry wastes is becoming a major problem in Nigeria and other countries due to the increasing concentration of fowls on poultry farms [27]. This study assessed the sustainable use of poultry wastes in compost bioremediation and the effects of sterile and non – sterile poultry waste on the bacterial degradation of petroleum in mangrove soil.

2. MATERIALS AND METHODS

2.1 Study Area

The hydrocarbon polluted soil was obtained from Eagle Island mangrove swamp located behind the River State University of Science and Technology, Nkpolu, Port Harcourt, Nigeria. The swamp is extensive covering a wide area of table land with a top layer of mud slurry overlying a relatively hard substratum. It is dominated with mangrove vegetation and conforms to the characteristics of mangrove swamps. This site was selected due to high level of pollution as a result of oil spillage from a pipelined owned by an upstream industry in Nigeria. The predominant mangrove plants in this area are *Rhizophora racemosa*, *Avicennia africana*, *Nypa fruticans* and *Paspalum vaginatum*. The major occupation of the Eagle Island people is fishing in the mangrove and farming in agricultural land [30].

2.2 Soil Sample Collection

The hydrocarbon polluted soil was collected with a sterile spade into plastic pail which was cleaned with cotton wool soaked in 70% alcohol to ensure that aseptic conditions are met during sampling [30,31]. Soil was collected from four sampling points and mixed together after excavation.

The excavated soil was transported to Environmental microbiology laboratory of the University of Port Harcourt for bioremediation study. Co-ordinates of the sampling points were determined using Global Positioning System (GPS). The co-ordinates were: 04°47'35.2"N; 006°58'24.9"E (Station 1), 04°47'34.9"N; 006°58'24.9" E (Station 2), 04°47'34.8" N; 006°58'24.9" E (Station 3) and 04°47'36.0" N; 006°58'24.9" E (Station 4). The sampling points are indicated in the map of Eagle Island (Fig. 1).

