ISSN 2449-8955 European Journal of Biological Research *Research Article*

DOI: http://dx.doi.org/10.5281/zenodo.2603912

Removal of Hydrocarbons from Crude Oil Contaminated Agricultural Soil by Phytoremediation Using *Mariscus alternifolius* and *Fimbristylis ferruginea*

C. C. Chukwuma, J. C. Ikewuchi*, M. O. Monanu

Department of Biochemistry, Faculty of Science, University of Port Harcourt, P.M.B. 5239, Choba, Rivers State, Nigeria

* Correspondence: E-mail: ecoli240733@yahoo.com

Received: 23 January 2019; **Revised submission:** 22 February 2019; **Accepted:** 21 March 2019

ABSTRACT: Crude oil extraction is one major route through which hydrocarbons are released into the environment and hydrocarbon contamination is highly hazardous to the ecosystem. This study investigated the removal of hydrocarbons from crude oil contaminated agricultural soils using *Mariscus alternifolius* Vahl. and *Fimbristylis ferruginea* plant species. Before planting, the polluted soil (negative control) had a total petroleum hydrocarbon concentration of 17962.11±1000.00 mg/kg and polycyclic aromatic hydrocarbon concentration of 440.97±1.00 mg/kg. Likewise, the soil oil and organic carbon contents were 3.25±0.10 ppm and 3.06±0.02% respectively. Results, 90 days after planting, indicated a significant decrease in the total petroleum hydrocarbon concentrations of *M. alternifolius* (100.82±46.31 mg/kg) and *F. ferruginea* (110.41±39.68 mg/kg) treated soils. Likewise, there was a significant decrease in the polycyclic aromatic hydrocarbon concentration of *M. alternifolius* treated soil (95.69±65.44 mg/kg). The oil content of the treated soils significantly decreased to 1.03±0.28 ppm and 0.84±0.33 ppm in *M. alternifolius* and *F. ferruginea* treated soils respectively, while the organic content of the treated soils significantly decreased to 2.16±0.09% and 2.20±0.20% in *M. alternifolius* and *F. ferruginea* treated soils respectively. Phytoremediation using *M. alternifolius* and *F. ferruginea* has proven to be potent in the remediation of hydrocarbon contaminated soil through enhancement and recovery of the polluted soil. These plant species which improved the cultivation and germination competence of the treated soils thus making the soil probable for agricultural and other related purposes are therefore recommended for used in the phytoremediation of crude oil contaminated soils.

Keywords: Crude oil; Polluted soil; Hydrocarbons; *Fimbristylis ferruginea*; *Mariscus alternifolius*.

1. INTRODUCTION

Crude oil is a complex mixture of organic compounds predominated by carbon and hydrogen atoms albeit containing smaller amounts of nitrogen, oxygen, sulfur and tinges of metallic constituents [1]. Its formation is a natural process resulting from geological deposits formed from organic decomposition products of ancient plants and animals under high temperature and pressure [2].

Crude oil extraction is one major route through which hydrocarbons are released into the environment. Such release sometimes emanates due to exploration, mining, transportation, pipeline rupture, or damage by saboteurs and hoodlums [3]. Hydrocarbon contamination is highly hazardous to the ecosystem and poses severe impact on plants and animals including human health [4]. This is because on reaching the environment,

petroleum hydrocarbons bind to soil components [5] causing biological damages by destructing the supply of water, nutrients, oxygen and light, hence, affecting soil fertility, plant growth and germination and making the soil unsuitable for agricultural and other investment purposes [4]. Pollution can result to imbalance in carbon-nitrogen ratio at site of spillage owing to the conglomeration of carbon and hydrogen in crude oil. Elevated levels of organic compounds on soil surface deplete oxygen reserves and diminish rates at which oxygen diffuses into deeper layers [6-7].

The fate and spread of petroleum hydrocarbons on subsurface is dependent on viscosity of the oil and its quantity. In soil, the fate of petroleum hydrocarbons is affected by the composition, chemical and physical properties of the soil as well as composition of the petroleum products. Likewise, the biodegradability of these petroleum hydrocarbons can be influenced by the availability and concentration of the contaminants. Petroleum hydrocarbons can be sequestered and fractionated within the soil via organic matter sorption or diffuse into the three dimensional structure of the organic matter. Thus, there is a proportional reduction in contaminant extraction and biodegradation as the interaction between particles of soil and pollutants increase [8].

