Bioremediation of Crude Oil Contaminated Niger Delta Rainforest Soil: A Focus on *Saccharum officinarum* Rhizoremediation

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Abstract: Quite a minute number of plants have been investigated for rhizoremediation of crude oil contaminated rainforest soils in the Niger Delta in relation to the overwhelmingly large number of plants. *S. officinarum*, one of the world's most propagated grasses was investigated by contaminating soils with oil at 3480 and 7050 mg/kg respectively and subjecting it to the following treatments: soil + oil [SO], soil + oil + fertilizer (NPK)[SOF], soil + oil + fertilizer + hydrocarbon utilizing bacteria and fungi [SOFM], Soil +oil + fertilizer + hydrocarbon utilizing bacteria and fungi + solarisation [SOFMS] (in triplicates). All contaminated soils were planted with *S. officinarum* (P) and monitored for 120 days to determine: population dynamics of culturable aerobic-mesophilic heterotrophic and hydrocarbon utilizing bacteria and fungi, and residual soil total petroleum hydrocarbon (TPH). Results indicated that while bacterial and fungal populations increased, residual TPH decreased with time in the rhizosphere of the plant at both concentrations. Degradation efficiency for the applied treatments was in order: PSOFM > PSOFMS > PSOF > PSO. Although, the highest attainable rates of degradation for PSOFM were 75.6 and 71.2 % within the study period, the cumulative TPH loss from soil were 2630 and 5020 mg/kg from the initial contamination levels of 3480 and 7050 mg/kg respectively. The occurrence of substantial remediation in the rhizosphere of *S. officinarum* indicates the plant holds enormous promise in the remediation of crude oil contaminated rainforest soils in the Niger Delta.

Keywords: Contamination; Crude oil; Rainforest soil; Rhizoremediation; Treatment; *Saccharum officinarum*.

INTRODUCTION

The world's major rainforest lies within the earth equatorial belt, located in South and Central America, South East Asia, Africa, New Pupa Guinea, Madagascar and in different tropical islands (Girton *et al.*, 1991). The world's rainforest occupies a total area of about 9.0 million sq/km (Hofsvan, 2014), though this size is relatively small in comparison to earth total surface area, the tropical rainforest biodiversity is amazingly great. Among the world 1.4 million plant and animal species that have been identified, over 50 % inhabit these ecosystems (Hofsvan, 2014). The importance of the rainforest is enormous. Its resources and ecosystem services are necessary for long term global energy and food security, health and environmental safety (Hofsvan, 2014).

The Niger Delta which consists of three main ecological zones: mangrove, freshwater forest, and low land rainforest (Izah, 2018), is classified as a tropical rainforest (Kadafa, 2012). This region which is renowned for its enormous oil deposit, is under extensive oil exploration activities that result in crude oil pollution of the environment. Approximately 13.0 million barrels of crude oil have been released into this environment in the past 50 years, equivalenting of 50 Exxon Valdez spill incidences (FME, 2006). The effects of oil spill on the environment are well documented. These includes biomagnification of toxic components of crude oil, soil flora and fauna destruction, surface and groundwater pollution, and degradation of arable land to barren soils (Obire and Anyanwu, 2009). The loss of tropical rainforest could result in remarkable widespread damaging effects on the world due to its biological diverse nature (Thompson and Thompson, 2009).

Soil, surface and groundwater contamination is a major global problem (Kuiper *et al.*, 2004).

The well-established engineering and physicochemical-based cleanup techniques such as excavation, chemical oxidation, thermal desorption, soil vapour extraction, soil washing, asphalt batching, incineration, hydrolysis and photolysis (Khan *et al*., 2004; Zhou *et al*., 2005; Do *et al*., 2009) are usually inadequate (Dixon, 1996; Gkorzis *et al*., 2016). These techniques are not only complex and costly; they are not ecofriendly thus, lacking public approbation (Niti *et al*., 2013; Gkorzis *et al*., 2016). Bioremediation technique which relies on the use of plants and microorganisms have become a more acceptable and environmentally friendly alternative. Although a substantial number of bioremediation studies on crude oil contaminated soils have been reported, using biostimulation and bioaugumentation techniques (Márquez-rocha *et al*., 2001; Tanee and Kinako, 2008; Chikere *et al*., 2009, Chorom *et al*., 2010; Syafruddin *et al*., 2010; Chang *et al*., 2011; Ibiene *et al*., 2011; Acuna *et al*., 2012; Roy *et al*., 2012; Turner *et al*., 2014; Ataikiru *et al*., 2018); rhizoremediation, the most evolved bioremediation options (Shukla *et al*., 2010), which involves the removal of pollutants from the contaminated site through mutual interactions of plant root and associated microorganisms (Gerhardta *et al*., 2009; Shukla *et al*., 2010), has become a generally acceptable practice over biostimulation and bioaugmentation in recent time in the field treatment of petroleum contaminated soils (Gerhardta *et al*., 2009). There is ample evidence that suggests that this option is better than the single application of either microorganisms or plants (Gurska *et al*., 2009; Xin *et al*., 2008; Escalante-Espinosa *et al*., 2005), even though the fact that plant and microorganisms can degrade petroleum pollutant independently (Frick *et al*., 1999). However, plants are devoid of the necessary catabolic enzymes required to accomplish complete mineralization of organic compounds in contrast to microorganisms (Eapen *et al*., 2007).

