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Exploring the Potentials of Nipa Palm (*Nypa fruticans*) Ash and Rabbit Droppings for Enhanced *Ex situ* Bioremediation of Crude Oil Contaminated Soil

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Authors' contributions

This paper was carried out by all authors. Author LS conceived the idea, collected the samples, carried out the laboratory analysis and wrote the initial draft of the manuscript. Author CJO wrote the second draft and proof-read the manuscript. Author CMA edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Crude oil exploration, production and refinement in the Niger Delta region of Nigeria have resulted in the contamination of soil. The study was aimed at exploring the potential of *Nypa fruticans* ash (NFA), and rabbit droppings (RD) as biostimulants for enhanced *ex-situ* bioremediation of crude oil contaminated soil (COCS) in Yorla. A microcosm was set up in three sets of containers, each having a surface area of 328 cm^2 and a volume of 1651 cm^3. Exactly 300 g COCS was weighed into each container (Sets A-C). SetA contained 150 g RD; SetB had 150 g NFA while SetC was not amended to serve as a control (CT). Monitoring was done for 5weeks (0, 1, 2, 3 and 4) with all parameters measured at an interval of 1week. SetA had hydrocarbon utilizing bacterial (HUB) and hydrocarbon utilizing fungal (HUF) counts reduced from $1.76x10^4$ to $0.55x10^3$ CFU/g and $1.43x10^3$ to $0.32x10^2$ CFU/g respectively after four weeks, while SetB had HUB reduced from $1.76x10^5$ to

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$1.42x10^4$ CFU/g and HUF reduced from $1.43x10^3$ to $0.51x10^3$ CFU/g by week 4. In SetA, total petroleum hydrocarbon (TPH) reduced by 57.9% while in SetB, it reduced by 39.6% and SetC by 0.59%. TPH reduced significantly (p<0.05) by week 5 in the order: RD>NFA>CT. Bacteria isolated included *Pseudomonas fluorescens, Micrococcus roseus, Escherichia coli* and *Bacillussubtilis* while the fungi counterpart were *Aspergillus* sp., *Candida lipolytica*, *Penicillium* sp.and *Rhizopus* sp. Reductions in physicochemical parameters could be due to their utilization by oil degraders. Next line of action will be to apply this technology *in situ* for enhanced remediation of COCS.

Keywords: Bioremediation; crude oil contaminated soil; Nypa fruticans ash; rabbit droppings.

1. INTRODUCTION

Crude oil pollution of agricultural soil is currently gaining research attention owing to the importance of soil resource in food production and healthy citizenry across the globe. The numerous hydrocarbons and chemicals present in crude oil represent a carcinogenic risk [1,2,3]. The negative impacts of crude oil pollution on environmental media include environmental degradation, poverty and depletion of natural resource base as well as health effect [4].

It appeared that the present generation is compromising the ability of the future generations to meet their needs by embarking on unsustainable environmental practices that are detrimental to the ecotype. Joint actions of the multi-national oil companies, the local communities and responsible partners in oil business are essential for effective policy implementation and sustainable development of the oil-producing region of the world [5,6,7]. Crude oil is by their nature biodegradable and to prevent significant health risks and further loss of biodiversity, enhanced bioremediation methods are necessary [8,9,10].

Enhanced bioremediation seeks to develop and apply a planned approach that removes, destroys, contains or otherwise reduce the availability of contaminants to people and the environment within a short period [11,12]. Using this method instead of excavation and mass change breaks down the contaminants into CO_2 and H_2O and hence, could reduce the emission of CO_2 during remediation [13,14,15,16]. The long-term aim of enhanced bioremediation technologies is to present a safe, ecologically friendly and cost-effective designs which reduce the pollutant level to a level referred to as: "As Low as Reasonable and Practicably Possible (ALARP)".

1.1 Significance of the Study

Several studies showed that over thirty craft items with materials sourced from nipa palm had been designed and perfected in Nigeria [17]. There is little or no report on its use in the clean-up of crude oil polluted soil. Rabbit droppings on the other hands; have been known to pose no threat to soil ecosystem, with little report on its use in bioremediation [18]. The present research, therefore, seeks to investigate the contribution and applicability of Nipa palm ash and rabbit droppings in enhancing the microbial activities during mineralization of hydrocarbons in crude oil contaminated agricultural soil by oil-degrading microbes.

2. MATERIALS AND METHODS

2.1 Study Location

The study site was Yorla farmland in Kpean community (Fig. 1) in Khana Local Government Area of Rivers State, Nigeria. The topsoil (15cm depth) was sampled using a manual soil auger into a clean polythene bag and transported to the laboratory for physicochemical, gas chromatographic and microbiological examination.

Nipa palm (*Nypa fruticans)*, an invasive species commonly found in the Niger Delta region, poses a severe threat to mangroves biodiversity in the coastal states of Nigeria and should be tackled before these large productive wetlands and tidal mudflats are irreversibly damaged. Nipa palm was collected from a wetland soil at Inter Wogba creek, Port Harcourt and identified at the University of Port Harcourt reference herbarium.

The palm bunch was dehusked, crushed and dried in oven (GallenKamp BS, 250, England) at 60^0C for 5days. Rabbit droppings were obtained in clean polythene bag from the Faculty of Agriculture Demonstration Farm (FADF) in the University of Port Harcourt. It was composted for 2 weeks to reduce its pathogenic impact on the environment [19].