Recently, the use of plants and associated microbes to decontaminate polluted soil has gained wide interest. This remediation technique, phytoremediation, established on the view of using nature to cleanse nature has been effectively used to tackle pollutants such as heavy metals and hydrocarbons [9]. Plants employ the mechanism of rhizospheric degradation of pollutants such as hydrocarbons that promote the increase in the microbial population in the root zone which sequentially breaks down pollutants [10].

Microorganisms are ubiquitously located almost in every part of the terrestrial ecosystem and are important in ecological and biodegradation functional processes in polluted soils [11]. They are furnished with metabolic machinery that enables them to utilize petroleum products as a carbon and energy source. There are enormous benefits of relying on indigenous microorganisms to degrade hydrocarbons. Foremost, natural populations must have evolved and developed through many years. These microorganisms adapt for survival and proliferation in that environment. Secondly, the capableness to utilize hydrocarbons is disseminated among a diverse microbial population. This population prevails in natural ecosystems and either independently or synergistically metabolizes several hydrocarbons [12]. This study was performed to ascertain the competence of *Mariscus alternifolius* Vahl. and *Fimbristylis ferruginea* in the removal of hydrocarbons from crude oil polluted agricultural soil.

2. MATERIALS AND METHODS

2.1. Experimental design

Crude oil polluted agricultural farmland, with over 10 years oil spill history, was identified in Bodo community of Ogoniland, Nigeria. A portion of the farmland was mapped and assessed to ascertain prevailing and physicochemical factors inherent in the site. Likewise, the prevailing indigenous plant community of the site was determined following collection and identification of the plant species. Two species: *Mariscus alternifolius* Vahl. and *Fimbristylis ferruginea*, were selected after the identification based on existing literature on their effectiveness in surviving and proliferating in extremely harsh soil environment and the scanty report on their phytoremediation capability. Mature and viable seeds of *M. alternifolius* and *F. ferruginea* were collected from the wild for nursery. Unpolluted soil for nursery which also served as the negative control soil in the study was collected from an agricultural farmland located in the premise of the University of Port Harcourt with no history of pollution while the polluted soil (positive control) was collected from the spill site. Collection was carried out using sterile airtight plastic bags and taken to Ecological Centre of the University of Port Harcourt for pot experiment study. The propagated seeds for nursery, at seedling level, were transferred into 8 kg pots containing polluted soils set up in triplicate. Each pot contained 4 seedlings. Non-vegetated positive and negative controls were likewise set up in triplicate and

placed under same condition and in proximity with the treatment groups, totaling 12 pots employed for the study.

2.2. Laboratory analyses

The total petroleum hydrocarbons (TPH) analysis followed EPA 8260c [13] and International Organization for Standardization ISO 16703 [14] standard methods. Polycyclic aromatic hydrocarbons (PAH) analysis followed EPA 8270 standard method as adopted [15-16]. Oil content was determined by the toluene extraction method [17-18]. Organic carbon was ascertained by loss of weight on ignition method while the pH of the soil samples was determined using a calibrated pH meter [19]. Moisture content determination followed the gravimetric method as described by [20]. Vapour phase transfer method [21-22] was employed for Hydrocarbon Utilizing Bacteria (HUB) and Fungi (HUF) estimation following decimal dilutions (10-fold) of the soil suspensions inoculated onto duplicate sterile Petri dishes containing mineral salt agar (MSA). The germination toxicity test was carried out by the method [22] using hydrocarbon sensitive plant seed, lettuce (*Lactuca sativa* L.).

2.3. Statistical analysis

Statistical analysis was carried out using the MS Excel and SPSS 20.0. Sampling and chemical analyses were examined in triplicate in order to decrease the experimental errors and to increase the experimental reproducibility, with results expressed as means ± standard deviation of the triplicate determinations. Using One way analysis of variance (ANOVA), data between groups were determined by the Bonferroni test at 95% ($p<0.05$) confidence level while data between periods were determined by the Student t-test.