Though, a considerable number of rhizoremediation studies exist for crude oil contaminated soil (Jussila, 2006; Gaskin, 2008; Soleimani *et al*., 2010; Tang *et al*., 2010; Ogbulie *et al*., 2011; Zand *et al*., 2011; Moreira *et al*., 2011; Kuo *et al*., 2014; Omotayo *et al*., 2014; Gouda *et al*., 2016), not much is known about tropical plant species that can be used for the remediation of oil polluted sites (Ogbo *et al*., 2009). With the overwhelming number of plants, a comparatively small number have been studied (Pivetz, 2001). For a plant to be selected for rhizoremediation purpose it must possess some desirable characteristics such as rapid growth, competitiveness, hardiness, ramified and long root system, a significant level of tolerance to pollutant(s) (Pilon-Smits, 2005), suitability for a wide range of soil type and endemic/native species, and tolerance to environmental conditions (Cook and Hesterberg, 2013). Perennial grasses and trees are generally engaged in rhizoremediation, the extremely large number of grasses in the literature indicates its wide range of success (Cook and Hesterberg, 2013). Vigorous growth upon establishment and branched root system which facilitates microbial adhesion, spread and activities justify the reasons for preferential selection of grasses over trees (Olson *et al*., 2007; Muratova *et al*., 2008). Furthermore, the root parts of grasses have antioxidant molecules which provide them with the extra ability to resist stress induced by pollutants (Olalekan, 2014).

The perennial grass, *S. officinarum* seems to show good promise for remediation of crude oil polluted soil in the rainforest. The reported survival and tolerance of the plant to some levels of crude oil in soil (Ubogu, 2017; Ubogu *et al*., 2018), lend credence to this assertion. However, there is a paucity of information in this regard. Although, *S. officinarum* needs fertile, well-drained soil for suitable growth; it is capable of growing in almost every classes of soil (Agriculture, Forestry and Fisheries, 2014).

The plant which is found growing mainly in Central, North and South America, Africa, Europe, Asia, Oceania and the Caribbean (FAOSTAT, 2008); is the most propagated crop in the world, cultivated in 120 countries (Agriculture, Forestry and Fisheries, 2014).

One major setback to rhizoremediation is the confinement of the process to root depth; quite a large number of plants have a shallow root system (Pivetz, 2001). Furthermore, Oxygen diffusion necessary for considerable biodegradation in soil exist within 30.0 cm of soil layer (Vidali, 2001), crude oil pollution also increases the carbon/nitrogen ratio in soil (Venosa and Zhu, 2003; Zhu *et al*., 2001; Leahy and Colwell, 1990). The combination of these factors can effectively impede the efficiency of the rhizoremediation process. However, the plant *S. officinarum* possesses some desirable qualities that may help ameliorate some of these impediments when used for rhizoremediation. First, the plant has a well-developed root system; root growth into soil depth can be up to 4.0 to 6.0 meters (Chopart *et al*., 2010). Second, the presence of specialize root vessels (aerenchyma) (Gilber et al., 2007; Munoz *et al*., 2013) in the plant can also accelerate the release of oxygen in the rhizosphere at much depth (Muratova, 2003; Zalesny, 2005). Third, nitrogen required for bioremediation may also be supplied by rhizosphere and stem colonizing nitrogen-fixing endophytic bacteria (Boddey *et al*., 1991; Dong *et al*., 1994; Hartemink, 2008).

It is in the bid of broadening the search for suitable plants for rhizoremediation of crude oil contaminated rainforest soils in the Niger Delta that *S. officanarum* with some known desirable characteristics was investigated.

MATERIALS AND METHODS

Evaluation of baseline physicochemical properties of rainforest soil

Rainforest soil baseline physicochemical properties were analyzed prior to crude oil contamination and *S. officinarum* propagation. Soil samples were obtained in triplicates within 0-15 cm depth and assessed for physicochemical properties. Soil textural components were determined by the hydrometer method as outlined by Aliyu and Oyeyiola (2011). While soil pH was determined using the method of Hendershot *et al*. (2006), nitrogen by the method of micro-Kjeldahl (van Reeuwijk, 2002), phosphorus by method of Bray and Kurtz No.1 (van Reeuwijk, 2002), total organic carbon (TOC) by the method of Skjemstand and Baldock (2006). Porosity and total petroleum hydrocarbon (TPH) contents were also determined using the methods of Ezzati et al. (2012), and US EPA - Method 8015C (2007) respectively.

Soil contamination and application of treatments

Soil from the rainforest (with no history of previous oil contamination) was obtained with the aid of a spade (within 0-15 cm soil vertical profile) and subsequently placed in plastic pots. Before plant propagation, soil was first subjected to the following treatments: soil + oil (SO) (control); soil + oil + fertilizer (SOF); soil + oil + fertilizer + hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) (SOFM); and soil + Oil + fertilizer + HUB and HUF + solarization (SOFMS) in two sets (A and B). All treatments in each set were prepared in triplicates.

Each treatment contained 4000 g of soil in a plastic pot (30.0 cm width and 15.0 cm depth). Crude oil (0.818 g/cm^3 specific gravity) at the amount of 120.0 g was added to each pot in set A, and 240.0 g to each pot in set B to obtain a concentration of 34800.0 and 7050 mg/kg of oil in the soil for set A and B respectively. Thereafter, stem cuttings of *S. officinarum* (P) were planted in each of the pots in both sets. Thus, the treatments: PSO, PSOF, PSOFM and PSOFMS.