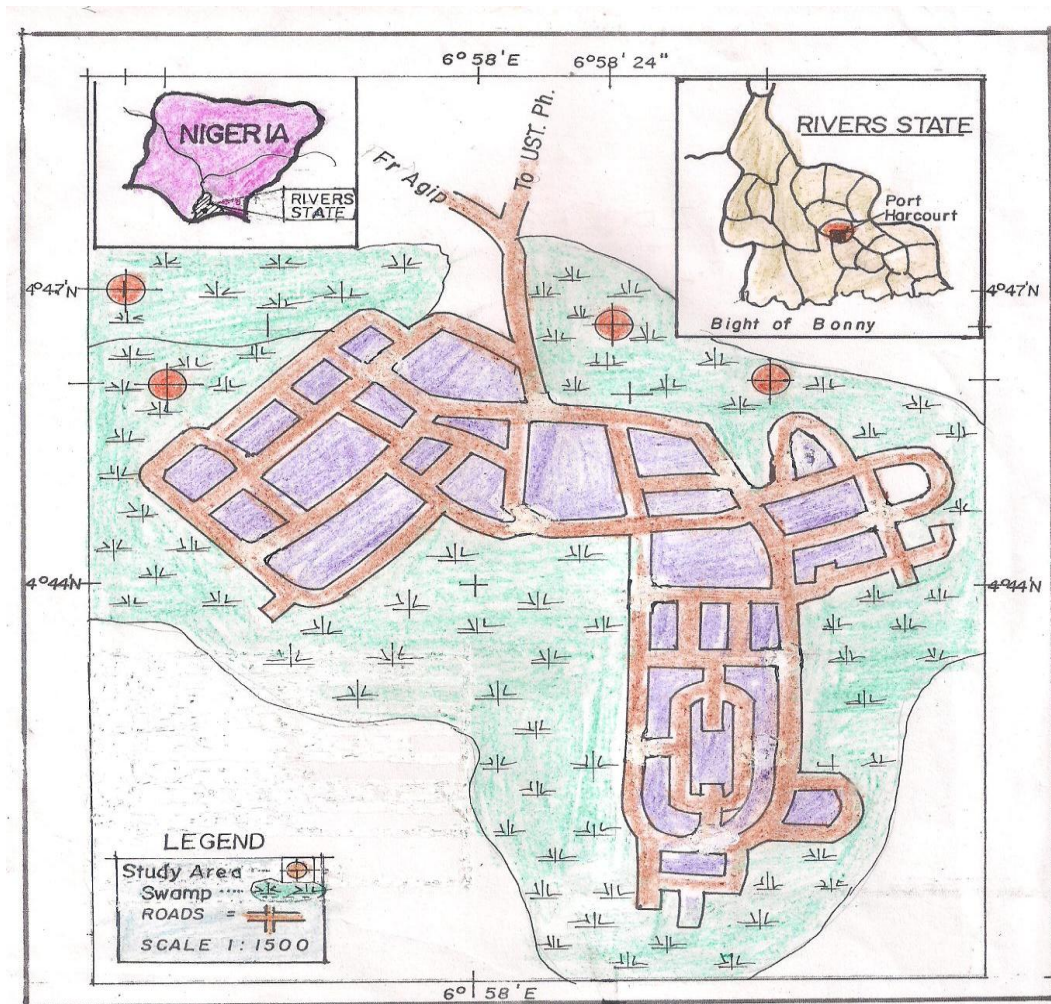


Fig. 1. Map of Eagle Island mangrove swamp showing soil sampling points

2.3 Soil Contamination

Five hundred grammes of mangrove soil sample were placed in each of four plastic containers. Fifty milliliters of Bonny light crude oil was poured in each container to stimulate a condition of a major spill [30]. Bonny light crude oil was obtained from NNPC (Eleme, Port Harcourt, Nigeria).

2.4 Nutrient Supplementation

The contaminated soil sample was supplemented with 50 g of poultry wastes according to Ezekoye et al. [30]. The baseline composition of the poultry wastes is shown in Table 3.

2.5 Preparation of Poultry Wastes

Poultry wastes of about 300 g were obtained from CEMAX Global Consult Ltd (CGCL), Aluu,

Rivers State and transported to the Environmental Microbiology laboratory. The Poultry waste was sundried for 5 days and was stored at room temperatures for usage. The sterile poultry waste was sterilized by autoclaving at 121°C, for 15 minutes at 15Psi.

2.6 Experimental Design

This is a laboratory based experiment that consist of three different set ups (SPW, NSPW and CTRL).

The SPW set up contain 500 g of mangrove soil, 50 mls of crude oil which constitute the polluted soil and 50 g of poultry wastes while NSPW contain 500 g of mangrove soil, 50 mls of crude oil (polluted soil) and 50 g of non-sterile poultry wastes. Also 500 g of the mangrove soil and 50 mls of crude oil (polluted soil) serve as the control (Table 1). The amendments used in the

laboratory based experiment are shown in Table 1.

2.7 Bioremediation Study

The experiment was set up in the laboratory. The soil samples collected were mixed thoroughly before use. The poultry wastes collected were mixed thoroughly, half of the quantity collected was sterilized by autoclaving at 121°C for 15 minutes while the other half was left unsterilized. Five hundred gramme each of the soil contained in four containers were separately contaminated with 50 ml of petroleum hydrocarbon, to give approximately 10% (v/w) pollution. Two of the set – ups designated treatments (SPW and NSPW) were treated with sterile and non – sterile poultry wastes, respectively, while the third set – up with no treatment were designated control (CTRL). Set ups SPW and NSPW were designed to determine the effects of sterile and non-sterile poultry wastes in bioremediation of polluted mangrove soil, respectively. However, the untreated (control) was designed to determine the contribution made by indigenous (autochthonous) soil microorganisms and natural attenuation to the soil. The two treatments and the untreated (control) designs were set up in three replicates and kept in the laboratory at room temperature (28±2°C) throughout the investigation periods (6 weeks). The different treatments (sterile and non-sterile poultry wastes and the control) were regularly tilled daily using different hand trowels and watered weekly with 20 ml sterile distilled water during the bioremediation study period. The samples were collected every two weeks for analysis.

2.8 Microbiological and Physicochemical Analyses

The spread plate method on nutrient agar (Antech Laboratories LTD) was used in the enumeration of total heterotrophic bacteria at fourteen days interval. A 10-fold serial dilution of the soil sample was carried out by weighing 1 g

of soil sample into a sterile test tube containing 9 ml of sterile physiological saline. Thereafter, a ten – fold serial dilution was performed to a dilution of 10⁻⁵. From each dilution, 0.1 ml was inoculated on nutrient agar plates. However, a triplicate plating of each dilution was employed. A sterile glass rod was used to spread the inoculums over the media. The plates were incubated at room temperature for 24 hours.

