Enhanced rhizoremediation of crude oil-contaminated mangrove swamp soil using two wetland plants (*Phragmites australis* and *Eichhornia crassipes*)

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Abstract

Comparative studies of enhanced rhizoremediation with biostimulation and bioaugmentation techniques in remediation of oil-contaminated mangrove environment were investigated. Contaminated soils at 7190 mg/kg of oil were subjected to the following treatments: soil (S), soil + oil (SO), soil + oil + fertilizer (NPK) (SOF), soil + oil + fertilizer + microorganisms (SOFM), soil + oil + fertilizer + microorganisms + solarization (SOFMS) (triplicates): two sets planted with *P. australis*, *E. crassipes*, and one unplanted. These were studied comparatively for 120 days for culturable (aerobic, mesophilic) heterotrophic and hydrocarbon-utilizing microbial populations, and soil residual TPH. Results showed culturable heterotrophic and hydrocarbon-utilizing microbial populations and TPH loss in planted soils were consistently higher than those in unplanted receiving corresponding treatments ($P < 0.05$). There were 44.4, 71.8, 74.7, and 67.5, and 50.5, 71.8, 82.3, and 71.8% reduction in residual TPH in soil planted with *P. australis* and *E. crassipes* respectively for treatments PSO, PSOF, PSOFM, and PSOFMS as against 20.0, 62.6, 67.5, and 67.5% losses in SO, SOF, SOFM, and SOFMS. Treatments PSOFM and SFOM recorded the highest TPH loss with daily residual TPH loss in the order as follows: *E. crassipes* (49.20 mg/kg/day) > *P. australis* (44.64 mg/kg/day) > unplanted soil (40.32 mg/kg/day). Enhanced rhizoremediation was more effective than biostimulation and bioaugmentation techniques.

Keywords Oil contamination · Bioremediation · Mangrove soil · Aquatic macrophyte · Rhizoremediation

Introduction

The Niger Delta whose wetland spans over 70,000 km² is ranked among the world's 10 most significant wetlands [1]. It also harbors the largest mangrove swamp (1900 km²) in Africa and fourth in the world [2]. Mangrove ecosystems are of great significance because of their rich biodiversity. These natural ecosystems are among the most productive on earth; hence, it is of great importance to the livelihood and wellbeing of the people of the Niger Delta [3].

However, the mangrove ecosystem is highly sensitive to oil spills [4, 5]. An estimated 13 million barrels of crude oil have been released through spillage within the last 50 years into Niger Delta environment, equivalent of 50 Exxon Valdez spill incidence [6], making it the top-list of five world's most negatively impacted environment by petroleum [7]. Majority of these spills are either left unclean or not completely cleaned making some of these areas to become wastelands [8].

Worldwide, the contamination of soil, surface, and groundwater is a severe problem because of its adverse effects on the environment and human health [9]. Conventional physiochemical and engineering-based clean-up techniques are insufficient [10], in addition to the fact that they are costly, complex, environmentally intrusive, and lacking public acceptance [11]. These have therefore spurred up the development of alternative technologies for in situ applications especially those based on biological remediation capacity of plants and microorganisms [12–14]. One of such technologies is rhizoremediation.

Rhizoremediation is one form of phytoremediation technique and involves the breakdown of organic pollutants in the root zones through shared interactions of plant root

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(exudates) and associated microorganisms. Desired characteristics required for the selection of a plant for phytoremediation purpose include hardiness, competitiveness, rapid growth, long and ramified root system, considerable degree of tolerance to pollutant(s) [13], tolerance to environmental conditions, suitability for a wide range of soil type, and endemic/native species [15]. Trees and perennial grasses are widely employed in rhizoremediation, and the overwhelming numbers of grasses in literatures suggest its greater range of effectiveness [15]. Robust growths upon establishment and ramified root system which may be necessary for microbial activities as a result of an enlarged adhesive surface area are the major reasons for the preferential selection of perennial grasses over trees [16, 17].