Soil solarization

Soil solarization was done in mid-January (time of intense sunlight) adapting the method of Elmore *et al*. (1997). Moistened crude oil contaminated soils in plastic pots were covered by transparent polyethylene sheets (0.025 mm thickness).

Polyethene sheets were allowed to make direct contact with the soil surface. To avoid heat escape, sheets were sealed to pots completely. Thereafter, pots were allowed to stand in the full glare of sunlight for two weeks. Soil temperature within this period of heating was assessed by inserting a thermometer into the soil at 10 cm depth. To avoid heat escape, the thermometer was sealed to polyethene sheet at the surface. The average soil temperature within the solarization period was 42.5 ± 3.2 ºC while unsolarized temperature was 30.5 ± 1.5 ºC.

Application of fertilizer

Six and 12.0 g of inorganic fertilizer (NPK-15:15:15) were respectively added to crude oil contaminated soils in set A (34800.0 mg/kg) and set B (7050 mg/kg), to attain the recommended 1.0 - 5.0 % nitrogen by weight of crude oil in the soil for effective nutrient enrichment amendment (Head and Swannell, 1999). Fertilizer was then carefully ploughed into the soil using a sterile hand trowel.

Presumptive Identification of culturable aerobic-mesophilic hydrocarbon utilizing bacteria (HUB) and fungi (HUF)

Culturable HUB and HUF used in this study were presumptively identified. HUB characterization was based on morphological, cultural and biochemical properties using Bergey's Manual of Systematic Bacteriology (Garrity et al., 2005; Cheesebrough, 2006; Vos *et al*., 2009 and Whiteman *et al*., 2012). On the other hand, HUF were characterized based on cultural and morphological properties using Barnett and Hunter, 1998; Watanabe, 2002; Humber, 2005; Ellis *et al*., 2007, identification schemes.

Confirmation of hydrocarbon utilizing ability of bacterial and fungal isolates and scaling-up

The following cuturable aerobic-mesophilic HUB: *Actinobacillus* sp*., Bacillus* sp*., Corynebacterium* sp., *Micrococcus* sp *Nocardia* sp., and HUF: *Aspergillus* sp^1., *Aspergillus* sp^2., *Paecilomyces* sp., *Penicillium* sp^1., *Penicillium* sp^2., *Penicillium* sp^3., *Trichoderma* sp. *Verricillium* sp., isolated from the rhizosphere of *S. officinarum* (from a previous crude oil contaminated soil) were confirmed for hydrocarbon utilizing ability through the Vapour Phase Transfere (VPT) technique (Chaudhry *et al*., 2014). Scale-up of pure culture of confirmed HUB and HUF was carried as outlined by Ubogu *et al*. (2018) before soil inoculation.

Soil inoculation

Each plastic pots containing crude oil contaminated rainforest soil was inoculated with 300 ml of physiological saline containing a mixture of HUB and HUF at the rate of 3.0 x 10^{10} and 4.5 x 10^8 cfu/mL respectively, following scale-up operation adopting the procedure of Ubogu *et al*. (2019). The inoculants mixture was thoroughly ploughed into soil using a sterile hand trowel.

Plant propagation in contaminated soil

S. officinarum stem cuttings were planted in all contaminated soils in pots. One stem cutting of 22.0 cm length and 3.5 ± 0.1 cm diameter was planted per pot. Plants were propagated in greenhouse and monitored for 120 days with regular watering (using distilled sterile water).

Treatment and oil concentration effects on bacterial and fungal populations in the rhizosphere

Soil dilution plate method was used for quantitative estimation of culturable aerobic-mesophilic heterotrophic bacteria and fungi, as well as percentage populations of HUB and HUF in the contaminated rhizosphere of *S. officinarum* at 3480.0 and 7050.0 mg/kg oil concentrations in soil. Culturable aerobic-mesophilic heterotrophic bacteria and fungi were estimated on nutrient agar (NA) and potato dextrose agar (PDA) plates respectively; while HUB and HUF on oil mineral salt agar (OMA) plates. Antibiotic (tetracycline) was incorporated into fungal plates for their selective isolation.

Population estimation was carried out for each treatment at the respective concentrations. In each treatment, triplicate soil samples were analyzed. Ten grams of each replica sample was obtained and homogenized; 1.0 g of this was then added to 9.0 mL diluents (physiological saline) in a sterile test tube. From this 5-fold serial dilutions were prepared; 0.1 mL aliquot of the dilutions was then platted out on the respective agar media plates (in triplicates) employing the pour plate technique. Culturable aerobic-mesophilic heterotrophic bacteria and fungi, HUB and HUF populations per gram of soil were expressed as the number of colony forming units on respective agar plates. Culturable aerobic-mesophilic heterotrophic bacterial and fungal counts were taken after 2- and 5-days incubation respectively at 28 ± 2 ºC (room temperature). HUB and HUF population estimation was conducted after 3- and 7-days period of incubation respectively, at 28 ± 2 ºC. Percentage populations of culturable aerobic-mesophilic HUB and HUF in the rhizosphere of *S. officinarum* at the various concentrations of oil tested were assessed by expressing the total culturable aerobic-mesophilic populations of HUB and HUF as percentage of culturable aerobic-mesophilic heterotrohic bacterial and fungal populations respectively.

