Original research papers

Phytoremediation of Crude Oil Polluted Agricultural Soil Using *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f.

ABSTRACT

Aims: To remove hydrocarbons from crude oil polluted agricultural soil using two selected plant species.

Study design: Mature seeds of *Schwenkia americana* L. and *Spermacoce ocymoides* Burm. f. were propagated onto a sterile unpolluted agricultural soil. Seedlings were transplanted into an 8 kg potted homogenized polluted soil for remediation.

Place and Duration of Study: Polluted agricultural soil collected from Ogoniland, Rivers State, Nigeria, Ecological Centre of the University of Port Harcourt Nigeria, between May 2017 and February 2018.

Methodology: Standard methods were employed for laboratory analyses. Reagents used were of analytical grades with high purity.

Results: Twelves weeks after planting (WAP), total petroleum hydrocarbons (TPH) decreased from 17962.11±1000.00 mg/kg to 117.64±30.27 and 117.45±14.76 mg/kg in *S. americana* and for *S. ocymoides* remediated groups respectively, while polycyclic aromatic hydrocarbons (PAH) decreased from 440.97±1.00 mg/kg to 120.99±50.05 and 181.97±158.69 mg/kg for *S. americana* and for *S. ocymoides* remediated groups respectively. While oil content decreased to 1.02±0.09 and 1.15±0.21 ppm for *S. americana* and *S. ocymoides* remediated groups respectively from an initial 3.19±0.10 ppm recorded at the onset of remediation, organic carbon decreased from 2.78±0.01 % to 2.16±0.06 and 2.07±0.18 % for *S. americana* and *S. ocymoides* remediated groups respectively. With regards to TPH, the percentage recovery moved towards normal values (510.27 and 509.02 %), however with regards PAH, the values nosedived indicating a failure in restoration. By 4, 8 and 12 WAP, the treatment restored the values towards normal values (1.08, 7.30, 10.58, 10.81 and 14.05 %), however, the treatment using *S. ocymoides*, by 12 WAP, plunged, indicating failure to restore the polluted soil, especially with regards to the oil content, while by 8 and 12 WAP, the treatments restored the polluted soil towards normal values (3.33, 11.11 and 13.33), with regards to the organic carbon.

Conclusion: The quality of the crude oil polluted agricultural soil was enhanced through phytoremediation with these plant species. This contention is supported by the diminution of hydrocarbons,organic carbon and the oil content of the remediated groups after the 12 weeks remediation.

Keywords: Phytoremediation, Hydrocarbons, *Schwenkia americana* L., *Spermacoce ocymoides* Burm. f., Restoration, Percentage recovery.

1. INTRODUCTION

The extraction of crude oil in Nigeria is one of the chief causes of pollution [1] [2]. Since 1960, more than 4000 spills have been estimated to occur in Nigeria and this has resulted to release of more than 2 million barrels of crude oil into the environments [2] [3] [4]. About 80% of crude oil pollution has been estimated to results from spillage. When oil spills, non-organic compounds, carcinogens, and

chemicals capable of inhibiting growth which are contained in the crude oil are released to the environment [5], and lengthened exposure to severe oil pollution could lead to the initiation of liver and kidney disease, damage of bone marrow and deepened risk of cancer [6]. To return the polluted environment back to its natural state so as to be wholesome for humans, it is vital for amelioration of the polluted environment to be effected to make the environment free from contaminants [7].

Crude oil is the basic mineral product which is acquired from the geological strata [8]. It is formed from natural processes emanating from geological deposits shaped from organic decomposition products of ancient animals and plants under high pressure and temperature [9]. Even though it is known to be a uniform mineral substance, it is certainly a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds prevailed by carbon and hydrogen atoms while containing smaller amounts of nitrogen, oxygen and sulphur [10]. It also comprised of other constituents such as alkanes (paraffin) and cycloalkanes, hydrogen sulphide gas, metals and heavy metals, naturally occurring radon materials (NORM), polycyclic aromatic hydrocarbon (PAHs) and volatile organic compounds (VOCs) [8]. Some chemicals present in petroleum hydrocarbons are represented by their common chemical characteristics such as boiling point range or the molecules size. Such chemicals include benzene, fluorine, hexane, naphthalene, toluene, xylene, and various constituents of mineral oils, jet fuels, gasoline, and other petroleum products [11]. The release of these petroleum hydrocarbons into the environment is the major source and cause of environmental pollution [9] [12].