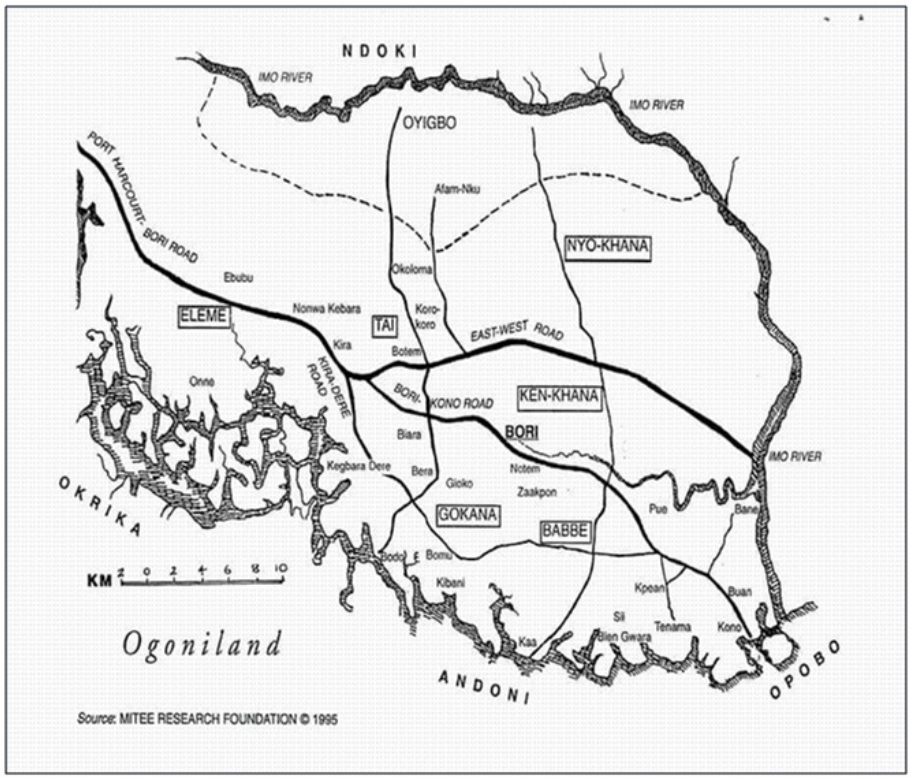

Fig. 1. Map showing study location (Yorla, Kpean) in Khana Local Government Area

2.2 Microcosm Description

Three hundred grams (300 g) of COCS were weighed and placed in three set of containers (SetA, SetB and SetC), each having a volume of 1651 cm^3 and a surface area of 328 cm^2. Sets A and B were amended with 150g of NFA and RD respectively while Set C was not amended to serve as control (CT). Monitoring was done for 5weeks (0, 1, 2, 3 and 4) with all parameters measured at interval of 1week. All treatments were moistened by sprinkling a moderate quantity of distilled water after an interval of two days and tilled to 15 cm depth to mix nutrients with polluted soil and enhance aeration and optimum microbial metabolism of the crude oil contaminants.

2.3 Enumeration of Total Culturable Heterotrophic Bacteria and Total Culturable Heterotrophic Fungi

The total heterotrophic bacterial count was determined using spread plate method on nutrient agar (oxoid) as described by Sylvia et al. [20]. A serial ten-fold dilution was prepared using 1g of soil and 10^{-4}—10^{-5} dilutions were spread-plated on media in triplicates. Culture plates were then incubated at 28±2℃ for 24 h. The same procedure was used for hydrocarbon utilizing fungi except that 1ml of lactic acid was added to Sabouraud dextrose agar (SDA, Antech) to inhibit the growth of bacteria. The fungal isolates were identified according to methods previously described [21 and 22].

2.4 Enumeration of Hydrocarbon Utilizing Bacteria and Fungi

Soil slurry was prepared by mixing 1g of wet soil with 9ml of sterile physiological saline. The hydrocarbon utilizing bacteria (HUB) were enumerated following the method of Hamamura et al. [23]. Mineral salts medium of Mill et al. [24] was used and crude oil was supplied by the vapour phase transfer method. For hydrocarbon utilizing fungi enumeration, the same procedure for total fungi was followed except that lactic acid was added to inhibit the growth of bacteria.

Discrete colonies that developed on media were purified by sub-culturing and identified based on microscopic, colonial morphology and biochemical characteristics with reference to

Bergey and Holt [25] and Cheesbrough [26]. Each purified isolate was placed on a clean and grease free slide and a drop of lactophenol was added. Slides were covered with coverslip and observed under x10 and x40 objective lenses.

2.5 Determination of Physicochemical Parameters

Various parameters (pH, phosphate content, nitrate content, moisture content, and total organic carbon) were analyzed in polluted soil during the five weeks study period at 1week intervals. All parameters were determined using standard laboratory procedures as described by the American Society for Testing and Material [27].

2.6 Extraction and Gas Chromatography

Each soil sample for gas chromatographic analysis was extracted with methylene chloride and an aliquot of the extract injected into a gas chromatograph (HP 5890, Hewlett Packard, Avondale, PA, USA) equipped with a flame ionization detector (FID). The extractable TPH has quantified accordingly (27). Percentage degradation was calculated as:

$$\%\ degradation = \frac{CTPH - TTPH}{CTPH} x100$$

Where,

CTPH is the weight of total petroleum hydrocarbons of the control and TTPH is the weight of total petroleum hydrocarbons (TPH) of the treatment.

2.7 Statistical Analysis of Data

Data obtained were subjected to statistical analysis to determine the significant difference among the data collected using the students' "t" test. A value of (p<0.05) was considered significant.

3. RESULTS AND DISCUSSION

The amount of total petroleum hydrocarbon (TPH) in the crude oil-contaminated soil before the addition of the NFA and RD as amendments was 97459 mg/kg. The baseline data was above the intervention TPH value of 5000 mg/kg [28]. The amount of nitrogen, phosphorus and potassium in the amendments (composted NFA and RD) are presented in Table 1. The results indicated that the amendments contain a significant amount of limiting nutrients needed to stimulate the microbes in utilizing the crude oil.

Figs. 2–5 show the changes in the microbial population of the different physiological groups during the 35 day enhanced *ex-situ* bioremediation of crude oil-contaminated soil (COCS) by supplementation with rabbit droppings (RD) and *Nypa fruticans* ash (NFA). Fig. 2 shows the changes in total heterotrophic bacterial counts (THBC) of COCS-amended with RD (SetA) and NFA (SetB) during the study period while Fig. 3 shows the changes in hydrocarbon utilizing bacterial counts (HUBC) of COCS-amended with RD and NFA during the study period and Fig. 3 indicated the changes in total fungal counts (TFC) of COCS-amended with RD and NFA during the study period.

Fig. 5 shows the changes in hydrocarbon utilizing fungal counts (HUFC) of COCS-amended with RD and NFA during the study period. In the control, the total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) counts increased from $1.98x10^2$ CFU/g in week zero to $1.93x10^3$ CFU/g and $1.76x10^4$ to $1.96x10^5$ CFU/g after week 4; while the total heterotrophic fungi (THF) and hydrocarbon utilizing fungi (HUF) counts increased from $1.54x10^4$ to $1.62x10^4$ CFU/g and $1.43x10^3$ to $1.51x10^3$ CFU/g respectively.

Table 1.Nutrients in the Soil before Addition of Organic Waste

Sample Code	Organic Carbon (mg/kg)	Nitrogen (mg/kg)	C/N Ratio	Organic matter (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)
NFA	1.23	2.13	0.58	2.07	2.44	1.342
RD	1.82	3.47	0.53	3.62	3.91	2.43

Key: NFA: Nipa fruticans ash, RD: Rabbit droppings

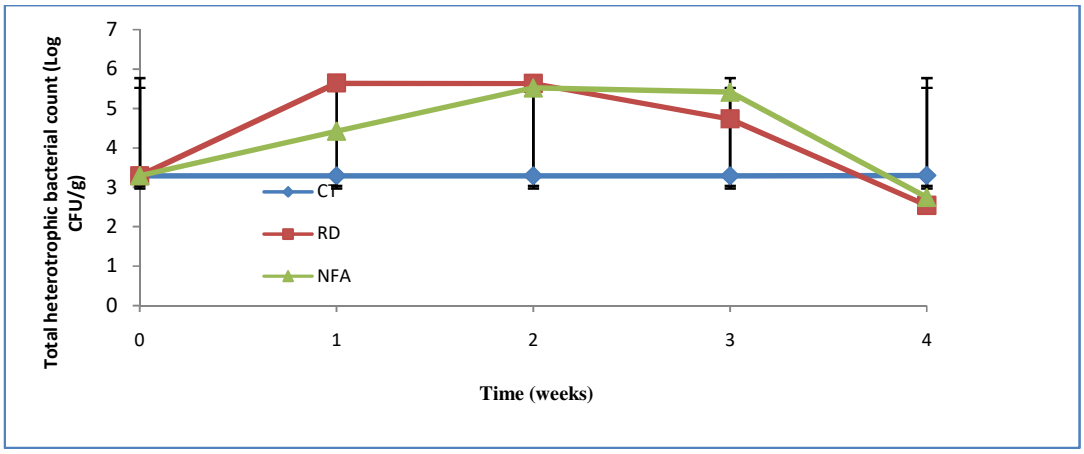

Fig. 2. Changes in total heterotrophic bacterial counts of COCS amended with RD and NFA during the study period

