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Biostimulation Potential of *Nypa fruticans* Ash on Indegenous Heterotrophic Bacteria and Fungi for Enhanced Remediation of Hydrocarbon Polluted Soil at B. Dere, Rivers State, Nigeria

***[1]GBOSIDOM, VL; [2]AMADI, N; [1]NGEREBARA, NN; [3]NWOKOMA, OAF; [1]OKOROAFOR, L**

**[1]Department of Science Laboratory Technology School of Applied Sciences, Kenule Beeson Saro-Wiwa Polytechnic Bori, Nigeria.*
[2]Department of Plant Science and Biotechnology, Rivers State University, Nigeria.
[3]Department of Biology, Ignatius Ajuru University of Education, Port Harcourt, Nigeria.

**Corresponding Authors Email: gbosidom.victor@kenpoly.edu.ng*
**ORCID: https://orcid.org/0000-0002-4786-969X*
**Tel: +2348036666056*

Co-Authors Email: noble.amadi1@ust.edu.ng; ngerenath@yahoo.com; okoriwokoma@yahoo.com; lilianokoroafor7@gmail.com

ABSTRACT: This study was designed to evaluate the biostimulation potential of *Nypa feuticans* ash on the growth of indigenous heterotrophic bacteria and fungi for enhanced remediation of hydrocarbon polluted soil at B. Dere, Rivers State, Nigeria in an ex-situ experiment, using the completely randomized split plot experimental design. 2kg of oil polluted soils were treated with 10g, 20g, 30g, 40g, 50g and 60g of *Nypa fruticans* ash and monitored for biostimulation and growth of indigenous biodegrading heterotrophic bacteria (THBC) and fungi (THFC). The results of four months post residual and cumulative effect on treated soil showed optimum improvement of microbial load at 60g/2kg treatment of *Nypa fruticans* ash. Findings on THBC for post residual treatment soil ranged from 0.1×10^5 to 8.0×10^4 cfu/g, while the THFC ranged from 0.1×10^4 to 3.0×10^4 cfu/g. THBC for post cumulative treated soil ranged from 2.7×10^5 to 9.0×10^4 cfu/g, while THFC ranged from 0.2×10^4 to 3.0×10^4 cfu/g. Three bacteria genera isolated from the post treated soil ware *Bacillus spp.* (36.3%), *Clostridium spp* (33.4%) and *staphylococcus spp.* (30.3%), while two fungi genera namely*Yeast spp*. (58%) and *Muccor spp*. (42%) were isolated from the post treated soil. The high-count THB and THF recorded in both residual and cumulative treated soil compared to the oil polluted untreated soil is attributed to *Nypa fruticans* ash capacity to neutralize the toxic effects of the crude oil on the microbial population by rapid improvement of the soil physiochemical properties, favoring the growth of indigenous microorganisms in the soil. This study has shown that treatment of crude oil polluted soil with *Nypa fruticans* ash at 60g/2kg showed optimum improvement which enhanced the biostimulation and growth of indigenous petroleum degrading microorganism.

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Petroleum hydrocarbons contamination of the environment associated with exploration, development and production operations are common features of oil producing nations around the world, especially in a developing country like Nigeria where the incidence of facilities sabotage, operational

**Corresponding Authors Email: gbosidom.victor@kenpoly.edu.ng*
**ORCID: https://orcid.org/0000-0002-4786-969X*
**Tel: +2348036666056*