Biotechnological techniques are brought into play with the goal of solving environmental contamination issues [13]. Conventional methods for cleaning up contaminants comprising of physical, chemical and thermal processes have been vital in the decontamination of oil polluted sites However, some of these processes possess some negative effects which have the tendency to cause more harm than the contaminant [14]. Aside this, these processes are expensive and may not be considered as lasting resolution to the problem as they either dilute or sequester the pollutants, and in some cases relocate them from one environmental area to another. This, however, does not solve the problem [15].

Phytoremediation can be expounded as the use of living green plants and/or associated microbes to detach, debase, suppress or proffer toxic materials non-toxic [14] in an effort to avert, diminish or alleviate damage to human health or the environment induced by these toxicants [5]. It is a potent, nonintrusive, economical, aesthetically affable and socially accepted means of remediating contaminated soil [16]. This remediation technique is established on the view of employing “nature to cleanse nature” [17] [18], and pollutants ranging from heavy metals and radionuclides to a broad range of organic pollutants have been effectively decontaminated using this method [14]. Plants employ several mechanisms for the eviction of contaminants ranging from uptake and sequestration, and transformation, to stabilization and rhizoshere degradation, the latter in which plants promote the increase in number of bacteria in the root zone which sequentially breaks down pollutants [19]. This study therefore seeks to evaluate the ability of *S. americana* and *S. ocymoides* to remove crude oil pollutants from contaminated agricultural soil.

2. METHODOLOGY

2.1 Experimental design

An agricultural farmland polluted as a result of crude oil spillage was identified in Bodo community, Gokana L.G.A. of Rivers State, Nigeria. The pollution was evaluated to establish the types of pollutants present and to implore most excellent technique for its restoration. In the evaluation, physical features and distribution of the pollutants were ascertained. Afterwards, indigenous plants from the polluted site were harvested and identified in the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt, Nigeria. Viable and mature seeds of two plants (*S. americana* and *S. ocymoides*), selected based on on-hand reports on their propensity to withstand polluted environments, were obtained from wild. The viability of the seeds was determined by wet paper germination method before they were propagated for nursery using sterile unpolluted agricultural soil and monitored from seed germination to seedling level. Prior to treatment of polluted soil, 4 seedlings each of the plants species were transplanted into an 8 kg potted homogenized polluted soil set up in triplicate. The polluted control group, the unpolluted control group and the treatment groups were all set up in triplicate. The polluted soil sample that was remediated in this study was collected from the identified crude oil polluted site while the unpolluted soil sample was

collected from an agricultural farmland located within the University of Port Harcourt. The soil samples were collected following method described by Motsara & Roy [20], where a sterilized soil auger was used to collect soils between 0 – 15 cm depth and transported using sterile plastic bags. For laboratory analyses, soil samples for baseline analyses were first collected before potting while subsequent sampling and analyses were carried out 4, 8, and 12 WAP. After the 12 weeks treatment period which lasted between November 2017 and January 2018 dry season, germination rate of the treated soils was determined using Lettuce (*Lactura sativa* L.) due to its sensitivity to crude oil pollution.

2.2 Laboratory analyses

All reagents used for this study were of analytical grades with high purity.

2.2.1 Total petroleum hydrocarbon (TPH)

The total petroleum hydrocarbons analyses was carried out using standard methods [21] [22], where homogenized soil sample is extracted by shaking mechanically or by sonication with acetone. 20 g each of homogenized soil sample was weighed and placed into a glass extraction vessel and to it was added 40 mL of acetone. The extraction vessel was briefly shaken by hand before 20 mL of the retention-time window (RTW) standard solution, comprising of n-tetracontane and n-decane, was added. Extraction was carried out for 1 hour after which the specimen was allowed to stand for the solid material to settle and the supernatant was transferred into a separatory funnel. The organic phase was washed twice by shaking thoroughly for 5 minutes with 100 mL of water to remove the acetone. The organic layer was collected in a glass tube following a thorough 5 minute double washing of the organic phase with 100 mL of water to remove acetone. Sufficient quantity of sodium sulphate was added so that no lumps were formed and 10 mL of the extract was transferred to a clean-up column filled with florisil and all the eluate was collected. An aliquot of the purified extract was then analyzed by gas chromatography-mass spectrometry (7890/5975).

