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Endophyte - Assisted Rhizoremediation of Petroleum By The Aquatic Macrophyte,*Commelina Benghalensis,*In The Wetlands of The Niger Delta Region, Nigeria

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Abstract: *Potted plant experiments in petroleum-spiked soils were carried out using the wetland plant, Commelina benghalensis of the Niger Delta Region, Nigeria. Petroleum degrading endophyte Providencia rettgeri, from an aquatic plant, Sacciolepsis africana, was inoculated in treatments using the root inoculation method. The treatments include sterilized and unsterilized soil, with or without the addition of nutrient (NPK). Results obtained showed a gradual decrease in plant growth and reduction in residual Total Petroleum Hydrocarbon with the endophyte-nutrient-unsterile soil treatment (CUSENPK) having the least with 48.77% of the oil degraded; and following in decreasing order of degradation; unsterile soil (CUS) 42.58%, endophyte-nutrient-sterile soil (CSSENPK) 41.97%, endophyte-sterile soil (CSSE) 38.28%, nutrient-unsterile soil (CUSNPK) 37.42%, sterile soil (CSS) 33.34% and lastly, endophyte-unsterile soil (CUSE) 30.75%. No significant difference (P>0.05) was however obtained in the treatments. Bacterial counts of the rhizosphere in the endophyte-sterile-soil treatments increased gradually before plant death while counts in the unsterile soil treatments fluctuated but no significant difference (P>0.05) occurred using the Duncan Multiple Range test statistic. The application of the endophyte in microbe-assisted phytoremediation strategies in wetland soils is recommended on further investigation.*

Keywords*: Commelina benghalensis,endophytes, petroleum,rhizoremediation, wetlands*

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I. Introduction

Crude oil leakages into the soil is estimated at 600,000 ± 200,000 metric tons per year [1].The Niger Delta Region of Nigeria, where oil and gas are produced, covers about 70,000 km^2 of wetlands and is estimated to be about 7.5% of Nigeria's land mass[2].Petroleum pollution of the region has become a recurrent phenomenon since oil was first discovered in 1956. Hundreds of spills occur yearly with millions of barrels lost to the environment. The number of oil spills that occur onshore are generally believed to be greater than the numbers recorded, notwithstanding, the figures are increasing as the years go by. The volumes of spills are also believed to be inaccurately recorded but in 2006 oil experts put figures at 9 to 13 million barrels lost over 50 years [3, 4]. The widespread contamination of petroleum in the environment is of great concern worldwide, especially in Africa and Asia [5].

Oil spills are usually damaging to the wetland ecosystem and any clean up exercise must be effective to minimize the ecological impact. The waterlogged nature of wetlands and marshy soil ecosystems makes it difficult to apply the conventional remediation techniques. Phytoremediation, the use of plants in conjunction with soil microorganisms, soil amendments and agronomic techniques to remove or render harmless, environmental contaminants [6], is a form of bioremediation that is non-invasive, cost effective, acceptable and environmentally friendly, and approximately 10 times cheaper than conventional remediation techniques [6,7, 8]. It is a green technology with much prospects in the remediation of both organic and inorganic environmental pollutants all over the world [9, 10].

Degradation of toxic organic compounds in soil by plant-associated bacteria can involve endophytic and rhizobacteria. Bacteria that inhabit the internal tissues of plants are called endophytes while the rhizosphere is the zone of soil in which microbes are influenced by the root. Rhizoremediation is defined as a specific form of phytoremediation involving plants and their associated rhizospheric microorganisms (bacteria and fungi) [11]. The association is beneficial for both plants and microorganisms where the microorganisms raise the bioavailability of compounds needed for plant growth and the plants help in the extraction and removal of such compound in extreme conditions [11].

Some endophytes associated with phytoremediation strategies of petroleum are: *Burkholderia cepacia* G4 in Yellow lupine (*Lupinus luteus* L.) - volatile organic compounds and toluene [12]; *B. cepacia* Bu61 (pTOM-Bu61) in poplar (*Populus)* – Toluene [13]. *Burkholderia*. p*hytofirmans*PsJN inoculation into Ryegrass improved plant biomass and diesel degradation [14].The phenanthrene degrading endophyte, *Pseudomonas putida* PD1 from poplar, inoculated into the plant symbionts (*Salixpurpurea* clone 94006 and *Salix discolor* clone S-365) reduced phytotoxicity of phenanthrene and improved removal of the pollutant from soils [15]. The mechanisms by which endophytic bacteria present in plants enhance pollutant degradation have been described [16].

Wetland plants; plants growing in water, soil or on a substrate that is periodically limited by oxygen due to high water content, could be important in oil bioremediation and wetland restoration [17].Very little information is found on the petroleum degradative abilities of endophytes of wetland plantsas few plant-microbe studies have been performed on them. One of such few studies has been reported [18] where endophytes of wetland plants degraded naphthalene. *Commelina benghalensis* is a common wetland plant of Bayelsa State of the Niger Delta Region, Nigeriaand found in petroleum-contaminated areas. No work has been reported on its endophyte-assisted-phytoremediation ability,therefore, the study was carried out to investigate the effect of endophyte inoculation and nutrient application on the degradation of crude oil by this plant in a simulated wetland environment.

