International Journal of Environment, Agriculture and Biotechnology, 5(3)
May-Jun, 2020 | Available: https://ijeab.com/

Microbial stimulating potential of Pineapple peel (*Ananas comosus*) and Coconut (*Cocos nucifera*) husk char in crude-oil polluted soil

Onuoha, E. M.[1*]; Ekpo, I. A.[1]; Anukwa, F. A.[2]; Nwagu, K. E.[3]

[1]Department of Genetics and Biotechnology, Faculty of Biological Science,University of Calabar, Calabar. Nigeria.
[2]Department of Botany, Faculty of Biological Science, University of Calabar, Calabar. Nigeria.
[3]Biological Science department, Alex Ekwueme Federal, Ndufu Alike Ikwo, Abakiliki, Ebonyi State, Nigeria
*Corresponding author

Abstract— *The bioremediation potential of ten different treatments formed from two organic sources of nutrients: coconut husk ash (CHA) and pineapple peel (PP) on 5kg of soil polluted with 400ml of crude oil were monitored for 84 days. The changes in the physicochemical properties of the soil were observed, the degradation process was monitored by; the measure of the total petroleum hydrocarbon (TPH) loss, the total bacterial and fungal counts, the crude oil utilizing bacterial and fungal counts. The result revealed that there was a reduction in the soil TPH with all treatments and also the polluted control, which may be owing to natural attenuation. The treatment, PP12 was seen to have the lowest TPH value of 40.40 ± 0.40 mg/kg at the 84th day with a percentage reduction of 89.90. This was followed by the PP8 (49.733±0.267mg/kg) and PP4 (70.000±0.577mg/kg), also the POC (polluted control) had a concentration of 245.333±1.453mg/kg at the 84th day which is a 38.67% reduction. The treatment, CHA12 influenced the TPH to a concentration of 78.000±1.528mg/kg which was an 80% reduction. The total bacterial count had the highest CFU/g of 2.06 x 10^7 ± 0.006 on the soil treated with CHAPP12 and the least count was at POC (2.3 x 10^6± 0.007cfu/g). Crude oil utilizing bacteria were least with the POC (0.21 x 10^4 ± 0.010 CFU/g) and were more at the PP12 treated soil (1.3 x 10^5 ± 0.012). Some of the probable bacterial isolates identified through biochemical testing included Bacillus spp., Serratia spp., Proteus spp., Pseudomonas spp., Enterobacter spp., Klebsiella spp., Aeromonas spp.and Staphylococci spp. The fungi isolates ranged from 3.0 x 10^4 CFU/g to 6.0 x 10^4CFU/g and identified fungi included Cephalosporiumspp, Coccidioides immitis, Aspergillus niger, Aspergillus fumigatus, Penicillium spp, Trichophyton mentagrophyte, and Moraxella spp.*

***Keywords*— bioremediation, crude oil utilizing bacteria, coconut husk ash, pineapple peel.**

INTRODUCTION

In most areas of the world, and Nigeria in particular, the case of land and water bodies being contaminated with hydrocarbons is now of serious concern. It is of global concern because of the potential consequence it has on the health of humans and also the ecosystem [1].

Hydrocarbon discharge into the environment sometimes happen accidentally, and it alters the physical and chemical state of the soil which in turn affects the population of the plant in such environment [2]. Hydrocarbons have a versatile purpose; they become pollutants when released to the environment, they do not easily biodegrade and are persistent in the environment causing serious ecological problems, also they are very toxic, carcinogenic and emit strong odours causing a public health risk [1]. For the sake of this, interest and focus are on the research for new schemes and approaches that are also environment-friendly that can be functional in the remediation of any part of the ecosystem exposed to hydrocarbon contamination. Amid the several approaches, biotechnological strategies that depend on the use of indigenous microorganisms to biodegrade organic pollutants are of particular relevance [1].

Biological methods of treatment have over time been seen to be more economical and promising compared to other methods of enhancing the loss of compounds in the environment that contains phenol. Some persons believe this method can lead to the breakdown of organic compounds completely and can cover wide concentrations of the compounds. The involvement of microorganisms in the bioremediation of hydrocarbons is principal for natural attenuation to occur at a contaminated site, but the reduction in the concentration of the contaminants can be attributed to physical and chemical (abiotic) factors like dispersion, dilution, sorption, and volatilization.

The clean-up technique, Bioremediation, has several techniques that have been designed to be deployed for use in enhancing the rate of degradation of hydrocarbons at the sites they contaminate [2]. bioremediation usually involves applying treatments or amendments in other to increase the bioavailability and invariably the degradation of petroleum hydrocarbons in polluted soils and sediments by autochthonous or allochthonous microorganism [1].

LITERATURE REVIEW

The soil is a very significant element of the natural ecosystem and a principal endpoint of wastes and chemicals used by the public naturally or accidentally [3]. Petroleum contaminants eventually end up mostly in soils and sediments, like the benzene, toluene, ethylbenzene, and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons (PAHs) [4].

The main cause of soil or water pollution is the conscious or accidental discharge due to anthropogenic activities of hydrocarbons containing compounds into the environment. Crude oil pollution adversely affects the soil ecosystem through soil particles adsorption, increase in carbon content that might be unavailable for microbial use and brings about limitation in soil nitrogen and phosphorus [5]. Soil contaminated with hydrocarbon can lead to widespread destruction of the natural state of an organism or the environment since the accumulation of contaminants in animals and plant tissue can lead to mutations or death [6].

2.1.Effect of crude oil pollution

Reference [7] showed that soil polluted with petroleum causes marked changes in soil properties which affect the physicochemical and microbiological characteristics of the soil. Crude oil in the soil results in the accumulation of essential (carbon, phosphorus, calcium, magnesium) and non-essential (magnesium, copper, lead, zinc, iron, cobalt,) elements in soil which are subsequently translocated or bioaccumulated in the tissues of plants. These change processes affect significantly the soil's enzymatic activities and result in a very slow process of degrading the crude oil in the soils. The effect of crude oil pollution is felt on:

A. Human health and the Environment:
B. The Economy
C. Tourism Industry
D. Soil Microorganism

2.2.Clean-Up Technologies

Oil spilled in the environment has a very intricate outcome. There are several processes both physical and chemical, that play a role in the dispersal and breakdown of crude oil components [8].