Effect of treatment and concentration of oil on residual TPH in the rhizosphere

Soil samples collected in triplicates from the rhizosphere of *S. officinarum* were evaluated for residual TPH at intervals of 30, 60, 90, and 120 days for all applied treatments and concentrations of oil tested. TPH was analyzed using US EPA-Method 8015C for non-halogenated hydrocarbon (US EPA, 2007). Percentage degradation rate of oil in soil within the 120 days period of the study were further determined thus: % degradation rate of oil =

$$\frac{\text{Total initial oil at day 0} - \text{Total residual oil at day 120}}{\text{Total initial oil at day 0}} \text{X100}$$

Data analysis

Microsoft Excel (Analysis Tool Pak) was used for statistical analysis of data collected in this study. Measure of central tendency and dispersion were determined for all replicate soil samples. Comparative analysis of the effect of oil concentration (at 3480 and 7050 $mg.kg^{-1}$) on THB, TF, HUB and HUF populations, as well as percentage degradation rate of oil for all corresponding treatments in the rhizosphere were evaluated using Student's *t*-Test. Analysis of variance (ANOVA) was used to determine the effect of treatments on microbial populations, and residual TPH in the rhizosphere of *S. officinarum*. Significant level for all analysis was set at confident limits of $p < 0.05$.

RESULTS

Baseline physicochemical properties of rainforest soil

Reference data on soil physicochemical properties engaged in this study revealed low levels of TOC (0.02 %) and TPH (85.00 mg/kg). The soil was moderately porous, slightly acidic in nature, and may be classified as loamy sand based on its textural constituents. Nitrogen and phosphorus content were 0.15 and 41.50 mg/kg respectively (Table 1).

Treatment and oil concentration effects on bacterial and fungal populations in the rhizosphere

The populations of culturable aerobic-mesophilic heterotrophic bacteria and fungi as well as HUB, and HUF in the rhizosphere of *S. officinarum* increased progressively throughout the period of study for all the applied treatments and concentrations of oil tested (Fig. 1 and 2). With the exception of treatments PSO and PSOFMS for culturable fungi, and PSOFMS for HUF, the populations of culturable aerobic-mesophilic heterotrophic bacteria, fungi, HUB, and HUF in the rhizosphere were comparatively higher at crude oil concentration of 7050 mg/kg than that of 3480 mg/kg for all the applied treatments. Furthermore, treatments PSOFM recorded the highest populations of THB, TF, HUB, and HUF among the applied treatments ($P < 0.05$) (Fig. 1 and 2).

Treatment and oil concentration effect on residual TPH in the rhizosphere

There was a steady decrease in the residual TPH in the rhizosphere of *S. officinarum* for all the applied treatments at both concentrations of oil tested with time within the study period (Fig. 3). Treatment PSOFM consistently recorded the lowest residual TPH in soil at both oil concentrations ($P < 0.05$). Residual TPH in soil for this treatment at day-120 were 850 and 2030 mg/kg from the initial 3480 and 7050 mg/kg at day-0 respectively. This translate to a total loss of 2630 and 5020 mg/kg of oil respectively within the period. However, higher percentage loss of oil from soil occurred at 3480 mg/kg than 7050 mg/kg of oil in soil for all applied treatments ($P < 0.05$) (Fig. 4). Percentage of oil loss from soil ranged from 41.7 to 75.6, and 37.6 to 71.2 % at 3480 and 7050 mg/kg of oil in soil respectively with treatment PSOFM, recording the highest degradation rate at both concentrations tested./

Table 1: Baseline physicochemical properties of rainforest soil

Characteristics	**Values** (Mean ± SE, n = 3)
TPH (mg/kg)	85.00 ± 2.0
TOC (%)	0.02 ± 0.0
Nitrogen (%)	0.15 ± 0.01
Phosphorus (mg/kg)	41.50 ± 2.5
Porosity (%)	60.0 ± 2.0
pH	6.0 ± 0.5
Sand (%)	90.0 ± 1.5
Clay (%)	6.0 ± 1.0
Silt (%)	4.0 ± 1.0

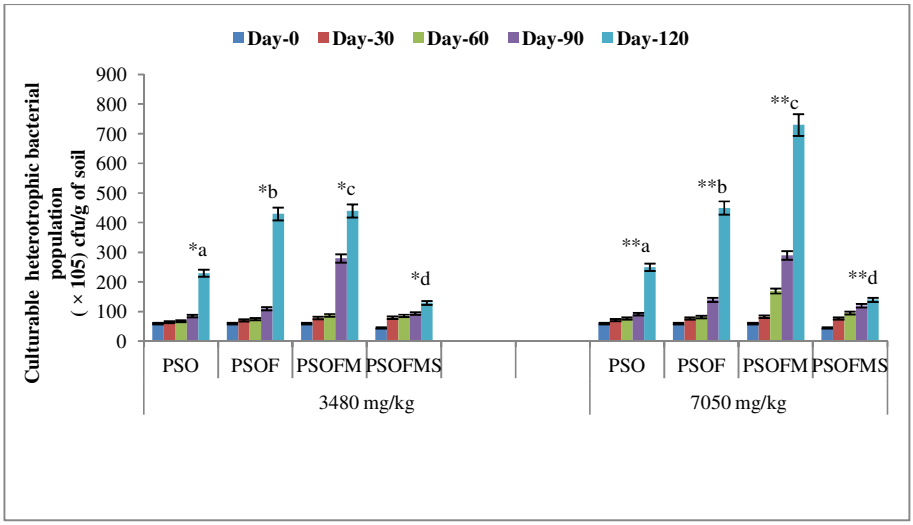

Figure 1a: Treatment and oil concentration effects on culturable aerobic-mesophilic heterotrophic bacterial populations in the rhizosphere of *S. officinarum*

Corresponding treatments at different oil concentrations with the same number of * are statistically the same (n = 15, Student's *t*-Test, $P < 0.05$). Different treatments at the same oil concentration with different letters differ significantly (n = 20, ANOVA, $P < 0.05$).

Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi

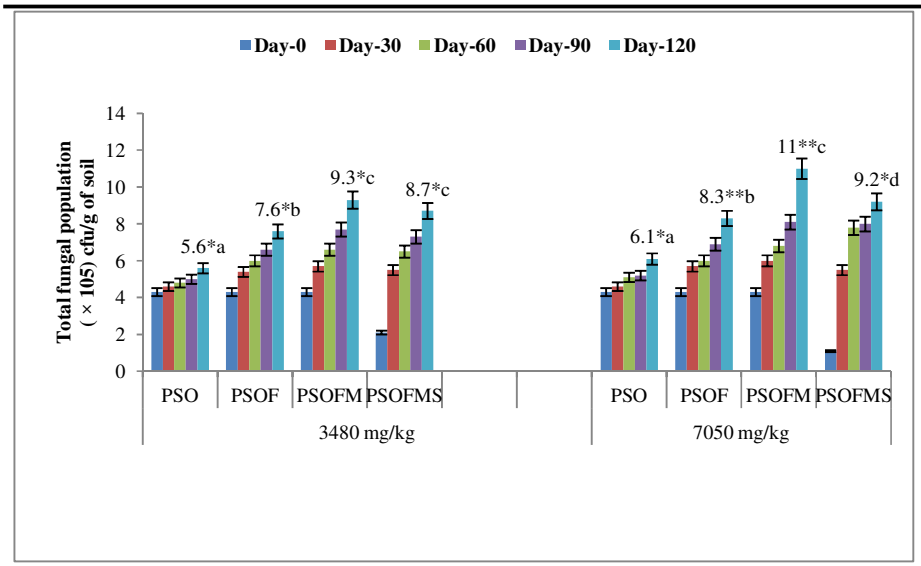

Figure 1b: Treatment and oil concentration effects on culturable mesophilic heterotrophic fungal populations in the rhizosphere of *S. officinarum*.
Corresponding treatments at different oil concentrations with the same number of * are statistically the same (n = 15, Student's *t*-Test, $P < 0.05$). Different treatments at the same oil concentration with different letter differ significantly (n = 20, ANOVA, $P < 0.05$).
Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi

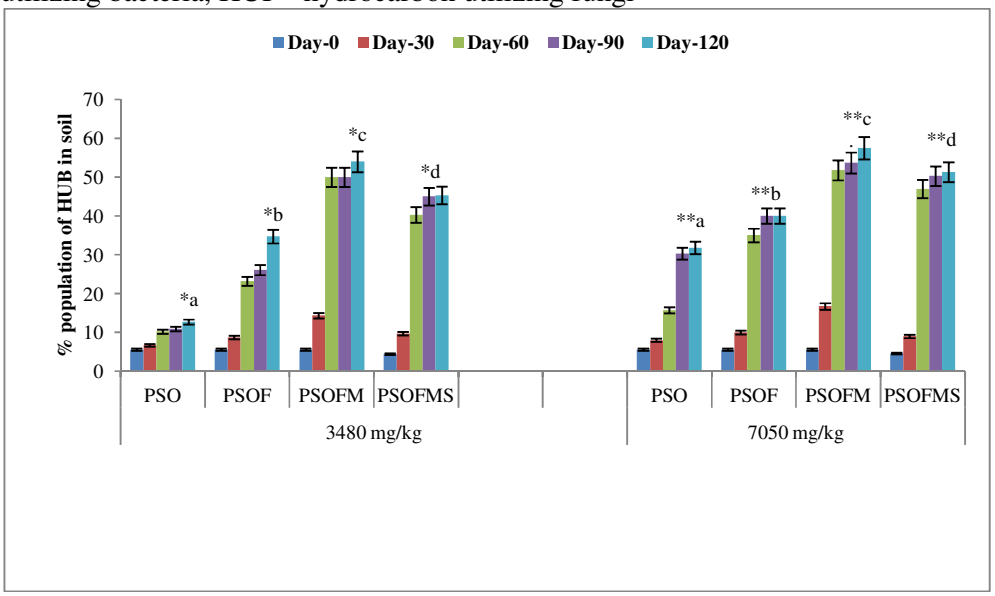

Figure 2a: Treatment and oil concentration effects on culturable mesophilic percentage populations of hydrocarbon utilizing bacteria (HUB) in the rhizosphere of *S. officinarum*.
Corresponding treatments at different oil concentrations with the same number of * are statistically the same (n =15, Student's *t*-Test, $P < 0.05$). Different treatments at the same oil concentration with different letter differ significantly (n = 20, ANOVA, $P < 0.05$).
Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi

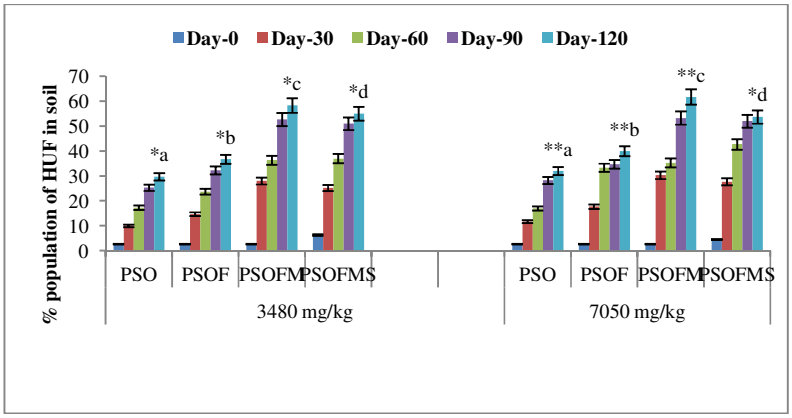

Figure 2b: Treatment and oil concentration effects on culturable mesophilic percentage populations of hydrocarbon utilizing fungi (HUF) in the rhizosphere of *S. officinarum.*

Corresponding treatments at different oil concentrations with the same number of * are statistically the same (n =153, Student's *t*-Test, $P < 0.05$). Different treatments at the same oil concentration with different letters differ significantly (n = 20, ANOVA, $P < 0.05$).

Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi.

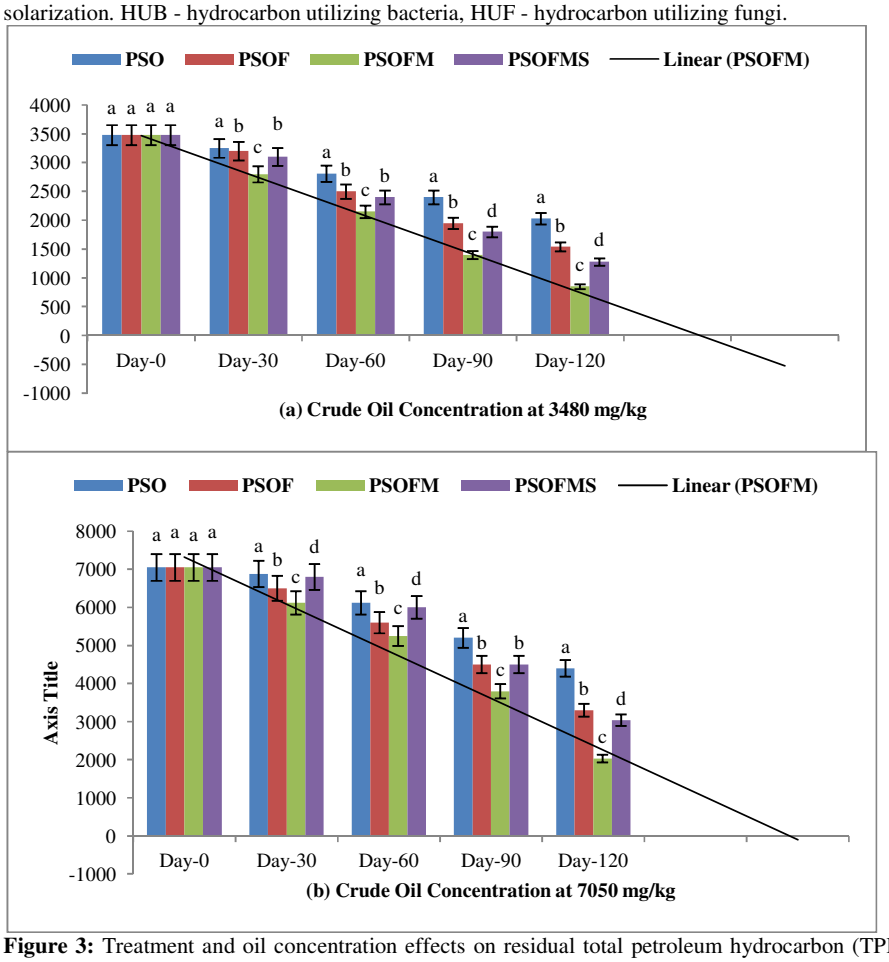

Figure 3: Treatment and oil concentration effects on residual total petroleum hydrocarbon (TPH) in the rhizosphere of *S. officinarum.*

Plotted values are means of triplicate samples. Values with different letter at same tested concentration differ significantly (n = 12, ANOVA, $P < 0.05$). Linear forecast trend-line shows probable time for complete soil remediation for the most effective treatment (PSOFM).

Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi

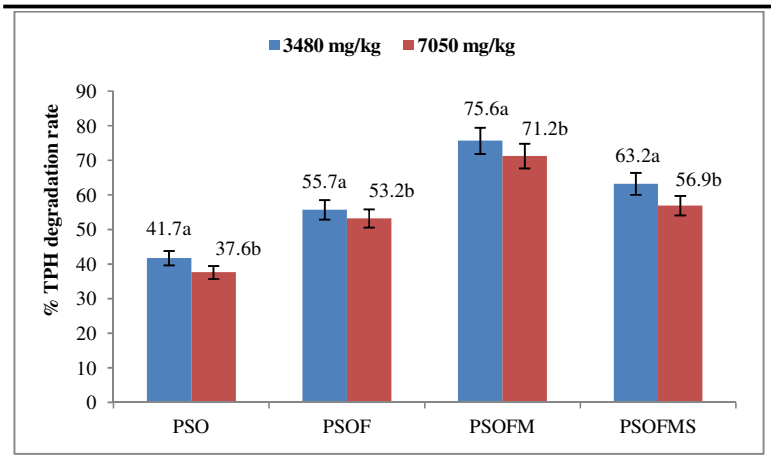

Figure 4: Treatment and oil concentration effects on percentage TPH degradation (at Day-120) in the rhizosphere of *S. officinarum.*

Corresponding treatment values with different letter are statistically different (n = 3, Student's *t*-Test, $P < 0.05$).