II. Materials And Methods

2.1 Study Site and Sample Collection

Soil at a depth of 0-30cm was collected from a non-petroleum contaminated wetland soil at Otuoke, Latitude 4.8019^{o}N Longitude 6.3189^{o}EBayelsa State-Nigeria. Soil was collected in clean plastic containers and sorted to remove debris. Seedlings (7-15 cm shoots and roots) of the experimental plant, *Commelina benghalensis* were obtained from non-petroleum contaminated areas in Yenagoa, Latitude 4.9212^{o}N Longitude 6.2748^{o}E Bayelsa State-Nigeria.

2.2 Inoculation of Aquatic Plants with Endophytic Bacteria

One ml of overnight culture of endophytic bacteria (*Providencia rettgeri*) was grown in 200ml LB medium with slight agitation at 25^{o}C ± 2^{o}C for approximately 24 hours after which the total bacterial count of 10^{8} cfu/ml was obtained.Seedlings of *Commelina benghalensis*were rinsed five to six times in sterile water to remove any attached sediments, the roots were dipped for 10 seconds in 70% ethanol and further rinsed twice in sterile water. Plant roots (3-6cm) were then put into 200ml of overnight culture of endophyte for 12 hours in the inoculating hood. Colonization of plant was allowed in the dark at room temperature.

2.3 Experimental Design

Pot experiments to simulate natural freshwater wetland soils were set up. The experimental design consisted of a water level flooded with 1-2cm of standing water to study the response to the wetland condition, saturated and partially submerged [19]. Each pot was filled with 1kg of wetland soil to a depth of 10cm. The soil was kept saturated and then planted with four seedlings of the wetland plant and plant inoculated endobacteria.Prior to planting, 30ml of Bonny Light crude oil was applied to the soil using a 10ml syringe to give a concentration of 3% (w/v). This was thoroughly mixed using a stainless steel stirring rod .and 400ml sterile water added. Water was added subsequently when required so as to keep the constant water level.

2.3.1Measurement of Plant Parameters

Plant parameters such as plant height (cm) and leaf area per plant (cm^2) were measured using a tape rule/ruler at the start of the experiment and weekly for a period of one month. Plant height (cm) were measured from the base of the plant at water level to the tip; leaf area (cm^2) per plant were determined non-destructively using the formula: ∑(LB x correction factor), where ∑ or sigma = the summation of all the leaf areas per plant; L = leaf length; B = leaf breadth at its widest [20].

2.3.2Plant Treatments

The treatments employed for growth monitoring were:

i. Crude oil + aquatic plant + sterile soil only, CSS (Control 1)
ii. Crude oil + aquatic plant + sterile soil + endobacteria, CSSE
iii. Crude oil + aquatic plant + sterile soil + endobacteria + fertilizer, CSSENPK
iv. Crude oil + aquatic plant + unsterile soil only, CUS (Control 2)
v. Crude oil + aquatic plant + unsterile soil + fertilizer, CUSNPK
vi Crude oil + aquatic plant + unsterile soil + endobacteria, CUSE
vii. Crude oil + aquatic plant + unsterile soil + endobacteria + fertilizer, CUSENPK

Triplicate treatments were done, and the experiment carried out in batches in a half-open house without environmental control.Two grams (0.2%) NPK (nitrogen, phosphorous, potassium) nutrient application started one week after the oil application and continued weekly for a period of four weeks. 5g of rhizosphere sediments were taken out for Total Petroleum Hydrocarbon analysis, Total Heterotrophic Bacteria (THB) and Hydrocarbon Utilising Bacteria (HUB) counts from week zero to week four, giving a total of five sampling points. The plants wereprocessed for endophytic bacteria at the end of the experiment.

2.4Determination of THB and HUB of the Rhizosphere Sediments

Serial dilutions of one gram rhizosphere sediments was done in sterile normal saline (0.85% NaCl), after which the total heterotrophic bacterial (THB) count of the rhizosphere sediments were determined using the drop plate method of Miles and Mishra. Here, 10µl of 10^{-5} dilution was dropped onto the plates in triplicates and allowed to diffuse into the medium for 20-30 minutes. Plates were incubated at room temperature for 18-24 hours and colonies counted using a colony counter. To get the count in one ml, the average number of colonies was taken, multiplied by100 and the dilution factor.

Hydrocarbon utilizing bacteria (HUB) were enumerated by the vapour phase transfer method using modified minerals salts medium containing 0.42g/l $MgSO_4.7H_2O$; 0.83g/l KH_2PO_4; 5.0g/l NaCl; 0.29g/l KCl; 1.25g/l Na_2HPO_4; 0.42g/l NH_4NO_3 and 15.0 g/l agar and deionized water [21]; pH was adjusted to 7.0 and the medium sterilized at 121^oC, 15 psi for 15 min. 10µl of diluted (10^{-5}) soil samples was dropped onto sterile mineral salts agar plates (in triplicates) and allowed to diffuse into medium for 20-30 minutes. Filter paper (Whatman No. 1) saturated with Bonny light crude oil was then placed aseptically onto covers of petridishes containing the medium. The saturated filter paper supplied hydrocarbon to the inoculum by vapour phase transfer. Plates were inverted and incubated at 25^oC ± 2^oC for 5-7 days after which colonies were counted.