The enumeration of total culturable hydrocarbon utilizing bacteria (HUB) was done using the vapour phase method reported by Hamamura et al. [32] and Ezekoye et al. [30]. Appropriate dilutions of the samples were inoculated into gelled mineral salt agar (MSA). Filter paper (Whatman NO 1) was saturated with bonny light crude oil and the crude oil impregnated papers were placed aseptically onto the covers of Petri dishes and inverted. The hydrocarbon saturated filter papers supply hydrocarbon by vapour phase transfer to the inocula [33,34,35,36]. The plates were incubated at 28°C±2°C for 7 days and colonies were counted from triplicates and mean values were recorded in colony forming units per gramme (Cfu/g). The pH of the samples was determined using a digital pH meter (Jenway 3015, United Kingdom). At each point, three values were obtained and the mean of these values was used. The conductivity of the soil samples was measured with a conductivity meter in triplicate. The conductivity values were measured in µs/cm, and it gives a surrogate value of level of salinity and total dissolved solids, (TDS). The brucine method reported by United Nations Environmental Programs [37] was adopted for the measurement of nitrate content. One millilitre of soil filtrate was measured into a clean test tube and 1ml of distilled water was measured into another test tube as blank solution. Brucine reagent (0.5 ml) was introduced into both test tubes using sterile pipettes. Concentrated sulphuric acid (2 ml) was added and shaken to homogenise the mixture. The resulting solution was allowed to cool to room temperature. The solution was measured at 470 nm on spectrophotometer.

Table 1. Bioremediation design of the study

| Experimental set-up | Text experiment |
|---------------------|---------------------------------------|
| SPW | 500 g of polluted soil + 50 g of PW |
| NSPW | 500 g of polluted soil +50 g of NSPW |
| CTRL | 500 g of polluted soil only (control) |

SPW: Sterile Poultry Waste, NSPW: Non-Sterile Poultry waste, CTRL: Control

Colorimetric method was adopted from the determination of phosphate content as described by United Nations Environmental Programme [37]. One – tenth of 2.5% of glacial acetic acid was prepared and used for the extraction of phosphate in 250ml conical flask. The mixture was stirred for 10 minutes. Fifty millilitres of sample extract was pipetted into a clean conical flask and autoclaved with $K_2S_2O_8$ and H_2SO_4 for 30 minutes at $121^\circ C$. Five millilitres of ammonium molybdate was added to the autoclaved mixture to form heteropoly molybdophosphoric acid and was reduced with stannous chloride in an aqueous sulphuric acid medium, at $30^\circ C$, to form a molybdenum blue complex. The resulting blue colour was measured spectrophotometrically at 660nm and compared to identically prepared standard (water).

Total organic carbon (TOC) was measured using the method of Nelson and Sommers [38]. One gramme of the sample was transferred into a clean Pyrex conical flask. Five millilitres of potassium chromate solution and 7.5 ml concentrated sulphuric acid was added. The mixture was heated on an electro thermal heater for 15 minutes to reflux. The sample was allowed to cool to room temperature and diluted to 100 mls with distilled water. Twenty five millilitres of the sample solution was titrated with 0.2 molar ferrous ammonium sulphate using Ferrion as indicator. A blank containing oxidant (Potassium chromate) and sulphuric acid was titrated as in the sample and the titre value was recorded. The Percentage of TOC was calculated as follows;

$$\% \text{ TOC} = (\text{Titre value of the blank} - \text{sample titre}) \times 0.003 \times 100 / \text{Sample weight}$$

2.9 Chemical Analyses

Total hydrocarbon content (THC) was determined as described by UNEP [37]. Five grammes of soil sample were weighed into a beaker and 10ml of xylen was added under the cork cover for 30 minutes. Aliquot of the extract was placed in an infrared spectrophotometer analyzer. The total hydrocarbon content (THC) value was determined by comparison to a calibration curve constructed from dilutions of a stock solution of 1:1 bonny light crude oil, and bonny medium. The spectrophotometric measurement was done at 420 nm using HACH DR 2400 spectrophotometer.