2.2.2 Polycyclic aromatic hydrocarbons (PAHs)

PAH of the soil samples was analyzed using EPA 8270 standard method [23] [24]. Each 10 g of homogenized soil sample was extracted in an extraction container (soxhlet apparatus) for 16 hours using 150ml of the extraction solvent, toluene. The extracts were concentrated to about 3 mL using the rotary evaporator and 3 mL residues obtained from the extraction step were added to the cartridge, and passed through at a low rate of 4-5 mL/min. The cartridge was finally eluted with 3×4 mL mixture of the dichloromethane and n-hexane (1:1) at a flow rate of 1 mL / min. The eluates were concentrated to 1 mL at 40°C and 335 mbar and then to dryness using gentle stream of nitrogen. The residues were dissolute in 1 mL n-hexane containing 1 µg/ml internal standard (1-Fluoronaphthalene), and 1 µl of the concentrated aromatic fraction was aspirated using a hypodermic syringe and injected through a rubber septum into the GC-vial of Gas chromatography (7890).

2.2.3 Determination of oil content

Oil content was determined spectrophotometrically according to toluene extraction method [25] [26]. 1 g of air dried and homogenized soil sample was weighed into a 50 mL conical flask and 10mLs of toluene (solvent) was added into it, shaken vigorously and allowed to stand for 10 minutes. This was filtered through Whatmann No. 1 filter paper, and the filtrate diluted appropriately with fresh toluene. The intensity was measured at a wavelength of 420 nm using a spectrophotometer.

2.2.4 Determination of organic carbon

Organic carbon was determined by loss of weight on ignition method [20]. 5 g of sieved (2 mm) soil was weighed into a crucible. The crucible with the soil was placed in a drying oven, set at 105 °C and allowed to dry. After 4 hours, the crucible was removed from the drying oven and placed in a dry atmosphere. When cooled, the crucible with the soil was weighed to the nearest 0.01 g. Again, the crucible with the dried soil was placed in a muffle furnace, set at 400 °C. After 4 hours of ashing, the crucible was removed from the muffle furnace, cooled in a dry atmosphere, and reweighed to the nearest 0.01 g.

2.2.5 Estimation of total nitrogen

To estimate for soil total nitrogen, the Kjeldahl method [20] with modification was adapted. For a gram of soil sample weighed into a flask, 0.7 g of $CuSO_4$, 1.5 g of K_2SO_4 and 30 mL of H_2SO_4 were added. The mixture was boiled briskly until it became clear (sky blue colour appeared) and then digested further for 30 minutes. The flask was removed from the heater, cooled and 50 mL of distilled water was added prior to distillation. 25 mL of standard acid (0.1 M HCl) was placed accurately in the receiving flask and 3 drops of methyl red indicator added. 30 mL of 35 % NaOH was added into the distilling flask in such a way that the contents did not mix. The contents were heated to distil the ammonia for about 30 minutes. The excess acid in the distillate was titrated with 0.1 M NaOH. Blank on reagents was determined using the same quantity of standard acid in a receiving conical flask.

2.2.6 Total culturable heterotrophic bacteria count (TCHBC)

TCHBC was estimated by the spread plate on nutrient agar (NA) method [27] [28]. 1 g of homogenized soil sample was added into 9 mL of sterile 0.85% normal saline. Decimal dilutions (5-fold) of the soil suspensions were plated out on agar medium, sealed with a film and incubated at 30 $^{\circ}$C for 24 hours. The colony forming units were counted and recorded.

2.2.7 Total heterotrophic fungi (THF)

THF count was estimated in duplicates using the spread plate method on potato dextrose agar (PDA) containing 1% lactic acid to inhibit the growth of bacteria [27] [28]. A 5-fold dilution of soil samples was inoculated on potato agar medium, sealed with a film and incubated at 28±2 $^{\circ}$C for a period of 3 days in the dark. Discrete colonies that formed on PDA were counted.