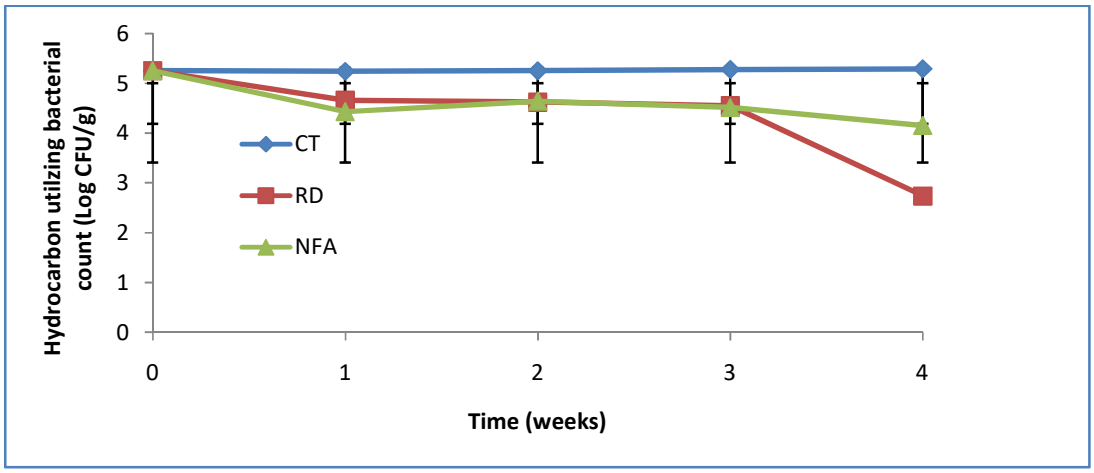

Fig. 3. Changes in hydrocarbon utilizing bacterial counts of COCS amended with RD and NFA during the study period

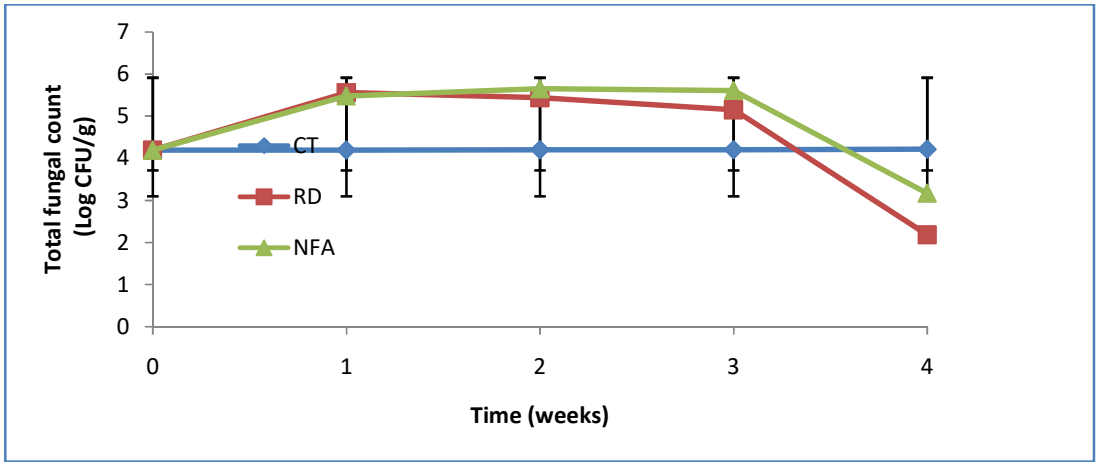

Fig. 4. Changes in total heterotrophic fungal counts (TFC) of COCS amended with RD and NFA during the study period

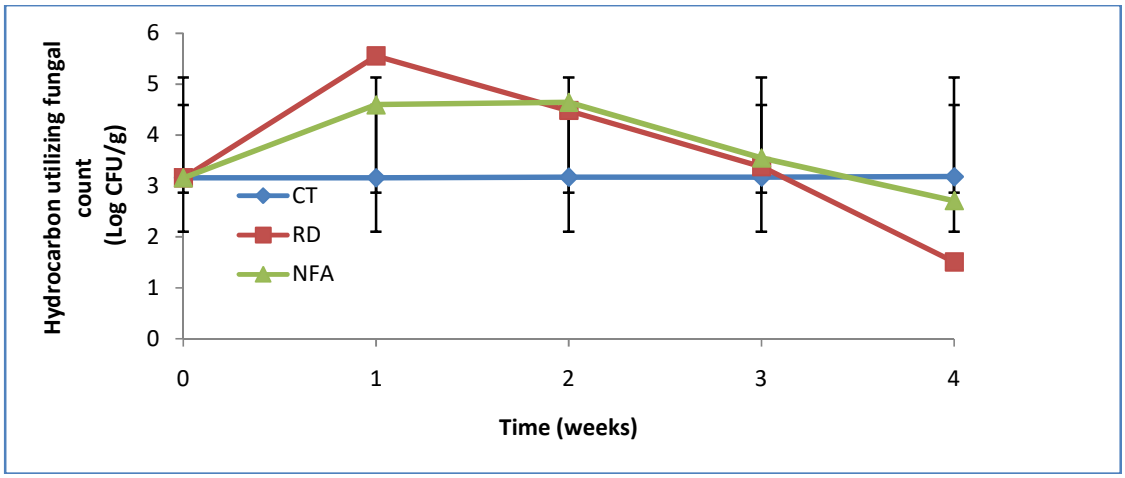

Fig. 5. Changes in hydrocarbon utilizing fungal counts of COCS amended with RD and NFA during the study period

There was a significant (p<0.05) increase in the microbial numbers within the second and third week of the study. The decrease could be attributable to nutrient depletion in the bioremediation set-ups. The positive effects of organic nutrient supplementation on enhanced bioremediation of crude oil impacted media have been reported [29,30] and [31] and these collaborate with the present study.

In the RD amended treatment (SetA); there was an increase in THB counts from $1.93x10^3$ CFU/g to $4.25x10^5$ CFU/g by week 3. Subsequently, a decrease in THB counts was obtained from $4.25x10^5$ CFU/g in week 3 to $0.35x10^3$ CFU/g in week 4. Likewise, the HUB counts increased from $1.76x10^4$ CFU/g at the outset to $4.13x10^4$ CFU/g by week 3 and later dropped to $1.42x10^4$ CFU/g by week the 4th week. By the 4th week, the soil nutrients may have been over-utilized, resulting in the decline in microbial population. The increased fungi population was also indicative of the positive effect of RD on biodegradation rate.