3. RESULTS AND DISCUSSION

The total petroleum hydrocarbons (TPH) of *M. alternifolius* and *F. ferruginea* treated soils are presented in Table 1. The polluted soil (negative control) before planting had TPH concentration of 17962.11±1000.00 mg/kg. This value was within the range of 126 to 52,200 mg/kg reported by United Nations Environment Programme (UNEP) along Shell Petroleum Development company (SPDC) pipeline rights of way in Ogoniland, Rivers State, Nigeria but falls above regulatory limits [23] target values of 50 mg/kg in farmland. The TPH concentrations of the treated soils, ninety days after planting (90 DAP), indicated *M. alternifolius* treated soil had TPH concentration of 100.82±46.31 mg/kg while *F. ferruginea* treated soil had TPH concentration of 110.41±39.68 mg/kg. Likewise, the polycyclic aromatic hydrocarbons (PAH) concentration of the polluted soil before planting as shown in Table 2, revealed PAH concentration of 440.97±1.00 mg/kg. This value falls above regulatory limits [23] target value of 1 mg/kg in farmlands. The PAH of the treated soil groups, 90 DAP, further revealed *M. alternifolius* treated soil had PAH concentration of 95.69±65.44 mg/kg while *F. ferruginea* treated soil had PAH concentration of 184.09±180.29 mg/kg. These findings corroborate the report [24] of reduced hydrocarbon concentration of petroleum hydrocarbon in oil impacted soil using Axonopus sp. and associated microorganisms. Likewise, Nwaichi et al. [25] reported similar significant decrease in PAH using *Fimbristylis littoralis, Hevea brasilensis*, Cymbopogom citratus, and *Vigna subterranean*. This finding further agrees with the reports [26-27] that phytoremediation can be successfully used to manage soil contaminated with petroleum hydrocarbons.

As could be observed in Tables 1 and 2, it may be pertinent to assert that natural biological processes could play important role in hydrocarbon reduction. If this is true, it may however account for the TPH concentration of 53.13±13.08 mg/kg and PAH concentration of 27.64±18.13 observed in the negative control 90 DAP albeit no significant difference ($p<0.05$) existed between this group and the others. This natural attenuation may have been hastened by atmospheric influence [26]. This finding agrees with previous reports

[28-32] on hydrocarbon degradation ability by natural attenuation. Similarly, pH, oxygen (aeration), soil nutrients, temperature, soil moisture, soil enzymes and various microorganisms can enhance hydrocarbon biodegradation [1] with previous reports [33-36] confirming this assertion. The percentage recovery [1, 3] of the polluted soil further revealed a failure in restoration as regards both TPH and PAH.

The oil content of *M. alternifolius* and *F. ferruginea* treated soils are presented in Table 3. The polluted soil (negative control) before planting had oil content concentration of 3.25±0.10 ppm. Nonetheless, a significant decrease in oil content of the treated soils was recorded over time. The oil content of the treated soil groups, 90 DAP, revealed a decrease to 1.03±0.28 ppm and 0.84±0.33 ppm in *M. alternifolius* and *F. ferruginea* treated soils, respectively. This result corroborates the report [1] of a similar decrease in oil content over time. Such degradation process follows a shifting order (1-0) [37]. With regards to oil content, by 30 and 60 DAP, the treatments restored the polluted soils towards normalcy (14.12, 7.07 and 0.54%). By 90 DAP the treatment using *F. ferruginea* restored the polluted soil towards normal value (2.47%). However, the value for the treatment using *M. alternifolius* nosedived indicating a failure in restoration.

Table 1. Total petroleum hydrocarbon (TPH) (in mg/kg) of the treated soils and their corresponding controls.

Group	BP	90 DAP	% R 90 DAP
Positive control	17.57±1.00^{a}	33.32±0.10^{a,*}	NA
Negative control	17962.11±1000.00^{b}	53.13±13.08^{a,*}	NA
M. alternifolius	17962.11±1000.00^{b}	100.82±46.31^{a,*}	-240.74
F. ferruginea	17962.11±1000.00^{b}	110.41±39.68^{a,*}	-289.148

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

Table 2. Polycyclic aromatic hydrocarbon (PAH) (in mg/kg) of the treated soils and their corresponding controls.

Group	BP	90 DAP	% R 90 DAP
Positive control	5.80±0.10^{a}	9.0368±0.49^{a,*}	NA
Negative control	440.97±1.00^{b}	27.64±18.13^{a,*}	NA
M. alternifolius	440.97±1.00^{b}	95.69±65.44^{a,*}	-365.77
F. ferruginea	440.97±1.00^{b}	184.09±180.29^{a}	-840.88

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

Table 3. Oil content (in ppm) of the treated soils and their corresponding controls.

Group	BP	30 DAP	60 DAP	90 DAP	% R 30 DAP	% R 60 DAP	% R 90 DAP
Positive control	0.13±0.01^{a}	0.09±0.01^{a*}	0.06±0.12^{a,*}	0.05±0.01^{a,*}	NA	NA	NA
Negative control	3.25±0.10^{b}	2.71±0.40^{b*}	1.90±0.17^{b,c,*}	0.86±0.09^{b,c,*}	NA	NA	NA
M. alternifolius	3.25±0.10^{b}	2.34±0.20^{b*}	1.77±0.42^{b,*}	1.03±0.28^{b,*}	14.12	7.07	-20.99
F. ferruginea	3.25±0.10^{b}	2.34±0.24^{b*}	1.89±0.03^{c,*}	0.84±0.33^{c,*}	14.12	0.54	2.47

