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Original research papers

Phytoremediation of Crude Oil Polluted Agricultural Soil Using *Schwenkia americana* and *Spermacoce ocymoides*

ABSTRACT

Aims: To remove hydrocarbons from crude oil polluted agricultural soils using two selected plant species.
Study design: Mature seeds of *Schwenkia americana* and *Spermacoce ocymoides* 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 Nigeria, ecological garden of the University of Port Harcourt Nigeria, between May 2017 and February 2018.
Methodology: Standard methods were employed for laboratory analyses. The hydrocarbons were analyzed by extraction method. Oil content, moisture content and organic content of the soil samples were determined spectrophotometrically, gravimetrically and by loss of weight on ignition methods, respectively. For estimation of the microbial population, the spread plate method was adapted.
Results: After 12 weeks remediation, TPH decreased from 17962±1000 mg/kg to 117±6413 mg/kg and 117.4540±1476 mg/kg in *S. americana* and for *S. ocymoides* remediated groups respectively, while PAH decreased from 440.9715±1.00 mg/kg to 120.9900±50.05 mg/kg and 181.9735±158.69 mg/kg for *S. americana* and for *S. ocymoides* remediated groups respectively. Oil content decreased to 1.0167±0.09 and 1.1513±0.21 for *S. americana* and *S. ocymoides* remediated groups respectively from the initial 3.1890±0.10 recorded at the onset of remediation, while organic carbon decreased from 2.7800±0.01% to 2.1633±0.06 % and 2.0733±0.18 % for *S. americana* and *S. ocymoides* remediated groups respectively. An increase in moisture content from the initial 8.67±0.01 % at the onset to 15.00±2.96 % and 14.22±0.51 % for *S. americana* and *S. ocymoides* remediated groups respectively was recorded while the soil microbial density of the remediated groups increased with time.
Conclusion: The quality of the crude oil polluted agricultural soil was enhanced through phytoremediation with these plant species. This assertion is based on the diminution of hydrocarbons and the oil content of the remediated groups after the 12 weeks remediation.

Keywords: Crude oil, Pollution, Hydrocarbons, Phytoremediation, *Schwenkia americana*, *Spermacoce ocymoides.*

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

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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, xylenes, 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 *Schwenkia americana* and *Spermacoce ocymoides* to remove crude oil pollutants from contaminated agricultural soil.

2. METHODOLOGY

2.1 Experimental design

Prior to identification of the species by the Department of Plant Science and Biotechnology, University of Port Harcourt, the plants were selected based on their diversity in a crude oil polluted agricultural soil of Bodo community, Ogoniland, Nigeria. The viable and mature seeds of the selected plants were obtained from wild and their viability was ascertained by wet paper germination method prior to propagation for nursery in a sterile unpolluted agricultural soil obtained from an agricultural farmland of the Department of Agricultural Science, University of Port Harcourt, Nigeria. The nursery was monitored during seed germination and at seedling levels transferred to the crude polluted soil collected following the standard method [20], where a sterilized soil auger was used to collect soils between 0 – 15 cm depth was used to collect soil from the polluted site using sterile plastic bags. For laboratory analyses, soil samples were collected using sample containers and aluminum foil and labeled appropriately. Where the analyses could not carried out immediately, the soil samples were preserved at 4 $^{\circ}$C.

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

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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 moisture content

The gravimetric method of moisture estimation, where the soil sample is placed in an oven at 105 °C and dried to a constant weight, was adapted [27]. An empty dish with lid was dried in an oven at 105 °C for 3 hours and transferred to a dessicator to cool. The empty dish with lid was weighed and recorded. 3 g of soil sample was weighed into the dish and spread with spatula then placed in the oven to dry to a constant weight for 3 hours at 105 °C. After drying, the dish with partially covered lid containing the dried sample was transferred to the dessicator to cool, and the reweighed.

2.2.5 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.

2.2.6 Total culturable heterotrophic bacteria count (TCHBC)

TCHBC was estimated by the spread plate on nutrient agar (NA) method [28] [29]. 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 °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 [28] [29]. A 5-fold dilution of soil samples

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was inoculated on potato agar medium, sealed with a film and incubated at 28±2 °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 [30] [31] 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 °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 [32] 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 [33]. 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.

3. RESULTS AND DISCUSSION

The TPH and PAH values of the soil samples are presented in Table 1. 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 [34] [35] [36] [37] [38] have suggested that evaporation and microbial degradation could enhance hydrocarbon reduction. The presence of oil-degrading microorganisms in soils with the ability to degrade nearly 100 % of the crude oil has been reported [39]. *Saccharomyces cerevisiae* isolate that could provide 49% degradation of crude oil has also been pointed out [6]. Plants release exudates which may enhance or hamper the biological activities of soils. Plant extracts of M. alternifolius and other plants inhibited the growth of certain fungi and bacteria, with *M. alternifolius* strongly inhibiting the fungi *P. chrysogenum* and bacteria *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* [40]. This could 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. More so, the reason for the increase in TPH and PAH values in the unpolluted control groups is not clear. Nonetheless, it 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

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during rainfall and the washing of hydrocarbons from the ecological garden to the experimental pots, which were perforated for aeration, thus leading to the absorption of the runoff water containing hydrocarbons by the remediating groups.