Thus, the THF and HUF counts increased from $1.54x10^4$ and $1.43x10^3$ CFU/g from the beginning of the experiment to $2.75x10^5$ and $3.05x10^4$ CFU/g by week 3 of the bioremediation study. Following week 3, there was a decrease in the THF and HUF counts to $0.15x10^3$ and 0.32 $x10^2$ CFU/g by the 4th week. These microbes have been known to be efficient in utilizing the residual crude oil as a source of carbon [32] and [33]. The NFA-amended set-up indicated that the THB and HUB counts increased from1.93 $x10^3$ and 1.76 $x10^5$ CFU/g respectively from the beginning to 3.32 $x10^5$ and $4.35x10^5$ CFU/g respectively by week 3. After that, a decrease in THB and HUB counts from $3.32x10^5$ and $4.35x10^5$ CFU/g to 0.55 $x10^3$ and $1.42x10^4$ CFU/g was obtained respectively by wk4 of the study.

The THF and HUF counts also increased drastically from 1.54 $x10^4$ and 1.43 $x10^3$ CFU/g to 4.43 $x10^5$ and $4.32x10^4$ CFU/g by week 3. Subsequently, the THF and HUF counts decreased to $1.49x10^3$ and 0.51 $x10^3$ CFU/g by the 4th week. The data obtained from the two treatments indicated that the microorganisms degrading the crude oil within the second and third weeks of bioremediation produced significant (p<0.05) results during the study period.

Hydrocarbon utilizing bacteria isolated were identified to the generic level. These hydrocarbonoclastic microorganisms included *Corynebacterium* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Achromobacter* sp., *Klebsiella* sp., *Serratia* sp., *Bacillus* sp., *Proteus* sp., *Micrococcus* sp., *Clostridium* sp., *Acinetobacter* sp., *Flavobacterium* sp., *Citrobacter* sp. and *Alcaligenes* sp. Their presence in polluted soil has been reported as being responsible for the development of adaptive features such as plasmid which support hydrocarbon co-metabolism [34].

Indigenous microorganisms are well adapted to their own environment. The present study shows that these isolates have the advantages of being well-adapted to the crude oil contaminated environment, leading to efficient biodegradation oil contaminants.

The hydrocarbon utilizing fungal isolates obtained belonged to the genera: *Candida* sp., *Rhodotorula* sp., *Saccharomyces* sp., *Trichosporium* sp., *Rhizopus* sp., *Microsporium* sp., *Geotrichum* sp., *Fusarium* sp.,*Cladosporium* sp., *Cephalosorium* sp., *Monosporium* sp., *Neurospora*sp., *Aspergillus* sp., *Penicillium* sp. and *Mucor* sp..

These genera have also been isolated from other workers [21,35] in Nigeria. In our present study, the fungal isolates were predominantly in the rabbit droppings (RD) amended treatment which also gave the highest rate (57.9%) of TPH biodegradation. Figs. 6 and 7 show the ratio of total heterotrophic bacterial to hydrocarbon utilizing bacterial (THB/HUB) counts and that of total fungal (THF) to hydrocarbon utilizing fungal (HUF) counts in the SetA, SetB and SetC (control experiment). The control (CT) set-up had THB/HUB ratio of 0.75 and THF/HUF ratio of 0.94 after the 4th week. In the RD treatment, the THB/HUB ratio was 2.45 while the THF/HUF gave 2.32, respectively.

The THB/HUB ratio obtained after the 4th week in NFA amended treatment was 0.98 while the THF/HUF was 1.06. An increase in the population density of microorganisms in crude oil contaminated media has been known to ensure rapid degradation of the pollutant [10,36] and [37].

In the present study, the different physiological groups increased both in number and population and that could be responsible for the reduction in TPH in the COCS. This indicated that the autochthonous microorganisms in the crude oil polluted soil environment have an efficient ability in utilizing the residual crude oil in the soil as their sole source of carbon.

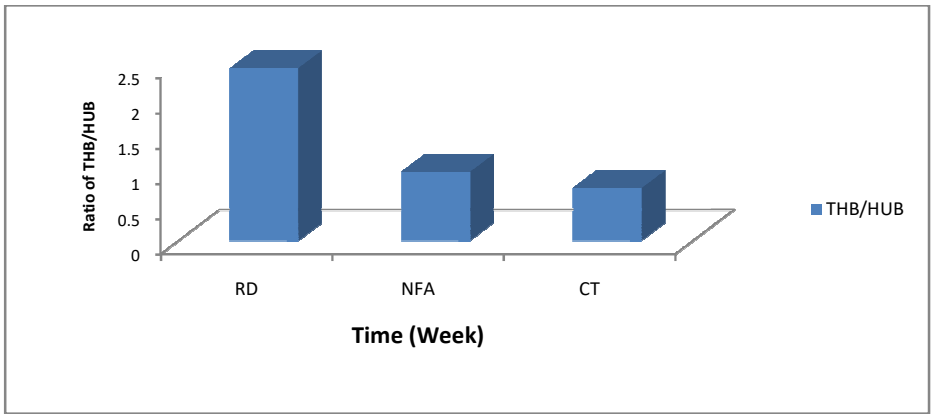

Fig. 6. Ratio of total heterotrophic bacteria (THB) to hydrocarbon utilizing bacteria (HUB) count during the study period

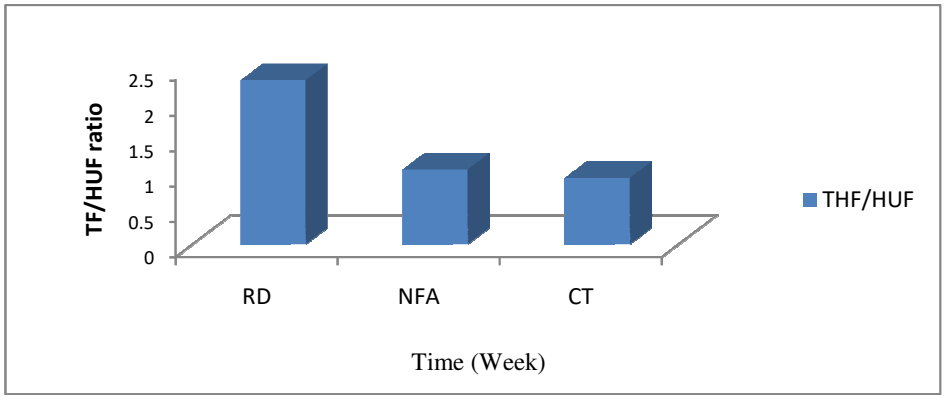

Fig. 7. Ratio of total heterotrophic fungal (TF) to hydrocarbon utilizing fungal (HUF) counts during the study period

Fig. 8 shows the reduction in total petroleum hydrocarbon (TPH) in COCS amended with RD and NFA, including the control (CT). In the CT experiment (SetC), the TPH value of 6706.76280 ppm at 15cm depth was reduced to 6666.7628 ppm after four weeks of bioremediation study. This represents 0.59% TPH loss to the environment.

The probable minimal reduction in TPH in the un-amended COCS could be attributable to natural attenuation processes of evaporation, volatilization, spreading, biodegradation and photo-oxidation. The loss of TPH due to natural attenuation at various time intervals have been reported [9,38,39]. The TPH in COCS amended with the RD decreased steadily throughout the study period from 6706.76280 ppm at the beginning of the experiment to 2818.42039 ppm by week 4. The data obtained indicated a drop in TPH of 57.9% in RD set-up after four weeks and there were significant differences (p<0.05) in the TPH values obtained during the weekly intervals.