Legend: PSO - plant + soil + oil, PSOF - plant + soil + oil + fertilizer (NPK), PSOFM - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF), PSOFMS - plant + soil + oil + fertilizer (NPK) + Microorganisms (HUB and HUF) + solarization. HUB - hydrocarbon utilizing bacteria, HUF - hydrocarbon utilizing fungi

DISCUSSION

In both inoculated and un-inoculated rhizospheres of *S. officinarum*, the culturable aerobic-mesophilic heterotrophic and hydrocarbon utilizing bacterial and fungal populations showed a steady increase with time throughout the study period irrespective of the concentrations of crude oil tested. These observed generalized increases may be attributed to the influence of plant root exudations, and additional carbon input emanating from the contaminating oil. A progressive rise in fungal populations in uncontaminated rhizospheres of *S. officinarum* (Al-Nur and Abdulmoneim, 2007), *Morus* spp. (Chowdary *et al*., 2014) and bacterial in *Morus* spp., *Hibicus esculentus, Arachis hypogeae* and *Amaranthus hybridicus* (Chowdary et al., 2014; Oyeyiola *et al*., 2013; Aliyu and Oyeyiola, 2011; Oyeyiola 2010) have been reported to occur with age to a certain period before the decline. Higher root exudation which stimulates a rise in microbial populations and activities in the rhizosphere, occurs with plant age (Atlas and Bartha, 1987; Shirley *et al*., 2009). The decline in microbial populations and activities correspond with a reduction in root exudation as plants attain maturity towards the end of their life cycle (Shirley *et al*., 2009). The absence of microbial population decline occasioned in the rhizosphere of *S. officinarum* within the 120 days (4 months) period of this investigation may be due to the fact that the plant has not yet reached the maturity stage in its life cycle. Growth retardation in *S. officinarum* begins 8 months after planting (Binbol *et al*., 2006). Crude oil addition to soil have also been reported to increase soil total microbial populations (Obayori *et al*., 2008; Chikere *et al*., 2009; Obire and Anyanwu, 2009; Abbasian *et al*. 2016), and particularly that segment of microbial community able to adapt and utilize the new substrate (Coulon *et al*., 2006; Hamamura, 2006).

Consistently, the culturable aerobic-mesophilic heterotrophic and hydrocarbon utilizing bacterial and fungal populations were significantly higher at oil concentration of 7050 mg/kg than that of 3480 mg/kg for all the applied treatments including control in the rhizosphere of *S. officinarum*. Induced elevated response in the population and activity of microorganisms have been shown to correlate positively with increasing quantities of crude oil in soil up to certain threshold before decline (Obire and Anyanwu, 2009; Ekpo and Ebeagwu, 2009; Rodriguez-Rodriguez *et al*., 2016; Ikuesan, 2018).

The positive correlation in microbial populations with increased oil concentration observed in this study may be ascribed to favourable conditions such as carbon abundance and increased nutrients availability. Lysates or exudates from roots could have lipophilic compounds which may enhance crude oil solubility in H_2O or facilitate the growth of microorganisms producing biosurfactants (Read *et al*., 2003; Pilon-Smits, 2005). Enzymes that are produced by microorganisms and plants can also impact solubility and permit organic pollutant bioavailability through side group alterations (Chaudhry *et al*., 2005), thus accelerating microbial metabolism and population rise.

Treatment PSOFM yielded the highest culturable aerobic-mesophilic heterotrophic and hydrocarbon utilizing bacterial and fungal populations among the applied treatments including the untreated control at both concentrations tested. This may be attributed to the synergistic effects of three interacting factors: NPK fertilization, inoculation of well-adapted hydrocarbon utilizing bacteria and fungi, and plant root exudation. Total bacterial population rise has been reported to occur exclusively with NPK fertilization (Odokuma and Dickson, 2003; Chikere *et al*., 2009) or in combination with hydrocarbon utilizing bacterial inoculation (Odokuma and Dickson, 2003; Chikere *et al*., 2009; Liu *et al*., 2009; Ibiene *et al*., 2011) in crude oil contaminated soils. Similarly, increased microbial population growth in the rhizosphere can be enhanced either through the application of organic manure or inorganic fertilization (Chaudhery *et al*., 2005). The common inorganic fertilizers such as NPK usually applied in agriculture stands out as the first option due its accessibility, affordability and effectiveness as evident in the extremely numerous literature (Zhu *et al*., 2004). Preferential inoculation of microbes with precise or wide-ranging biodegrading characteristics into the rhizosphere (Bioaugumented Rhizoremediation) provides the necessary microbial populations which could speed-up remediation of soils contaminated with organic pollutants (Chaudhery *et al*., 2005). The most-suited microorganisms for bioremediation are usually those isolated from contaminated locations with a specific target substrate. Rhizospheres inoculation with (a) specific contaminant-degrading microbe(s) has been reported to improve biological remediation (Mishra, 2001; Chaudhery *et al*., 2005 Gurska *et al*., 2009). Liberated phenolics from plant roots may precisely stimulate microbial genes involved in the breakdown of organic compounds such as polyaromatic hydrocarbons or serve as a co-metabolite to accelerate microbial breakdown (Leigh *et al*., 2002; Pilon-Smits, 2005). Microorganisms that can utilize phenolic substrates as carbon source usually possess enzymes that co-metabolize contaminants with the same structures (Chaudhry *et al*., 2005).