2.5Measurement of Total Petroleum Hydrocarbon (TPH) of Sediments

To extract the residual oil from soil, one gram of the sample was put into 50mls of Methylene chloride, followed by vigorous shaking for 10mins and filtered into a clean conical flask using Whatman no.1 filter paper [22].The TPH was then analyzed using gas chromatography equipped with single flame ionization detector (GC/FID).

2.6Sample Processing for Endophytic Bacteria

After the removal of rhizosphere soil, plant samples from each site were rinsed with sterilized water to wash out sediments. The roots, stems and leaves taken separately from different plants within each treatment, were cut into 1cm pieces, mixed and divided into 3 subsamples. Each subsample was washed in sterilized water for 5 minutes, surface-sterilized with a solution containing 5% active chloride (w/v, added as a NaOCl solution) for 3 min and 70% ethanol for 1 min and then rinsed 4 to 5 times in sterile deionized water. They were then further cut into 0.3 - 0.5 cm pieces separately with sterile blades and ground using a sterilized glass rod in a surface sterilized (cotton wool dipped in 70% ethanol) mortar with 0.1ml of sterile deionized water. Each plant slurry of the roots, stems and leaves was then spread onto Luria-Bertani (LB) agar plates in triplicate. 100µl of the last rinse of plant parts were plated for sterility check [18, 23 modified]. All plates were incubated at 25^oC ± 2^oC for 24 – 48 hours after which isolates were identified and stored at -70^oC in 20% glycerol.

2.7Identification and Characterization of Isolates

Colonial morphology, cultural characteristics and biochemical tests in the identification of isolates were carried out using standard procedures. Characterization of isolates were based on their morphology on gram staining, biochemical tests [24, 25, and 26]. Gram negatives were further identified using API 20 Enterobacteriaceae (Biomereux, France).

2.8 Statistical Analyses

Statistical calculations of mean values, standard deviations, bar charts and pictorial representations were determined using Microsoft excel (version 2013) and SPSS (version 20.0). Analysis of variance (ANOVA) and post-hoc analysis of sources of variations using least significant difference (LSD) and Duncan's multiple range (DMR) were determined using SPSS (version 20.0).

III. Results

3.1 Plant Growth in the Petroleum-SpikedSoil Treatments

The leaf area measured weekly after plant growth in crude oil in the sterile and unsterile soil revealed a general decline in all the treatments. No results were recorded for CSS after week 1, CSSE leaf area decreased up to week 4 while there were no recorded values for CSSENPK at weeks 3 and 4. In the unsterile soil treatments, CUS and CUSE leaf area decreased up to week 4 while no result was obtained for CUSNPK and CUSENPK at week 4 (Fig. 1).

The stem height also decreased in all the treatments. In the sterile soil treatments, no results were obtained in CSS at weeks 2-4, CSSE decreased up to week 4 while there were no results for CSSENPK at weeks 3 and 4. For the unsterile soil treatments, CUS increased at week 1, decreased at weeks 2 and 3 and further increased by week 4. CUSNPK decreased and none recorded at week 4. CUSE decreased at week 1, increased at week 2 and further decreased to week 4. CUSENPK decreased continuously with no leaf area recorded at week 4 (Fig. 2). There was no statistically significant difference (P>0.05) in the leaf areas and stem heights of the different treatments.

3.2 Comparison of THB and HUB of the Plant Treatments

In the sterile soil treatments, CSS the bacterial counts (THB and HUB) increased slightly but reduced after the second week. CSSE (endophyte treated), showed increase in bacterial counts with a decline in the 4th week of growth. The result for CSSENPK indicated a gradual increase in bacterial counts with a slight decrease at week 4. The THB and HUB at the start of the experiment was low (3±0.17 X 10^4 and < 10^4 respectively) for the sterile soil treatments.

For the unsterile soil treatments, the initial THB and HUB counts were 9.3 X 10^7 and 4.8±0.07 X 10^5 respectively. CUS bacterial counts increased at week 1 and week 2 after which it decreased gradually but an increase in HUB was noted at week 4. CUSNPK counts increased for the first two weeks before declining (TABLES 1 and 2).

In the endophyte treated soil CUSE, THB decreased slightly at the start of the experiment before increasing at week 2 and week 3, with a slight drop at week 4 (but remained high). The HUB counts in this treatment increased and remained high for up to week 3 before reducing. For the nutrient treated soil, CUSENPK the THB decreased in the first week, increased at week 2 then reduced slightly, but remained high. The HUB counts increased up to week 2 and then reduced afterwards but remained high also (TABLES 1 and 2).

Statistically, there was no significant difference (P>0.05) in the total heterotrophic bacterial count (THB) and in the hydrocarbon when comparing the effect of each treatment on the total viable counts.

3.3 Comparison of Residual TPH of the Plant treatments After Growth in Petroleum-Spiked Soils

The residual TPH values in all the treatments showed a general decline with increasing weeks of plant growth except for CSSENPK at week 1 where the TPH increased slightly due to accumulation of the oil on the top soil layer (Fig. 3). Results at the end of the experiment showed that the lowest residual TPH was obtained in CUSENPK where 48.77% of the oil was degraded and following in decreasing order of degradation; CUS 42.58%, CSSENPK 41.97%, CSSE 38.28%, CUSNPK 37.42%, CSS 33.34% and lastly, CUSE 30.75% (table not shown). There was however, no significant difference between the treatments in the degradation of the crude oil.