failures, accidental discharges, pipeline vandalization and leakages, bunkering and artisanal refining is very common (Aniefiok *et al.,* 2018). When an oil spill occurs, it results in a huge influx of hydrocarbon into the affected environment which in turn alter soil physical and chemical properties by reducing the availability of soil nutrients, particularly nitrogen and phosphorus (Gojgic-Cvijovic *et al*., 2012). Bioremediation has proven to be an effective, environmentally friendly and less expensive treatment option for the remediation of environments contaminated with hydrocarbons (Walworth *et al*., 2007). The technology enhances the degradation of soil toxicity through the addition of materials such as nutrients, microbial products or microorganisms and aeration to contaminated soils (Chuma, 2010). The fundamental principle behind microbial bioremediation is biodegradation of oils: that is, the use of microorganisms to break down crude oil and to change the structure of the oil from large, harmful molecules to smaller, harmless substances (Yosef and Tamiru, 2016). The use of microorganisms for remediation purposes is thus a possible solution for hydrocarbon pollution as it is a sustainable remediation technology that helps to rectify and re-establish the natural condition of both aquatic and terrestrial ecosystem (Chuma, 2010). *Nypa fruticans* is a mangrove palm that grows well in calm estuaries and coastal zones. This species can prevail in a simple channel or complex tributaries, bays, tidal flats and creeks, as long as there is a tide and a freshwater outflow action (Okugbo, 2012). It is a palm with tall erect fronds and underground rhizomatous stem that possesses an extensive root system, well suited to resist swift running water (Shahidullah, 2001). Biostimulation technique involves nutritional amendment (N and P), oxygenation, temperature and pH control, and addition of surfactant in contaminated sites to stimulate the growth of indigenous hydrocarbon degraders, thus enhancing biodegradation rates (Abed *et al*., 2014). This procedure results in the stimulation of the growth and activities of the indigenous microorganisms present at contaminated sites through the addition of nutrients in order to accelerate the rate of natural biodegradation (Nikolopoulou and Kalogerakis, 2010). Studies has showed that high concentrations of petroleum hydrocarbon containing around 80% carbon can lead to a rapid reduction in the concentration of inorganic nutrients in the soil (Walworth et al., 2007). Biostimulation often includes the addition of nutrients and electron acceptors such as P, C, N, and O_2, representing an effective technology for restoring oil polluted and nutrient deficient sites (Li *et al*., 2007). However, care must be taken in the amount of nutrients added as excess quantities of nitrogen may result in inhibition of the soil microbial community. The main advantage of biostimulation is that enhanced biodegradation takes place by the native microbial communities which have already acclimatized to their environment (Chaillan *et al*., 2006). Microorganisms has been reported to adapt their catabolic activities to the use of toxic organic pollutants from soil as food source, to mineralize complex organic compounds to simpler compounds, carbon dioxide and water (Iranzo *et al*., 2001). Bacteria and fungi are known to metabolize complex molecules depending on their abilities to produce specific enzymes (Zhu *et al*., 2001). The concentration of the contaminants, biodegradability, soil properties at the contaminated site, selected treatment technique, natural indigenous microbial population, longevity; toxicity and bioavailability of the contaminants, and water mobility in the soil may influence the degree of bioremediation (Cunningham and Philip, 2000).

Studies has indicated that bacteria, yeasts, actinomycetes and filamentous fungi are capable of breaking down hydrocarbons, but bacteria have been applauded as the most important in the biodegradative process. According to Atlas (2012), the more frequently isolated bacteria from hydrocarbon-polluted sites belong to the genera *Pseudomonas, Rhodococcus, Bacillus, Sphingomonas, Acinetobacter, Alcaligenes, Micrococcus, Flavobacterium, Arthrobacter, Mycobacterium, Actinobacter* and *Alcanivorax.* In order to enhance effective natural biodegradation, this study was designed to evaluate the biostimulation potential of *Nypa fruticans* ash on growth of indigenous heterotrophic bacteria and fungi for enhanced remediation of hydrocarbon polluted soil at B. Dere, Rivers State, Nigeria.

MATERIALS AND METHODS

Study area: Soil sample for this study was collected from B-Dere community, located at latitude $4^0 40^1 24.6^{11}$N and longitude $7^0 22^0 4.3^{11}$E in Gokana Local Government Area of Rivers State, while the ex-situ study was conducted at the Green house in the Botanical garden of Kenule Beeson Saro-Wiwa Polytechnic, Bori.

Soil and Plant sampling: Hydrocarbon polluted soil samples was randomly collected from oil impacted site at the study area and bulked as composite samples, then transported to the green house at the Botanical garden of the Department of Science Laboratory Technology, Kenule Beeson Polytechnic, Bori for ex-situ experimentation.

Nypa fruticans leave and stem samples were collected from B-Dere mangrove forest and stored in clean polyethene bags and transported to the Botanical garden for experimentation.

