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Enhanced Microbial and Total petroleum hydrocarbon degradation in Crude-Oil Polluted Soils using Agro-Wastes

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ABSTRACT

Bioremediation has become a trending and developing field in environmental restoration through the use of micro-organisms to utilize and reduced the concentration and toxicity of various chemical pollutants. This study is on bioremediation of hydrocarbon-polluted soils using some agricultural wastes. Ninety (90) plastic buckets were filled with 4kg each of the composite soil. The soil contained in the plastic buckets was spiked with 250ml crude oil, except in the unpolluted plastic buckets (0%) crude oil. The agro-wastes (plantain stem sap, bush mango peels, and fruited pumpkin husk powder) in single and combined forms were applied after 14 days soil pollution. The amendments were applied as follows: Pristine control (0% agro-wastes), crude-oil control (0% agro-wastes), 150g, 250g, and 350g of the agro-wastes. Soil samples were collected at 90 days for soil microbial counts and the total hydrocarbon content of the soil. Data collected were subjected to 2-way ANOVA. The result showed that the microbial population in the crude-oil polluted soil amended with different agricultural wastes significantly increased ($p<0.05$) the total heterotrophic and crude oil utilizing bacterial and fungal counts in the soils and the increase in microbial population result in a significant reduction in total hydrocarbon content (THC) of the soils. The reduction in the THC of the soil was treatment dependent. It is, therefore concluded that based on the efficiency of these agro-wastes in enhancing microbial degradation, further studies should be carried out on the enzyme activities and production of bio-surfactant from the wastes to shorten the degradation time.

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INTRODUCTION

Crude oil is formed from the decomposition of dead organic matter buried beneath the earth's crust for millions of years which have been transformed into a mixture of comparatively volatile liquid hydrocarbons (alkanes, naphthene's, and aromatics). These compounds are composed of hydrogen and carbon, although these compounds also contain nitrogen, sulfur, and oxygen in a minute quantity. These elements form large and different types of molecular structures. Crude oil composition varies and these are dependent on the geographical area of which the source of oil is discovered (USEPA, 2000). Crude oil has five molecular compositions although it varies widely from formation to formation, the type and source of the oil; the proposition of the chemical

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constituent in them varies over fairly narrow limits. The chemistry of petroleum can be traced to a complex mixture of hydrocarbons, and the molecules found mostly are alkanes (paraffin), cycloalkanes (naphthene), aromatic hydrocarbons, and complicated chemical mixtures called asphaltenes. These petroleum products have their unique mixture of molecules based on their physical and chemical properties such as color and viscosity. Oil spilled in the environment through anthropogenic activities such as drilling of rigs and wells, pipeline vandalization, oil transportation and reckless handling of waste oil has resulted in vast contamination of the environment. The effect of this soil pollution affects the growth and productivity of the plants, due largely to the accumulation of heavy metals and other toxic chemicals in the plants. The degradation of hydrocarbons in the soil environment is made possible with the introduction of microbial substrates with specific abilities to utilize the hydrocarbons as their sole energy source. Petroleum products affect the soil's textural organization and render it unproductive for agricultural activities. Over the years, it has been assumed that petroleum products are mostly degraded in aerobic conditions, especially using aerobic microorganisms. The abundance and bioavailability of the microorganisms to the pollutants make them accessible to the microorganism to utilize (Besalatpour et al., 2011). Bioremediation though embraces the use of microbes for pollutant breakdown but when certain environmental conditions such as temperature, moisture, and nutrients are lacking or inadequate, the effectiveness of the microbes in the degradation of petroleum products is reduced. Petroleum products are also derived from crude oil, which has a very high value in the development of the economic structure of the nation, but in turn, causes harm to the environment through mishandling of its refining process (Zhang *et al*., 2020). Petroleum hydrocarbons such as phthalate esters, polycyclic aromatic hydrocarbons, nitroaromatic compounds, polychlorinated biphenyls, pesticides, industrial solvents, etc. can be reduced in their concentration through the use of micro-organisms as a sequential process. The drastic reduction of the concentration of these pollutants by the interaction of micro-organisms results in biotransformation from a greater effect to a lesser effect in the environment. Gradual biodegradation of petroleum hydrocarbons mostly occurs through the processes of bio-stimulation and bioaugmentation (Tanee & Albert 2011; Bassey *et al*., 2021). However, in some affected areas, the approach of initiating the right strategy in the contaminated sides is dependent upon three principles which include the amenability of the product (biochemistry), contaminants accessibility to the micro-organisms (bioavailability), and the ability to optimize the biological activity (bioactivity) (Owaid *et al*., 2022; Dua *et al*., 2002). The effective and efficient initiation of the bioremediation process in the environment is a goal getting achievement in environmental biotechnology through the combination of microbiological, ecological, and biochemical mechanisms (Ainon *et al*., 2010, Elkhouly *et al* 2021). Adams *et al*., (2017) observed that the addition of rice husk to the polluted soil removed more petroleum hydrocarbons compared to chicken manure and their combination. The use of agricultural waste, especially manual composting increases the microbial quantity in the soil, thereby reducing the hydrocarbon content (Chao *et al*., 2020; Abdelzaher *et al*., 2022). Liu *et al*., (2015) noted that the combination of agricultural wastes influences the proliferation of microorganisms in soils with a resultant decrease in the hydrocarbon content in soils. These visibility and dramatic experiences of oil spillage require urgent attention by the public to ameliorate this pollution to a lesser effect and rejuvenate the environment in a capacity that can harbor plants and animals again. The main parameters evaluated during the study were: Microbial count and identification of isolates using molecular tools and the total hydrocarbon content of the soil.