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

Table 4 reveals the Organic carbon (OC) content of the treated soils with their corresponding controls. The organic carbon content of the polluted soil (negative control) was significantly higher ($p<0.05$) than the unpolluted soil (positive control). This finding corroborates some previous reports [18, 38-40] and such observed difference may be due to metabolic processes following the oil spill that facilitate agronomical addition of organic carbon from petroleum hydrocarbon [38, 41]. The decrease in organic carbon content of the treated soils over time (see Table 4) agrees with the report [42] which showed similar changes in total organic carbon during bioremediation of crude oil impacted soil. According to Tanee and Albert [43], increased microbial population implies increased energy (carbon) demand since the microbial oil degraders use the carbon content for the provision of energy. With regards to organic carbon, the treatments, 30 DAP, nosedived indicating failure in restoration. However, by 90 DAP, treatments using *M. alternifolius* and *F. ferruginea* restored the polluted soil towards normalcy at 10.40 and 5.58% respectively.

Table 4. Organic carbon (OC) (in %) of the treated soils and their corresponding controls.

Group	BP	30 DAP	90 DAP	% R 30 DAP	% R 90 DAP
Positive control	1.64 ± 0.10^{a}	1.60 ± 0.35^{a}	$1.35\pm0.15^{a,*}$	NA	NA
Negative control	3.06 ± 0.02^{b}	2.69 ± 0.23^{b}	$2.25\pm0.17^{b,*}$	NA	NA
M. alternifolius	3.06 ± 0.02^{b}	2.77 ± 0.23^{b}	$2.16\pm0.09^{b,*}$	-7.03	10.40
F. ferruginea	3.06 ± 0.02^{b}	$2.75\pm0.07^{b,*}$	$2.20\pm0.20^{b,*}$	-5.50	5.58

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

The pH of the treated soils and the corresponding controls (Table 5) revealed a significant decrease in the pH of the polluted soil (negative control) before planting when compared with the unpolluted soil (positive control). This finding corresponds with reports [44-45] on positive correlation between acidic pH and crude oil concentration in soil and opined that crude oil pollution could make soils acidic thereby increasing the toxicity of the soil. The pH values (5.50±0.42 and 5.50±0.04) of the treated soils obtained 90 DAP agrees with Hatami et al. [46] who reported a decrease in pH of soil samples treated with alfalfa powder and associated such a decrease with the release of organic acids during decomposition process. This is because organic matter is capable of lowering pH by releasing H^{+} associated with organic anions or through nitrification process [47]. Nonetheless, the obtained pH values fall within the standard limit of 5.5 to 6.5 as stipulated [23].

Table 5. pH of the treated soils and their corresponding controls.

Group	BP	30 DAP	60 DAP	90 DAP
Positive control	6.75 ± 0.10^{a}	6.71 ± 0.74^{a}	$6.43\pm0.32^{a,*}$	6.46 ± 0.11^{a}
Negative control	5.69 ± 0.10^{b}	5.81 ± 0.47^{a}	5.71 ± 0.30^{b}	5.55 ± 0.42^{b}
M. alternifolius	5.69 ± 0.10^{b}	6.08 ± 0.78^{a}	$5.91\pm0.61^{a,b}$	5.50 ± 0.42^{c}
F. ferruginea	5.69 ± 0.10^{b}	6.19 ± 1.15^{a}	5.72 ± 0.34^{b}	$5.50\pm0.04^{b,c,*}$

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

The moisture content of the treated soils as shown in Table 6 revealed a significant decrease in moisture content of the polluted soil (negative control) when compared with unpolluted soil (positive control) before planting. This result agrees with Essien and John [48] who reported significantly low moisture content in polluted soil compared to unpolluted soil. It has also been reported that high crude oil concentrations in soil could clog soil pores and reduce water and oxygen penetration [49-50]. According to Abosede [51], crude oil might have negative effects on some soil physical properties such as decreased pore spaces. This may be due to the presence of less dissolved materials present for plant uptake and subsequent metabolism, as well as the blockage of soils emanating from crude oil contamination of the soil. It has been reported that crude oil spillage reduces soil moisture availability or holding capacity, or increase moisture deficit in agricultural soils thereby damaging plant growth and yield [52]. The observed increase in the moisture content of the treated soils agrees with the reports [44, 53, 54]. Since crude oil can bind soil particles together [44] and decrease water permeability, such an increase in moisture content may be a result of the decrease in hydrocarbon contents of the soil.