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

	TPH		PAH	
GROUP	BEFORE	WEEK 12	BEFORE	WEEK 12
Unpolluted control	17.57±1.00[a]	56.2878±8.57[a]*	5.80±0.10[a]	19.7396±7.00*[a]
Polluted control	17962.11±1000.00[b]	41.3335±3.94[a]*	440.9715±1.00[b]	47.2598±2.75*[a]
Schwenkia americana	17962.11±1000.00[b]	117.6413±30.27[b]*	440.9715±1.00[b]	120.99±50.05*[a]
Spermacoce ocymoides	17962.11±1000.00[b]	117.4540±14.76[b]*	440.9715±1.00[b]	181.9735±158.69[a]

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at P = .05.
**P = .05 compared to the corresponding values before treatment.*

Compared to the baseline values, the oil content (Table 2) of the remediated and control groups reduced with time which is typical of any degradation process. This degradation process follows a shifting order (1-0) similarly reported [42].

Table 2. Oil content (in ppm) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 4	WEEK 8	WEEK 12
Unpolluted control	0.1010±0.01[a]	0.0813±0.01[a]	0.0570±0.01[a*]	0.0313±0.01[a*]
Polluted control	3.1890±0.10[b]	2.817±0.14[b*]	1.9067±0.17[c,d*]	1.1443±0.13[b*]
Schwenkia americana	3.1890±0.10[b]	2.534±0.05[c*]	1.6543±0.06[c*]	1.0167±0.09[b*]
Spermacoce ocymoides	3.1890±0.10[b]	2.6203±0.23[b,c*]	1.894±0.04[b*]	1.1513±0.21[b*]

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at P = .05.
**P = .05 compared to the corresponding values before treatment.*

As presented in Table 3, the moisture content of the remediated groups increased across the period when compared with the baseline values. As reported [43], crude oil might have negative effects on some soil physical properties such as decreased pore spaces. Crude oil spillage could reduce soil moisture availability or holding capacity, or increase moisture deficit in agricultural soils thereby damaging plant growth and yield [44]. It is also reported [45] [46] that high crude oil concentrations in soil could clog soil pores and reduce water and oxygen penetration. These reports therefore confirm the reason for the low MC recorded in the polluted soil at the onset of the experiment. The increased moisture content indicates the reduction of crude oil present in the soils and corroborates with previous reports [47] [48] [49].

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Table 3. Moisture content (MC) (%) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 4	WEEK 8	WEEK 12
Unpolluted control	11.67±1.00[a]	10.11±0.19[a*]	18.00±0.33[a*]	14.44±1.39[a]
Polluted control	8.67±0.10[b]	8.89±2.01[a]	16.63±6.80[a,b*]	15.76±2.14[a*]
Schwenkia americana	8.67±0.10[b]	16.22±1.02[b*]	23.78±1.02[b*]	15.00±2.96[a*]
Spermacoce ocymoides	8.67±0.10[b]	15.33±2.00[b*]	21.89±2.37[a,b*]	14.22±0.51[a*]

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at P = .05.
**P = .05 compared to the corresponding values before treatment.*

The soil organic carbon is presented in Table 4. 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 [50] that increased microbial population implies increased energy (carbon) demand since the microbial oil degraders use the carbon content for the provision of energy.

Table 4. Organic carbon content (%) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 8	WEEK 12
Unpolluted control	1.4400±0.10[a]	1.4333±0.12[a]	1.2900±0.11[a]
Polluted control	2.7800±0.01[b]	2.3333±0.05[b*]	2.1867±0.08[b*]
Schwenkia americana	2.7800±0.01[b]	2.2333±0.14[b*]	2.1633±0.06[b*]
Spermacoce ocymoides	2.7800±0.01[b]	2.2067±0.15[b*]	2.0733±0.18[b*]

Values are mean ± standard deviations of triplicate determinations.
Values in the same column with different letters (a,b) are significantly different at P = .05.
**P = .05 compared to the corresponding values before treatment.*

The total culturable heterotrophic bacteria count (TCHBC), total fungi count (TFC), hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) are presented in Tables 5 to 8. 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 [51]. It has been reported [5] that higher microbial counts 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 [52] which attributed such a difference to the soil nutritional status and the presence of toxic components.