Some technologies that are frequently used in remediating polluted soil include washing, burying, dispersion, evaporation, and the mechanical approach. Though, these methods are quite costly and may not lead to a complete breakdown of the contaminants.

Here are the basic lines of action it takes to clean up the oil spill:

(1) Leaving it to naturally disperse

(2) The use of booms to accumulate the oil on the water's surface and

skimmers to collect them.

(3) Using dispersants to split up the oil and enhance its degradation

4) Involving microorganisms like bacteria and fungi to augment the biodegradation process

The search for a treatment technology that is more suitable in remediating crude oil-contaminated soil and water has not been easy. Several techniques exist and most are not cost-effective, so the need for a practical and cheaper procedure for the clean-up process has made several researchers give in to the goal of studying different processes used in soil pollutions and to give considerations to the best applicable solution. Amongst several approaches to soil clean-up, few are briefly explained below:

2.2.1. Bioremediation Strategy

The technology, bioremediation was derived to combat the problem of environmental pollution [9]. Bioremediation technology is believed to be sustainable [10], non-invasive, cost-effective and relatively cheaper than other remediation technologies [6].

Bioremediationis a technology that involves the use of treatments for the clean-up of polluted sites. It uses several processes just to enhance the process of contaminant biodegradation.This technology involves using indigenous or foreign microorganisms that are added for the decontamination and degradation of contaminants in the environment. This technique also is established around strategies that apply moisture, aeration, and nutrients to the contaminated environment to enhance and augment microbial activity and the degradation of the pollutant[11]Numerous microorganisms have the capability of metabolically making use of undesirable pollutants in the environment as their source of food and energy, in so doing they reduce the pollutants in the environment from an energy-rich state to an energy-poor one.So, microorganisms can bioremediate the environment even as they biodegrade pollutants to acquire energy [12] A disproportion in the carbon-nitrogen ratio is one result of the crude oil spill in the soil. Knowing that crude oil is a composition of hydrogen and carbon, it also results in a limitation of nitrogen and phosphorus in a soil soaked with oil, which causes retardation in microbial growth and the use of the carbon as an energy source. Microbes and nutrients have been identified as some of the factors that can reduce the rate of degrading petroleum hydrocarbon**.** Thus, bioremediation technologies are developed for soils and coastline areas by the addition of nutrients and microorganism [1].

Under conditions suitable to bioremediation, contaminants are transformed into harmless substances such as water and carbon dioxide via diverse metabolic capabilities of microorganism. The degree and magnitude at which bioremediation occurs are influenced by the state of the contaminated environment and the interfaces between the organisms present [13]

To successfully carry out oil spill bioremediation it is pertinent to notes that conditions that favour and enhances the process of oil degradation in the polluted environment ought to beestablished and maintained. There are several reviews on the factors that affect the rate of oil biodegradation. One very significant requisite is the presence of microorganisms with suitable metabolic abilities**.** If themicroorganisms are present, then the growth rate can be enhanced for the biodegradation process to be sustained, this can be done by making sure that sufficient concentrations of the basic nutrients and ample oxygen are present and also the pH should range from six to nine. The physicochemical characteristics and surface area of the oil are also an essential determining factor of successful bioremediation. Furthermore, the use of indigenous microbial consortium will ensure that the organisms have a greater tolerance to the toxic effect of hydrocarbons and are resilient to environmental changes [14]. The application of bioremediation can be controlled and optimized where the condition of the environment permits microbial growth and activity; it may also include manipulating environmental factors in other to increase microbial growth and for the degradation process to occur faster. Enhanced bioremediation involves technologies that support the addition of electron acceptors or donors to promote the growth of naturally occurring microorganism (biostimulation) or may involve introducing specific microorganisms (bioaugmentation) to augment the process of biodegrading the targeted compound [15].

The Bioremediation has major two approaches;

A Bioaugmentation: A method in which identified microbes are applied to support the existing microbial population. It involves introducing microorganisms with the capability to biodegrade a contaminant into an environment with such contaminant for them to assist the indigenous microorganisms with the process of biodegradation. This may sometimes involve adding genetically designed microorganisms created specifically for biodegradation into the contaminated soil [16].The ability of allochthonous microorganisms to degrade contaminants in soil is likely to be affected by the physicochemical and biological characteristics of the soil because the soil environment is very complicated and at times, adding foreign microorganisms even those with the capacity to degrade the pollutants can still fail the bioaugmentation approach.There are cases where engineered or laboratory strains of microorganisms are unable to survive and degrade xenobiotics as much as the autochthonous microorganisms, this has made suggestions that the bioaugmentation approach may not at all times be effectual in the remediation of polluted soils, also bioaugmentation approach has not yet been accepted by the public and particularly when it comes to using genetically engineered microorganisms because of the belief that GEMs applied to contaminated soil, they may alter the ecosystem and if they persist in the soil after the remediation process, they can become threats to environmental health[16].

B, Biostimulation Involves stimulating the growth of inherent microorganisms with oil-degrading abilities via adding soil treatments or other soil nutrient-enhancing co-substrates[6].

Microorganisms are present in the soil even when it is contaminated, yet for the remediation of such soil to be effective, the growth of the microbial community has to be influenced. Biostimulation entails adding nutrients, electron acceptor, and oxygen to act as growth stimulants to already present bacteria involved in soil remediation. It also involves improving the environmental condition of the polluted area [17]. Biostimulation is well recommended as a suitable approach to bioremediation for crude oil removal in soils and this also calls for monitoring the degrading capacities of the autochthonous microorganisms and the ecological factors that work together in the kinetics of the *in situ* process [1].

Composting technologies can also be used to achieve biostimulation. This technology relies on mixing the contaminated soil with constituents of compost such that as the compost matures it serves as a nutrient to the microbes, and the pollutants will be degraded by the microorganisms that are active inside the mixture [16].

In biostimulation, it is required that both the inherent capacities of the indigenous microorganisms to degrade pollutants and environmental factors involved in the process be evaluated, one of which is aeration, which can be improved in the remediation process by using of plant crop residues that can function as bulking agents [18].