Total petroleum hydrocarbon (TPH) was determined using the method of Ezekoye et al. [30]. The extraction was done with dichloromethane (DCM) using cold extraction method with ASTM – D- 3694 heavy machine for 1 hour. Twenty grammes of dried soil sample were weighed into 100 mL conical flask. Twenty grammes of activated anhydrous sodium sulphate and 20 mL of DCM were gently added into the barrier containing the test soil sample. This was allowed to stand for 1 hour and then filtered into 50 mL conical flask using filtration plugged/packed with cotton wool. The procedure was repeated on the residual soil until a colourless solution was obtained. The extract was analysed by gas chromatography, using Hp Agilent 6890 gas chromatography (Agilent technologies, 610 Wharfdale Road, Wokingham, Berkshire, United Kingdom) equipped with a FID detector, an agilent 7673 auto sampler and 5 capillary column (15 m x 0.25 mm) with a nominal film thickness of 0.24 μm , split less injection method (all in batch). Injection volume was 1 μl and injection temperature was $33^\circ C$. Helium was used as a carrier gas (2 mL/min). The column was held at $35^\circ C$ for 1.50 min. Real values of TPH were calculated as product of raw data on FID table or graph and dilution factor used for each sample [30].

Calibration of Hp Agilent 6890 gas chromatography (Agilent technologies, Berkshire, United Kingdom) was done using Bonny light crude oil, acetone and mixture of Bonny light crude oil and acetone. The percentage loss in TPH is calculated as follows:

$$\% \text{ loss in TPH} = (\text{Original concentration} - \text{Initial concentration}) \times 100 / \text{Initial concentration}$$

N.B: Original concentration is the concentration of the total petroleum hydrocarbon in the soil (i.e. Concentration at a point) while Initial concentration is the previous concentration of the total petroleum hydrocarbon in the soil.

2.9.1 Gas chromatography flame - ionization detection system

The soil extracts were analysed by gas chromatography, using Hp Agilent 6890 gas chromatography (Agilent technologies, Berkshire, United Kingdom) equipped with a FID detector, an Agilent 7673 auto sampler and 5 capillary

column (15 m x 0.25 nm) with a nominal film thickness of 0.25 μm , split less injection method (all in batch). Injection volume was 1 μl and injection temperature was 330°C. Helium was used as a carrier gas (2 mlmin^{-1}). The column was held at 35°C for 1.50 minutes. The temperature was increased from 15°C/minutes, to 310°C/minutes and held for 10 minutes. This enabled complete run within 27 minutes. The amount of total petroleum hydrocarbon (TPH) was then determined as a sum total of resolved and unresolved components eluted from the GC capillary column between retention times of 5 minutes to 35 minutes. This method called peak sum calculates TPH by summing up all components of crude oil from C₁₀ and upwards. Real values of TPH were calculated as product of raw data on FID table or graph and dilution factor used for each sample.

2.10 Statistical Analysis

Statistical analyses were carried out using Statistical package for Social Sciences (SPSS, Version 17.0). Analysis of variance (ANOVA) was carried out at 95% level of confidence using statistical package for social sciences.

3. RESULTS

The physicochemical properties and microbial load of the mangrove soil and poultry wastes used as organic nutrient in the bioremediation of hydrocarbon polluted mangrove soil are as shown in Tables 2 and 3, respectively.

Table 3 shows the baseline composition of the poultry wastes. These have shown that the

poultry wastes are alkaline, contain nutrients, high conductivity, bacterial populations and as well polluted with hydrocarbon which may be insignificant.

The poultry wastes contain considerable adequate amounts of phosphate and nitrate (Table 3) which are limiting nutrients that are essential for microbial growth as well as adequate population of hydrocarbon utilizing bacteria ($4.65 \pm 0.58 \text{ Log}_{10}\text{Cfu/ml}$). These hydrocarbon utilizers were presumptively identified to be *Pseudomonas* spp., *Bacillus* spp., *Escherichia* spp., and *Salmonella* spp. (Table 4).

Table 4 shows the biochemical characteristics of bacteria populations that were isolated from the poultry wastes capable of using hydrocarbon as sole source of carbon. They are mostly Gram Positive and Negative rods.

Biodegradation of petroleum hydrocarbon in the mangrove polluted soil ecosystems were monitored by periodic evaluation of changes in total heterotrophic bacterial populations and reduction in total petroleum hydrocarbon (TPH) concentration. Figs. 2 and 3 presents the changes in logarithmic THBC and THUBC of hydrocarbon polluted mangrove swamp soil. In Fig. 2 there was a progressive increase in total heterotrophic bacterial populations in the treatment options and in the control. Similarly in Fig. 3 the total hydrocarbon utilizing bacterial population increases progressively in the treatment options and the control but there was a sharp decrease after 28 days bioremediation study which might be attributed to limited nutrients.