The NFA amended treatment had TPH reduced from 6706.76280 ppm at the beginning to 4054.55278 ppm by week 4, representing 39.6 % TPH loss from soil environment. The results obtained indicated that rabbit dropping is more efficient in enhancing biostimulation of hydrocarbonoclastic microorganisms in mineralizing the total petroleum hydrocarbon in crude oil-contaminated soil than Nipa palm (*Nypa fruticans)* ash. The order of biodegradation efficiency of the two supplements (biostimulants) when compared with the control set-up is given as RM>NFA>CT.

This loses in TPH from oil-contaminated soil have been attributable to the biodegradability of the residual crude in the contaminated soil by indigenous microbes in the soil and our findings collaborated with similar trends [40,41,42,43,44] and [45].

Figs. 9—11 show the chromatograms obtained for the un-amended (CT) and amended (RD and NFA) COCS. Fig. 9 is the chromatogram of the control (CT), indicating the extent of the different carbon fractions of the crude oil after week zero (a) and week 4 (b) of the experiment while Fig.10 shows the chromatogram of COCS amended with RD showing the extent of the different carbon fractions of the crude oil after weeks 1 (a), 2 (b), 3(c) and 4 (d).

Furthermore, Fig.11 is the chromatogram of COCS amended with NFA showing the extent of the different carbon fractions of the crude oil after weeks 1(a), 2(b), 3(c) and 4(d) of treatment. In all the treatments, there was a reduction in the levels of the light and heavy fractions of the crude oil after four weeks, as shown in the chromatograms. The C_1—C_{16} fractions were non-existent in the COCS soil while the heavier fractions (C_{17}—C_{40}) predominated. However, the two treatments were able to reduce the C_{17}—C_{40} fractions appreciably though, at different rates.

The highest reduction in the fractions was obtained with the RD-amended COCS followed by the NFA amended COCS which indicated a slight reduction after four weeks. The more mobile and volatile fractions were easily degradable and removed from the soil. Studies have shown that the remaining components, which were not readily degraded, can still pose a high risk to microbial population and to the immediate vicinity of the area in which they remain or persisted [37] and [45].

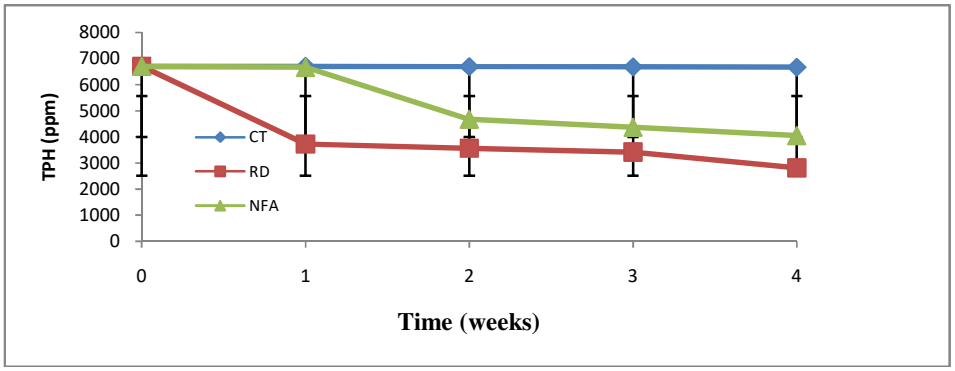

Fig. 8. Changes in total petroleum hydrocarbon (TPH) content of COCS amended with RD, NFA during the study period

Figs. 12–16 show the results of other physicochemical parameters analyzed in the CT, RD and NFA-amended treatments during the study period. In the control (CT), the total organic carbon (%TOC) that was 32.48 mg/kg at the onset of the study reduced to 26.48 mg/kg by week 4 (Fig. 12).

The nitrate (Fig. 13) and phosphate (Fig. 14) contents reduced from 17.82 mg/kg and 24.12 mg/kg respectively to 11.82 mg/kg and 17.12 mg/kg after the 4th week. The moisture (Fig. 15) content increased from 13.43 at the onset to 14.43 while the pH (Fig. 16) value decreased from 5.34 to 4.74 after week 4 of the experiment.

In the RD amended COCS, the nitrate content decreased from 17.82mg/kg from the outset to 16.14 mg/kg by week 4. Similarly, the phosphate content and TOC contents decreased from 24.12 and 32.48mg/kg from the beginning of the study to 14.71 and 22.41 kg/kg by the 4th week.