Sample preparation: The *Nypa fruticans* leave and stem samples were air dried and ashed by combustion. The ash was collected and stored in sterile containers. Soil samples were sieved to remove woods, roots and stones.

Treatment of Polluted Soil: A 2kg of sieved soil sample was weighed into plastic buckets and treated with 10g, 20g, 30g, 40g, 50g and 60g of *Nypa fruticans* ash in six replications each. The ash was applied by incorporating appropriate quantities into the hydrocarbon polluted soils and properly mixed to ensure even distribution within the soil. Three replications of 2kg hydrocarbon polluted untreated soils were kept as control. All treatments were monitored, watered uniformly and aerated intermittently at 3 days interval.
At the end of 2 months post-treatment, the set up was split into two with three replications each. One set of three replications served as residual treatment, while the second set was re-treated, serving as cumulative treatment.

Cumulative treatment: The second half of experiment 1 was re-treated with 10g, 20g, 30g, 40g, 50g and 60g of *Nypa fruticans* ash with three replications each, serving as cumulative treatment. The experiment was monitored for 1 month.

Post treatment soil sampling: Representative soil samples were collected from the respective post treated soils and control. Samples from each treatment was bulked together, stored in clean polytene bags and labeled with sample identity for assessment of microbiological properties.

Preparation of media: The media used for microbial analysis are nutrient agar and potato dextrose agar.

Nutrient Agar: This medium was prepared from commercially available dehydrated powder, available from suppliers of culture media. In the preparation, 2.8g of nutrient powder was dissolved in 100ml of distilled water in a conical flask covered with cotton wool and aluminum foil paper. This was stirred and autoclaved at 121^0C for 15 minutes and then cooled to 45^0C, before pouring into Petri dishes.

Potato dextrose agar: This medium was used for isolation of fungi from the samples and for the preparation of pure cultures. The medium was prepared from commercially produced dehydrated medium following the manufacturer's instruction. 3.9g of Potato Dextrose agar powder was dissolved in 100ml of distilled water in a sterile conical flask covered with cotton wool and aluminum foil paper. It was mixed thoroughly and autoclaved at 121^0C for 15 minutes. The medium was cooled after autoclaving to 45^0C, before dispensing into Petri dishes.

Sterilization process: The glass wares were washed with water and liquid soap, then sterilized using the hot air oven at the temperature of 160^0C for 1 hour. The glass rod and wire loop were sterilized using absolute ethanol and this was done by dipping into the solution and flaming directly through Bunsen flame and allowed to cool for few seconds prior to spreading.

Serial dilution: 10grams of the soil sample was weighed and added to 90ml of sterile distilled water to get an aliquot. Six test tubes were pre-labeled 10^{-1} to 10^{-6} and kept in a test tube rack; 9ml of distilled water was then measured into the six test tubes. 1ml of the diluted soil sample was then introduced into the first test tube labeled 10^{-1} and mixed thoroughly, 1ml was taken from the test tube and transferred to the second test tube labelled 10^{-2}. This was continued until the 10^{-6} dilution was obtained.

Inoculation and incubation: The molten Nutrient Agar media were poured into ten sterile petri dishes. This was amended with nystatin to suppress the growth of fungi. The molten Potato Dextrose Agar media was amended with tetracycline to inhibit the growth of bacteria, then poured into four sterile petri-dishes.
0.1ml of the 10^{-3} to 10^{-6} dilution of each soil samples was plated on nutrient agar and Potato Dextrose Agar medium using the spread plate method respectively.

Bacteria cultures were incubated at 37^0C for 24 hours. After 24 hours, growth of bacteria colonies on the agar were counted and recorded in colony forming unit per milliliter (cfu/ml), while the potato dextrose agar plates were incubated at 37^0C for 48 hours.

Enumeration and isolation of bacteria and fungi colonies: After the period of incubation, discrete colony forming unit from both bacteria and fungi colonies were randomly picked with sterile inoculating wire loop and was streaked on freshly prepared nutrient agar and potato dextrose agar media respectively for further purification. The bacteria culture plates were then incubated at 37^0C for 24 hours, while the fungi cultures were at 37^0C for 72 hours.