Table 6. Moisture content (in %) of the treated soils and their corresponding controls.

Group	BP	30 DAP	60 DAP	90 DAP
Positive control	10.33 ± 0.10^{a}	$21.00\pm3.18^{a,*}$	7.55 ± 2.34^{a}	$19.11\pm1.95^{a,*}$
Negative control	9.67 ± 0.01^{b}	$5.89\pm0.38^{b,*}$	$17.11\pm1.64^{b,*}$	$28.33\pm0.67^{b,*}$
M. alternifolius	9.67 ± 0.01^{b}	$13.44\pm1.84^{c,*}$	$17.11\pm1.17^{b,*}$	$16.33\pm2.52^{c,*}$
F. ferruginea	9.67 ± 0.01^{b}	$13.56\pm2.50^{c,*}$	$15.89\pm2.27^{b,*}$	$21.00\pm2.02^{a,c,*}$

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

Table 7. Hydrocarbon utilizing bacteria (HUB) (in Log_{10} cfu/g) of the treated soils and their corresponding controls.

Group	BP	45 DAP	90 DAP
Positive control	4.38 ± 0.16^{a}	5.04 ± 0.31^{a}	$6.32\pm0.20^{a,*}$
Negative control	4.81 ± 0.01^{b}	$5.41\pm0.01^{a,*}$	$6.91\pm0.10^{b,*}$
M. alternifolius	4.81 ± 0.01^{b}	5.67 ± 0.25^{a}	$6.87\pm0.06^{b,*}$
F. ferruginea	4.81 ± 0.01^{b}	$5.60\pm0.20^{a*}$	$6.85\pm0.06^{b,*}$

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

The microbiological profiles of the treated soils are presented in Tables 7 and 8. The polluted soil (negative control) before planting had hydrocarbon utilizing bacteria (HUB) of 6.50×10^{4} cfu/g as compared to 2.42×10^{4} cfu/g contained in the unpolluted soil (positive control) (Table 7). Likewise, the polluted soil (negative control) before planting had hydrocarbon utilizing fungi (HUF) of 6.45×10^{4} cfu/g as against 3.20×10^{3} cfu/g recorded in the unpolluted soil (positive control) (Table 8). These results agree with the findings [22, 55] which showed higher population of crude oil degrading microbes in the polluted soils than the unpolluted soils and associated such a difference with the presence of crude oil which could boost carbon supply in the soils and therefore favour the growth of the organisms including certain changes in the physicochemical properties of the soils especially the provision of essential nutrients required for microbial growth. According to Ataikiru et al. [21], it is known that colony forming unit (cfu) counts are higher in

polluted soil than unpolluted soil and microbial counting of a contaminated site is the easiest method that can be employed for bioremediation. As could be observed (See Tables 7 and 8), the significant increase in bacteria and fungi count over time agrees with some findings [1, 56, 57]. This may be an indication of increased biodegradation and utilization of the hydrocarbons by the microbial community [1].

Table 8. Hydrocarbon utilizing fungi (HUF) (in Log_{10} cfu/g) of the treated soils and their corresponding controls.

Group	BP	45 DAP	90 DAP
Positive control	3.51±0.16[a]	4.27±0.18[a,*]	5.34±0.08[a,*]
Negative control	3.81±0.01[b]	4.75±0.13[a,b,*]	5.69±0.04[b,*]
M. alternifolius	3.81±0.01[b]	4.80±0.08[b,*]	5.65±0.14[b,*]
F. ferruginea	3.81±0.01[b]	4.99±0.27[a]	5.70±0.14[a,b,*]

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable.

The higher percentage germination recorded in the treated soils compared to the corresponding negative control (See Table 9) indicated that the treatment of the soils with the plants species improved the soil germination capacity. This finding corroborates Chukwuma et al. [1] who reported the germination of lettuce seed in crude oil polluted soil treated with *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f. Likewise, Abioye et al. [33] reported seed germination on remediated soil previously contaminated with lubricating oil. Although the TPH and PAH levels of the negative control were higher than those of the treated soils after the 90 days treatment, and given that lettuce is hydrocarbon sensitive, it could be that the treatment plants secreted exudates which potentially impacted the soil thus providing the treated soils with properties that enhanced the germination of the lettuce in the treated soils. It may however be that the removal of pollutants, other than hydrocarbons, was enhanced more in the treated soils.

Table 9. Germination toxicity test of the treated soils and their corresponding controls.