3.4 Endophytic Bacteria from *Commelina benghalensis* after Plant Growth Experiment

Endophytic bacteria re-isolated from the roots, stems and leaves after plant growth in the petroleum-spiked treatmentsare shown in TABLE 3. *Ps. aeruginosa* and other *Pseudomonas* spp., *Chryseobacterium indologenes, Enterobacter cloaceae, and Corynebacterium* sp, were isolated from the endophyte and non-endophytetreated plants while*Serratia mercescens* and *Serratia odorifera* were isolated from only the endophyte treated plants of the sterile soil. More endophytes were isolated from the leaves and stems than the roots of the plants.

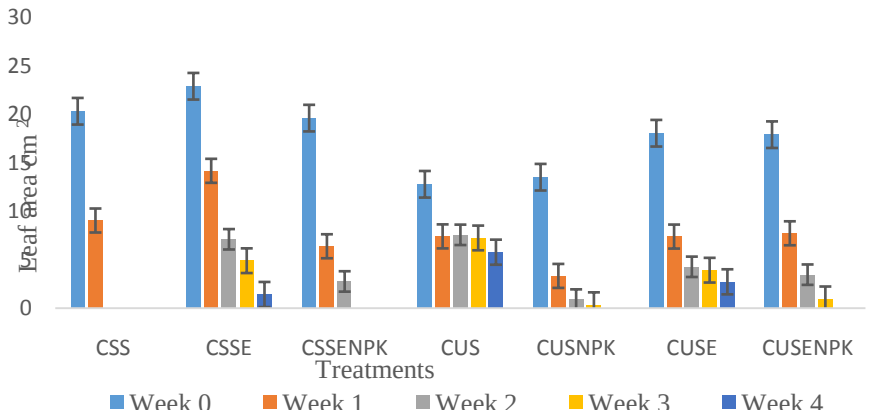

Figure 1: Plant growth (leaf area) in the petroleum-spiked treatments.

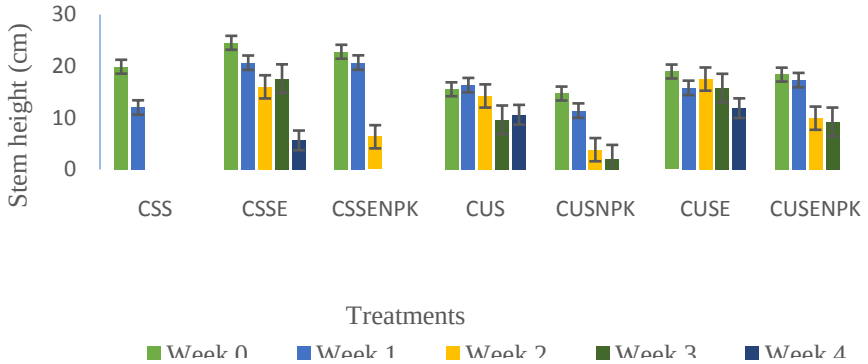

Figure .2: Plant growth (stem height) in the petroleum-spiked treatments.

Table 1: Changes in Total Heterotrophic Bacterial (THB) counts of Rhizosphere bacteria during growth of *Commelina benghalensis* in the petroleum-contaminated treatments

Treatments	Week 0	Week 1	Week 2	Week 3	Week 4
CSS	4.30±0.17a	6.10±0.00a	6.30±0.04a	6.04±0.04a	5.61±0.15a
CSSE	4.30±0.17a	5.94±0.17a	7.11±0.14a	7.57±0.12ab	7.42±0.23ab
CSSENPK	4.30±0.17a	5.92±0.54a	6.78±0.66a	7.68±0.47ab	7.23±0.14ab
CUS	7.93±0.46b	8.11±0.15b	8.06±0.31b	7.75±0.27ab	7.73±0.29c
CUSNPK	7.93±0.46b	8.14±0.02b	8.44±0.02b	7.85±0.46ab	7.61±0.45b
CUSE	7.93±0.46b	7.84±0.14b	8.23±0.07b	8.33±0.37c	8.02±0.33c
CUSENPK	7.93±0.46b	7.77±0.05b	8.38±0.17b	8.00±0.15c	7.77±0.29c

Data is expressed as mean ± standard error (n=3); the same alphabets along the column indicate no significant difference (P>0.05) according to Duncan multiple Range Test statistics

Key: CSS= crude oil in sterile soil; CSSE= crude oil plus bacteria in sterile soil; CSSENPK= crude oil, bacteria and fertilizer in sterile soil; CUS = crude oil and unsterile soil; CUSNPK= crude oil plus fertilizer; CUSE= crude oil plus bacteria in unsterile soil; CUSENPK= crude oil, bacteria and fertilizer in unsterile soil.