Biochemical test: Biochemical test carried out include: Gram's reaction, Catalase test, Indole test, Methyl Red and sugar fermentation test.

Gram's reaction: Respective isolate was smeared on a clean grease free slide and fixed by passing the slide over a Bunsen flame three times. This was followed by flooding with crystal violet for 60 seconds before draining of excess. This was gently washed with tap water. A mordant (Lugol's iodine) was applied and allowed for 1 minute then washed off and decolorized with an alcohol (acetone). The smear was counterstained (using neutral red), washed off after 60 seconds and air dried and examined under the microscope using the oil immersion objective (X100). Isolate morphology and reaction to Gram's reagent was noted. The test differentiates bacteria into Gram +ve and Gram –ve.

Catalase test: This was used to test the ability of organism to breakdown hydrogen to liberate oxygen. A wire loop was used to pick the organism (colony) on a sterile slide after which a few drops of hydrogen peroxide (H_2O_2) was placed on it and it was observed. The appearance of bubble indicates a positive response for *Pseudomonas species* and the absence of bubbles indicates negative reaction.

Indole test: This is used to determine the ability of organisms to breakdown the amino acid tryptophan into indole.
1g of tryptophan broth was dissolved in 100ml of distilled water, swirled and autoclaved at 121^0C for 15minutes, then allowed to cool. The media was dispensed into test tubes and a loopful of the isolates was inoculated into the test tubes. The tubes were incubated for 24hours at 37^0C. after incubation 2ml of Kovac's reagent was added and shaken gently and allowed to stand for 5 minutes.
The present of a ring pink/red colour, indicates positive (*Micrococcus* species) response and yellow colour indicates negative reaction.

Methyl red: This test is used for the determination of the conversion of glucose into acidic products.
1.7g of methyl broth was dissolved in 100ml of water and autoclaved at 121^0C for 15minutes and allowed to cool before dispensing it into test tubes. A small amount of culture was picked with sterile wire loop and inoculated into the test tube containing the methyl red medium and incubated for 24hours at 37^0C, after which 2 drops of methyl red indicator was dropped inside the tubes and mixed. Bright pink colour indicates (*Bacillus species*) positive reaction, yellow colour indicates negative reaction.

Sugar fermentation test: 3.2g of triple sugar iron agar (TSIA) was dissolved in 50ml of distilled water and autoclaved at 121^0C for 15minutes and allowed to cool before dispensing it into test tubes at slant position. An inoculating needle was used to touch the top of the isolated colony and stabbed through the center into the medium to get to the bottom of the test tube. Wire loop was used to pick colonies from the cultured plates and strike on top of the TSIA. The test tube was placed inside a beaker and incubated for 24 hours. The changes of colour to yellow and spaced at the bottom of the tube indicates Acid and Gas production, while no colour change or spaced is an indication that the organism is negative to Acid and Gas production.

Staining technique for fungi: Inoculating wire loop were flamed over the burning Bunsen burner. Then a small portion of the growth on the culture plate was transferred into the drop of lacto-phenol in cotton blue on the slide with the help of wire loop. The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding for the mycelium. The specimen was then observed under the microscope for microscopic identification.

Identification of fungi isolate: Identification of the fungal species were based on morphological characteristics of the colony and microscopic examinations. The colony growth which include length and width of the colony, the presence or absence of aerial mycelium, the color, wrinkles furrows and any other pigment production were the macro morphological characteristic evaluated.

RESULTS AND DISCUSSION

The results of the microbial load of the pre and post treated soil samples are presented in Tables 1 and 2.
Pre-treatment (control): The mean results of Total Heterotrophic bacteria count (THBC) of oil polluted soil sample from the study area ranged from 2.6×10^7 to 5.7×10^4Cfu/g. Five bacteria genera namely: *Clostridium spp.*, *Bacillus spp.*, *Staphylococcus spp*., *E. coli* and *Pseudomonas spp* were isolated from the oil polluted soil samples (Table 1).