The perennial aquatic macrophytes *P. australis* and *E. crassipes* possess most of the aforementioned characteristics. *P. australis* [18, 19] and *E. crassipes* [20–22] have been reported to tolerate some degree of oil contamination in soil and water. *P. australis*, a cosmopolitan [23], highly competitive wetland plant [24], spreads profusely through its systems of stolons and rhizomes [25], occurring in every continent save for Antarctica [26]. Similarly, *E. crassipes*, a highly prolific aquatic plant [21] due to its rapid reproductive capacity [27] and intricate root system [28] with a remarkable ability to grow in severely polluted waters [29], spreads across 62 countries in the world within latitude 40° N and 45° S [30].

To protect the naturally endowed mangrove ecosystem of the Niger Delta for sustainable development, environmentally friendly, efficient, and economical clean-up techniques are required to mitigate the damaging consequences of crude oil pollution. Numerous studies on bioremediation of crude oil, its fractionates and derivatives, have been conducted in mangrove swamp soils [31–36], and other soil ecosystems [37–47], employing biostimulation and bioaugmentation techniques.

Although a substantial number of rhizoremediation studies on petroleum hydrocarbon have also been reported [48–57], very few of such studies occurred in the mangrove swamp. There is also a dearth of studies comparing the efficiency of biostimulation and bioaugmentation techniques with rhizoremediation of petroleum hydrocarbon involving aquatic macrophytes in the mangrove environment. It is on the premise of providing some of this information in the remediation of crude oil-contaminated mangrove soil in the Niger Delta that this study was carried out.

Materials and methods

Sampling location

Soil samples and plants used in this study were collected from a mangrove swamp forest located in Warri, Delta State, Nigeria, within latitude 5° 31' 2.53" N and longitude 5° 45' 0.22 " E of the equator (Fig. 1).

Analysis of baseline physicochemical features of mangrove swamp soil

Mangrove swamp soil employed for this study was evaluated to determine its baseline physicochemical characteristics before crude oil contamination and propagation of plants. Triplicate soil samples were collected and analyzed for each physicochemical characteristic. The hydrometer technique as described by Aliyu and Oyeyiola [58] was employed for the assessment of textural components of soil. Nitrogen content (N) was determined by the methods of micro-Kjeldahl (van Reeuwijk [59]). The method of Hendershot et al. [60] was used to determine soil pH, while that of Bray and Kurtz No.1 (van Reeuwijk [59]) was used for soil phosphorus. Soil

Fig. 1 Global Positioning System (GPS) coordinates of Warri, Delta State, Nigeria: location of sample collection

total petroleum hydrocarbon (TPH), total organic carbon (TOC), and porosity were assessed using US EPA–Method 8015C [61], Skjemstand and Baldock [62], and Ezzati et al. [63], respectively.

Soil contamination and treatments

Mangrove swamp soil employed in this study was collected from a mangrove swamp forest in Warri (Niger Delta) within 0 to 15 cm of soil vertical profile with the aid of a spade at ebb tide. Soil collected was then subjected to the following treatments: soil + oil (SO); 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 three separate sets (A, B, and C). Each of the treatment pot (plastic pots) contained a thorough mixture of soil (4000 g) and crude oil (240 g of 0.818 g/cm³ specific gravity). From this, a final concentration of 7190 mg/kg of oil in soil was obtained. Every treatment in each set including control (S) (uncontaminated soil) was prepared in triplicate. All the treatments in set A were planted with *P. australis*, while B with *E. crassipes*. Control pots (S) were also planted with *P. australis* and *E. crassipes* respectively. All treatments in set C, including its control (S), were unplanted.

Soil solarization

Solarization of soil was carried out mid-January (period of enormous sunlight) adapting the technique described by Elmore et al. [64]. A transparent polyethylene sheet (0.025-mm thickness) was placed over moist contaminated soil in plastic pots with sheets lying and contacting directly the soil surface, and completely sealed to prevent heat escape. Pots were then placed under direct sunlight for heating for 2 weeks. Soil temperature was closely observed during the solarization process through the insertion of a thermometer at a soil depth of 10 cm. Soil within the period attained average solarization temperature of 42.5 ± 3.2 °C, while non-solarized soil had a mean temperature of 30.0 ± 2.0 °C.