Table 2: Changes in Hydrocarbon Utilising Bacterial (HUB) counts of Rhizosphere bacteria during growth of *Commelina benghalensis* in the petroleum-contaminated treatments

Treatments	Week 0	Week 1	Week 2	Week 3	Week 4
CSS	4.00±0.00a	5.70±0.00a	6.16±0.04a	5.11±0.07a	4.24±0.18a
CSSE	4.00±0.00a	5.74±0.07a	7.06±0.15b	7.41±0.15bc	7.16±0.13bc
CSSENPK	4.00±0.00a	5.76±0.14a	6.08±0.17a	6.67±0.38b	6.41±0.38b
CUS	5.48±0.07b	7.64±0.03b	8.04±0.31c	7.32±0.12bc	7.70±0.26c
CUSNPK	5.48±0.07b	7.53±0.05b	8.22±0.14c	7.14±0.58bc	6.96±0.52bc
CUSE	5.48±0.07b	7.78±0.11b	8.07±0.09c	7.93±0.43c	7.54±0.31c
CUSENPK	5.48±0.07b	7.66±0.11b	8.32±0.20c	7.19±0.09bc	6.89±0.18c

Data is expressed as mean ± standard error (n=3); the same alphabets along the column indicate no significant difference (P>0.05) according to Duncan multiple Range Test statistics

Key: CSS= crude oil in sterile soil; CSSE= crude oil plus bacteria in sterile soil; CSSENPK= crude oil, bacteria and fertilizer in sterile soil; CUS = crude oil and unsterile soil; CUSNPK= crude oil plus fertilizer; CUSE= crude oil plus bacteria in unsterile soil; CUSENPK= crude oil, bacteria and fertilizer in unsterile soil.

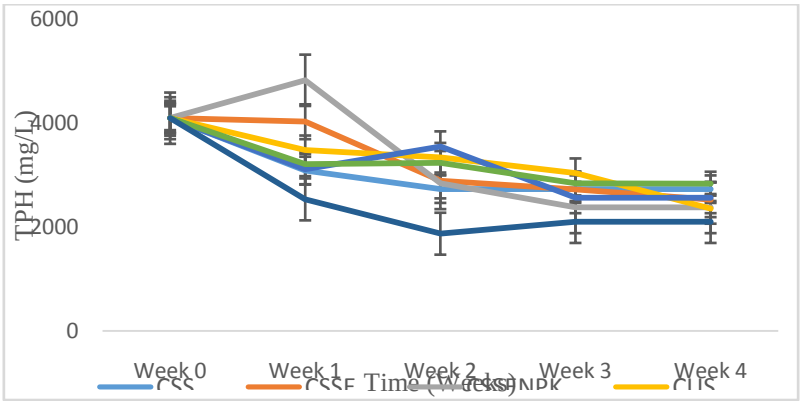

Figure 3: Petroleum degradation by *Commelina benghalensis* in the treatments.

Table 3: Culturable Endophytes after Plant Reprocessing

Treatment	Endophyte	Root	Stem	Leaf
CSS	*Aeromonas* spp.	-	+	+
	Enterobacter cloacae	-	+	+
	Chryseobacterium indologenes	+	-	-
CSSE	*Serratia mercescens*	-	+	+
	Serratia odorifera	-	+	+
	Pseudomonas sp.	+	-	-
	Chryseobacterium indologenes	+	+	+
CSSENPK	*Serratia mercescens*	-	+	+
	Chryseobacterium indologenes	+	+	+
	Pseudomonas sp.	+	-	-
CUS	*Pseudomonas* spp.	-	+	+
	Chryseobacterium indologenes	+	+	+
CUSNPK	*Pseudomonas* spp.	-	+	+
	Chryseobacterium indologenes	+	+	+
CUSE	*Enterobacter cloacae*	-	+	+
	Pseudomonas aeruginosa	+	-	-
	Chryseobacterium indologenes	-	+	+
	Corynebacterium	-	+	+
CUSENPK	*Chryseobacterium indologenes*	+	+	+
	Pseudomonas sp.	-	-	+
Total		9	15	16

Key: CSS= crude oil in sterile soil; CSSE= crude oil plus bacteria in sterile soil; CSSENPK= crude oil, bacteria and fertilizer in sterile soil; CUS = crude oil and unsterile soil; CUSNPK= crude oil plus fertilizer; CUSE= crude oil plus bacteria in unsterile soil; CUSENPK= crude oil, bacteria and fertilizer in unsterile soil.

IV. Discussion

Petroleum bioremediation of wetland soils are usually complicated due to insufficient oxygen supply needed for this activity, and the marshy and impenetrable nature of the ecosystem makes it difficult to apply conventional techniques of remediation. The most preferred method of wetland clean up would be bioremediation, particularly phytoremediation, using wetland plants so as to maintain the rich and valuable ecosystem. The results showed that there was a decline in plant growth in the sterile soil (CSS) treatment with the seedlings not surviving after the 2nd week but degraded the oil as the TPH was lower. The THB and HUB counts increased slightly before plant death which could have resulted from the toxicity of the hydrocarbon to the young seedlings and/or the inability of the naturally occurring oil microbes to establish themselves in the plant root due to their low numbers.

The endophyte treated sterile soil (CSSE) plants survived the entire experimental period with decrease in plant growth and TPH. The THB increased after week one due to the presence of the endophyte. Both THB and HUB increased in the course of the experiment. The drop in bacterial counts at week 4 signified the possible death of plant after this period. CSSE appeared to be better than CSS probably because of the presence of the endophyte which increased the microbial population and may have aided in the establishment of the interaction by attaching to the plant root. The colonizing capacity of the endophyte would have been increased, as there seemed to have been little competition with the indigenous soil bacteria (from the crude oil) because of their low number [27]. The endophyte colonization patterns and their activities which is in turn determined by the host plant influence the phytoremediation process [28]. The endophytic bacteria may also have possessed plant growth-promoting abilities and petroleum degradative enzymes which could have enhanced the plant's adaptation and growth in the petroleum contaminated soil [16].