2.2.2. Natural attenuation

Natural attenuation is said to occur when physical, chemical and biological activities work together and causes a reduction in the toxic form of contaminants as well as hinders the spreading on the contaminants to other areas around the polluted site [19]. Natural attenuation can be let to take its course when the natural conditions support bioremediation to take place without human intervention because the hydrocarbons degradation process is quite complex and it is influenced by nature and concentration of the hydrocarbons present.One other essential factor that limits the process of degrading oil contaminants in the environment is that they are not readily available to microorganisms [6].

2.2.3. Advantages of Biological Remediation

Bioremediation in comparison with other treatment technologies is of greater advantage in the removal of a contaminant. Some advantages include;

1. It destroys the contaminants instead of transferring them to some other place
2. The exposure of workers to the contaminants is very minimal
3. It has a longer span of public health protection
4. It can also reduce the duration of the process of remediation [16].

2.3. Factors Affecting Hydrocarbon Biodegradation Processes

For bioremediation technology to be successfully applied to a contaminated area, certain factors that affect the process are taken into consideration. They include the characteristic of the contaminated site, the characteristic of the soil and the contaminant, the bioavailability of the contaminant to the microorganism, the number of microorganism present during the contamination period and the catabolic reactions they undergo. These limiting factors ought to be considered and properly monitored and understood to adopt and implement any bioremediation strategy.

2.2. Agricultural Waste

Wastes from fruits and vegetables from the agricultural and food industries are usually in very large quantities. Because they are highly degradable, they cause a form of nuisance in the environment when dumped at landfills. These waste can serve as adsorbents and through the process of biosorption, they can be used effectively in removing toxic heavy metals was contaminated environment especially wastewater [20].

More attention in the use of microorganisms for production is on the increased because the microbial community has the capability of using various kinds of wastes which are sources of environmental pollution and health hazards.Agricultural wastes are good renewable resources of energy and they have biotechnological benefits. Waste products such as bagasse, rice straw, rice hulls, and starch residues have been applied as growth enhancers for microorganisms. The use of agro-waste is economical for use as a tool to salvage pollution problems and reduce their further disposal[21].

Agricultural wastes have high potency for use in producing and stimulating the growth of microorganism, yet they have to satisfy the following conditions; they must not be toxic, they ought to be in abundance, cheap and readily available, they also must be completely regenerable, and must be able to stimulate and enhance faster growth and proliferation of microbial population, which will lead to the production of biomass with good quality[21].

Even though microorganisms are found in contaminated soil, they may not be in the quantities that area need for the bioremediation of the soil. The growth and activity of the microorganism need to be stimulated. Carbon is the most basic form of nutrient required for living organisms and also, the microbes require macronutrient like nitrogen and phosphorous to ensure optimum growth and effective degradation of the oil. The ideal nutrient balance required for hydrocarbon remediation is carbon: nitrogen: phosphorus equals 100:10:4. In general, at least, 1 ppm of ammonium nitrogen and 0.4 ppm of orthophosphate needs to be present. The remediation pathways can be influenced by further adjusting the quantity of the bio-nutrients [17].

In most soil bioremediation studies, inorganic chemical fertilizers have been extensively used as stimulating agents, though, they are relatively scarce and expensive and not usually enough for use in agriculture because of their high demand, how much more being sufficient for cleaning oil spills. Therefore, the search for more affordable and environment-friendly options for the enhancement of petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent [22]. One such option is the use of organic wastes derived from plant and animals. Few research persons have reported potential applications of plant organic wastes in bioremediation, such as rice husk and coconut shell [23], plantain peels and cocoa pod husk [9], *Moringa oleifera* and soya beans [24] poultry manure and goat dung [25] as stimulating agents for microbial growth in soils contaminated with petroleum hydrocarbons and they all showed a positive effect in reducing the hydrocarbon concentration in the soils. However, the search for a more cost-effective and environment-friendly technique of increasing the degradation of petroleum hydrocarbon in soils needs more advancement.

Information on the use of coconut husk ash and pineapple peels as soil amendments and stimulants of indigenous microorganism in petroleum hydrocarbons contaminated soils has not been previously done. Reference [26] also investigated the permeability of adsorbents made from pineapple peel and reported an existing relationship between biosorption efficiency and perviousness. Bulking agents such as rice husk, coconut shells, and sawdust have been used in biodegradation of hydrocarbons. The efficiency of hydrocarbon degradation by the addition of bulking agents and fertilizer NPK was about the same with no significance in a short period of incubation (up to 24 days) [18].

Another study by [27] showed coconut shell led to the higher percentage loss of petroleum hydrocarbon than rice husks when used as a treatment in a 9 percent diesel polluted soil.

MATERIALS & METHODS

3.1. Source of materials

The crude oil was obtained from Bonny, Port Harcourt, River State, Nigeria, while the coconut husk (CH), and pineapple peel (PP) were obtained from local farmers in River State, Nigeria.

3.2. Production of treatment

The collected agro-waste; coconut husk was allowed to dry to facilitate proper grinding. The dried coconut husk was ashed in a muffle furnace at 500°C for five minutes, allowed to cool and stored in a container. The Pineapple peel was sun-dried for two weeks and pulverized into powder using Master Chef 7 in 1 Blender with model No. ML 810. The powdered substance was sieved through a two-millimetre sieve, labelled and stored in a container.

3.3. Macronutrient analysis of the treatment

The powdered samples of ashed coconut husk and pineapple peel wereanalyzed for the macronutrients; nitrogen, phosphorus, magnesium, potassium, calcium, sodium and organic carbon contents as a requisite for use in this study.

3.4. Soil Sample collection

Surface soil from a depth of 0 to 25cm was randomly collected from four points with an auger. The collected soil was bulked to form a composite sample. The soil was air-dried and passed through a 2mm sieve. The buckets were arranged in triplicates using the completely randomized design (CRD). This site was used to use agricultural soil that has not been exposed to intentional hydrocarbon.

3.5. Artificial pollution of soil

The soil in each bucket except the pristine control was contaminated with 0.4 litres of crude oil artificially. The polluted soil in the plastic bucket was tilled and allowed to stand for 2 weeks (to allow for the acclimatization of indigenous microorganism in the soil to the new soil condition). Tilling of the soil for proper mixing was done weekly to ensure proper aeration and dispersion of the hydrocarbons, making them readily available for microbial attack.