2.2.8 Hydrocarbon utilizing bacteria (HUB) and fungi (HUF)

The vapour phase transfer method [29] [30] was employed for HUB and HUF estimation. Decimal dilution (5-fold) of the soil suspensions were inoculated onto duplicate sterile Petri dishes containing mineral salt agar (MSA). The MSA comprised of 3.27 g of Bushnell Haas Broth and 15 g of agar agar dissolved in 1000 mL of distilled water. The MSA containing 1 % of nystatin solution was poured onto the dishes designated for HUB, while the MSA containing 1 % lactic acid was poured onto the plates designated for HUF. The media were allowed to solidify. Sterile filter paper (Whatman No 1) was saturated with filtered and sterilized crude oil and placed inside the cover of the Petri dish. The dishes were closed, sealed, inverted and incubated at 30 $^{\circ}$C for 24 hours for bacteria and 3 – 7 days at room temperature for fungi. The Whatmann No. 1 filter paper saturated with filtered and sterilized crude oil served as a sole carbon source.

2.2.9 Plant height and number of leaves

The metric method [31] was adapted for plant height measurement. The plant height was measured from soil level to terminal bud using a meter rule. The number of leaves was determined by counting.

2.2.10 Germination toxicity test

The germination toxicity test was carried out by the method as described [32]. Lettuce was employed for the study owing to its sensitivity to crude oil pollutants. The germination test was conducted over a 5-day period. The seeds of lettuce were obtained commercially. For each soil sample, 150 g of remediated soil was mixed thoroughly and placed in 100 x 15mm petri dish. Ten (10) viable seeds of lettuce (*Lactura sativa* L.) were evenly placed throughout each petri dish and covered with 10 g of dry sand. The samples were prepared in triplicates and moisture content of soil was maintained at 80 % water holding capacity. The petri dishes were placed in a room with 16 hours light and 8 hours darkness for 5 days after which the number of seedlings that emerged from the surface of soil was counted and recorded prior to the calculation of the germination index.

2.3 Statistical analysis

Results of all the studies are expressed as means ± standard deviation of triplicate determination. To detect a significant difference between the groups, statistical analysis was carried out using one way analysis of variance (ANOVA). Data between groups were analyzed by the Bonferroni test using

Statistical Package for the Social Science (SPSS®) Version 20 statistics software at 95% (P = .05) confidence level, while data between periods were analyzed using Student t-test.

3. RESULTS AND DISCUSSION

The TPH and PAH values of the soil samples are presented in Tables 1 – 2. Compared to the corresponding baseline values, the TPH values of the remediated groups decreased after 12 weeks remediation. This may be due to physical and biological factors necessary for the biodegradation of petroleum hydrocarbons. Some authors [33] [34] [35] [36] [37] have suggested that evaporation and microbial degradation could enhance hydrocarbon reduction. Atagana *et al*. [38] also reported that soil with C:N 10:1 would be adequate to stimulate microbial growth thereby leading to the degradation of hydrocarbons. The presence of oil-degrading microorganisms in soils with the ability to degrade nearly 100 % of the crude oil has been reported [39], while *Saccharomyces cerevisiae* isolate that could provide 49% degradation of crude oil has also been indicated [6]. According to Adeniyi *et al*. [40], plants release exudates could enhance or hamper the biological activities in soils, and plant extracts have the tendency to inhibit the growth of certain fungi and bacteria. This may be the reason why the TPH and PAH values of the treated groups had a higher value than the polluted control group after 12 weeks. The higher TPH and PAH values in the unpolluted control groups the 12 weeks remediation period may be due to the transport of hydrocarbons by motile microorganisms from the polluted control pots since they were in close proximity with each other. It has been reported [41] that microbial carriers could enhance mass transfer of hydrophobic organic chemicals (HOCs), and microbes could enhance PAH mass transfer up to hundred fold. It may however be due to run off during rainfall and the washing of hydrocarbons within the ecological centre to the experimental pots, which were perforated for aeration, thus leading to the absorption of the runoff water containing hydrocarbons by the remediating groups. By 12 WAP, the percentage recovery of the remediated soils, calculated as: % recovery = {[Parameter in consideration] × [Test (polluted) Control] / [Normal (unpolluted) control] × [Test (polluted) Control]} × 100, [42], showed that the treatments restored the polluted soil towards normal values (510.27 % and 509.02 %), especially with regards to TPH. However with regards PAH, the values nosedived indicating a failure in restoration.