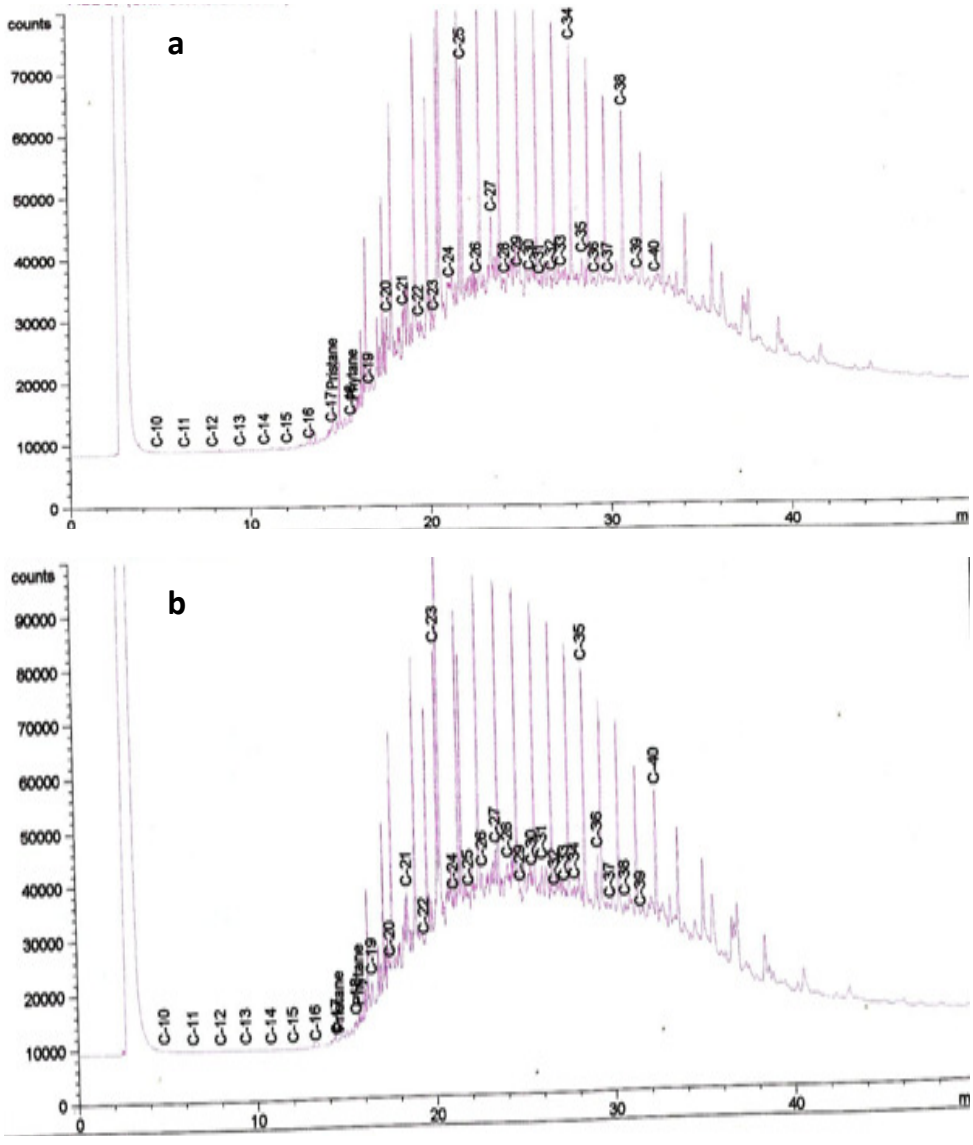

Fig.9. The chromatogram of un-amended COCS (control) showing the extent of the different carbon fractions of the crude oil after week zero (a) and week 4 (d)

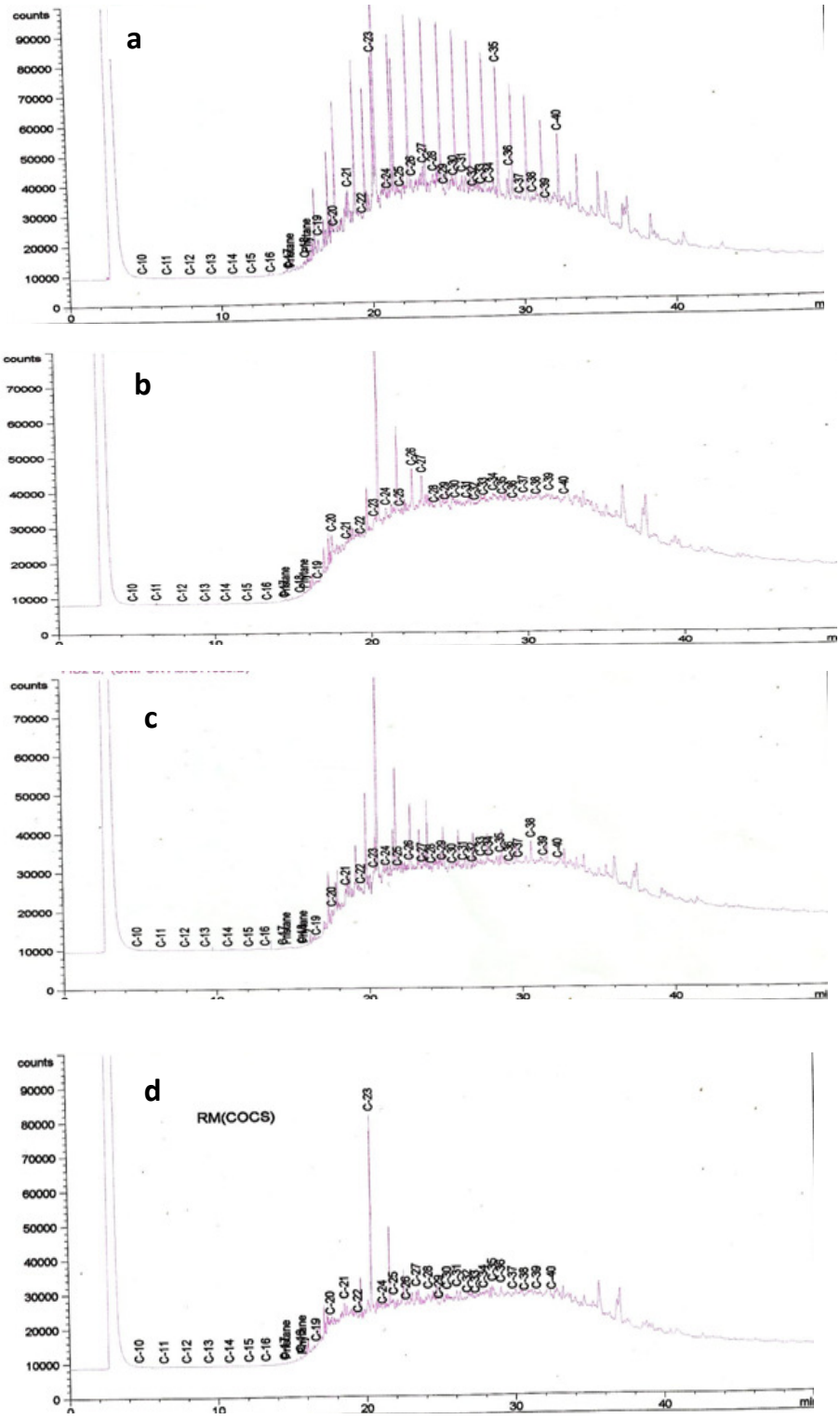

Fig.10. The chromatogram of COCS amended with RD showing the extent of the different carbon fractions of the crude after weeks 1 (a), 2 (b), 3(c) and 4 (d)