3.5.1. Treatment application

The polluted soil was treated with selected agro-wastes in single and combined forms as follows:

Table 1. Treatment Composition and Codes

Treatment Code	Treatment
PC	Pristine Control
POC	Polluted + No treatment
CHA4	Polluted Soil + 4% Coconut husk Ash
CHA8	Polluted Soil + 8% Coconut husk Ash
CHA12	Polluted Soil + 12% Coconut husk Ash
PP4	Polluted Soil + 4% Pineapple Peel
PP8	Polluted Soil + 8% Pineapple Peel
PP12	Polluted Soil + 12% Pineapple Peel
CHAPP4	Polluted Soil + 4% CHA + Pineapple Peel
CHAPP8	Polluted Soil + 8% CHA + Pineapple Peel
CHAPP12	Polluted Soil + 12% CHA + Pineapple Peel

CHA – Coconut Husk Ash; PP - Pineapple Peel

3.6. Experimental Design

The experiment was conducted using a 10x3 factorial experimental unit in a completely randomized design (CRD) with 3 replicates

Factor 1: 10 Treatments (POC, CHA4, CHA8, CHA12, PP4, PP8, PP12, CHAPP4, CHAPP8, CHAPP12)

Factor 2: Duration (28D, 56D, 84D)

Calculation of treatment percentage

$$Percentage of treatment = \frac{quantity of organic wastes}{Quantity of soil} \times 100$$

$$4\%\ treatment = \frac{200g}{5000g} \times 100$$

$$8\%\ treatment = \frac{400g}{5000g} \times 100$$

$$12\%\ treatment = \frac{600g}{5000g} \times 100$$

3.7. Laboratory Analysis

3.7.1. Physicochemical analysis of the soil

The physicochemical properties of the soil samples were determined using the methods of [28] and the [29]. The parameters analyzed included: moisture content, pH, organic carbon, nitrogen, phosphorus, potassium, calcium, magnesium, hydrogen ion, aluminium, cation exchange capacity, and base saturation.

3.7.2. Microbiological analysis of the soil sample

The collected soil sample was analyzed for the bacterial and fungal population in the soil using a surface plating method.

3.7.2.1. Bacterial counts and isolation

3.7.2.1.1. Enumeration of total heterotrophic bacteria

Total heterotrophic bacterial (THB) was counted by applying the spread plate method on nutrient agar (NA) according to [30]

3.7.2.1.2. Enumeration and isolation of crude oil-degrading bacteria

The Crude oil-utilizing bacteria in the soil samples were counted by the viable count method using the surface spreading technique and the mineral salts medium as done by [31].

3.7.2.2. Bacterial counts and isolation

3.7.2.2.1. Fungi Enumeration

The total number of fungi present in the soil was enumerated using surface spreading techniques following[32].

3.7.2.2.2. Enumeration and isolation of crude oil utilizing fungi

This was done using the surface spreading technique. The same procedure used in counting crude oil-degrading bacteria. But in this case, 0.1ml of 10^{-6} dilution was plated onto mineral salt agar medium.

3.7.2.3. Characterization and identification of the isolates

Standard inoculums were prepared from preserved cultures by taking loop full of the isolates and aseptically inoculating onto sterile nutrient agar (NA) plates. The plates were incubated at 28°C for 24 hours. The isolates were characterized using Gram staining, oxidase, catalase, citrate, urease, coagulase, triple sugar iron agar, Mobility indole ornithine and methyl red tests as explained in [33]. The fungal isolates were examined macroscopically and then microscopically using the wet mount method (cotton blue in lactopherol) before the fungal identification.

3.7.3. Determination of total petroleum hydrocarbons

The following calculations were done using their appropriate equations below:

i. Percentage hydrocarbon saturation during the 84 days = $TPH_{84} \div TPH_{initial}) \, x \, 100$

ii. Percentage of hydrocarbon degradation = $100 - (TPH_{84} \div TPH_{initial}) \, x \, 100$

iii. Time required for 100% TPH degradation (year) = (84 days ÷ % TPH_{84}) x 100 ÷ 365 days

iv. The degradation rate of TPH per day ($TPH_{initial}$ – TPH_{84}) ÷ T

Source: [34]

3.8. Statistical Analysis

Analysis of variance (ANOVA) was carried out on the data collected using a 10x3 factorial in a Completely Randomized Design (CRD). The significant means were using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Table 2. Characteristics of coconut husk ash and pineapple peel as a requisite for use in remediation study

Nutrients	Coconut Husk Ash (%)	Pineapple Peels (%)
Nitrogen (mg/kg)	0.29	0.98
Phosphorus (mg/kg)	0.26	0.05
Potassium (cmol/kg)	1.72	0.56
Calcium (cmol/kg)	0.64	0.88
Magnesium (cmol/kg)	0.43	0.29

Table 3.Baseline Assessment of the soil

Physicochemical Properties	Before contamination	After contamination
Moisture (%)	8.27 ± 0.0173	10.3 ± 0.05
pH	5.307±0.097	8.233±0.088
Organic Carbon (%)	1.283±0.012	3.233±0.133
Total Nitrogen (%)	0.137±0.022	0.070±0.012
Phosphorus (mg/kg)	78.650±0.180	36.297±0.179
Calcium (mg/kg)	7.833±0.145	4.500±0.252
Magnesium (mg/kg)	2.400±0.115	1.400±0.115
Potassium (cmol/kg)	0.110±0.006	0.077±0.009
Sodium (cmol/kg)	0.080±0.006	0.060±0.006
Aluminium (cmol/kg)	0.193±0.007	0.923±0.018
Hydrogen (cmol/kg)	1.193±0.007	1.613±0.009
ECEC (cmol/kg)	10.327±0.015	8.163±0.007
Base Saturation (%)	83.167±0.120	62.833±0.441
Clay (%)	12.067±0.120	6.900±0.058
Silt (%)	5.667±0.060	10.683±0.009
Sand (%)	81.453±0.174	75.283±0.012
TPH (mg/kg)	2.500 ± 0.058	400.000 ± 4.509
THB (cfu/g)	$5.30 \pm 0.05 \times 10^6$	$2.2 \pm 0.15 \times 10^4$
CRUB(cfu/g)	$2.30 \pm 0.03 \times 10^3$	$1.8 \pm 0.02 \times 10^4$
THF(cfu/g)	$4.21 \pm 0.10 \times 10^6$	$1.5 \pm 0.06 \times 10^2$
CRUF (cfu/g)	$1.19 \pm 0.002 \times 10^4$	$0.8 \pm 0.02 \times 10^3$