Table 2. Physicochemical and microbiological properties of hydrocarbon impacted Mangrove soil

| Parameter | Baseline | Hydrocarbon polluted soil |
|--|------------------|---------------------------|
| pH | 3.69 \pm 0.58 | 7.31 \pm 0.58 |
| Moisture | 26.3 \pm 0.06 | 35.0 \pm 0.06 |
| Conductivity ($\mu\text{s/cm}$) | 3,520 \pm 0.58 | 6240 \pm 0.58 |
| Nitrate (mg/kg) | 4.7 \pm 0.58 | 4.6 \pm 0.06 |
| Phosphate (mg/kg) | 31.1 \pm 0.58 | 30.5 \pm 0.06 |
| THC (mg/kg) | 49.5 \pm 0.06 | 2000.45 \pm 0.58 |
| THBC ($\text{Log}_{10}\text{Cfu/ml}$) | 5.36 \pm 0.58 | 5.31 \pm 0.58 |
| THUBC ($\text{Log}_{10}\text{Cfu/ml}$) | 5.18 \pm 0.58 | 4.65 \pm 0.58 |

THBC: Total Heterotrophic Bacterial Count; THUBC: Total Hydrocarbon Utilizing Bacteria Count, $\text{Log}_{10}\text{Cfu/ml}$: Logarithmic Colony Forming Unit

Table 3. Baseline physicochemical composition of poultry wastes

| Parameter | Poultry wastes |
|----------------------------------|----------------|
| pH | 8.4±0.06 |
| Moisture | 5.76±0.58 |
| Conductivity (µs/cm) | 15760±0.58 |
| Nitrate (mg/kg) | 3.20±0.58 |
| Phosphate (mg/kg) | 103.13±0.58 |
| THC (mg/kg) | 26.94±0.58 |
| THBC (Log ₁₀ Cfu/ml) | 5.31±0.58 |
| THUBC (Log ₁₀ Cfu/ml) | 4.65±0.58 |

There was an initial decrease in total heterotrophic bacterial count (THBC) and Total hydrocarbon utilizing Bacteria from 5.36±0.58 Log₁₀Cfu/ml to 5.31±0.58 Log₁₀Cfu/ml and from 5.18±0.58 Log₁₀Cfu/ml to 4.65±0.58 Log₁₀Cfu/ml, respectively, between the baseline and Hydrocarbon polluted soil (Table 2); thus showing the toxic effect of the petroleum hydrocarbon on the indigenous microorganisms. However, there was a subsequent increase in bacterial population (THBC and HUBC) which was highly significant in NSPW followed by SPW supplemented with non-sterile and sterile poultry

wastes compared with the control (CTRL) that is not supplemented with poultry wastes which results in progressive decrease of the HUB at day 28 due to lack of limiting nutrients (Fig. 3). The total petroleum hydrocarbon (TPH) decreased progressively from 1540.36±36 ppm and 1049±.68±0.01 ppm to 498.14±0.01ppm and 389.42±0.01 ppm in SPW and NSPW, respectively, while in the corresponding control (CTRL), it decreased from 1540.36±0.01 ppm to 1087.00±0.01 ppm during the study period. There was a significant mean difference between SPW, NSPW and the CTRL ($p < 0.05$) (Fig. 4).

4. DISCUSSION

Biostimulation is the most frequent used bioremediation technique as the contaminant introduces enormous amount of carbon source which tends to result in rapid depletion of the available nitrogen and phosphorus which are essential for microbial growth [39]. In view of this, we investigated the effects of poultry wastes (SPW and NSPW) on bacterial degradation of petroleum hydrocarbon in soil.