Table 1: Heterotrophic Bacteria (THB) isolate from oil polluted samples

Sample	Dilution factor	Mean THB	Cfu/ml
Crude oil polluted sample	10^{-3}	57	5.7×10^4
	10^{-4}	48	4.8×10^5
	$10.^{-5}$	36	3.6×10^6
	10^{-6}	26	2.6×10^7

Key: THB = Total Heterotrophic Bacteria; Cfu = Colony forming unit.

The mean results of Total Heterotrophic fungi count (THFC) for oil polluted soil samples from the study area ranged from 0.4 X 10^7 to 1.3 X 10^4 Cfu/g. Six fungi genera that were isolated from the oil polluted soil samples include *Aspergillus spp., Fusarium spp., Penicillium spp., Rhizopus spp., Mucor spp. and Yeast* (Table 2).

Table 2: Heterotrophic fungi (THF) isolate from oil polluted samples

Sample	Dilution factor	Mean THB	Cfu/ml
Crude oil polluted sample	10^{-3}	13	1. X 10^4
	10^{-4}	10	1.0 X 10^5
	10. $^{-5}$	5	0. X 10^4
	10^{-6}	4	0.4 X 10^7

Key: THF = Total Heterotrophic Fungi; Cfu = Colony forming unit.

Table 3: Total Heterotrophic Bacteria count (THBC) of isolate from post Residual treated soil with *Nypa fruticans* ash amendment

Sample	Sample size	Dilution factor	Mean THBC	Cfu/g
Residual soil samples	10g	10^{-3}	57	5.7 X 10^4
		10^{-4}	45	4.5 X 10^5
	20g	10^{-3}	50	5.0 X 10^4
		10^{-4}	55	5.5 X 10^5
	30g	10^{-3}	55	5.5 X 10^4
		10^{-4}	10	1.0 X 10^5
	40g	10^{-3}	60	6.0 X 10^4
		10^{-4}	70	7.0 X 10^5
	50g	10^{-3}	55	5.5 X 10^4
		10^{-4}	35	3.5 X 10^5
	60g	10^{-3}	80	8.0 X 10^4
		10^{-4}	75	7.5 X 10^5

Table 4: Total Heterotrophic Bacteria count (THBC) isolate from Cumulative treated soil samples with *Nypa fruticans* ash amendment

Sample	Sample size	Dilution factor	Mean THBC	Cfu/ml
Cumulative soil samples	**10g**	10^{-3}	50	5.0 X 10^4
		10^{-4}	47	4.7 X 10^5
	20g	10^{-3}	51	5.1 X 10^4
		10^{-4}	27	2.7 X 10^5
	30g	10^{-3}	54	5.4 X 10^4
		10^{-4}	51	5.1 X 10^5
	40g	10^{-3}	50	5.0 X 10^4
		10^{-4}	29	2.9 X 10^5
	50g	10^{-3}	45	4.5 X 10^4
		10^{-4}	28	2.8 X 10^5
	60g	10^{-3}	64	6.4 X 10^4
		10^{-4}	60	6.0 X 10^5

Residual and cumulative treatment: The mean Total Heterotrophic bacteria count (THBC) for Residual treated soil samples ranged from 1.0 X 10^5 to 8.0 X 10^4 Cfu/g, while the mean THC for Cumulative treated soil samples ranged from 2.7 X 10^5 to 6.4 X 10^4 Cfu/g. The cultural, morphological and biochemical characteristics of bacteria isolates from the post-treated soil samples are presented in Table 3 and 4.

he Higher THBC in both residual and cumulative treated soil samples might be due to the presence and bioavailability of more N and P into the soil that contributed to the stimulation of the microbial flora in the soil. The mean THFC for Residual soil samples ranged from 0.1 X 10^4 to 0.5 X 10^4 Cfu/g, while the mean THFC for Cumulative soil samples ranged from 0.2 X 10^4 to 0.8 X 10^4 Cfu/g. The macroscopic and microscopic characteristics of fungi isolates from the residual and cumulative soil samples are presented in Table 5 and 6.