Table 4.Effect of coconut husk ash and pineapple peels on the total petroleum hydrocarbon in a crude oil-polluted soil

TREATMENT	DURATION		
	Day 28	Day 56	Day 84
POC	349.667[h] ±2.906	302.333[j] ± 1.453	245.333[h] ±1.453
CHA4	317.933[g] ±1.507	168.333[i] ±0.882	119.433[g] ±0.567
CHA8	291.167[f] ±4.512	149.300[g] ±0.700	80.400[e] ±0.833
CHA12	101.133[b] ±1.988	84.033[c] ±0.578	70.000[c] ±0.577
PP4	119.733[c] ±0.267	89.800[d] ±0.200	74.933[d] ±0.067
PP8	99.567[b] ±0.433	59.533[b] ±0.467	49.733[b] ±0.267
PP12	79.700[a] ±0.300	49.333[a] ±0.667	40.400[a] ±0.400
CHAPP4	241.000[e] ±2.082	160.700[h] ±1.179	80.400[e] ±0.833
CHAPP8	140.233[d] ±0.960	103.333[f] ±1.202	88.800[f] ±1.114
CHAPP12	100.567[b] ±0.977	96.667[e] ±1.202	78.000[e] ±1.528

Mean ± S.D

POC – Polluted control; CHA- Coconut Husk Ash; PP- Pineapple Peel; CHAPP- Coconut Husk & Pineapple peel;

Mean with the same superscript along the horizontal arrays indicate no significant difference (p>0.05)

Table 5:Effects of coconut husk ash and pineapple peels on the total bacterial and fungal count in the polluted soil 84 days after treatment

	POC	CHA4	CHA8	CHA12	PP4	PP8	PP12	CHAPP 4	CHAPP 8	CHAPP 12
THB ($X10^7$) CFU/g	0.24±0.006[a]	0.42±0.015[c]	0.78±0.012[d]	1.52±0.012[h]	1.17±0.016[e]	1.26±0.001[f]	1.34±0.014[g]	0.37±0.012[b]	1.30±0.006[e]	2.06±0.000[i]
CRUB ($X10^4$) CFU/g	1.12±0.012[a]	1.21±0.009[b]	3.01±0.009[d]	8.01±0.010[h]	2.01±0.013[c]	5.02±0.012[f]	9.03±0.015[i]	4.03±0.018[e]	8.00±0.003[g]	13.03±0.20[j]

THF ($X10^6$) CFU/g	1.25±0.050[a]	2.84±0.01[b]	2.94±0.037[c]	3.31±0.013[f]	3.01±0.010[d]	3.22±0.020[e]	3.81±0.007[g]	2.80±0.003[b]	2.94±0.037[c]	3.42±0.017[ef]
CRUF ($X10^4$) CFU/g	0.32±0.017[a]	0.84±0.003[b]	0.92±0.003[c]	1.12±0.023[f]	0.87±0.010[b]	1.12±0.017[e]	1.46±0.007[h]	0.85±0.007[b]	1.07±0.017[d]	1.41±0.007[g]

Mean ± Std. Deviation

POC- Polluted control;PP- Pineapple peel; CHA- Coconut husk Ash4- 200g; 8- 400g; 12-600g

*a, b, c significantly different means $p<0.05$

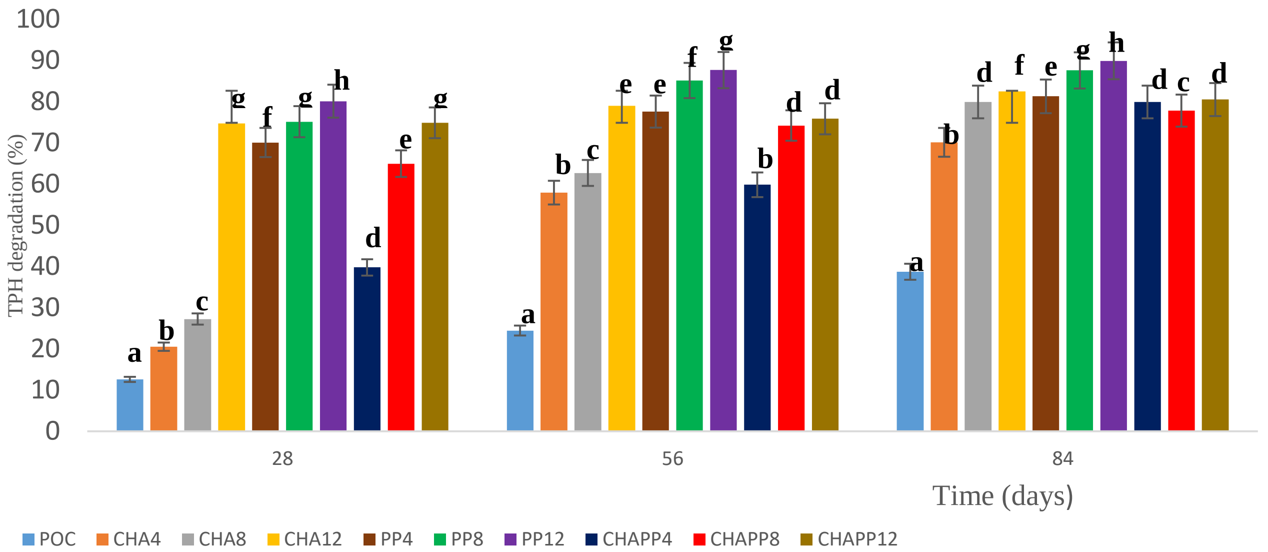

Fig.1: Effect of coconut husk char and pineapple peel on the degradation of crude oil in the soil

POC- Polluted control; CHA- Coconut husk ash; PP Pineapple peel; Different letters indicate significant difference at $p<0.05$

Table 5: Rate of TPH degradation

Treatment/ Parameter	TPH Saturation (%)	TPH Degradation (%)	Time required for 100 TPH degradation (Years)	Rate of degradation per day (mg/kg)
POC	61.33	38.67	0.60	1.84
CHA4	29.86	70.14	0.33	3.34
CHA8	20.10	79.90	0.29	3.80
CHA12	17.50	82.50	0.28	3.93
PP4	18.73	81.27	0.28	3.87
PP8	12.43	87.57	0.26	4.17
PP12	10.10	89.90	0.26	4.28
CHAPP4	20.10	79.90	0.29	3.80
CHAPP8	22.20	77.80	0.30	3.70
CHAPP12	19.50	80.50	0.26	3.83