Table 1. Total petroleum hydrocarbon (mg/kg) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	12 WAP	% R 12 WAP
Unpolluted control	17.57±1.00^{a}	56.29±8.57^{a,*}	NA
Polluted control	17962.11±1000.00^{b}	41.33±3.94^{a,*}	NA
S. americana	17962.11±1000.00^{b}	117.64±30.27^{b,*}	510.27
S. ocymoides	17962.11±1000.00^{b}	117.45±14.76^{b,*}	509.02

Values are mean ± standard deviations of triplicate determinations.
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; % R = Percentage Recovery; NA = Not Applicable.

Table 2. Polycyclic aromatic hydrocarbons (mg/kg) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	12 WAP	% R 12 WAP
Unpolluted control	5.80±0.10^{a}	19.74±7.00^{a,*}	NA

Polluted control	440.97±1.00[b]	47.26±2.75[a,*]	NA
S. americana	440.97±1.00[b]	120.99±50.05[a,*]	-267.91
S. ocymoides	440.97±1.00[b]	181.97±158.69[a]	-489.50

Values are mean ± standard deviations of triplicate determinations.
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; % R = Percentage Recovery; NA = Not Applicable.

Compared to the baseline values, the oil content (Table 3) of the remediated groups reduced with time which is typical of any degradation process. This degradation process follows a shifting order (1-0) similarly reported [43]. Nonetheless, the treatments restored the polluted soil towards normal values (1.08 %, 7.30 %, 10.58 % and 14.05 %), especially with regards to the oil content.

Table 3. Oil content (in ppm) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	4 WAP	8 WAP	12 WAP	% R 4 WAP	% R 8 WAP
Unpolluted control	0.10±0.01[a]	0.08±0.01[a]	0.06±0.01[a*]	0.03±0.01[a*]	NA	NA
Polluted control	3.19±0.10[b]	2.82±0.14[b*]	1.91±0.17[c,d*]	1.14±0.13[b*]	NA	NA
S. americana	3.19±0.10[b]	2.53±0.05[c*]	1.65±0.06[c*]	1.02±0.09[b*]	10.58	14.05
S. ocymoides	3.19±0.10[b]	2.62±0.23[b,c*]	1.89±0.04[b*]	1.15±0.21[b*]	7.30	1.08

Values are mean ± standard deviations of triplicate determinations.
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; % R = Percentage Recovery; NA = Not Applicable.

The soil organic carbon is presented in Table 5. Compared to baseline values, the organic carbon content of the remediated groups reduced over time. This may be due to reduction in the hydrocarbon content of the groups resulting from the proliferation of the microbial population and their utilization of the carbon as energy source. It has been reported [44] that increased microbial population implies increased energy (carbon) demand since the microbial oil degraders use the carbon content for the provision of energy. Also by 8 and 12 WAP, the treatments restored the polluted soil towards normal values (3.33, 11.11 and 13.33), with regards to the organic carbon.

Table 5. Organic carbon content (%) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	8 WAP	12 WAP	% R 8 WAP	% R 12 WAP
Unpolluted control	1.44±0.10[a]	1.43±0.12[a]	1.29±0.11[a]	NA	NA
Polluted control	2.78±0.01[b]	2.33±0.05[b*]	2.19±0.08[b*]	NA	NA
S. americana	2.78±0.01[b]	2.23±0.14[b*]	2.16±0.06[b*]	11.11	3.33
S. ocymoides	2.78±0.01[b]	2.21±0.15[b*]	2.07±0.18[b*]	13.33	13.33

Values are mean ± standard deviations of triplicate determinations.
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; % R = Percentage Recovery; NA = Not Applicable.