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Table 5: Total culturable heterotrophic bacteria count (TCHBC) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 6	WEEK 12
Unpolluted control	6.3100±0.00^{a}	7.7533±0.07^{a*}	6.6267±0.13^{a*}
Polluted control	6.6233±0.01^{b}	8.0600±0.04^{b*}	6.6733±0.14^{a,b*}
Schwenkia americana	6.6233±0.01^{b}	7.8633±0.28^{a,b}	6.8333±0.09^{b*}
Spermacoce ocymoides	6.6233±0.01^{b}	8.0733±0.18^{a,b*}	6.8900±0.10^{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 = .05.
**P = .05 compared to the corresponding values before treatment.*

Table 6. Total fungi count (TFC) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 6	WEEK 12
Unpolluted control	6.1467±0.04^{a}	6.9867±0.10^{a*}	6.5467±0.11^{a*}
Polluted control	5.1433±0.57^{b}	6.5500±0.16^{b*}	6.7200±0.23^{a}
Schwenkia americana	5.1433±0.57^{b}	6.7067±0.35^{b*}	6.5267±0.16^{a*}
Spermacoce ocymoides	5.1433±0.57^{b}	6.6667±0.07^{b*}	6.6567±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 = .05.
**P = .05 compared to the corresponding values before treatment.*

Table 7. Hydrocarbon Utilyzing Bacteria (HUB) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 6	WEEK 12
Unpolluted control	5.3800±0.02^{a}	6.5467±0.43^{a}	6.1800±0.20^{a,b*}
Polluted control	5.9767±0.01^{b}	6.5200±0.24^{a}	6.2667±0.16^{c}
Schwenkia americana	5.9767±0.01^{b}	6.5000±0.41^{a}	6.6567±0.17^{a,b*}
Spermacoce ocymoides	5.9767±0.01^{b}	6.6800±0.30^{a}	6.7833±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 = .05.
**P = .05 compared to the corresponding values before treatment.*

Table 8. Hydrocarbon Utilyzing Fungi (HUF) (Log_{10} cfu/g) of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	BEFORE	WEEK 6	WEEK 12
Unpolluted control	4.3200±0.02^{a}	5.3367±0.18^{a*}	5.7867±0.12^{a*}
Polluted control	4.7200±0.01^{b}	5.6800±0.10^{a*}	5.7233±0.18^{a,b*}
Schwenkia	4.7200±0.01^{b}	5.5700±0.11^{a*}	5.4200±0.21^{b*}

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americana			
Spermacoce ocymoides	4.7200±0.01[b]	5.6467±0.20[a*]	5.4700±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 = .05.
**P = .05 compared to the corresponding values before treatment.*

The percentage germination and germination index (Table 9) of the remediated groups was higher that the polluted control group. Although the polluted control group recorded a 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 9. Germination toxicity test of unpolluted control, polluted control, *S. americana* remediated and *S. ocymoides* remediated soils

GROUP	PERCENTAGE GERMINATION (%)	PERCENTAGE GERMINATION INDEX (%)
Unpolluted control	95.0000±5.00[a]	NA
Polluted control	65.0000±0.00[b]	27.3333±3.21[a]
Schwenkia americana	75.0000±5.00[c,d]	58.3333±8.02[b]
Spermacoce ocymoides	73.3333±10.41[b,d]	49.0000±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 = .05.*

The plants' height and number of leaves are presented in Table 10. 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 [53]. 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 [54]. As reported [55], 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 [56]. An evidence of growth retardation as a result of increased demand for oxygen by oil decomposing organisms has also been shown [57]. 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 10. Plant Height and Number of leaves of *S. americana* and *S. ocymoides* species.

	Plant Height		Number of Leaves	
PERIOD	*Schwenkia americana*	*Spermacoce ocymoides*	*Schwenkia americana*	*Spermacoce ocymoides*
WEEK 0	8.9000±2.16	5.2788±0.75	7.0833±1.01	2.9667±1.68
WEEK 1	10.5583±3.28	5.3333±1.53	7.0000±0.66	3.0500±1.67
WEEK 2	13.8667±4.56*	7.7777±4.02	7.2500±0.43	3.5167±1.90*
WEEK 3	16.0333±4.66*	7.6389±3.22	6.7500±0.75	3.7833±1.91*
WEEK 4	16.6500±4.77*	7.3889±2.55	6.4167±0.52	3.8333±2.00*
WEEK 5	16.9167±4.83*	7.7500±3.38	6.4167±0.29	3.6667±1.87
WEEK 6	17.4750±4.90*	6.7222±1.93	6.5000±0.25	3.9250±1.96*
WEEK 7	18.0667±4.91*	5.7500±1.54	6.5000±1.00	3.9000±2.14

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WEEK 8	18.9250±4.78*	6.2222±2.04	8.5000±2.41	4.0583±2.28
WEEK 9	18.9333±5.63*	4.4444±1.07	10.7500±1.64	3.6917±2.37
WEEK 10	21.0500±3.55*	0.9167±1.59*	15.5833±1.28*	2.5583±2.36
WEEK 11	24.1667±0.86*	00.00±00.00*	18.3333±2.13*	00.00±00.00*
WEEK 12	24.0417±3.83*	00.00±00.00*	21.7500±4.21*	00.00±00.00*

Values are mean ± standard deviations of triplicate determinations.
**P = .05* compared to the corresponding values on week 0.

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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