CHA – Coconut Husk Ash; PP - Pineapple Peel

DISCUSSION

Soil physicochemical properties

The addition of organic materials to enhancing the physicochemical characteristic (pH, organic carbon, nitrogen, available P, Ca, K, and Mg) of crude oil-contaminated soil will increase the solubility and loss of the contaminants and thus, improve crude oil biodegradation rates [3].

pH in a way affects the plant growth by increasing or decreasing nutrient availability, toxins, and microbial growth. At pH lower than 5.5, there is a reduction in the soil nutrient and the activities of the decomposing organisms, this can slow down the supply of nutrients from organic minerals in the soil to support the growth of plants. With time, the subsequent addition of manure to the soil can make it acidic. So it is very necessary to monitor the pH level. In an organic system, wood ash can be used to increase soil pH. Wood ash just like coconut husk ash also is a good potassium supply to the soil, but it should not be applied as a potassium source if the pH is already high. At soil pH between 7 and 8.3 microbial activities are highly enhanced, but then it may cause a reduction in the availability of phosphorus [35]. From Table 2 coconut husk ash amendments which are high in potassium were able to raise the pH of the soil from 8.23±0.088 to 11.133±0.067 (CHA12). This is in line with [36] who showed that coconut husk ash is very good mineral fertilizer, rich in potassium particularly and can perform as potassium chloride (KCl) causing 92 percent of coconut palms to be fertilized as against 26 percent of control palms. The pineapple peels amendment caused a reduction in the soil pH from 8.23±0.088 to 7.133±0.03 (PP12). This may be due to the acidic property of pineapple fruit.

The results obtained show that the organic carbon in crude oil-contaminated soil was higher than in the uncontaminated soil. This is possibly due to the effect of contamination with hydrocarbon in the soil as crude oil is essentially a combination of different hydrocarbons with little nitrogen, sulfur, and oxygen. As reported by [3], and observed increase in organic carbon in crude oil-contaminated soil following an initial scarcity will lead to a reduction in the nitrogen content of soil soaked with oil, this will also hinder the growth of microorganisms, making them unable to make use of the carbon source for energy, as well as a deficiency in other micronutrients like phosphorus which may be growth-rate-limiting. This was also observed in this study. The organic carbon in the baseline soil (1.283 ± 0.012 percent) was increased to 3.233±0.133 percent after pollution with crude oil. The different treatments were significantly different $P<0.05$ as regards the soil organic carbon. The soil with the PP12 amendment had the lowest organic carbon content of 1.900±0.02mg/kg. Organic wastes such as pineapple peel and coconut husk ash can cause a reduction in the organic content of the soil; this may be by increasing the organic matter and nutrient in the soil and increasing the ability of soil microorganisms to use up the carbon in the soil for energy.

Nitrogen is one of the regulating nutrients necessary for the effective breakdown of organic pollutants in soil [37]. The use of soil amendment with appreciable nitrogen and phosphorus content can stimulate microbial growth in the soil for the attack of the pollutants. The nitrogen, phosphorus and potassium concentration between the treatments were significantly different ($P>0.05$). Nitrogen content of PP12 was 0.827 percent having increased from POC (0.07 percent), the CHA12 treatment showed the highest phosphorus and potassium content of 112.48mg/kg and 1.193cmol/kg respectively as against 36.297mg/kg and 0.077cmol/kg respectively in the polluted soil. The increase in the phosphorus content of the soil of the amendments is in line with the work of [38] who reported that available phosphorus increased as the soil was amended with organic compost.

The soil properties are indispensable in controlling and monitoring the effect of hydrocarbon contaminants on the activities of the soil microbial communities [39].

Several reports indicate that biological treatments are more effectual and cost-effective other chemical and physical methods, thus, bioremediation technique is deployed for the breakdown of crude oil in soil medium by using microorganisms with the capability of converting complex petroleum hydrocarbons into less toxic substances. However, high molecular weight hydrocarbons are not soluble and difficult to absorb, this makes them less available to microorganisms to act upon. Hence, applying organic materials such as pineapple peels singly or in combination with good bulking agents like the coconut husk ash in enhancing the physicochemical properties (pH, organic carbon, nitrogen, available P, Ca, K, and Mg) of the soil will increase the solubility and subsequent loss of these contaminants, and oil biodegradation rates will be increased also [38].

5.1.2 Total petroleum hydrocarbon

From the data obtained (Table 4), there was a significant difference between the pineapple peel amendments and the coconut husk ash amendments. The highest loss of Hydrocarbon was where the PP treatment was used by the 84th day of treatment. The significant difference can be attributed to the presence of the available nutrient elements like N (0.98 percent) and P (0.9 percent) in PP than in CHA (Table 2). The rate at which the petroleum hydrocarbon (TPH) in the soil was degrading was 1.84mgkg-1/day, for the polluted control soil, 3.34 – 3.93 mgkg-1/day when using coconut husk ash amendment and 3.87-4.28 mgkg-1/day when using pineapple peels, the combined effect of coconut husk ash and pineapple peel was at the rate between 3.80 – 3.83 mgkg-1/day. From this result, it shows that the rate at which the petroleum hydrocarbon in the soil was degrading in a day was significantly higher in the amended soil than the crude oil control where there was no amendment, also the pineapple peel (PP) and the combination of the amendments (CHAPP) degraded more hydrocarbons in the polluted soil and at a faster rate than coconut husk ash.

5.1.3 Soil microorganism

From the result, a mixed consortium of bacteria and fungi was seen. Several workers showed that a mixed bacterial consortium was able to degrade between 28 to 51 percent of saturates and 0 to18 percent of aromatics and up to 60 percent in crude oil [40]. Another study by Rahmam et al. (2002), for a mixed bacterial consortium of Micrococcus sp., Bacillus sp., Corynebacterium sp., Flavobacterium sp., and Pseudomonas sp. carried out on the degradation of crude oil, a maximum of 78 percent reduction in the hydrocarbon was recorded 20 days post-incubation, Bacillus sp. and Micrococcus sp. had degradations of 59 percent and 49 percent, respectively. The mixed bacterial consortium gave the maximum breakdown because no single bacteria have the metabolic capacity to degrade all the components found in crude oil [41].