MATERIALS AND METHODS

The soil microbial analysis was conducted at Microbiology Laboratory, University of

Calabar, while, the total petroleum hydrocarbon (TPH) was conducted at Mifor Consult Nig. Ltd Laboratory Marine Road Calabar. Bonny light crude oil was purchased from Nigeria Agip Oil Company, Port Harcourt, Rivers State, Nigeria. The agro-wastes (plantain stem, bush mango peels, and fruited pumpkin husk) were collected from Bendeghe Ekiem Village, Etung LGA, Cross River State. The agro-wastes were air-dried for 10 days and then pulverized to powder using an electric blender (Model 4250 Braun Germany). The powdered samples were sieved in a 2mm sieve, to remove grits and other foreign materials, the sieved powdered samples were stored in containers.

Air-dried soil samples were collected from four points, at a depth of 0-25cm, bulked to form composite soil samples. Four kilograms each of the dried soils were measured and transferred into ninety (90) labeled experimental buckets with drainage holes at the base while soil retention was made possible by plugging cotton wool; the experimental buckets were arranged in triplicate. A 5x6 factorial in a Completely Randomized Design (CRD) was adopted as the experimental design. However, agro-wastes were at six levels (plantain stem powder (PSP), bush mango peels powder (BMPP), fruited pumpkin husk powder (FPHP), PSSP + BMPP, PSSP + FPHP, and BMPP+ FPHP). While, the concentrations of the agro-wastes were at five (5) levels (Pristine soil (unpolluted, 0 gram), crude oil polluted control (Polluted, 0 gram), 150g, 250g, and 350g of the amendments).

Water, oil application, application of agro-wastes, and mixing

Bioremediation of crude oil-polluted soils was carried out in the laboratory. The ninety (90) plastic buckets including the polluted and unpolluted soil samples were watered every two days with 200ml of water for one week before oil application. The main reason for this initial watering process was to ensure 60% homogeneity of the moisture content of each sample and also to ensure that the soil moisture holding capacity which was the limit established as optimum for extraction and biodegradation of hydrocarbon products in soil was achieved. All plastic buckets were polluted with 12.5% of crude oil i.e. 250ml of crude oil, except the unpolluted plastic buckets (0% crude oil).

After oil application, the content of each plastic bucket was thoroughly mixed using a sterile spatula to provide aeration, as would be achieved in the field site through tilling. After mixing, all plastic buckets were left undisturbed for two weeks before applications of agro-wastes in single and combined form (plantain stem sap, bush mango peels, and fruited pumpkin husk). However, before the application of the amendments, the amendments were dissolved in 200ml of distilled water and applied in a liquid form to guarantee uniform distribution within the soil. Mixing of the various plastic buckets was done every week. Mixing of the plastic buckets was important because it keeps the environment aerobic and disperses hydrocarbons making them more readily available for microbial attack (USEPA, 2000). After treatment, the experiment was allowed for 90 days before soil samples were collected for laboratory analysis.

Enumeration of microbial count

The spread plate method was used to determine the bacteria and fungi counts on nutrient and Sabouraud dextrose agar respectively. A ten (10) fold serial dilution using one gram (1g) of soil was suspended on the test tube and 0.1ml 10^{-6} and 10^{-7} dilution, was spread on the Petri dishes in triplicates and incubated at 28^0C for 18 hours for bacterial while the fungi plates were incubated at 37^0 C for 72 hours. The plate counts were done and expressed as colony-forming units per gram (CFU/g) a detailed procedure as previously reported by (APHA 1998). The hydrocarbon utilizing bacteria and fungi counts in soil were determined using the viable count's procedure and mineral salt agar was used as previously reported by (Hamamura *et al*., 2006). The characterization and identification of the bacterial isolates were carried out as previously described by (John *et al*., 1994). The Bergey's manual for bacteriological determination, 9th

Edition was used as a presumptive identification tool. The fungi isolates were characterized and identified macroscopically and microscopically using lactophenol-in-cotton blue in a method previously reported by (John et al. 1994).