Table 6. Carbon:Nitrogen ratio of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP			8 WAP			12 WAP		
	% C	% N	C:N	% C	% N	C:N	% C	% N	C:N
Unpolluted control	1.44	0.83	1.73	1.43	0.39	3.67	1.29	0.21	6.14
Polluted control	2.78	0.57	4.88	2.33	0.25	9.32	2.19	0.14	15.64
S. americana	2.78	0.57	4.88	2.23	0.26	8.58	2.16	0.15	14.40
S. ocymoides	2.78	0.57	4.88	2.21	0.25	8.84	2.07	0.14	14.79

Note: BP = Before Planting; WAP = Week(s) After Planting, % C = Percentage Carbon, % N = Percentage Nitrogen, C:N = Carbon-Nitrogen Ratio.

The total culturable heterotrophic bacteria count (TCHBC), total fungi count (TFC), hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) are presented in Tables 7 to 10. Compared to the baseline values, the TCHBC, TFC, HUB and HUF of the remediated groups increased over time. This observed increase in the population of bacteria and fungi is not surprising as this has shown the progressive utilization of organic matter and hydrocarbon, with the hydrocarbons functioning as primary substrate [45]. It has been reported [5] that a higher microbial count recorded over time is an indication of increased biodegradation by the microbial community. The higher TCHBC and HUB over TFC and HUF respectively may be due to the soil nutrient status of the soils and other toxic components that may not favour the growth of fungi. This corroborates the report [46] which attributed such a difference to the soil nutritional status and the presence of toxic components.

Table 7. Total culturable heterotrophic bacteria count (TCHBC) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	6 WAP	12 WAP
Unpolluted control	6.31 ± 0.00^{a}	$7.75\pm0.07^{a*}$	$6.63\pm0.13^{a*}$
Polluted control	6.62 ± 0.01^{b}	$8.06\pm0.04^{b*}$	$6.67\pm0.14^{a,b*}$
S americana	6.62 ± 0.01^{b}	$7.86\pm0.28^{a,b}$	$6.83\pm0.09^{b*}$
S. ocymoides	6.62 ± 0.01^{b}	$8.07\pm0.18^{a,b*}$	$6.89\pm0.10^{a,b*}$

Values are mean ± standard deviations of triplicate determinations.
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

Table 8. Total fungi count (TFC) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	6 WAP	12 WAP
Unpolluted control	6.15±0.04[a]	6.99±0.10[a*]	6.55±0.11[a*]
Polluted control	5.14±0.57[b]	6.55±0.16[b*]	6.72±0.23[a]
S. americana	5.14±0.57[b]	6.71±0.35[b*]	6.53±0.16[a*]
S. ocymoides	5.14±0.57[b]	6.67±0.07[b*]	6.66±0.11[a*]

Values are mean ± standard deviations of triplicate determinations.
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

Table 9. Hydrocarbon utilizing bacteria (HUB) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	6 WAP	12 WAP
Unpolluted control	5.38±0.02[a]	6.55±0.43[a]	6.18±0.20[a,b*]
Polluted control	5.98±0.01[b]	6.52±0.24[a]	6.27±0.16[c]
S. americana	5.98±0.01[b]	6.50±0.41[a]	6.66±0.17[a,b*]
S. ocymoides	5.98±0.01[b]	6.68±0.30[a]	6.78±0.11[b*]

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b,c) are significantly different at p = 0.05.
**p = 0.05 compared to the corresponding values before treatment.*
Note: BP = Before Planting; WAP = Week(s) After Planting

Table 10. Hydrocarbon utilizing fungi (HUF) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BP	6 WAP	12 WAP
Unpolluted control	4.32±0.02[a]	5.34±0.18[a*]	5.79±0.12[a*]
Polluted control	4.72±0.01[b]	5.68±0.10[a*]	5.72±0.18[a,b*]
S. americana	4.72±0.01[b]	5.57±0.11[a*]	5.42±0.21[b*]
S. ocymoides	4.72±0.01[b]	5.65±0.20[a*]	5.47±0.25[a,b*]

Values are mean ± standard deviations of triplicate determinations.
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 treatment.*
Note: BP = Before Planting; WAP = Week(s) After Planting

The percentage germination (Table 11) of the group treated with *S. americana* showed a significantly (p<0.05) higher value when compared with the polluted control group. Nonetheless, there was no significant difference between the percentage germination indexes of the remediated groups. Although the polluted control group recorded lower TPH and PAH values compared to the remediated groups after 12 weeks remediation, it could be that the presence of other pollutants in the polluted control group reduced its germination rate. However, exudates from the treatment plants may have positively enhanced the germination rate of the remediated groups. This finding corroborates the report [6] that seed germination on remediated soil previously contaminated with lubricating oil.