Fertilizer application

Upon the completion of soil solarization, 12.0 g of inorganic fertilizer (NPK-15:15:15) were incorporated into contaminated soil to obtain the usual 1.0 to 5.0% nitrogen by weight of crude oil in soil needed for desired nutrient enhancement amendment as stated by Head and Swannell [65]. Fertilizer was thoroughly worked into the soil with the aid of a hand trowel (sterilized).

Hydrocarbon-utilizing microorganisms scale-up and soil inoculation

The following culturable aerobic mesophilic HUB, *Actinobacillus* sp., *Bacillus* sp., *Klebsiella* sp., *Micrococcus* sp., and *Pseudomonas* sp., and HUF, *Aspergillus* sp¹., *Aspergillus* sp²., *Aspergillus* sp³., *Mucor* sp., *Penicillium* sp., *Trichoderma* sp¹., and *Trichoderma* sp²., isolated from crude oil-contaminated, untreated rhizosphere of *P. australis* and *E. crassipes* in the mangrove swamp of the Niger Delta (Ogunu, Warri South Local Area of Delta State) (Ubogu [22]) were purified and confirmed for their hydrocarbon-utilizing ability, employing the vapor phase transfer (VPT) method as described by Chaudhry et al. [66] and subsequently scaled up for soil inoculation.

These aerobic mesophilic hydrocarbon-utilizing bacteria and fungi were presumptively characterized. HUB was characterized on the basis of cultural, morphological, and biochemical properties using Bergey's Manual of Determinative Bacteriology [67, 68] and Cheesebrough [69] while HUF were characterized based on morphological and cultural properties using Barnett and Hunter [70], Humber [71], and Ellis et al. [72] identification schemes.

Oil mineral broth was employed in the scale-up operation. This was constituted as reported in Dutta and Singh [73], with the exclusion of a gelling agent (agar). The composition includes NaCl, 10.0 g; Na₂HPO₄, 1.25 g; KH₂PO₄, 0.83 g; MgSO₄·7H₂O, 0.42; KCl, 0.29 g; NaNO₃, 0.42 g; distilled water, 1 l; and pH of 7.2. Filter-sterilized crude oil (Millipore filter with pore size 0.22 µm) was introduced into the above constituents at 1% v/v after sterilization for 15.0 min at 121.0 °C, 15 psi in an autoclave.

The method of Odokuma and Dickson [31] was adapted for the scale-up operation. Young pure culture of each microbial species from their respective slant was scrapped using a wire loop into 200.0 ml of oil mineral salt broth in a 500.0-ml flask: one microbial species per flask making a total of 12 flasks. Flasks containing bacteria and fungi were incubated under aerobic condition (opening in flasks plugged with sterile cotton) in a shaker at 28.0 ± 2.0 °C for 10 and 14 days respectively. The content of each flask was then transferred separately into a 4-l jerrycan that has been previously sanitized (using 70% alcohol) containing 800.0 ml of freshly prepared oil mineral salt broth. Jerrycans were incubated as earlier described for flasks with regular vigorous handshaking for aeration.

After incubation, aliquots of the content of each jerrycan were centrifuged to obtain cell mass of hydrocarbon utilizers. All bacterial and fungal species were then mixed together by shaking vigorously in a 20.0 l jerrycan containing normal physiological saline (0.85% NaCl). Each treatment pot in PSOFM, PSOFMS, SOFM, and SOFMS was respectively inoculated with a 350.0 ml microbial suspension at the rate

of 3.2×10^{10} cfu/ml and 5.0×10^8 cfu/ml of bacteria and fungi respectively.

Plant propagation

P. australis and *E. crassipes* were grown in mangrove swamp soil contaminated with crude oil (both treated and untreated) as well as uncontaminated control. Planted as well as unplanted soils were maintained in a greenhouse throughout the study period (120 days). Thirty-centimeter stems of *P. australis* with a diameter of 0.8 ± 0.1 cm without leaves were propagated, two stems per pots in triplicate. In the case of *E. crassipes*, tender plants obtained from the wild were transplanted into pots (each plant made up of five bulb-leaves with an average height of 12.5 ± 1.5 cm and root length of 7.5 ± 1.5 cm), one plant per pot in triplicate.