DNA Extraction and Sequencing

The Microbial isolates obtained from the pure culture were used for the extraction of the DNA. The DNA was extracted from the growth of microbial isolates that lasted for 24 hours in nutrient broth and was harvested through centrifugation of 14,000 x g for 10 minutes; the other procedure used was based on methods previously reported by (Agbor *et al*. 2021). However, after the extraction of the DNA, the extract was transported in an ice cube to Inqaba Biotechnology PTY Laboratory, Pretoria, South Africa for sequencing. The Methods of Sanger (Dideoxy) were used in the determination of the nucleotide sequence of the isolated bacteria and fungi via an automated PCR cycle-Sanger sequencer™ 3730/3730 XL DNA analyzers. The result was obtained as nucleotides as previously described by (Agbor *et al*., 2021). The sequence data were downloaded and read using FINCH TV Software and blasting was carried out on every set of the isolates obtained as reported by (Weiburg *et al*., 1991).

Determination of soil total petroleum hydrocarbon

The total petroleum hydrocarbon in soil samples of the polluted, pristine, and agro-waste amended soils was carried out. The soil samples were homogenized into finer textures using mortar and Pistle, thereafter, the pebbles, sticks, and rock particles were removed. The adjusted EPA 418.1 method involved a soil sample, 3-10 grams, the addition of 1-5grams of sodium, and the addition of the extracting solvent (Freon 113, 20-30ml), a detailed procedure as previously described by (Agbor et al., 2019). The hydrocarbons in the representative soil sample were determined using the Varian Model BV CP 3800 GC-FID as previously described by (Kachienga et al., 2018).

Percentage of hydrocarbon saturation during the 3 months (90 days)
$(TPH_{90} \div TPH_{initial}) \times 100$
(i) Percentage hydrocarbon degradation
$100 - (TPH_{90} \div TPH_{initial}) \times 100$
(ii) The required time for 100% TPH degradation
$(90 \text{ days} \div \% TPH_{90}) \times 100 \div 365 \text{ days}$
(iii) Degradation per day of total petroleum hydrocarbon
$(TPH_{initial} - TPH_{90}) \div T$
Source: (Jidere and Akamigbo 2009).

Statistical analysis

Data generated from the study were subjected to a two-way analysis of variance (2 ANOVA) test while significant means were separated using least significant difference (LSD) at 5% and 1% probability.

RESULTS AND DISCUSSION

Microbial Population in Soils

The bacteria load after pollution was observed to be higher in the soil before pollution. The increase in microbial load after pollution could be attributed to the re-activation of the hydrocarbon-utilizing organisms, which solely depend on hydrocarbons as their source of carbon and energy.

Total fungi count in Agro-wastes amended soils

Fungi species with specific degradation potentials have been known over the years through

extensive research to break down hydrocarbons in soils. The abundance of fungi species in soils does not determine their degradation abilities because not all fungi are hydrocarbon degraders. The amendment of the soil with 150gBMPP, 150g PSSP, and 150g FPHP produces significantly high ($P<0.05$) fungi counts with no observable differences among the means obtained (Table 1). The counts obtained from polluted soils ameliorated with 250g BMPP, 250 FPHP, 350g FPHP, 150g FPHP + 150g PSSP, 250g FPHP +250gPSSP and 150g BMPP + 150g FPHP decreases with no significant differences ($P>0.05$) in the mean fungi counts but higher ($P<0.05$) than the mean fungi count obtained from polluted-soils amended with 350g BMPP and 250g PSSP with no difference in the mean counts. Significantly reduced fungi counts were obtained in untreated crude-oil-polluted soils while the pristine soil recorded high ($P<0.05$) fungi count than the crude oil control. The abundance of fungi counts among the different treatment groups, shows that BMPP (2.81 x 10^5CFU/g) and FPHP (2.88 x 10^5CFU/g) had the highest counts, while a decrease