Table 11. Germination toxicity test of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	PERCENTAGE GERMINATION (%)	PERCENTAGE GERMINATION INDEX (%)
Unpolluted control	95.00±5.00[a]	NA
Polluted control	65.00±0.00[b]	NA
S. americana	75.00±5.00[c,d]	58.33±8.02[b]
S. ocymoides	73.33±10.41[b,d]	49.00±15.13[b]

Values are mean ± standard deviations of triplicate determinations.
Values in the same row with different letters (a,b,c,d) are significantly different at $p = 0.05$.
Note: NA = Not Applicable

The plants' height and number of leaves are presented in Table 12. Plant height as a plant growth character and yield index is vital. This is because, the taller a plant, the higher the amount of light energy absorbed by such plant and invariably, the higher the rate of photosynthesis and consequently the amount of assimilates produced by the leaves [47]. Compared to week 0, the height of *M. alternifolius* Vahl increased over time. Enhanced growth may be related to the ability of plants to metabolize hydrocarbons [48]. As reported [49], some plants can oxidize many hydrocarbons and their derivatives which occur naturally in them. Likewise, *S. ocymoides* increased over time, retarded after 6 weeks and went into extinction 2 weeks before the end of the remediation. Growth retardation is possible with oil pollution of soil due to insufficient aeration caused by displacement of air from pore spaces [50]. An evidence of growth retardation as a result of increased demand for oxygen by oil decomposing organisms has also been shown [51]. On the other hand, the number of leaves of the *S. americana* increased after 10 weeks while *S. ocymoides* went into complete extinction from week 11.

Table 12. Plant height and number of leaves of *S. americana* and *S. ocymoides* species.

	Plant Height		Number of Leaves	
PERIOD	*S. americana*	*S. ocymoides*	*S. americana*	*S. ocymoides*
0 WAP	8.90±2.16	2.97±1.68	7.08±1.01	5.28±0.75
1 WAP	10.56±3.28	3.05±1.67	7.00±0.66	5.33±1.53
2 WAP	13.87±4.56*	3.52±1.90*	7.25±0.43	7.78±4.02
3 WAP	16.03±4.66*	3.78±1.91*	6.75±0.75	7.64±3.22
4 WAP	16.65±4.77*	3.83±2.00*	6.42±0.52	7.39±2.55
5 WAP	16.92±4.83*	3.67±1.87	6.42±0.29	7.75±3.38
6 WAP	17.48±4.90*	3.93±1.96*	6.50±0.25	6.72±1.93
7 WAP	18.07±4.91*	3.90±2.14	6.50±1.00	5.75±1.54
8 WAP	18.93±4.78*	4.06±2.28	8.50±2.41	6.22±2.04
9 WAP	18.93±5.63*	3.69±2.37	10.75±1.64	4.44±1.07
10 WAP	21.05±3.55*	2.56±2.36	15.58±1.28*	0.92±1.59*
11 WAP	24.17±0.86*	0.00±0.00*	18.33±2.13*	0.00±0.00*
12 WAP	24.04±3.83*	0.00±0.00*	21.75±4.21*	0.00±0.00*

Values are mean ± standard deviations of triplicate determinations.
*$p = 0.05$ compared to the corresponding values 0 WAP.
Note: WAP = Week(s) After Planting

4. CONCLUSION

Largely, the quality of the crude oil polluted agricultural soil was enhanced through phytoremediation with these plant species. Aside the use of these plant species, aeration, microbial activity and other favourable environmental factors may have contributed to the reduction of these pollutants in the unvegetated soil. This assertion is based on the diminution of hydrocarbons observed during the remediation period.

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