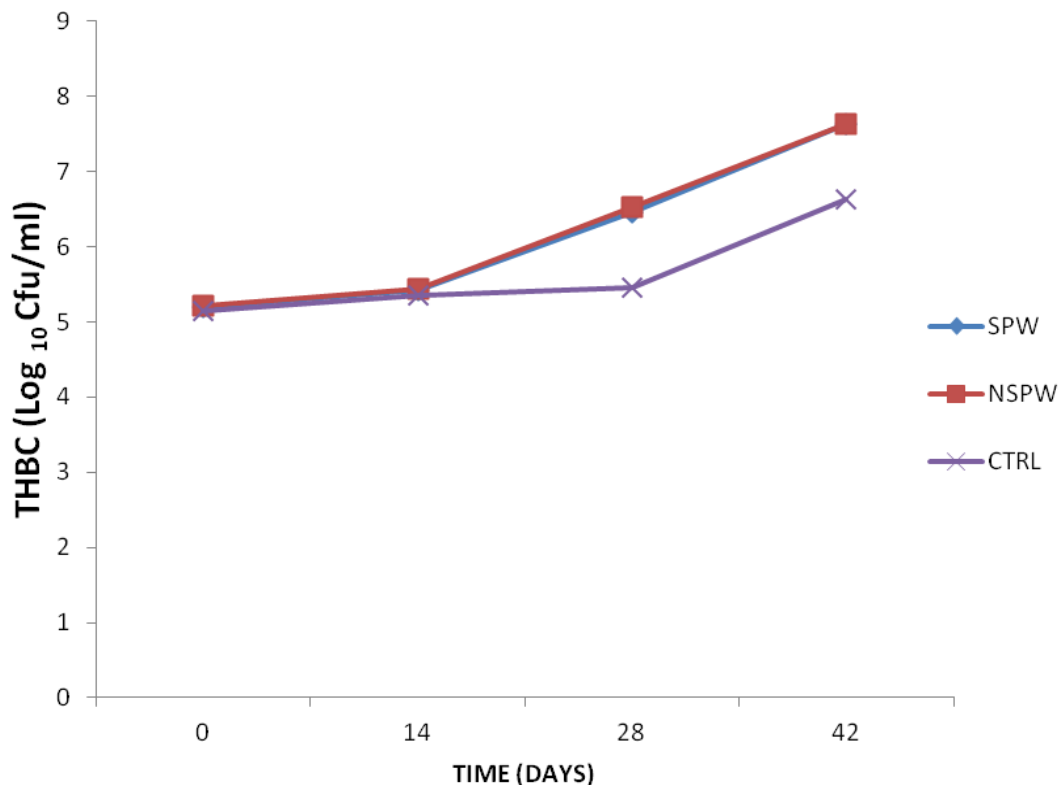


Fig. 2. Changes in total culturable heterotrophic bacterial count (THBC) of hydrocarbon polluted soil during the 42 day bioremediation

SPW: Polluted soil + sterile poultry wastes, NSPW: Polluted soil + non-sterile poultry wastes, NPK: Polluted soil + NPK, CTRL: Control

Table 4. Biochemical characteristics of hydrocarbon utilizing bacteria isolates from poultry wastes

| S/No | Isolate No | Morphology and gram reaction | Motility | Citrate | Catalase | Indole | Methyl red | Voges Proskauer | Starch hydrolysis | H ₂ S production | Oxidase | Glucose | Lactose | Mannitol | Sucrose | Most probable bacteria |
|------|-------------------|------------------------------|----------|---------|----------|--------|------------|-----------------|-------------------|-----------------------------|---------|---------|---------|----------|---------|------------------------|
| 1. | HUB ₁ | Rods- | + | V | + | - | - | - | - | - | + | +G | + | V | + | <i>Pseudomonas</i> sp. |
| 2. | HUB ₂ | Rods- | + | V | + | - | - | - | - | - | + | +G | + | V | + | <i>Pseudomonas</i> sp. |
| 3. | HUB ₃ | Rods+ | + | + | + | - | V+ | + | + | - | - | + | + | + | - | <i>Bacillus</i> sp. |
| 4. | HUB ₄ | Rods+ | + | + | + | - | V+ | + | + | - | - | + | + | + | - | <i>Bacillus</i> sp. |
| 5. | HUB ₅ | Rods+ | + | + | + | - | V+ | + | - | - | - | + | + | + | - | <i>Bacillus</i> sp. |
| 6. | HUB ₆ | Rods- | + | - | + | + | + | - | + | - | + | + | + | + | + | <i>Escherichia</i> sp. |
| 7. | HUB ₇ | Rods- | + | - | + | + | + | - | + | - | + | + | + | + | + | <i>Escherichia</i> sp. |
| 8. | HUB ₈ | Rods- | + | - | + | - | + | - | - | + | + | + | - | + | - | <i>Salmonella</i> sp |
| 9. | HUB ₉ | Rods- | + | - | + | - | - | + | + | + | - | + | - | + | - | <i>Salmonella</i> sp |
| 10. | HUB ₁₀ | Rods- | + | - | + | + | + | - | + | - | + | +G | + | + | + | <i>Escherichia</i> sp. |