Group	Percentage Germination (%)	Percentage Germination Index (%)	% R Percentage Germination (%)
Positive control	95.00±5.00[a]	NA	NA
Negative control	68.33±2.89[b]	NA	NA
M. alternifolius	80.00±5.00[c]	67.00±7.21[b]	43.75
F. ferruginea	80.00±5.00[c]	69.00±7.00[b]	43.75

Values are mean ± standard deviation of triplicate determination. Values in the same column with different letters (a, b...) are significantly different at $p<0.05$. *$p<0.05$ compared to the corresponding values before planting.

Note: BP = Before Planting; WAP = Week(s) After Planting; NA = Not Applicable

4. CONCLUSION

The application of *M. alternifolius* and *F. ferruginea* plant species has proven to possess the potential for remediation of hydrocarbon contaminated soil through the enhancement and recovery of the polluted soil and improved the cultivation and germination competence of the treated soils thus making the soil probable for agricultural and other related purposes. These plant species are therefore recommended for used in the phytoremediation of crude oil contaminated soils.

Author Contributions: This work was carried out in collaboration between all authors. All authors designed the study and wrote the protocol. Author CCC wrote the first draft of the manuscript and managed the literature searches. Author JCI performed the statistical analysis. Author MOM managed analysis of the study. All authors read and approved the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Chukwuma CC, Ikewuchi JC, Ekeke C, Monanu MO. Phytoremediation of crude oil polluted agricultural soil using *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f. Int J Biochem Res Rev. 2018; 23: 1-12.
2. Singh K, Chandra S. Treatment of petroleum hydrocarbon polluted environment through bioremediation: A review. Pak J Biol Sci. 2014; 19: 1-8.
3. Chukwuma CC, Monanu MO, Ikewuchi JC, Ekeke C. Variance in protease, dehydrogenase, phosphatase and respiratory activities during phytoremediation of crude oil polluted agricultural soil using *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f. Annu Res Rev Biol. 2018; 28: 1-9.
4. Chukwuma CC, Onuah CL, Nwauche KT, Ohanador R, Chukwu CN, Enobong, E. Periodic effects of crude oil pollution on some nutrient elements of soils treated over a 90 day period using *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f. Int J Adv Sci Res Engin. 2018; 4: 7-15.
5. Barathi S, Vasudevan N. Utilization of petroleum hydrocarbons by *Pseudomonas fluorescens* isolated from a petroleum-contaminated soil. Environ Int. 2001; 26: 413-416.
6. Jain PK, Gupta VK, Pathak H, Lowry M, Jaroli DP. Characterization of 2T engine oil degrading indigenous bacteria, collected from high altitude Mussoorie region of India. World J Microbiol Biotechnol. 2010; 26: 1419-1426.
7. Dhaker AK, Jain PK. Sewage pollutants and their bioremediation by using different microorganism In: Jain PK, VK Gupta, V. Bajpai, eds. Recent advances in environmental biotechnology. LAP lambert academic publishing AG and Co. KG, Germany. 2011: 189-204.
8. William SD, Ladd DE, Farmer JJ. Fate and transport of petroleum hydrocarbons in soil and ground water at Big South Fork National River and Recreation Area, Tennessee and Kentucky, 2002-2003: U.S. Geological Survey Scientific Investigations Report, 2005-5104. 2006: 1–29.
9. Njoku KL, Akinola MO, Oboh BO. Phytoremediation of crude oil contaminated soil: The effect of growth of glycine max on the physic-chemistry and crude oil contents of soil. Nature Sci. 2009: 7: 79-87.
10. Joseph D, Travis L, Arash B, Christopher K, Kendal HO. Phytoremediation with native plants. Final Report to the Zumberge fund for Innovation. 2015.
11. Peng M, Zi X, Wang Q. Bacterial community diversity of oil-contaminated soil assessed by high throughput sequencing of 16s rRNA genes. Int J Environ Res Public Health. 2015; 12: 12002-12015.
12. Olajire AA, Essien JP. Aerobic degradation of petroleum components by microbial consortia. J Petroleum Environ Biotechnol. 2014; 5: 1-22.
13. United States Environmental Protection Agency (US EPA). Method 8260C: Volatile Organic Compounds by Gas Chromatography. Mass Spectrometry (GC/MS), Revision, 2. 1996.
14. International Organization for Standardization (ISO). Soil quality determination of content of hydrocarbon in the range C10-C40 by gas chromatography, Geneva, Switzerland. 2004.
15. Alawi MA, Azeez AL. Study of polycyclic aromatic hydrocarbons (PAHs) in soil samples frol Al-Ahdab

oil field in Waset Region, Iraq. Toxin Rev. 2016; 35: 69-76.