The endophyte-nutrient treated sterile soil (CSSENPK) plants declined in growth with oil degradation and by the 3rd week, the plants were dead. The THB and HUB counts also increased throughout the experiment. The total population density of endophytes has been reported to increase with the application of organic fertilizers (29). Again, high concentrations of fertilizer could have negative effects over endophytic populations due to changes in the plant's physiology [30] and on the colonization process [27]. The bacterial counts were observed to decline with nutrient application as compared with the treatment where none was used (CSSE). The weekly addition of fertilizer (NPK) may have caused plant toxicity due to increased concentrations of toxic compounds and ammonia build-up leading to early death [31, 32].

Plant growth of the petroleum treated unsterile soil (CUS) generally decreased gradually while degradation was going on, although periods of increased plant height were noticed after the 1st and 4th weeks. The bacterial counts also fluctuated during the course of the experiment, then increased at the 4th week with increase in plant height. This indicated that the untreated plants survived the oil concentration and continued growing and degrading the oil. Growth continued for up to two months after the oil pollution (data not shown). For the endophyte treated soil (CUSE), the plants continued to survive and degrade the oil with increasing bacterial counts until the 4th week when the counts declined signifying gradual death of plant. The plants also

survived a further two weeks (data not shown) and may have been unable to withstand competition with indigenous microorganisms before dying. Natural selection of competitive microorganisms capable of surviving root exudates occur in the rhizosphere [27]. The endophytic bacteria may also have had growth-promoting abilities that enhanced the plant's adaptation and survival in the oil polluted soil [16].

The THB for all the treatments of the unsterile soil decreased after the first week of growth while the HUB counts increased signifying possible switch to hydrocarbon utilization. The nutrient treated endophyte (CUSENPK) and non endophyte (CUSNPK) soils survived and degraded the oil for three weeks after which the plants died. Bacterial counts (HUB) also increased at the first two weeks and decreased before the plants died. CUSENPK recorded the highest bacterial counts in all the treatments which was as a result of the presence of the endophyte and the initial nutrient addition to boost growth. This treatment also recorded the lowest TPH at week 3 before the plants died. Death could have occurred early due to the frequent application of fertilizer in the two treatments (CUSENPK and CUSNPK). Again, it implies that nutrient addition could be desirable but its frequency of application should be with caution [31, 33] as this may lead to the accumulation of toxic byproducts of metabolism from the plant-microbe partnership leading to their death. The increase followed by gradual decline or fluctuations in bacterial populations with period of plant growth in all the treatments (though higher with nutrient addition) of this study is in agreement with documented reports of biodegradation experiments in wetland systems [32, 34, 35]. Petroleum degradation patterns were also comparable to the results of low level water oil treated wetland soil experiments [19]. The residual TPH at the end of the experiment indicated that CUSENPK was the best treatment employed.

Reprocessing of plant inner parts for the presence of the inoculated endophyte was not successful as *Pseudomonas* sp, *Pseudomonas aeruginosa*,*Enterobacter cloacae*, *Chryseobacterium indologenes*, *Aeromonas* sp,*Serratia mercescens*, *Serratia odorifera* and *Corynebacterium* were the species isolated. Interestingly, *Serratia* species were isolated from the endophyte and nutrient treated sterile soils only.*Providencia rettgeri* could not be isolated inside the plant probably because more time would have been required for it to penetrate into the plant or it was unable to establish itself on the plant roots (due to competition from other soil microorganisms) to enable penetration, but remained as a rhizosphere organism. The presence of *Serratia* spp. as endophytes of only the sterile soil treatments could mean that the inoculated endophyte may have altered the microbial community of the plant. The specificity of endophytes to plants may probably be another reason why the inoculated endophyte was not established in *Commelina benghalensis* since it was not isolated from any of the treatments.

The inoculation method was probably inadequate to ensure maximum colonization of plant roots and another inoculation method may likely be more successful. *Providencia rettgeri* may not be a natural inhabitant of *Commelina benghalensis.* However, it is possible that inoculation with the endophyte may have altered the rhizosphere community composition as this phenomenon occurred where inoculation with the endophyte *P. putida* W619-TCE altered community composition of poplar plants and of parts of the plant where it was not established (36). Though the ages of the seedlings were not determined in this study, the establishment of petroleum degrading bacteria differs with age of plant as older plants adapt better than younger plants [37].

Commelina benghalensis has an extensive root system. The plant is a perennial that grows very fast with creeping or ascending stems, and continuous rooting from nodes meaning it can spread a wide area of the contaminated site when established, therefore, soil inoculation of endophyte would likely have been more appropriate for endophyte colonization. One of the conditions for effective rhizoremediation of petroleum polluted environments is that the plant must be tolerant enough to the contaminant so as to stimulate microbial degradation at the rhizosphere. The plant, though aquatic, is also able to withstand high periods of drought when established [38]. These characteristics of the plant makes it suitable for rhizoremediation.