Residual TPH measurements taken at designated time intervals in the contaminated rhizosphere of *S. officinarum* indicated a steady and gradual depletion with time for all applied treatments including untreated control at 3480 and 7050 mg/kg. The observed gradual reduction in residual TPH is the resultant effects of the applied treatments together with the natural process of attenuation such as evaporation and photodegradation (Dadrasnia and Agamuthu, 2013); hence there was a reduction in residual TPH in both treated and untreated control with higher TPH loss occurring in treated soils. Similar findings have earlier been reported (Odokuma and Dickson, 2003; Obayori *et al*., 2008; Chikere et al., 2009; Tang *et al*., 2010; Chorom *et al*., 2010; Zand *et al*., 2011; Ubogu *et al*., 2019).

Notwithstanding that reduction in residual TPH occurred in all applied treatments and untreated control, treatment PSOFM proved to be more efficient in the remediation of the crude oil contaminated soil at the tested concentrations.

The accelerated loss witnessed in treatment PSOFM over the other treatments is inherent in the inoculation of well-adapted hydrocarbon utilizing bacteria and fungi. The rationale of inoculating well-adapted hydrocarbon degraders in the remediation of the oil-polluted site is premise on the fact that microbial populations which naturally resides in the polluted environment may lack the capacity to breakdown a large number of possible substrates available in petroleum mixture (Leahy and Cowell, 1990) or that the microorganisms may be in a state of stress due to the non-distant exposure to the pollutant (Zhu *et al*., 2004), or that the resident soil populations of microorganisms capable of breaking down hydrocarbon may not be high enough, therefore microbial seeding is employed to shorten the lag phase so that bioremediation will commence immediately since the speed of remediation is of primary concern (Forsyth *et al*., 1995). Although, similar inoculations existed in treatment PSOFMS, the solarization process in the presence of oil may have reduced a significant number of resident hydrocarbon degraders, and thus lowering the overall populations in comparison to treatment PSOFM. Although, soil solarization was used to kill or weakened soil resident microorganisms (Elmore *et al*., 1997), provide resistance to protozoan predation (Bashan 1998), and confer competitive advantage for the soil inoculants in this study, the process does not discriminate against resident soil hydrocarbon degrading microorganisms in its general action.

In this study, though the overall quantum of oil loss from soil within the period was cumulatively more at the higher concentration, comparatively the percentage of oil loss from soil from the initial oil concentration was more at the lower concentration. This implies that contaminant cleanup was faster at a low rate of oil contamination in soil. Higher crude oil degradation rates have been reported to occur at lower concentrations than at higher concentrations in vegetated and un-vegetated soils (Tang *et al*., 2010; Abioye *et al*., 2012; Akpe *et al*., 2015; Chen et al., 2017). Reduced degradation at higher levels of oil contamination is linked to increased hydrocarbon toxicity to rhizosphere microorganisms and the associated plant (Tang *et al*., 2010). Quite a large number of hydrocarbons present in crude oil particularly the aromatics are toxic to plants and microorganisms (Ziołkowska and Wyszkowski, 2010). Within a certain threshold, the potential toxicity or inhibitory effect of petroleum hydrocarbon does not necessarily manifest in soils where biodegradation conditions are favourable (Atlas, 1984). Why high levels of crude oil in soil may be inhibitory, moderate levels can trigger enzymatic activities of alkaline phosphatase, urease, dehydrogenases, and nitrogen fixation processes (Wyszkowska and Wyszkowski, 2006; Kucharski and Jastrzębska, 2006), which ultimately enhances biodegradation. The degree of activities of dehydrogenase enzyme in soil has been reported to be dependent on concentration of petroleum hydrocarbon in soil (Xu and Johnson, 1997). Generally, petroleum hydrocarbons concentration that is above 25,000 ppm in soil usually viewed as toxic or inhibitory to aerobic bacteria (US EPA, 1994).

CONCLUSION

Substantial remediation occurred in the contaminated rhizosphere of *S. officinarum* at the tested concentrations of crude oil. Treatment PSFOM was more efficient among the applied treatments. Though, lower percentage degradation rate occurred at 7050 mg/kg in comparison to 3480 mg/kg crude oil contamination in the soil; the 75.6 and 71.2 % degradation rate respectively attained within the 120 days of the study provide a promising prospect.Thus, the propagation of *S. officinarum* in combination with NPK fertilization, and inoculation of rhizosphere-competent hydrocarbon utilizing bacteria and fungi offers veritable means of cleaning up of crude oil contaminated rainforest soil in the Niger Delta.

Recommendation

Rhizoremediation of crude oil contaminated rainforest soil in the Niger Delta can be undertaken using one of the world most propagated grasses, *S. officinarum* in combination with NPK fertilization, and inoculation of rhizosphere-competent hydrocarbon utilizing bacteria and fungi. However, the issue of bioaccumulation and toxicity that may arise from the use of *S. officinarum* in the rhizoremediation of crude oil contaminated soil can be addressed by confining plant employed for this purpose to bio-ethanol production for gasoline blend.

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