Table 1. Microbial population of the soil ameliorated with agro-wastes

Agro-wastes	THFC (X10^6 CFU/g)	THBC (X10^5 CFU/g)	HUBC (X10^5 CFU/g)	HUFC (X10^5 CFU/g)
PS	0.39^{h}±0.67	0.70^{d}±1.16	1.20^{h}±1.16	0.53^{h}±0.67
COPSC	0.87^{g}±0.67	2.97^{c}±0.88	2.60^{g}±1.16	0.87^{g}±0.67
150g BMPP	3.43^{a}±1.16	6.83^{a}±0.33	4.87^{d}±0.67	1.93^{e}±0.67
250g BMPP	3.10^{b}±0.58	6.20^{b}±1.16	5.77^{d}±1.20	2.43^{d}±0.88
350g BMPP	2.73^{c}±0.67	5.83^{b}±0.88	6.80^{c}±0.58	2.80^{d}±1.16
150g PSSP	3.40^{a}±1.16	6.00^{b}±0.58	3.93^{f}±0.67	2.40^{d}±0.58
250g PSSP	2.73^{c}±0.67	5.00^{c}±1.16	5.80^{d}±0.58	3.30^{c}±1.53
350g PSSP	2.37^{e}±0.33	4.60^{c}±1.16	7.20^{b}±1.16	3.67^{b}±1.33
150g FPHP	3.53^{a}±0.67	6.00^{b}±1.16	3.93^{f}±0.67	2.20^{e}±1.16
250g FPHP	3.20^{b}±1.16	5.60^{b}±1.16	5.40^{d}±1.16	3.00^{d}±1.16
350g FPHP	2.87^{b}±0.67	5.07^{c}±1.45	6.57^{c}±0.88	3.93^{a}±0.67
150g BMPP + 150g PSSP	2.53^{d}±0.67	3.80^{c}±1.16	6.00^{d}±1.16	2.50^{d}±0.58
250g BMPP+250g PSSP	2.13^{f}±0.88	3.33^{c}±0.67	7.47^{b}±0.67	3.00^{d}±1.16
350g BMPP+350gPSSP	2.07^{f}±1.76	2.97^{c}±0.88	8.00^{a}±1.16	3.07^{d}±2.67
150g FPHP + 150g PSSP	3.27^{b}±0.67	4.57^{c}±0.88	3.63^{f}±0.88	2.87^{d}±0.67
250g FPHP+250g PSSP	2.87^{b}±0.67	4.13^{c}±0.67	4.27^{e}±0.67	3.70^{b}±0.58
350g FPHP+350gPSSP	2.23^{f}±1.20	3.47^{c}±0.67	5.20^{d}±1.16	4.10^{a}±0.58
150g BMPP + 150g FPHP	2.97^{b}±0.33	4.20^{c}±1.16	3.27^{f}±1.33	1.63^{f}±0.88
250g BMPP+250g FPHP	2.60^{d}±1.16	3.63^{c}±0.88	4.30^{e}±0.58	1.97^{e}±0.88
350g BMPP+350g FPHP	2.00^{f}±1.16	3.13^{c}±1.76	5.17^{d}±0.88	2.63^{d}±1.45
LSD	0.11	0.48	0.37	0.18

Means with the same superscript along the same vertical arrays indicate no significant difference ($P>0.05$)
Legend:
BMPP: Bush mango peels powder
FPHP: Fluted pumpkin husk powder
PSSP: Plantain stem sap powder

Table 2. Comparison of the microbial population of the soil ameliorated with agro-wastes

Agro-wastes	THFC (X10^5 CFU/g)	THBC (X10^5 CFU/g)	HUBC (X10^5 CFU/g)	HUFC (X10^5 CFU/g)
BMPP	2.81^a±2.80	5.11^b±3.65	4.15^b±5.77	2.15^b±3.39
PSSP	2.66^b±2.81	5.77^a±3.92	4.25^b±5.52	1.71^c±2.37
FPHP	2.88^a±2.87	5.33^b±3.61	3.94^b±5.13	2.11^b±3.43
BMPP + PSSP	2.31^d±2.66	4.01^c±4.09	5.05^a±7.21	1.99^b±2.93
PSP+FPHP	2.63^b±2.80	4.43^c±3.75	3.38^c±3.71	2.41^a±3.91
BMPP+FPHP	2.47^c±2.74	4.19^c±3.96	3.31^c±3.68	1.53^d±2.05
LSD	0.08	0.31	0.25	0.17

Means with the same superscript along the same vertical arrays indicate no significant difference (P>0.05)
Legend:
BMPP: Bush mango peels powder
FPHP: Fluted pumpkin husk powder
PSSP: Plantain stem sap powder