The addition of either organic or inorganic nutrient source causes a significant increase in bacterial count as microbes can use hydrocarbons as a carbon source and other supplied nutrients for continuous growth and sustain higher biomass. The higher the number of organisms presents leads to greater biodegradation of the hydrocarbons. Besides supplying nutrient to the microbes, the addition of bulking agent can also help the adaptation process for microbes by absorbing the excess of hydrocarbon in the soil when the concentration of crude oil is too [18]. This is responsible for the choice of combining coconut husk ash (CHA) and pineapple peels (PP). The coconut husk ash is to function as a bulking agent and the pineapple peels as core organic source of nutrient for the microorganism. There was a significant difference $p<0.05$ between the treatments as regards the total heterotrophic bacteria, crude oil utilizing bacteria, total heterotrophic fungi, and crude oil utilizing fungal counts showing that the synergy of both treatments (CHAPP) resulted in a higher population of the bacterial and fungal counts as seen in Tables 5. Thus, each of the amendments increases the microbial population in the soil. However, among the single amendments used the result shows that the microbial count in the soil treated with pineapple peels was significantly higher than coconut husk ash but the combination of the coconut husk and pineapple peel shows a much higher microbial count than the single amendments. This is an indication that the combination of these treatments has stronger degrading potentials since it possesses a mixed culture of the microorganisms found in the single amendment.

The crude oil utilizing bacterial isolates identified in the study belonged to genera Bacillus spp, Proteus spp., Serratia spp., Pseudomonas spp., Enterobacter sp, Staphylococcus spp, and Micrococcus sp. The crude oil utilizing fungal species isolated include Cephalosporiumsp, Aspergillus fumigatus, Penicillium spp., Coccidiodesimmitis, Aspergillus niger and Penicillium sp.

As reported by [6], Pseudomonas sp. are the best bacteria because they can make good use of the hydrocarbons and still be used in producing biosurfactants.

It is, therefore, possible to attain a 100 percent hydrocarbon degradation, even though the microbial counts in the soil will be reduced as a result of a drop in the hydrocarbon contents in the soil which is a sole energy source of the organisms and an influencer to the degraders.

CONCLUSION

The usefulness of wastes in this present time is gaining worldwide attention for more research and the bioremediation of crude oil-polluted soil with agro-wastes (coconut husk ash and pineapple peel) through this study is seen to be an effective method for the removal of petroleum hydrocarbons in soil. The endpoint of bioremediation is the complete breakdown of contaminants, i.e. converting them to water, carbon dioxide, nitrogen, and HCl. From studies, there is no known toxicity implication of coconut husk ash and pineapple peels on the soil microbial activity but rather stabilizing and microbe-stimulating potentials due to their nutrient properties which retain soil nutrient and increase microbial growth in contaminated soil.

Polluted soil is always low in organic nutrient and thus has reduced microbial activity. The addition of agricultural waste that serves as a bulking agent and source of nutrient fertilizer enhanced microbial growth and thus concurrently increased the degradation of TPH. Since the availability of micronutrients, especially nitrogen, is one of the factors that affect bacterial growth in soil, therefore the use of N, P, K-rich organic substance can enhance up to 100 percent biodegradation of hydrocarbon.

REFERENCES

[1] Agarry, S. E., Aremu, M. O.& Aworanti, O. A. (2013). Biodegradation of 2, 6-Dichlorophenol Wastewater in Soil Column Reactor in the Presence of Pineapple Peels-Derived Activated Carbon, Palm Kernel Oil, and Inorganic Fertilizer. *Journal of Environmental Protection*, *4*, 537–547.

[2] Dadrasnia, A. & Ismail, S. B. (2015). Bio-Enrichment of Waste Crude Oil Polluted Soil: Amended with Bacillus 139SI and Organic Waste. *International Journal of Environmental Science and Development*, *6*(4).

[3] Isitekhale, H. H., Aboh, S. I., Edion, R. I., &Abhanzioya, M. I. (2013). Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop. *Journal of Environment and Earth Science*, *3*(7), 116–121

[4] Ite, E. A., Ibok, J. U., Ite, M. U, & Petters, S. W. (2013). Petroleum Exploration and Production: Past and Present Environmental Issues in Nigeria's Niger Delta. *American Journal of Environmental Protection*, *1*(4), 78–90.

[5] Obasi, N. A., Eze, E., Anyanwu, D. I., & Okorie, U. C. (2013). Effects of organic manures on the physicochemical properties of crude oil polluted soils. *Global Journal of Environmental Biochemistry*, *1*(1), 66–74.

[6] Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*. 11, 1-13.

[7] Okonokhua, B. O., Ikhajiagbe, B., Anoliefo, G. O., & Emede, T. O. (2007). The Effects of Spent Engine Oil on Soil Properties and Growth of Maize (*Zea mays* L.). *Journal of Applied Science and Environmental Management*,11 (3), 147 – 152.

[8] Namose (2014). Microorganisms, facts, approaches at MetaMicrobe. Retrieved from http://www.metamicrobe.com/petroleum-microbiology/oil-bioremediation-introduction.html

[9] Agbor, R. B., Ekpo, I. A., Osuagwu, A. N., Udofia, U. U., Okpako, E. C. &Antai, S. P. (2012). Biostimulation of microbial degradation of crude oil polluted soil using cocoa pod husk and plantain peels. *Journal of Microbiology and Biotechnology Research*, *2*(3), 464–469.

[10] Stephen, E., Job, O. S., &Abioye, O. (2013). Study on Biodegradation of Diesel Contaminated Soil Amended with Cowpea Chaff. *Journal of Science & Multidisciplinary Research*, *2*(1), 14–18.

[11] Chikere, C. B., Okpokwasili, G. C.&Chikere, B. O. (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *Journal of Biotechnology*, *8*(11), 2535–2540.

[12] Bonaventura, C. & Johnson, F. M. (1997). Healthy environments for healthy people: Bioremediation today and tomorrow. *Environmental Health Perspectives*, 105 (1), 5-20.

[13] Mohajeri, L., Aziz, H. A., Isa, M. H., Zahed, M. A., &Mohajeri, S. (2013). Effect of remediation strategy on crude oil biodegradation kinetics and half-life times in shoreline sediment samples. *International Journal of Marine Science &Engineering*, *3*(2), 99–104.