16. Barshick SA, Worthy S, Griest WH. EPA method 8270 (semivolatiles) using a GC/ion trap spectrometer (No. CONF-9505261-), ASMS, East Lansing, MI (United States). 1995.
17. Odu CTI, Nwoboshi LC, Fagade SO, Awani PE. Post impact study of SPDC's Nun River 8" delivery line oil spillage. Final report. SPDC, Nig. 1989: 95.
18. Okolo JC, Amadi EN, Odu CTI. Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. Appl Ecol Environ Res. 2005; 3: 47-53.
19. Motsara MR, Roy RN. Guide to laboratory establishment for plant nutrient analysis, Vol. 19, Rome: Food and Agriculture Organization of the United Nations. 2008.
20. Cunniff P, ed. Official methods of analysis of: current through: march 1999 revision (p. 10), Gaithersburg, Maryland: Association of Official Analytical Chemists (AOAC) International. 1999.
21. Ataikiru TL, Okorhi-Damisa BF, Akpaiboh JI. Microbial community structure of an oil polluted site in Effurun, Nigeria. Int Res J Public Environ Health; 2017; 4: 41-47.
22. Abioye OP, Agamuthu P, Abdul Aziz AR. Biodegradation of used motor oil in soil using organic waste amendments. Biotechnol Res Int. 2012: 1-8.
23. Chikere CB, Ekwuabu CB. Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil impacted sites in Bodo, Ogoniland, Nigeria. Afr J Environ Sci Technolo. 2014; 8: 401-406.
24. Department of Petroleum Resources (DPR). Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN)', Ministry of Petroleum and Natural Resources, Abuja, Nigeria. 2002: 314.
25. Ighovie1 ES, Ikechukwu EE. Phytoremediation of crude oil contaminated soil with *Axonopus compressus* in the Niger Delta Region of Nigeria. Nat Res. 2014; 5: 59-67.
26. Nwaichi EO, Frac M, Nwoha PA, Eragbor P. Enhanced phytoremediation of crude oil-polluted soil by four plant species: effect of inorganic and organic bioaugmentation. Int J Phytoremed. 2015; 17: 1253-1261.
27. Efe SI, Okpali AE. Management of petroleum impacted soil with phytoremediation and soil amendments in Ekpan Delta State, Nigeria. J Environ Prot. 2012: 3: 386-393.
28. McClutchen S, Schnoor J. Phytoremediation transformation and control of contaminants, John Wiley and Sons Inc., Hoboken. 2003.
29. Phillips LA, Greer CW, Germida JJ. Culture-based and culture-independent assessment of the impact of mixed and single plant treatments on rhizosphere microbial communities in hydrocarbon contaminated flare-pit soil. Soil Biol Biochem. 2006; 38: 2823-2833.
30. Eulis K, Ho C, Schwah AP, Rock S, Banks MK. Greenhouse and field assessment of phytoremediation for petroleum contaminant in a riparian zone. Biores Technol. 2008; 99: 1961-1971.
31. Xin L, Li XJ, Li PJ, Li F, Lei Z, Zhou QX. Evaluation of plant-microorganism synergy for the remediation of diesel fuel contaminated soil. Bull Environ Cont Toxicol. 2008; 81: 19-24.
32. Gurska J, Wang WX, Gerhardt KE, Khalid AM, Isherwood DM, Huang XD, et al. Three year field test of a plant growth promoting Rhizobacteria enhanced phytoremediation system at a land farm for treatment of hydrocarbon waste. Environ Sci Technol. 2009; 43: 4472-4479.
33. Phillips LA, Charles WG, Farrell RE, Germida, JJ. Field-scale assessment of weathered hydrocarbon degradation by mixed and single plant treatments. Appl Soil Ecol. 2009; 42: 9-17.
34. Abioye OP, Akinsola RO, Aransiola SA, Damisa D, Auta SH. Biodegradation of crude oil by

saccharomyces cerevisiae isolated from fermented zobo (locally fermented beverage in Nigeria). Pak J Biol Sci. 2013; 16: 2058-2061.