HUB: Hydrocarbon utilizing bacteria; +G: Positive with gas production positive; - Negative and + Positive

In Table 2 the pH of the baseline study of the soil was acidic and after spiking of the soil with 50mls of crude oil, the soil acidity was raised to alkaline by addition of limewater. Also there was decrease in the nutrient composition and bacterial populations while the total hydrocarbon content, moisture content and conductivity increases. The high pH in the poultry wastes also help to maintain the alkalinity of the polluted soil during the study period. This rise in pH of the treatments soil may favour crude oil degradation by micro – organisms as observed in similar studies that higher pH range (6 – 9) provides better conditions for mineralization of hydrocarbons since most bacteria capable of metabolizing hydrocarbons developed best at pH conditions close to neutrality [40].

The baseline physico-chemical composition of poultry wastes as revealed in Table 3 showed that there are essential nutrients in poultry wastes especially nitrate and phosphate necessary for microbial growth. This is in agreement with the work of Umanu and Babade [29] who documented the presence of nitrate and phosphate in poultry wastes.

The findings obtained from the study showed that biostimulation of hydrocarbon polluted mangrove soil with nutrient amendments (poultry wastes) induced an increase in the bacterial community with concomitant degradation of the hydrocarbons. This was in – line with the work done by Ohiri et al. [36]. They found that nutrient availability and the presence of high microbial population in poultry droppings increases the percentage remediation of both aliphatic and polycyclic aromatic hydrocarbon. In this study hydrocarbon utilizers maintained a steady rise in counts in the biostimulated treatments throughout the experimental period. The control experimental set-up also maintained a steady but slow increase in counts within 14th day till 28th day of the experiment before it begins to decrease progressively. Fig. 3 revealed a steady increase in the population density of hydrocarbon utilizing bacteria especially in the treatments, thus the higher reduction in petroleum hydrocarbon observed in the treatments especially those amended with NSPW as compared to the control. The same trend was observed by Calomiris et al. [41]; Nwachukwu [42] and Umanu and Babade [29], who reported that there is always an increase in the population density of hydrocarbon utilizers in the

ecosystems exposed to crude petroleum and petroleum products. Also the populations densities of hydrocarbon utilizers present in treatments fortified with NSPW were higher and significantly different when compared with the treatments amended with SPW and the control (without amendment). This finding is in accordance with the reports by Obire et al. [43] and Umanu and Nwachukwu [44]. Increases in bacterial counts (for both TCHB and TCHUB) in crude-oil polluted soil amended with organic nutrient sources have been reported by other researchers. Roling et al. [45] examined bacterial dynamics and crude oil degradation after biostimulation and found that nutrient enhancement increased bacterial counts which correlated significantly with hydrocarbon attenuation. A similar observation has been reported by other workers [46,47,48,49,50,51].

The results showed that there was a marked significant decrease in Total petroleum hydrocarbon (TPH) of the treatments amended with SPW and NSPW relative to the control. Highest loss of total petroleum hydrocarbon was evident in NSPW followed by SPW treatment when compared with the control (Fig. 4). This reduction in the TPH of the treatments amended soils is in line with the reports of Obasi et al. [52] who observed highest significant loss of TPH in treatments amended with Poultry manure and Cow dung (PM + CM) followed by Poultry manure (PM) treatment.

The baseline characteristics of the polluted mangrove soil as shown in Table 2 showed that logarithmic total culturable heterotrophic bacterial count and total culturable hydrocarbon utilizing bacterial count in the mangrove polluted soil were 5.36 ± 0.58 Cfu/ml and 5.18 ± 0.58 Cfu/ml, respectively. This indicates that the hydrocarbon utilizing bacteria in the mangrove soil was relatively adequate for bioremediation. This observation was in-line with Ebuehi et al. [53]. The amount of limiting nutrients such as nitrogen and phosphorus present in this polluted soil was very low. Managing poultry wastes from poultry farms is a common problem in developing countries like Nigeria. Putting this waste into effective use such as in bioremediation should be a welcome development in Sub-Saharan Africa and other parts of the world where waste management has been limited by resources [54].