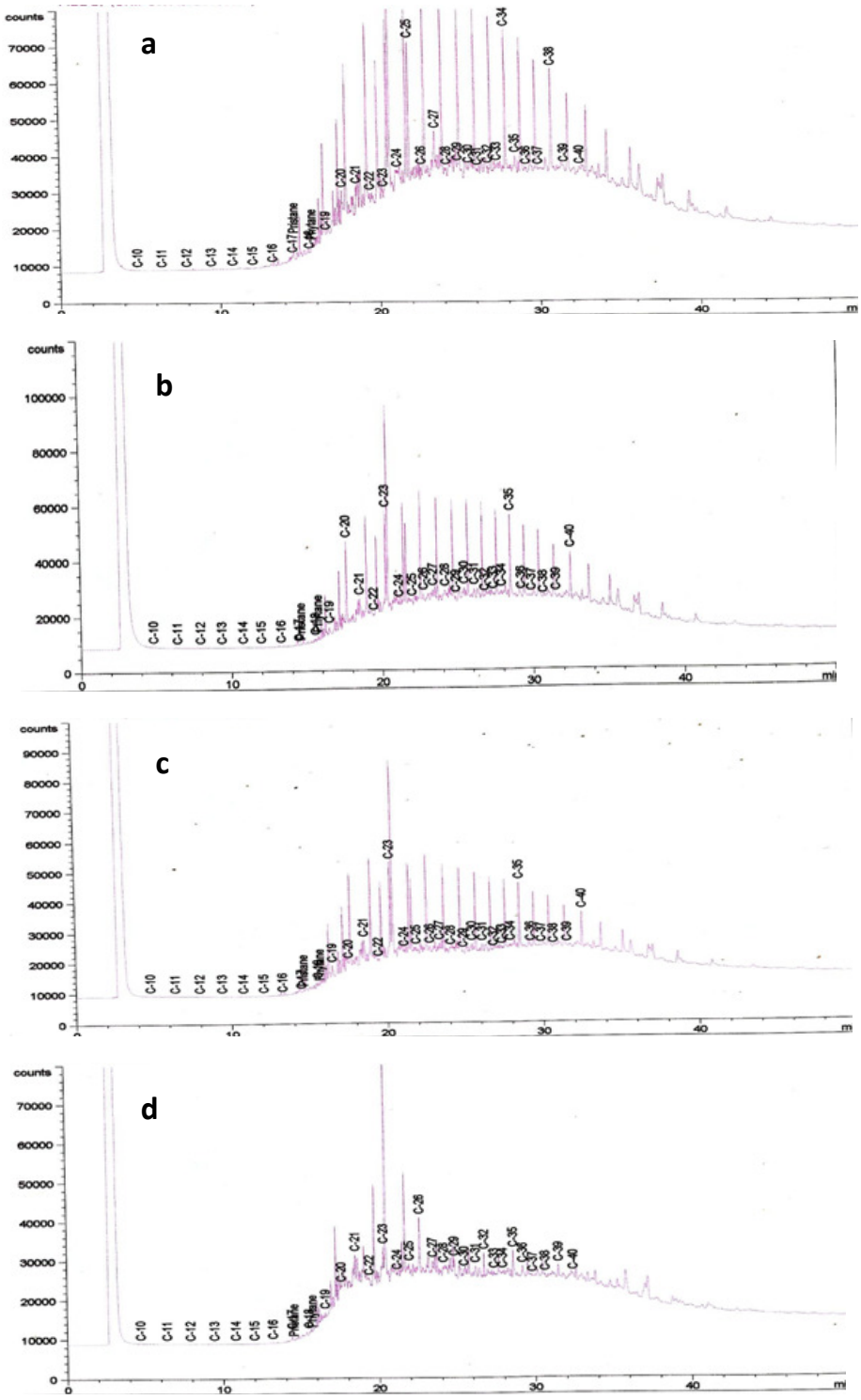

Fig.11. The chromatogram of COCS amended with NFA showing the extent of the different carbon fractions of the crude oil after weeks 1(a), 2(b), 3(c) and 4(d)

The moisture content increased from 6.48 to 13.43. The pH values at pre-amendment were in acidic range (5.34). There was further shift to high acidity (4.74). The shift may be probably due to increase in the number of oil-degrading microbes which are believed to have deposited their waste product (organic acid) of metabolism in the soil environment.

There was significant (p <0.05) effect of pH on soil environment which collaborated the results of previous studies that a decrease in TPH concentrations in soil could lower pH values [46, 47,48]. In the NFA amended COCS, both nitrate and phosphate contents decreased from 17.82 mg/kg and 24.12 kg/mg to 13.42mg/kg and 13.92 mg/kg respectively by week 4. It has been reported that microorganisms make use of nitrate and phosphate in the degradation of crude oil contaminants [32,33] and the decrease in the various limiting nutrients studied in this research is in conformity with their finding.

The total organic carbon (%TOC) decreased from 32.48 to 26.50 after week 4. The pH decreased from 5.34 to 4.60 by the 4th week. Furthermore, the moisture content increased from 8.15 to 13.43 from the onset of the study to week 4 in the NFA amended COCS treatment set up. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron (48). These nutrients could become limiting factor thus affecting the biodegradation processes [12].

The reductions in organics nutrients (total organic carbon, nitrate and phosphate) could be attributed to their utilization by resident crude oil-degrading microorganisms in soil environment [11,12,44,33,40]. The increase in moisture could be due to water added to moisturized soil. This is in agreement with the finding of Skipper [10] who reported an increase in water availability prior to a constant water supply to the make organic sorbent bio-available to microbes in the oil-contaminated soil. Temperature plays a vital role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas [49] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons was reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons [50].

Although it has been reported that hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with a decreasing temperature [50]. The concentrations of total petroleum hydrocarbon (TPH) in the crude oil contaminated soil significantly (p <0.05) affected soil temperature. The highest degradation rates that generally occur in soil environments are in the temperature range of 30–40°C [51,52].

Venosa and Zhu [53] reported that ambient temperature of the contaminated soil environment affected both the properties of spilt oil and the activity of the microorganisms [53]. Significant (p <0.05) biodegradation of hydrocarbons has been reported in psychrophilic environments in temperate regions [50,54].

The observed shift in pH in this study from low acidity to high acidity could be attributable to the deposition of the waste product (organic acid) of metabolism and cell materials (biomass) by resident hydrocarbon-degrading microbial populations in the crude oil-contaminated soil environment [1,9,43].

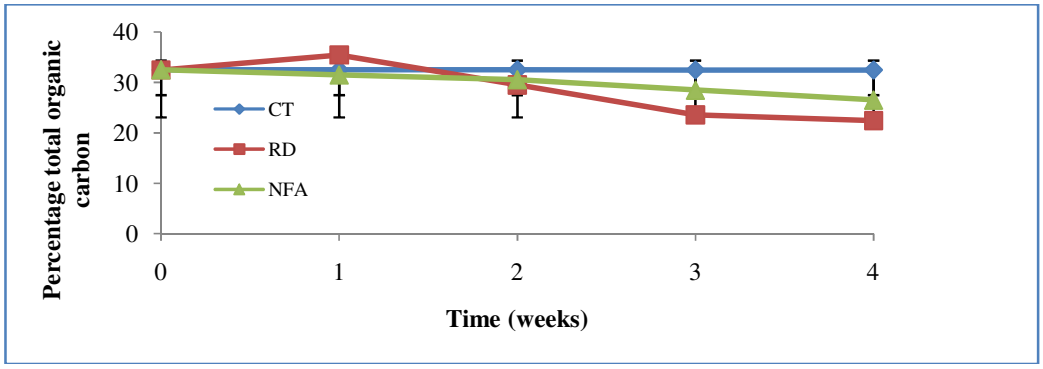

Fig. 12. Changes in total organic carbon of COCS amended with RD and NFA during the study period

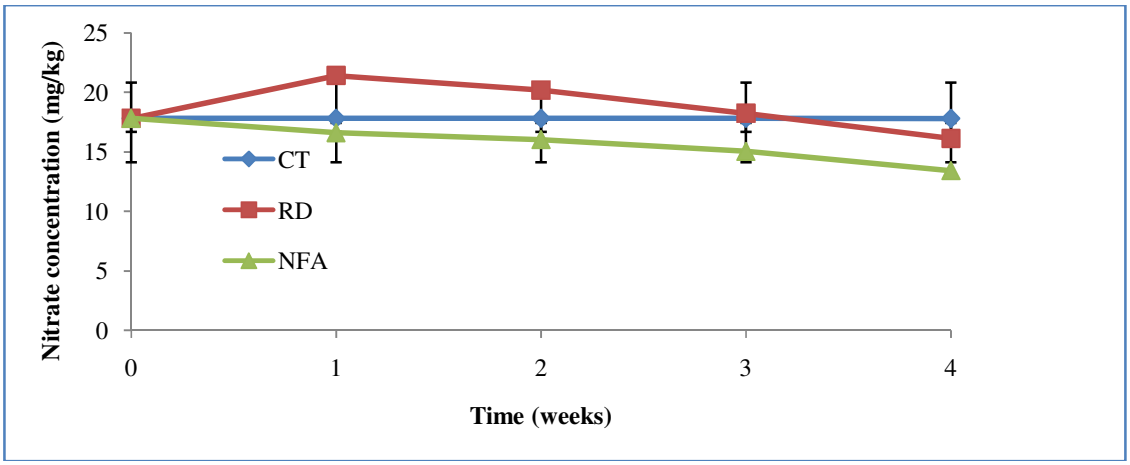

Fig. 13. Changes in nitrate content of COCS amended with RD and NFA during the study