[14] Adibarata, T. H. &Achibana, S. T. (2009). Microbial Degradation of Crude Oil by Fungi Pre-Grown on Wood Meal. *Interdisciplinary Studies On Environmental Chemistry*, 5(1), 317–322.

[15] Sharma, S. (2012). Bioremediation: Features, Strategies and applications. *Asian Journal of Pharmacy and Life Science*, *2*(2), 202–213.

[16] Abioye, O. P. (2011). Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil. *Soil Contamination*, 127–142

[17] Thapa, B., Kc, A. K., & Ghimire, A. (2012). A Review on Bioremediation of Petroleum Hydrocarbon Contaminants in Soil. *Kathmandu University Journal of Science, Engineering and Technology*, *8*(1), 164–170.

[18] Hamzah, A., Salleh, Siti N. M., &Sarmani, S. (2014). Enhancing Biodegradation of Crude Oil in Soil Using Fertilizer and Empty Fruit Bunch of Oil Palm. *SainsMalaysiana*, 43(9), 1327–1332.

[19] Azad, M. A. K., Amin, L.& Sidik, N. M. (2014). Genetically engineered organisms for bioremediation of pollutants in contaminated sites. *Chinese Science Bulletin*, *59*(8), 703–714.

[20] Patel, S. (2012). Potential of fruit and vegetable wastes as novel biosorbents: summarizing the recent studies. *Reviews in Environmental Science and Bio/Technology*,11(4): 365–380.

[21] Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N., & Panneerselvam, A. (2011). Production Of Single Cell Protein From Pineapple Waste. *Innovative Romanian Food Biotechnology*, *8*, 26–32.

[22] Agarry, S.E. &Jimoda, L. A. (2013). Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in In-situ Soil Bioremediation of Diesel Oil: Experimental Analysis and Kinetic Modelling. *Journal of Environment and Earth Science*, *3*(7), 51–63.

[23] Nyankanga, R. O., Onwonga, R. N., Wekesa, F. S., Nakimbugwe, D., Masinde, D. & Mugisha, J. (2012). Effect of inorganic and organic fertilizers on the performance and profitability of grain Amaranth (*Amaranthus caudatus* L.) in Western Kenya. *Journal of Agricultural Science*, 4 (1), 223 - 232

[24] Danjuma, B. Y., Abdulsalam, S. &Sulaiman, A. D. I. (2012). Kinetic investigation of Escravos crude oil contaminated soil using natural stimulants of plant sources. *International Journal of Emerging Trends in Engineering & Development*, 2 (5), 478-486

[25] Agarry S. E, Owabor, C. N. & Yusuf, R. O. (2010). Bioremediation of soil artificially contaminated with petroleum hydrocarbon mixtures: Evaluation of the use of animal manure and chemical fertilizer. *Bioremediation Journal*, 14, 189–195.

[26] Mishra V., Balomajumder C., & Agarwal, V. K. (2010). Biosorption of Zn (II) onto the surface of non-living biomasses: a comparative study of adsorbent particle size and removal capacity of three different biomasses. *Water Air Soil Pollution* 211, 489 – 500

[27] Pala, D. M., de Souza, J. A., De Carvalho, D. D. & Sant' Anna Jr, G.L. (2005). Effect of bulking agents and clay content on bioremediation of diesel-contaminated soils. *Mercosur Congress on Process Systems Engineering*, 2, 1-10.

[28] Udo, E. J. &Ogunwale, J. A. (1986). *Laboratory Manual for the Analysis of Soil, Plant and Water Samples*, 2nd Edition, University of Ibadan, Nigeria

[29] Association of Official Analytical Chemists (AOAC) (1990). *Methods of Analysis*, 12th Edition, AOAC, Washington, DC, USA

[30] APHA (1998). Standard Methods for the Examination of Water and Waste Water. 20th edition APHA – AWWA - WPCF. Washington., DC

[31] Hamamura, N., Olson, S. H., Ward, D.M. & Inskeep, W.P. (2006). Microbial population dynamics associated with crude oil biodegradation in diverse soils. *Applied and Environmental Microbiology*, 72, 6316–6324

[32] Sexton, A. J & Atlas, R. M., (1997). The response of Microbial Populations in Arctic Tundra Soil Crude oil. *CanadianJournal of Microbiology*, 23, 1327-1333.

[33] Holt, G. J., Noel, R. K., Sneath P. H., Staley J., & Williams S. T. (1994). *Bergey's Manual of Determinative Bacteriology*, (9th ed.), Williams and Wilkins Publishers: London.

[34] Jidere, C. M &Akamigbo, F. O. R. (2009). Hydrocarbon Degradation in Poultry Droppings and Cassava Peels-Amended Typic Paleustults in Southeastern, Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension*, 8(1), 24 – 30

[35] Rosen, C. J., & Bierman, P. M. (2005). Nutrient Management for Fruit & Vegetable Crop Production using manure and compost. *Agricultural, Food and Environmental Services*, 1–10.

[36] Bonneau X., Haryantos I. &Karsiwan T. (2010). Coconut husk ash as a fertilizer for coconut palms on peat *Experimental Agriculture*, 46 (3), 401-414

[37] Dadrasnia, A. &Agamuthu, P. (2013). Potential of biowastes to remediate diesel fuel contaminated soil. *Global NEST Journal*, *15*(4), 474–484.

[38] Eneje R. C., Nwagbara, C., &Uwumarongie-Ilori, E. G. (2012). Amelioration of chemical properties of crude oil contaminated soil using compost from *Calapoigoniummucunoides* and poultry manure. *International Research Journal of Agricultural Science and Soil Science*, *2*(6), 246–251.

[39] Alrumman, S. A., Standing, D. B.& Paton, G. I. (2015). Effects of hydrocarbon contamination on soil microbial community and enzyme activity. *Journal of King Saud University - Science*, *27*(1), 31–41.

[40] Vasudevan N. &Rajaram, P. (2001) Bioremediation of oil sludge- contaminated soil, *Environment International*, 26 (5-6), 409–411.

[41] Al-Wasify, R. S., & Hamed, S. R. (2014). Bacterial Biodegradation of Crude Oil Using Local Isolates. *International Journal of Bacteriology*, 1–8.