Table 5: Total Heterotrophic Fungi count (THFC) isolate from post Residual soil with *Nypa fruticans* ash amendment

Sample	Sample size	Dilution factor	Mean THF	Cfu/ml
Residual soil samples	**10g**	10^{-3}	1	0.1 X 10^4
		10^{-4}	-	-
	20g	10^{-3}	4	0.4 X 10^4
		10^{-4}	-	-
	30g	10^{-3}	3	0.3 X 10^4
		10^{-4}	-	-
	40g	10^{-3}	1	0.1 X 10^4
		10^{-4}	-	-
	50g	10^{-3}	1	0.1 X 10^4
		10^{-4}	-	-
	60g	10^{-3}	5	0.5 X 10^4
		10^{-4}	-	-

Table 6: Total Heterotrophic Fungi count (THFC) of isolate from post Cumulative treated soil with *Nypa fruticans* ash amendment

Sample	Sample size	Dilution factor	Mean THF	Cfu/ml
Cumulative soil samples	**10g**	10^{-3}	-	-
		10^{-4}	-	-
	20g	10^{-3}	-	-
		10^{-4}	-	-
	30g	10^{-3}	2	0.2 X 10^4
		10^{-4}	-	-
	40g	10^{-3}	3	0.3 X 10^4
		10^{-4}	-	-
	50g	10^{-3}	2	0.2 X 10^4
		10^{-4}	-	-
	60g	10^{-3}	8	0.8 X 10^4
		10^{-4}	-	-

Isolation of heterotrophic bacteria and fungi in post hydrocarbon polluted soil treated with Nypa fruticans ash: Three bacteria genera namely *Bacillus spp., Clostridium spp.* and *Staphylococcus spp*.

were isolated from post treated (residual and cumulative) samples with percentage occurrence as *Bacillus spp.*< *Clostridium spp.*< *Staphylococcus* (fig 1), while two fungi genera namely *Yeast* and *Muccor spp.* were isolated from post treated (residual and cumulative) samples in the range of *Yeast* < *Muccor spp.* percentage occurrence (fig 2).

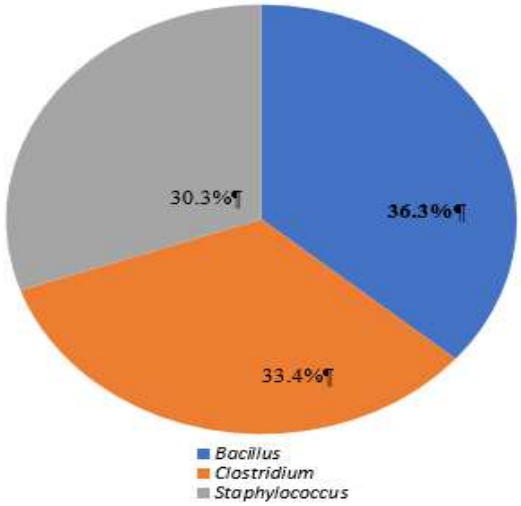

Fig. 1: Percentages of hydrotrophic bacteria isolated from post hydrocarbon polluted soil treated with *Nypa fruticans* ash.

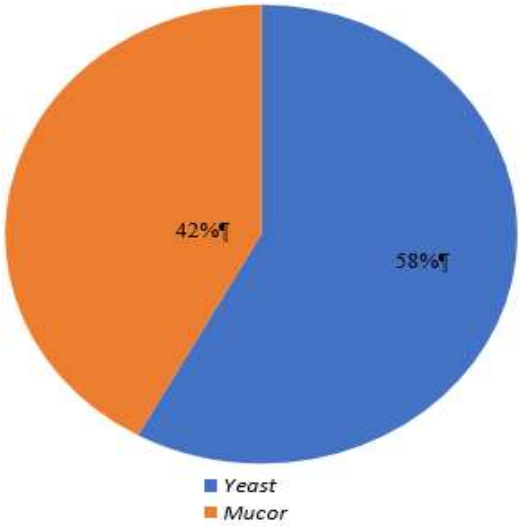

Fig. 2: Percentage of isolated fungi from post hydrocarbon polluted soil treated with *Nypa fruticans* ash.