An oil pollution level of 50mg per gram of soil used in the present study was tolerant to the growth of *Commelina benghalensis*. While successive plantings at four week intervals were done to achieve substantial amounts of petroleum reduction in a previous study(20), the present study was able to achieve some degree of petroleum degradation within four weeks, with the addition of the endophyte. It is also possible that the average leaf areas and stem lengths of the plants would improve and even yield better results with successive plantings.

V. Conclusion

Rhizoremediation, a process occurring naturally in plants, can be improved by deliberately altering the conditions of the rhizosphere for optimum plant growth and functional activity e.g. in the degradation of organic pollutants. Such alterations could involve the introduction of plant growth promoting rhizobacteria (PGPR), or contaminant-degrading microorganisms. The present study is the first attempt at endophyte inoculation of the wetland plant, *Commelina benghalensis* for the rhizoremediation of petroleum in the wetland soil of Bayelsa-Nigeria with promising results. The results obtained showed that petroleum degradation was enhanced by augmenting the plant with endobacteria, but the cautious application of chemical nutrient would be required to avoid possible toxicity to plants. Further studies on the bacterial assisted rhizoremediation abilities of the plant

association, using molecular and other related approaches, is required to improve on its efficacy and to understand the mechanisms governing this association with subsequent application as enhanced in-situ bioremediation in wetland soils.

References

[1]. N. Das and P. Chandran. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International*, 2011, 1 – 13.

[2]. K. C. N. Onyechi, C. Eseadi, F. O. Ugwuozor, J. C.Omeje, D. U. Ngwoke. Probable Psychological Impacts of Environmental Pollution (Oil Spills) in the Coastal Areas of the Niger Delta of Nigeria: a Philosophical Discourse. *American-Eurasian Journal of Agriculture and Environmental Science, 16 (2),* 2016,374 – 379.

[3]. Amnesty International. *Nigeria: Petroleum pollution and poverty in the Niger Delta* (Amnesty International Publications London, 2009).

[4]. Amnesty International. *Bad Information: Oil Spill Investigations in the Niger Delta* (Amnesty International Publications London, 2013).

[5]. P. Agamuthu, O. P. Abioye and A, Abdul Aziz. Phytoremediation of soil contaminated with used lubricating oil using *Jatropha curcas. Journal of Hazardous Materials, 179,* 2010,891 – 894.

[6]. P. M. White, C. W. Duane, G. J., Thoma and C. M. Reynolds. Phytoremediation of alkylated polycyclicaromatic hydrocarbons in a crude oil-contaminated soil. *Water, Air and Soil Pollution, 169,* 2005,207 – 220.

[7]. S. L. Doty. Review*:* Enhancing phytoremediation through the use of transgenics and endophytes. *New Phytologist, 179,* 2008,318 – 333.

[8]. M. McGuinness and D. Dowling. Plant-Associated Bacterial Degradation of Toxic Organic Compounds in Soil. *International Journal of Environmental Research and Public Health, 6,* 2009,2226 – 2247.

[9]. M. I. Lone, L. He, P. J. Stoffella and X. Yang. Phytoremediation of heavy metal polluted soils and water: Progresses and perspectives. *Journal of Zhejiang University Science, 9(3),* 2008, 210 – 220.

[10]. A. D. Zand, G. N. Bidhendi and N. Mehrdad. Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species inIran.*Turkish Journal of Agriculture and Forestry, 34,* 2010, *429 – 438.*

[11]. B. Divya and M. D. Kumar. Review: Plant - Microbe Interaction with Enhanced Bioremediation. *Research Journal of Biotechnology, 6 (4),* 2011,72 – 79.

[12]. T.Barac, S. Taghavi, B. Borremans, A. Provoost, L. Oeyen, J. V. Colpaert, J. Vangronsveld and D. van der Lelie. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. *Nature Biotechnology, 22(5),* 2004, 583 – 588.

[13]. S. Taghavi, T. Barac, B. Greenberg, B. Borremans, J. Vangronsveld and D. van der Lelie. Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Applied and Environmental Microbiology, 71,* 2005, 8500 - 8505.

[14]. M. Afzal, S. Khan, S. Iqbal, M. S. Mirza and Q. M. Khan. Inoculation method affects colonization and activity of *Burkholderia phytofirmans* PsJN during phytoremediation of diesel-contaminated soil. *International Biodeterioration and Biodegradation, 85,* 2013,331-336.

[15]. Z. Khan, D. Roman, T. Kintz, M. delas Alas, R. Yap and S. Doty. Degradation, Phytoprotection and Phytoremediation of Phenanthrene by Endophyte *Pseudomonas putida*, PD1. *Environmental Science and Technology, 48,* 2014, 12221−12228.

[16]. Afzal, M., Khan, Q. M., Sessitsch, A.). Endophytic bacteria: Prospects and applications for the phytoremediation of organic pollutants. *Chemosphere 117,* 2014, *232–242.*

[17]. X. Zhu, A. D. Venosa, M. T. Suidan and K. Lee. *Guidelines for the bioremediation of marine shorelines and freshwater wetlands* (U.S. Environmental Protection Agency, Cincinnati, 2001).

[18]. W-M. Chen, Y-Q. Tang, K. M. Mori and X-L. Wu. Distribution of culturable endophytic bacteria in aquatic plants and their potential for bioremediation in polluted waters. *Aquatic Biology, 15,* 2012, 99 – 110.