35. Maddela NR, Masabanda M, Leiva-Mora M. Novel diesel-oil degrading bacteria and fungi from the Ecuadorian Amazon rainforest. Water Sci Technol. 2015; 71: 1554-1561.
36. Mansur AA, Adetutu EM, Makadia T, Morrison PD, Ball AS. Assessment of the hydrocarbon degrading abilities of three bioaugmentation agents for the bioremediation of crude oil tank bottom sludge contaminated Libyan soil. Int J Environ Bioremed Biodegrad. 2015; 3: 1-9.
37. El-Hanafy AAEME, Anwar Y, Mohamed SA, Al-Garni SMS, Sabir JSM, AbuZinadah OA, et al. Isolation and identification of bacterial consortia responsible for degrading oil spills from the coastal area of Yanbu, Saudi Arabia. Biotechnol Biotechnol Equip. 2016; 30: 69-74.
38. Abdusalam S, Adefila SS, Bugaje IM, Ibrahim S. Bioremediation of soil contaminated with used motor oil in a closed system. J Bioremed Biodegrad. 2012; 3(12): 1-7.
39. Osuji LC, Onojake CM. Field reconnaissance and estimation of petroleum hydrocarbon and heavy metal contents of soils affected by the Ebocha-8 oil spillage in Niger Delta, Nigeria. J Environ Manag. 2006; 79: 133-139.
40. Ekundayo EO, Obuekwe CA. Effect of oil spill on soil physio-chemical properties of a spill site in a typic paledult of Midwestern Nigeria. Environ Monit Asses. 1997; 45: 209-221.
41. Lee IS, Kim OK, Chang YY, Bae B, Kim HH, Baek KH. Heavy metal concentrations and enzyme activities in soil from a contaminated Korean shooting range. J Biosci Bioengin. 2002; 94: 406-411.
42. Wang XY, Feng J, Wang J. Petroleum hydrocarbon contamination and impact on soil characteristics from oilfield Momoge Wetland. Huan jing ke xue= Huanjing kexue. 2009; 30: 2394-2401.
43. Okoro SE, Adoki A. Bioremediation of crude oil impacted soil utilizing surfactant, nutrient and enzyme amendments. J Biodiv Environ Sci. 2014; 4: 41-50.
44. Tanee FBG, Albert E. Reconnaissance assessment of long term effects of crude oil spill on soil chemical properties and plant composition at Kwawa, Ogoni, Nigeria. J Environ Sci Technol. 2015; 8: 320-329.
45. Njoku KL, Akinola MO, Oboh BO. Does crude oil affect pH, moisture and organic matter content of soils. Ecol Environ Conser. 2008; 14: 731-736.
46. Ifemeje JC, Egbuna C. Effect of crude oil pollution on soil and proximate composition of Cassava from Owaza in Ukwa West Local Government Area of Abia State, Nigeria. Int J Innov Appl Stud. 2016; 15: 72.
47. Hatami E, Abbaspour A. Dorostkar V. Phytoremediation of a petroleum-polluted soil by native plant species in Lorestan Province, Iran. Environ Sci Poll Res. 2018. https://doi.org/10.1007/s11356-018-1297-7
48. Abbaspour A, Kalbasi M, Hajrasuliha S, Fotovat, A. Effect of organic matter and salinity on ethylenediaminetetraacetic acid–extractable and solution species of cadmium and lead in three agricultural soils. Common Soil Sci Plant Anal. 2008; 39: 983-1005.
49. Essien OE, John IA. Impact of crude-oil spillage pollution and chemical remediation on agricultural soil. J Appl Sci Environ Manag. 2010; 14: 147-154.
50. Kuyukina MS, Ivshina IB, Makarov SO, Litvinenko LV, Cunningham CJ, Philp JC. Effect of biosurfactants on crude oil desorption and mobilization in a soil system. Environ Int. 2005; 31: 155-161.
51. Khamehchiyan M, Charkhabi AH, Tajik M. Effects of crude oil contamination on geotechnical properties of clayey and sandy soils. Engin Geol. 2007; 89: 220-229.
52. Abosede EE. Effect of crude oil pollution on some soil physical properties. J Agric Vet Sci. 2013; 6: 14-

17.

53. Osuji LC, Onojake CM. Trace heavy metals associated with crude oil: A case study of Ebocho-8-oil-spill-polluted site in Niger Delta, Nigeria. Chem Biochem. 2004; 1: 1612-1880.
54. Zhang H, Lian C, Shen Z. Proteomic identification of small, copper-responsive proteins in germinating embryos of *Oryza sativa*. Ann Bot. 2009; 103: 923-930.
55. Ikuesan FA. Microbial response to varying concentrations of crude oil pollution of agricultural soils in Ondo State, Nigeria. Microb Res J Int. 2017; 22: 1-8.
56. Ebuehi OAT, Abibo IB, Shekwolo PD, Sigismund KI, Adoki A, Okoro IC. Remediation of crude oil contaminated soil by enhanced natural attenuation technique. J Appl Sci Environ Manag. 2005; 9: 103-106.
57. Ibiene AA, Orji FA, Orji-Nwosu EC. Microbial population dynamics in crude oil-polluted soils in the Niger Delta. Nigerian J Agric Food Environ. 2011; 7: 8-13.