While *P. australis* was grown in pots with 25.0-cm width and 14.0-cm depth, *E. crassipes* were grown in pots with 25.0-cm width and 30.0-cm depth. Planted and unplanted soils were kept in a continuous flooded state using tap water. The larger pot depth in *E. crassipes* was meant to allow plant buoyancy. Water levels in pots were sustained by routine water top-up (using distilled sterile water) to make up for evaporative loss.

Estimation of culturable heterotrophic and percentage hydrocarbon-utilizing bacteria and fungi

The culturable (aerobic and mesophilic) heterotrophic and percentage hydrocarbon-utilizing bacteria and fungi in the rhizospheres of *P. australis* and *E. crassipes*, and the unplanted mangrove swamp soil were estimated using the soil dilution plate method on potato dextrose agar (PDA) (for fungi), nutrient agar (NA) (for heterotrophic bacteria), and oil mineral salt agar (OMA) (for hydrocarbon-utilizing bacteria and fungi). The OMA was constituted as reported in Dutta and Singh [73].

Microbial population estimation was carried out for the respective treatments. For each treatment, triplicate soil samples were analyzed. Ten grams (10.0 g) of each replica sample was obtained and homogenized; 1.0 g of this was added to 9.0 ml diluents (physiological saline) in sterile test tube. Fivefold serial dilutions were then prepared. From these dilutions, aliquots of 0.1 ml were collected and plated out in triplicate on the respective agar media using the pour plate technique (tetracycline incorporated for the selective isolation of fungi).

Bacterial and fungal growth on agar plates were counted and expressed as colony forming unit per gram of soil (cfu/g). Culturable heterotrophic aerobic mesophilic bacterial and fungal colonies on plates were counted and estimated after incubation at room temperature (28 ± 2 °C) for 2 and 5 days

respectively. While culturable aerobic mesophilic HUB and HUF were counted and estimated after incubation for 3 and 7 days respectively at room temperature. Furthermore, percentage populations of HUB and HUF in the rhizospheres of *P. australis* and *E. crassipes*, and unplanted soil were determined by expressing the entire counts of HUB and HUF as percentage population of the overall THB and TF counts respectively.

Evaluation of residual TPH in crude oil-contaminated rhizosphere and unplanted mangrove swamp soil

Triplicate soil samples for each treatment were collected from the oil-contaminated rhizospheres of *P. australis* and *E. crassipes*, and unplanted soil (for both treated and untreated soils) for residual TPH analysis using US EPA–Method 8015C [61]. Contaminated soil sample was first dried using anhydrous sodium sulfate. Ten grams of the dried soil sample was transferred to a clean extracting amber glass bottle to which 10.0 ml of extractant (n-Hexane) was added. Extraction of oil from soil samples was carried out by shaking the mixture of extractant and soil sample in a mechanical shaker for 30.0 min. Extract was collected and stored in a dry organic free vial, noting the volume of the extract. From the vial, 1.0 µl of the extract was collected and injected into already calibrated gas chromatograph (DANI MASTER GC), equipped with capillary column, identification, and data processing software (Data Apex Clarity) for TPH quantification.

Quantification of soil residual TPH was done at intervals of 30 days during a 120-day period to determine the progression pattern in residual TPH loss with time. Furthermore, daily average rate of crude oil degradation in contaminated rhizospheres and unplanted mangrove swamp soil were also estimated thus:

$$\text{Degradation Rate (mg/kg/day)} = \frac{\text{Total amount of oil degraded}}{\text{Time taken (days)}}$$

Data analysis

Data obtained from this study were statistically analyzed using Microsoft Excel (Analysis Tool Pak). Measures of central tendency and dispersion were determined for all replicate soil samples collected. Student's *t* test was employed in the comparative analysis of paired treatments. The effects of the applied treatments on residual TPH and degradation rates in the rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove soil were determined using analysis of variance (ANOVA). Significant levels for all analysis were set at confident limits of $P < 0.05$.