Microbial population of pre-treated oil polluted soil: Findings of bacteriological analysis on hydrocarbon polluted field soil samples (control) without amendments with *Nypa fruticans* ash contained isolated populations of bacteria namely *Clostridium spp.*, *Bacillus spp.*, *Staphylococcus spp*., *E. coli* and *Pseudomonas spp.*, while the mycological analysis indicated six fungi genera isolates namely *Aspergillus spp., Fusarium spp., Penicillium ssp., Rhizopus spp., Mucor spp. and Yeast.* This observation portrays the fact that hydrocarbon polluted soil contains indigenous hydrocarbon degrading microorganisms that can support bioremediation, thus collaborating the report of Coates and Anderson (2000), who posited that appropriate specific pollutant-degrading microorganisms are always present at polluted sites, but at low concentration. Similarly, Uzoamaka *et al*. (2009) reported the presence of hydrocarbon utilizing microbes in oil polluted soil, while Van Gestel (2001) observed significant increment in bacteria population remediation in oil polluted soil.

Microbial population of post-treated oil polluted soil: The study on microbial population following the amendment treatment of hydrocarbon polluted soils with varying concentrations of *Nypa fruticans* ash enhanced the growth of naturally occurring heterotrophic bacteria and fungi population based on amendment concentrations, with residual treatment showing optimum growth of bacteria at 60g/2kg amendment level, while fungi optimum growth was observed at 20g/2kg and 60g/2kg amendment levels. The observed population growth increases in heterotrophic bacteria and fungi could be attributed to the metabolism of organic compounds that are inherent in crude oil by the enhanced population of indigenous heterotrophic hydrocarbon-degrading bacteria and fungi following the amendment of *Nypa fruticans* ash for the release of secondary substrates that triggered the increase in their population. This finding justifies the report of Banat, (2004) who stated that organic compounds in crude oil could be metabolized by oil-degrading bacteria to release secondary substrate that would support the growth and activities of other bacteria. Furthermore, cumulative treatment study also indicated a significant increase in the population of heterotrophic bacteria and fungi, with 60g/2kg amendment levels as optimum performance in population growth. This observation is an indication that microbial population is associated with increase in amendment levels of *Nypa fruticans* ash. This finding is in conformity with Abioye *et al.* (2010), who observed that oil polluted soil amended with organic matter stimulates the growth of indigenous oil-degrading microbiota in it. Similar observation was reported (Song and Bartha, 1990; Al-Kindi and Aded, 2016). The higher counts of Total Heterotrophic bacteria counts recorded in both residual and cumulative treated samples compared to the polluted untreated soil might be attributed to the presence of appreciable quantities of macro and micro elements in the *Nypa fruticans* ash which served as a nutrient source for the growth and maintenance of microbial community. This finding is in agreement with Gbosidom and Teme, (2015) who reported that treatment of hydrocarbon polluted soil with palm

bunch ash rapidly improved the soil physicochemical properties which consequently favored the growth of indigenous microorganisms in the soil.

Isolation of heterotrophic bacteria and fungi study: Findings on isolation showed that three bacteria genera namely *Clostridium spp.*, *Bacillus spp*. and *Staphylococcus spp*. were isolated and identified in post oil polluted treated soils (residual and cumulative) with *Nypa fruticans* ash., while two fungi genera namely *Yeast* and *Muccor spp.* were also isolated and identified. The low fungi population in residual and cumulative treated soil might be attributed to the appreciable effect of *Nypa fruticans ash* on the oil polluted soil which eventually results to the decrease in the population of the hydrocarbon utilizing fungi population. This finding is in line with Abioye *et al*. (2009) who reported that the treatment of crude oil polluted soil with *Nypa fruticans* ash supported the bio stimulation of *Yeast* and *Muccor spp*.

Conclusion: This study has demonstrated the biostimulation potential of *Nypa fruticans* ash on growth of indigenous *Clostridium spp*, *Bacillus spp* and *Stephylococcu spp* heterotrophic bacteria, as well as Yeast and Muccor heterotrophic fungi, which enhanced the bioremediation of hydrocarbon polluted soil. The optimum growth and remediation performance were recorded at 60g/2kg treatment level. Consequently, with proper manipulation of environmental conditions and monitoring, *Nypa fruticans* ash can be used for bioremediation of hydrocarbon impacted soil.

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