in the counts was observed in the polluted soils amended with PSSP (2.66 x 10^{5}CFU/g) and PSSP + FPHP (2.63 x 10^{5}CFU/g) with no variation in the mean counts, while, the fungi count in soil amended with BMPP+FPHP (2.47 x 10^{5}CFU/g) and soils amended with BMPP+PSSP (2.31 x 10^{5}CFU/g) were the lowest. This result implies that the amended soils were not dose-dependent since the lower agro-wastes concentrations had the highest fungi counts in soils. High HUF was obtained in soil amended with 350gFPHP+350g PSSP (4.10x10^{5}CFU/g) and 350g FPHP (3.93x10^{5}CFU/g) with no difference (P>0.05) in the mean counts, this was followed by a decrease in the fungi counts in soil amended with 250g FPHP+250g PSSP (3.70x10^{5}CFU/g) higher than the count obtained in soil amended with 250gPSP, the soils amended with 250g and 350g BMPP, 150g PSSP, 250gFPSP, 150g, 250g, 350g (BMPP+PSSP), 150gFPHP+150gPSSP and 350g BMPP+350gFPHP with no difference (P>0.05) in mean counts. It was also observed that the crude oil control (without amendment) had high fungi counts than the pristine soils. However, the ameliorated soils with high agro-waste concentrations both in combined and single forms enhance the growth of the fungi population in soils as observed in both positive and negative controls. The HUFC as shown in Table 2 indicates that the polluted soils amended with PSSP +FPHP were the highest, with a corresponding decrease in soils amended with BMPP, FPPP, and BMPP+PSSP with no significant difference (P>0.05) in the mean values.

Total bacteria count in the soil amended with some agro-wastes

The abundance of bacteria in an environment or soil ecosystem is most times determined by the richness of the soil. Soil enrichment is one of the surest ways of increasing the bacteria population because their survivability depends on the available substrate for growth and multiplicity. The availability and abundance of bacteria engineered the process of biodegradation of hydrocarbons in polluted soils. The polluted soils amended with 150g BMPP (6.83x10^5 CFU/g) had the highest bacteria counts, the polluted soils ameliorated with 250g BMPP, 350g BMPP, 150g PSSP, 150g FPHP, 250g FPHP decreases with no difference (P>0.05) in the mean bacteria counts. This was followed by a corresponding decrease in the bacteria counts obtained in polluted soils amended with 250g PSSP, 350g PSSP, 350g FPHP, 150g BMPP +150gPSSP, 250g BMPP +250g PSSP, 350g BMPP + 350g PSSP, 150g BMPP+150g FPHP, 250g BMPP+250g FPPP and 350g BMPP+350g FPHP and the crude oil control (without amendment) with no difference (P>0.05) in the mean counts obtained (Table 1). This result implied that the single amendment increases the bacteria counts of the soils more than the combined amendments while the lower treatment concentrations increase the bacteria counts more than the high treatment concentrations. In nature, not all bacteria present in soil can degrade

hydrocarbons but bacteria with specific abilities utilize the hydrocarbons as their sole source of carbon. The population of the hydrocarbon degraders on freshly polluted soils is often low and may take some periods for the available utilizers to recuperate, multiple before degradation will commence. The engineered process of bio-stimulating the hydrocarbon-utilizing bacteria is one of the surest ways of achieving speedy degradation within a shorter time as observed during this study. The polluted soil amended with 350g BMPP +350g PSSP ($8.00x10^5$ CFU/g) had the highest HUBC in the soil, this was followed by a decrease in the counts obtained in polluted soils ameliorated with 250g BMPP +250g PSSP (7.47×10^5 CFU/g) and 350g PSSP (7.20×10^5 CFU/g) higher ($P<0.05$) than the HUBC obtained in soil amended with 350gBMPP (6.80×10^5 CFU/g) and 350g FPHP (6.57×10^5 CFU/g) with no variation in mean but also, higher ($P<0.05$) than the counts obtained in soil amended with 150g & 250g BMPP, 250gPSSP, 250gFPHP, 150gBMPP+150g PSSP, 350g FPHP +350gPSSP and 350g BMPP+350g FPHP with no difference ($P>0.05$) in mean bacteria counts. However, among the positive and negative controls, it was observed that the counts in crude-oil control (without amendment) were higher than the count in pristine soils. The result implies that the amended soils with the various agro-wastes at varying concentrations improve the growth and multiplicity of the hydrocarbon-degrading bacteria more than the unamended soils. The comparative analysis of the efficacy of the different agro-wastes in advancing bacteria growth in soils shows that the amended soil with BMPP+ PSSP had the highest HUBC, with a decrease in the polluted soils amended with the single amendments (BMPP, PSSP, FPHP), while soils ameliorated with PSSP +FPHP and BMPP+FPHP had the lowest counts (Table 2).