[19]. J. A. Purandare, T. Huang, M. T. Suidan, , B. Johnston, A. D. Venosa and P. Pier. Microcosm study of bioremediation of oil-contaminated freshwater wetlands, *Proc. 1999 International Oil Spill Conf*. American Petroleum Institute, Washington DC. 1999, 489 – 495.

[20]. L. A. Daniel-Kalio and S. F. Pepple. Effect of Bonny Light Crude Oil Pollution of Soil on the Growth of dayflower (*Commelina benghalensis L.*) in the Niger Delta., Nigeria. *Journal of Applied Science and Environmental Management, 10(2),* 2006,111 – 114.

[21]. S. C. Eziuzor and G. C. Okpokwasili. Bioremediation of Hydrocarbon Contaminated Mangrove Soil in a Bioreactor. *Nigerian Journal of Microbiology, 23(1),* 2009, 1777 – 1791.

[22]. A. D. Eaton, L. S. Clesceri and A. E. Greenberg. *Standard methods for the examination of water and waste water* (The American Public Health Association, APHA, Washington, 1995).

[23]. S. J. Bhore, R. Nithya and C. Y. Loh. Screening of endophytic bacteria isolated from leaves of Sambung Nyawa [*Gynura procumbens* (Lour.) Merr.] for cytokinin-like compounds. *Bioinformatics, 5(5),* 2010,191 – 197.

[24]. S. L. Abbott, W. K. W. Cheung and J. M. Janda. The Genus *Aeromonas*: Biochemical Characteristics, Atypical Reactions, and Phenotypic Identification Schemes. *Journal of Clinical Microbiology, 41 (6),* 2003,2348 – 2357.

[25]. J. Manos and R. Belas. The Genera *Proteus, Providencia*, and *Morganella.Prokaryotes 6*, 2006, 245 – 269.

[26]. Public Health England. Identification of *Pseudomonas* species and other NonGlucose Fermenters. *UK Standards for Microbiology Investigations. 17 (3),* 2015, 1-41.

[27]. M. Rosenblueth and E. Martinez-Romero. Bacterial endophytes and their interactions with hosts. *Molecular Plant Microbe Interactions, 19,* 2006,827 – 837.

[28]. K. Fatima, A. Imran, I. Amin, Q. M. Khan and M. Afzal. Plant species affect colonization patterns and metabolic activity of associated endophytes during phytoremediation of crude oil-contaminated soil. *Environmental Science and Pollution Research International*, 23(7), 2016, 6188-96.

[29]. Z.,Stępniewska and A. Kuźniar. Endophytic microorganisms—promising applications in bioremediation of greenhouse gases. *Applied Microbiology and Biotechnology, 97,* 2013,9589 – 9596.

[30]. A. Quadt-Hallmann, N. Benhamou and J. W. Kloepper. Bacterial endophytes in cotton: mechanisms of entering the plant. *Canadian Journal of Microbiology, 43,* 1997,577 – 582.

[31]. D. C. Mueller, J. S. Bonner, R. L. Autenreth, K. Lee and K. Doe. The toxicity of oil-contaminated sediments during bioremediation of a wetland. *Proc. 1999 International Oil Spill Conf.* American Petroleum Institute, Washington DC, 1999, 1049 - 1052.

[32]. M. A. Mills, J. S. Bonner, C. A. Page and R. L. Autenrieth. Evaluation of bioremediation strategies of a controlled oil release in a wetland. *Marine Pollution Bulletin, 49,* 2004,425 – 435.
[33]. D. Mueller, J. Bonner, S. McDonald, R. Autenrieth, K. Donnelly, K. Lee, K. Doe and J. Anderson. The Use of toxicity bioassays to monitor the recovery of oiled wetland sediments. *Environmental Toxicology and Chemistry 22 (9),* 2003,1945–1955.
[34]. R. T. Townsend, J. S. Bonner and R. I. Autenrieth. The effect of bioremediation on microbial populations in an oil-contaminated coastal wetland. *Proc. 1999 International Oil Spill Conf.* American Petroleum Institute, Washington DC, 1999, 477 – 483.
[35]. R. T. Townsend, J. S. Bonner and R. L. Autenrieth. Microbial dynamics during bioremediation of a crude oil-contaminated coastal wetland. *Bioremediation Journal, 4 (3),* 2000, 203
[36]. N. Weyens, D. Van Der Lelie, T. Artois, T. Smeets, S. Taghavi, L. C. Newman and J. Vangronsveld. Bioaugmentation with Engineered Endophytic Bacteria Improves Contaminant Fate in Phytoremediation. *Environmental Science and Technology, 43,* 2009,9413 – 9418.
[37]. M., Afzal, S. Yousaf, T. G. Reichenauer, M. Kuffner and A. Sessitsch. Soil type affects plant colonization, activity and catabolic gene expression of inoculated bacterial strains during phytoremediation of diesel. *Journal of Hazardous Materials, 186,* 2011,1568 - 1575
[38]. D.Emmanuel, E. Udoh and P. Akansi. Phytoremediation of xylene polluted environment, using a macrophyte *Commelina benghalensis* L. *Asian Journal of Plant Science and Research, 4(3),* 2014,1 – 4.

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