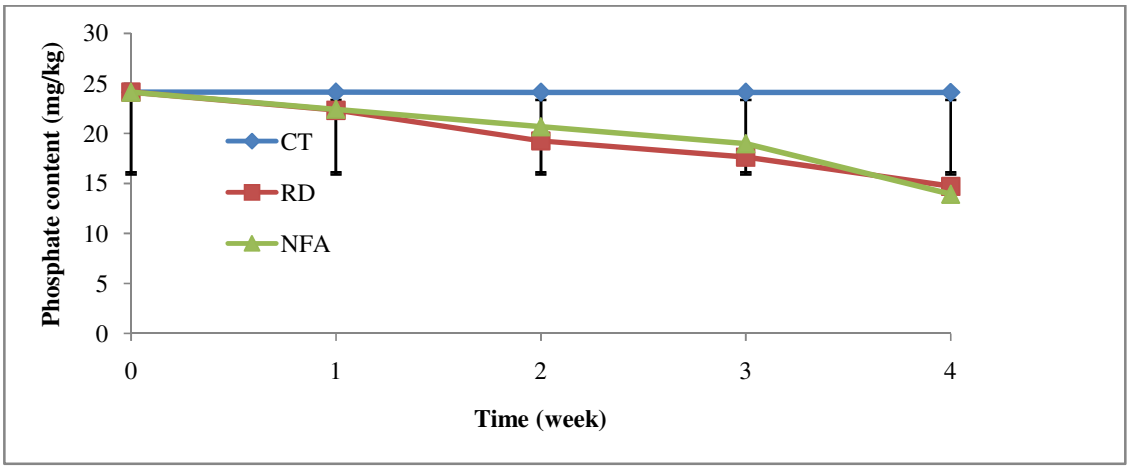

Fig. 14. Changes phosphate of COCS amended with RD and NFA during the study

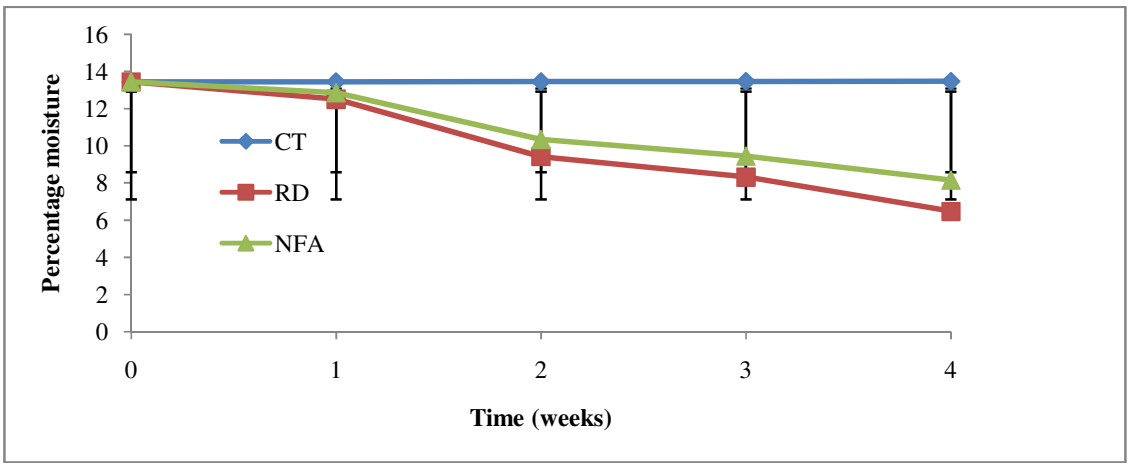

Fig. 15. Changes in moisture of COCS amended with RD and NFA during the study

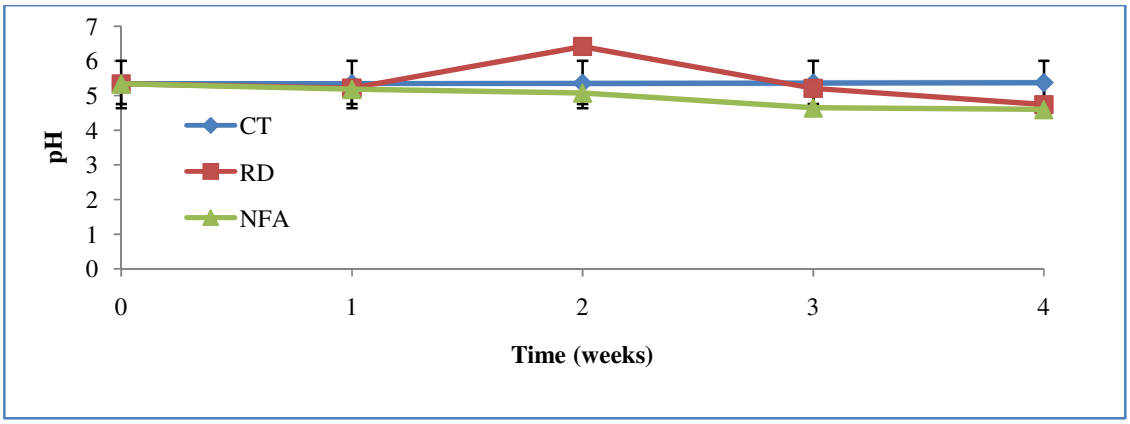

Fig. 16. Changes in pH of COCS amended with RD and NFA during the study

All the physicochemical parameters studied indicated decrease except moisture content which gave a marked increase and may be attributable to the distilled water used in moistening the COCS soil sample. Bioremediation treatment approaches offer the best environmentally friendly method for remediating hydrocarbon contaminated soil because it utilizes the capability of the indigenous microbes in the soil environment to break down the hydrocarbons into innocuous substances [44,53,54,55].

4. CONCLUSION

The study showed the order of biodegradation efficiency of rabbit droppings (RD) and *Nypa fruticans* ash (NFA) supplements as RM>NFA>CT. This indicated that RM had a higher enhancement capacity than NFA. The results demonstrated an ALARP (As Low as Reasonable and Practicably Possible) condition, which explains that the residual crude in soil has been reduced to a level where if bioremediation proceeds, it becomes economical and sustainable.

5. RECOMMENDATIONS

Different enhancements which must be ecologically friendly, widely available and cost-effective are recommended for effective cleanup of crude oil-contaminated soil *ex-situ*. This will definitely restore the soil's health and reduce any existing hazards to human health, safety and the environment to an acceptable level. The next line of action will be to transfer the technology to pilot-scale study for *in situ* eco-restoration of crude oil-contaminated land. This will certainly enhance the healthy lifestyle of citizenry across the globe and further boost sustainable agriculture and consequently, food supply in the locality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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