DNA Sequence Result for Bacteria and Fungi Species

The result of the microbial isolates identified through DNA sequencing during the bioremediation studies revealed that the following bacterial and fungal species were involved in the degradation of hydrocarbons in the soils: *Proteus mirabilis, Serratia marcescens, Bacillus cereus, Providencia rettgeri, Enterobacteria asburiae Pseudomonas aeruginosa, and Proteus penneri* (Table 3). *Aspergillus oryzae, Penicillium daleae, Cunninghamella polymorpha Penicillium citrinum, Cunninghamella bertholletiae, Aspergillus flavus, and Penicillium sp. Cs/2/5* (Table 4*).*

Total petroleum hydrocarbon (TPH) in the soil after bioremediation

The result as presented in Table 5 shows that the soil amended with the combined wastes at 350gBMPP+350gPSSP, 350g FPHP + 350g PSSP, and 350g BMPP + 350g FPHP had reduced TPH of 71.65mg/kg, 63.49mg/kg and 61.23mg/kg respectively with no difference ($P>0.05$) in the mean values. While soils amended with 350g FPHP shows a reduction in the TPH of the soil with a mean of 81.97mg/kg. A corresponding decrease in TPH was observed in soils amended with 350g BMPP (109.11mg/kg), 250gFPHP +250g PSSP (119.64mg/kg),

Table 3. Sequence identification of fungal species detected in agro-wastes amended soils

Sample No.	Query No.	Gene bank accession no.	Identity of isolate obtained
1.	891	KJ767060.1	*Penicillium daleae*
2.	558	HQ596918.1	*Penicillium citrinum*
3.	490	KF619561.1	*Aspergillus oryzae*
4.	951	KM067097.1	*Aspergillus flavus*
5.	757	JN585934.1	*Penicillium* sp. *Cs/2/5*
6	979	KF983475.1	*Cunninghamella polymorpha*
7.	993	DQ681328.1	*Penicillium purpurogenus*
9	974	AF252930.1	*Cunninghamella bertholletiae*

Total 4. Sequence identification of bacterial species detected in agro-wastes amended soils

Sample No.	Query Length	Gene bank accession No	The identity of the Isolate obtained
1	1122	JQ308547.1	*Bacillus cereus*
2	1022	EF633995.1	*Bacillus cereus*
3	993	EF434507.1	*Pseudomonas aeruginosa*
4	696	FR717839.1	*Proteus mirabilis*
5	1136	KC150144.1	*Proteus mirabilis*
6	1144	KC344360.1	*Proteus mirabilis*
7	1104	HQ259936.1	*Proteus penneri*
8	1141	KF938667.1	*Serratia marcescens*
9	1148	KC172019.1	*Providencia rettgeri*
10	1138	KJ877656.1	*Enterobacter asburiae*

Table 5. Total hydrocarbon content of the soil ameliorated with agro-wastes

Agro-wastes	THC (mg/kg)	% Degradation	% Saturation	Time for 100% degradation
PS	411.75^{i}±1.76	75.06	24.94	0.06
CPSC	713.97^{k}±2.79	56.81	43.19	0.03
150g BMPP	270.04^{g}±6.91	83.64	16.36	0.09
250g BMPP	186.76^{f}±2.52	88.69	11.31	0.13
350g BMPP	109.11^{c}±2.93	93.39	6.61	0.22
150g PSSP	449.2^{j}±4.01	72.79	27.21	0.05
250g PSSP	291.98^{h}±3.38	82.31	17.69	0.08
350g PSSP	263.27^{g}±4.38	84.05	15.95	0.09
150g FPHP	278.85^{g}±3.66	83.11	16.88	0.09
250g FPHP	177.53^{f}±1.71	89.28	10.72	0.13
350g FPHP	81.97^{b}±0.85	95.04	4.96	0.30
150g BMPP + 150g PSSP	168.29^{f}±1.73	89.81	10.19	0.15
250g BMPP+250g PSSP	155.65^{e}±2.74	90.57	9.43	0.16
350g BMPP+350gPSSP	71.65^{a}±0.43	95.66	4.34	0.34
150g FPHP + 150g PSSP	134.29^{d}±2.42	91.87	8.13	0.18
250g FPHP+250g PSSP	119.64^{c}±0.58	92.75	7.25	0.21
350g FPHP+350gPSSP	63.49^{a}±1.16	96.15	3.85	0.39
150g BMPP + 150g FPHP	116.53^{c}±1.20	92.94	7.06	0.21
250g BMPP+250g FPHP	111.10^{c}±1.67	93.27	6.73	0.22
350g BMPP+350g FPHP	61.23^{a}±0.44	96.29	3.71	0.40
LSD	0.56			

Means with the same superscript along the same vertical arrays indicate no significant difference (P>0.05)

250g BMPP+250FPHP (110.10mg/kg), and 150g BMPP+150FPHP (116.53) with no difference (P>0.05) in the mean values. A significant reduction in the TPH level in soil amended with 150g FPHP+ 150g PSSP with a mean of 134.29mg/kg was also observed (Table 5). The reduction of TPH in the amended soil polluted with crude oil is significantly greater than in the polluted soils without the amendment. This implies that the application of the waste at high treatment levels reduces the TPH of the soils more than the low treatment levels. The reduction in TPH