Results

Baseline physicochemical features of mangrove swamp soil

Soil physicochemical reference data indicated a low level of TPH (394.0 mg/kg) and TOC (0.08%). The soil was acidic (pH 5.09) with a low level of porosity and may be classified on the basis of its textural components as sandy clay loam. Its phosphorus and nitrogen contents were 43.03 mg/kg and 0.14% respectively (Table 1).

Culturable heterotrophic aerobic mesophilic bacterial and fungal populations in oil-contaminated rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove swamp soil

The culturable heterotrophic bacterial and fungal populations in the contaminated rhizospheres of *P. australis* and *E. crassipes*, and the unplanted mangrove swamp soil increased progressively with time within the period of study in both treated and untreated soils. However, while the uncontaminated rhizospheres of the two plants witnessed similar progressive heterotrophic bacterial and fungal population increase with time, the unplanted uncontaminated soil did not show any remarkable population shift. With the exception of fungal populations in treatments SOFMS/PSOFMS, consistently, the heterotrophic bacterial and fungal populations were significantly higher in the rhizospheres of both plants than their corresponding unplanted soils for both treated and untreated soils ($P < 0.05$) (Figs. 2 and 3). In general, higher populations of heterotrophs existed in the contaminated soils than the uncontaminated soils. Treatment PSOFM recorded the highest heterotrophic bacterial and fungal populations in the rhizospheres of *P. australis* and *E. crassipes* ($P < 0.05$).

Table 1 Baseline physicochemical features of mangrove swamp soil

Characteristics	Value (mean $\pm$ SE, $n = 3$)
TPH (mg/kg)	394.0 $\pm$ 2.0
TOC (%)	0.08 $\pm$ 0.01
Nitrogen (%)	0.14 $\pm$ 0.01
Phosphorus (mg/kg)	43.03 $\pm$ 1.5
Porosity (%)	35.0 $\pm$ 1.0
pH	5.09 $\pm$ 0.5
Sand (%)	47.5 $\pm$ 1.5
Silt (%)	24.7 $\pm$ 1.0
Clay (%)	27.8 $\pm$ 1.5

Culturable aerobic mesophilic populations of HUB and HUF in oil-contaminated rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove swamp soil

The percentage of culturable populations of HUB and HUF showed a progressive increase up to day 90 before undergoing a steady decline in contaminated rhizospheres of both plants and the unplanted soils. In contrast, the percentage populations of HUB and HUF in the uncontaminated rhizospheres of *P. australis* and *E. crassipes* experienced a steady population increase throughout the study period, while the uncontaminated unplanted soil recorded no discernable increase or decrease (Figs. 4 and 5). However, the percentage populations of culturable HUB and HUF were found to be consistently higher in the rhizospheres of *P. australis* and *E. crassipes* than their corresponding unplanted soils in both contaminated and uncontaminated soils ($P < 0.05$). Similar to the heterotrophs, the highest culturable percentage populations of HUB and HUF in the rhizospheres of both plants were recorded in treatment PSOFM.

Residual TPH in oil-contaminated rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove swamp soil

Residual soil TPH measurements in the contaminated rhizospheres of *P. australis* and *E. crassipes* showed a downward progression from the initial amount of 7190 mg/kg at day 0 to 4000.0, 2030.0, 1820.0, and 2340.0 mg/kg in *P. australis*, and 3560.0, 2030.0, 1280.0, and 2030.0 mg/kg in *E. crassipes* for PSO, PSOF, PSOFM, and PSOFMS respectively at day 120 (Fig. 6a, b). These decreases represent 44.4, 71.8, 74.7, and 67.5, and 50.5, 71.8, 82.3, and 71.8% reduction in soil residual TPH in the rhizospheres of *P. australis* and *E. crassipes* respectively for PSO, PSOF, PSOFM, and PSOFMS treatments.

In unplanted contaminated mangrove soil, the residual TPH also decreased from 7190.0 mg/kg at day 0 to 5750.0, 2690.0, 2340.0 and 2340.0 mg/kg respectively for SO, SOF, SOFM, and SOFMS at day 120 (Fig. 6c). These decreases represent 20.0, 62.6, 67.5, and 67.5% reduction in soil residual TPH respectively.