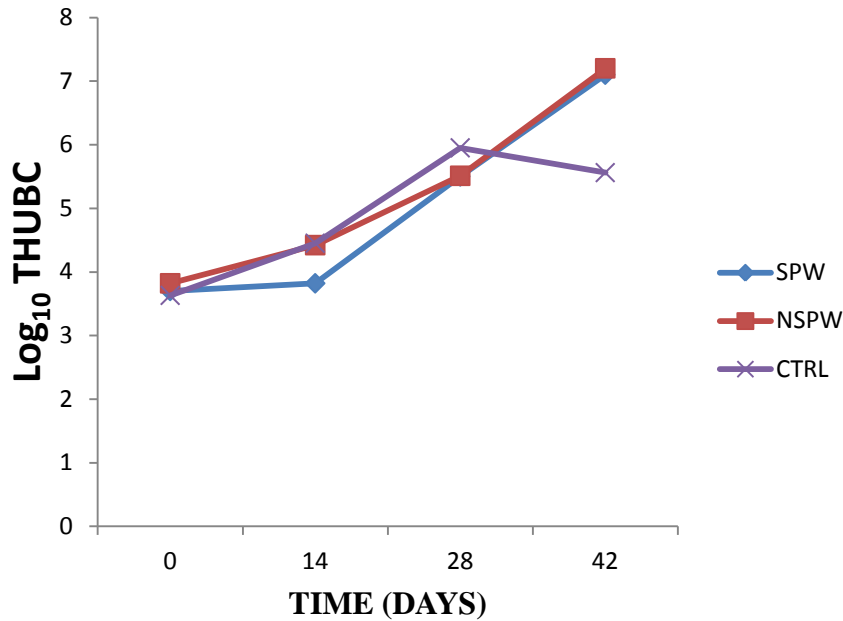


Fig. 3. Changes in the total culturable hydrocarbon utilizing bacterial count during the 42 day bioremediation

Legend: SPW: Polluted soil + sterile poultry wastes, NSPW: Polluted soil + non-sterile poultry wastes, NPK: Polluted soil + NPK, CTRL: Control

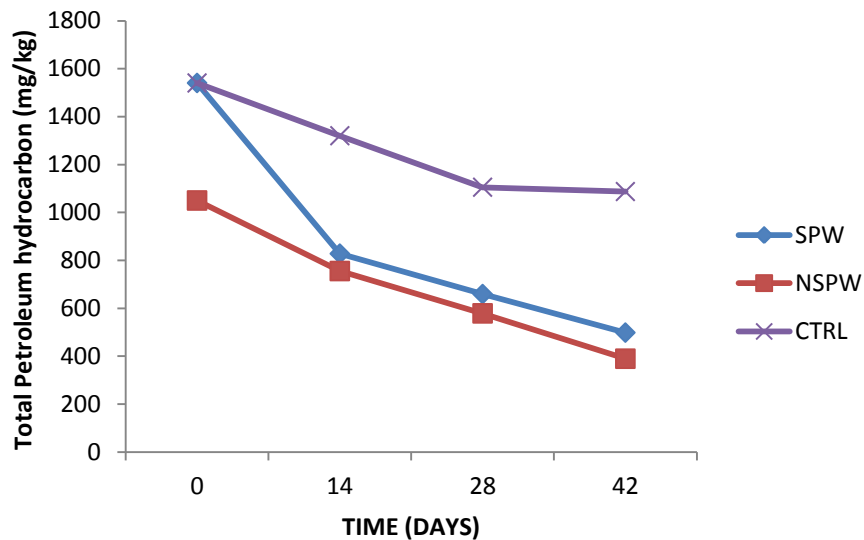


Fig. 4. Changes in Total Petroleum Hydrocarbon (TPH) of hydrocarbon polluted soil during the 42 day bioremediation

SPW: Polluted soil + sterile poultry wastes, NSPW: Polluted soil + non-sterile poultry wastes, NPK: Polluted soil + NPK, CTRL: Control

5. CONCLUSION

This study has shown that proper use of poultry wastes especially non-sterile poultry waste in bioremediation can effectively and efficiently enhance removal of petroleum from polluted site

as it contains essential nutrients such as nitrate and phosphate needed for microbial growth and metabolism as well as significant population of hydrocarbon utilizing bacteria. Furthermore, pilot study using these inorganic nutrients should be recommended.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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