Table 6. Comparison of Total hydrocarbon content of the soil ameliorated with agro-wastes

Agro-wastes	THC	% Degradation	% Saturation
BMPP	338.33^{b}±56.92	79.51	20.49
PSSP	426.03^{a}±42.80	74.20	25.80
FPHP	332.81^{b}±58.74	79.84	20.16
BMPP + PSSP	411.75^{a}±1.76	75.06	24.94
PSSP+FPHP	288.62^{c}±65.38	82.52	17.48
BMPP+FPHP	282.82^{c}±66.42	82.87	17.13
LSD	20.6		

Legend:
BMPP: Bush mango peels powder
FPHP: Fluted pumpkin husk powder
PSSP: Plantain stem Sap powder

in polluted soil bearing the amended wastes implies that the waste materials possess a strong ameliorating property informed by their levels of microbial load. The percentage hydrocarbon degradation as presented in Table 6 shows that the combined amendment (350g BMPP + 350g PSSP) enhanced 96.29% hydrocarbon degradation in soils, followed by 350gFPHP+350g PSSP, 350gBMPP + 350gPSP, 350gFPHP and 350gBMPP which percentage hydrocarbon degradation of 96.15%, 95.66%, 95.04% and 93.39% respectively. The crude-oil control had the lowest percentage of hydrocarbon degradation value of 56.81 with a percentage saturation value of 43.19%. However, the waste with the highest percentage of hydrocarbon degradation is BMPP + FPHP (80.87%) as shown in Table 6.

DISCUSSION

Soil remediation is an aspect of soil reclamation and restoration. It ensures the removal of the pollutants in the soil, thereby restoring the hope of agriculturists in the re-utilization of the soil ecosystem (Antai *et al.* 2023). Primarily, when soil is polluted the micro and macro-organisms lose their activeness, functionality, and dependency in soils, due to the depletion of essential micro and macronutrient. Enhanced microbial degradation is one of the surest ways of ensuring the complete mineralization of pollutants in an environment (Liu et al., 2015). The proliferation of microbes in soils is mostly possible with the amelioration of suitable agronomic materials with required nutrients. The depreciation in the amount and volume of microbes in soil reduces the degradation rate of hydrocarbons in soils (Hesnawi & Mogadami 2013). The baseline result proves that the addition of crude oil in soils increases microbial counts, compared to the low counts obtained in the pristine soils. The increase in the bacteria and fungi counts may be a result of the utilization of the hydrocarbons as an energy source by the hydrocarbon-utilizing microorganisms with a resultant increase in the growth of the microbes. This present study shows that the amenability of the soil with the various agricultural wastes impacted positively the proliferation of hydrocarbon-utilizing microorganisms. The increase in the microorganisms was high in soils amended with the combined agro-wastes than in the single amendment. (Besalatpour et al., 2011; Antai *et al*., 2023) equally observed that the reason for high bacteria counts in amended soils was based on the availability of appreciable quantities of nitrogen and phosphorus in agro-waste. The availability of organic wastes promotes soil aeration, thereby, allowing aerobic microorganisms to perform at their peak. However, the presence of high nitrogen could be beneficial to the proliferation and biodegradability activities of microorganisms. The bioavailability of the nutrient to the indigenous microbes and the accessibility of the microbes to the pollutants for possible degradation was highly necessary for hydrocarbon breakdown. Abioye *et al*., (2009) observed that an appreciable increase in bacteria counts when spent-

engine oil was inoculated in soil and treated with a banana skin, brewery spent grain, and spent mushroom. The increase observed in the heterotrophic bacteria counts was an indication of the effectiveness of the different agro-wastes supplementation which increases nutrient value for optimum utilization by indigenous soil microbes. The amelioration of the soil with agro-waste improves its performance when compared with the pristine control soil. The increase in the fungi counts negates the earlier findings of (Liu et al. 2015) that the addition of agro-wastes in the polluted soil was treatment dependent (the higher the concentration of the amendments, the higher the fungi count). Microbial diversity varies in different soils which most times affects their abundance in the ecosystem an effective application of nutrient supplements to enhance the abundance of the microbes revealed that the most populated microbial species were Gram-positive bacteria (Chikere *et al*., 2011). Evans *et al*., (2004) also reported that the abundance of Gram-positive bacteria contributed significantly during the bioremediation of polluted soils. It was also reported by (Olabisi 2009) that *Bacillus, Micrococcus, Pseudomonas,* and *Acinetobacter* were the most frequently occurring bacterial Genera in petroleum hydrocarbon-polluted soils ameliorated with melon shell powder. However, the report from several studies shows that microbes with high degrading potentials in both terrestrial and aquatic ecosystems are Gram-positive bacteria, because of their metabolic capabilities The Gram-positive bacterial have a high advantage over the gram-negative bacterial due to their metabolic abilities, and resilience in highly polluted environments and as good materials for the production of bio-surfactant (Hamamura *et al*., 2006). Ekanem & Ogunjobi (2017) observed also that "total microbial biomass was a poor predictor for determining biodegradation potential mainly because the active biomass may differ in species composition and metabolic regimes". The hydrocarbon degradation in soils depends on the specific abilities of the microbial biomass to utilize the hydrocarbons as a source of carbon for growth and energy. The functionality of the microbes in polluted soil is an indication that they are hydrocarbon utilizers, since, the non-hydrocarbon utilizers are limited in strength and survival is unaccounted for. The sequenced results revealed that microbial species such as *Proteus mirabilis, Serratia marcescens, Bacillus cereus, Providencia rettgeri, Enterobacteria asburiae Pseudomonas aeruginosa, and Proteus penneri* (Table 3). *Aspergillus oryzae, Penicillium daleae, Cunninghamella polymorpha Penicillium citrinum, Cunninghamella bertholletiae, Aspergillus flavus, and Penicillium sp. Cs/2/5,* were present (Table 4). The identified organisms have been proven in several studies to possess a strong degradation potential in breaking hydrocarbon chains. (Pathak *et al*., 2015) also reported on the abilities of *Pseudomonas aeruginosa, Pseudomonas putida,* and *Acinetobacter iwoffi* to degrade oil components of C_{12} to C_{13} in polluted soils. The abundance of total petroleum hydrocarbon in soil reduces the availability of soil microbes that lack specific abilities to tolerate the effect of hydrocarbons. The elevated quantity of hydrocarbons in the soil ecosystem affects the performance of crops and plants found in such areas. Many terrestrial environments are under threat and habitat loss due to the abundance of hydrocarbon products in soils. The degradation of TPH in the ecosystem is solely dependent on several factors such as temperature (evaporation and degradation of hydrocarbon molecules is made possible at higher temperatures, especially the low molecular weight compounds). Microbial degradation is supreme when it comes to bioremediation. Microbial degradation is one of several methods that have been classed to degrade hydrocarbon compounds either the low or high molecular weight (Streche *et al*., 2018). Interestingly, microbial degradation brings about a speedy hydrocarbon reduction in the polluted environment. The higher the microbial load the more the breakdown of the hydrocarbons. Microbial degradation is dependent on the specific microbes, with specific abilities to degrade the hydrocarbons. The TPH in soils amended with the highest concentrations of the combined agro-wastes shows the lowest values. This result is in line with the findings of (Antai *et al*., 2023), that the higher the concentrations of the amendments the lower the hydrocarbon content in soils. Benson *et al*. (2016) recorded a remarkable reduction

in the total hydrocarbon content which is attributed to the increase in hydrocarbon-degrading microbes in the amended soil. Eigbuluese *et al*., (2021) revealed that plant parts or domestic wastes have the potential for sustainable remediation of crude oil-contaminated soils; they observed a significant reduction in the TPH of the soils. However, Venkateswaran & Harayama (1995) opine that nutrient supplementation to an ecosystem could lead to a selective increase in microorganisms with specific hydrocarbon degradation potentials. Metzenberg 2003; Russel 2002) reported a high reduction in the hydrocarbon content of the polluted soils, when wheat bran and swine wastewater were utilized for bioremediation of oil-contaminated soils, the percentage of TPH degraded was high. The result of this study equally revealed a significant reduction in the hydrocarbon content, due to high microbial proliferation in the amended soils.

CONCLUSION

Enhanced microbial degradation of hydrocarbon-polluted soil with the agro-wastes (plantain stem sap, bush mango peels, and fluted pumpkin husk) in single and combined forms during the piloted bioremediation study shows increased soil enrichment with a concomitant increase in the microbial community. The degradation of the crude oil was achieved with the availability of *Pseudomonas aeruginosa, Bacillus cereus, Aspergillus* sp, and *Penicillium sp.* These microbes possess specific abilities in the utilization of hydrocarbons as their sole energy source. The biostimulation of the soil with the combined treatment of the crude oil-polluted soil with bush mango peels (BMP) + fluted pumpkin peels and plantain stem sap + fluted pumpkin husk enhances the microbial degradation of hydrocarbons at 82.87% and 82.52% in the soil. It is therefore concluded that based on the efficiency of these agro-wastes in enhancing microbial degradation, further studies should be carried out on the enzyme activities and production of Bio-surfactant from the wastes.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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