The highest daily losses in residual TPH in the rhizospheres of *P. australis* and *E. crassipes*, and unplanted soil were recorded in treatments PSOFM and SOFM. The daily residual TPH losses were in the order *E. crassipes* (49.20 mg/kg/day) > *P. australis* (44.64 mg/kg/day) > unplanted soil (40.32 mg/kg/day) (Table 2).

Discussion

The culturable heterotrophic aerobic mesophilic bacterial and fungal populations increased with time throughout the study period in the rhizospheres of *P. australis* and *E. crassipes* in both uncontaminated and contaminated (treated and untreated) soils. These

Fig. 2 **a** Culturable heterotrophic aerobic mesophilic bacterial populations in crude oil-contaminated rhizospheres of *P. australis* and unplanted mangrove swamp soil. **b** Culturable heterotrophic aerobic mesophilic bacterial populations in crude oil-contaminated rhizospheres of *E. crassipes* and unplanted mangrove swamp soil. Plotted values are means of triplicate samples. Paired treatments with different alphabet (a, b) differ significantly ($n = 15$, Student's *t* test, $P < 0.05$). S, soil; SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization; PS, plant + soil; PSO, plant + soil + oil; PSOF, plant + soil + oil + fertilizer; PSOFM, plant + soil + oil + fertilizer + microorganisms; PSOFMS, plant + soil + oil + fertilizer + microorganisms + solarization

findings are in agreement with earlier reports on microbial population dynamics in the rhizospheres. Oyeyiola [74], Aliyu and Oyeyiola [58], and Oyeyiola et al. [75] reported steady bacterial population rise in the rhizospheres of *Amaranthus hybridicus*, *Arachis hypogaeae*, and *Hibiscus esculentus* respectively with age up to certain periods before a progressive decline in population. Al-Nur and Abdulmoneim [76] also reported an increase in fungal activities in the rhizosphere of *Saccharum officinarum* with age and a decline in colony counts when close to maturity. However, Oyeyiola et al. [77] reported the absence of a definite pattern in the colonization of rhizosphere and rhizoplane of *Capsicum frutescens* by bacteria as the plant ages.

The differential quantitative response of bacteria and fungi among different plant species in the rhizosphere to aging may be attributed to the quality and quantity of plant root exudation. Differentiation of root exudates chemical composition is related to genus, species, cultivar, age, and a lot of abiotic and biotic factors [78]. A linear correlation has been established between rhizosphere enzyme activities and microbial abundance [79]. The progressive increase in microbial populations reported in this study may be due to the increase in root exudation into the rhizosphere as plant ages, which further stimulated microbial population increase. At early stage, carbohydrate exudates and mucilaginous materials in the rhizosphere

Fig. 3 **a** Culturable mesophilic fungal populations in crude oil-contaminated rhizospheres of *P. australis* and unplanted mangrove swamp soil. **b** Culturable mesophilic fungal populations in crude oil-contaminated rhizospheres of *E. crassipes* and unplanted mangrove swamp soil. Plotted values are means of triplicate samples. Paired treatments with different alphabet (a, b) differ significantly ($n = 15$, Student's t test, $P < 0.05$). S, soil; SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization; PS, plant + soil; PSO, plant + soil + oil; PSOF, plant + soil + oil + fertilizer; PSOFM, plant + soil + oil + fertilizer + microorganisms; PSOFMS, plant + soil + oil + fertilizer + microorganisms + solarization

support the growth of a large population of microorganisms [80]. The absence of a decline phase in the rhizosphere of *P. australis* and *E. crassipes* within the period of study may be attributed to the fact that the plants were yet to reach the climax of their growth. Low microbial activities as plants aged occur around the time when the slowdown in active exudates released from the root is anticipated as plants attained the final part of their full life cycle [81].

The unplanted uncontaminated soil did not show any remarkable culturable heterotrophic aerobic mesophilic bacterial and fungal population shift throughout the study period. Similar observations were made by Oyeyiola [74] and Oyeyiola et al. [75] while comparing the bacterial populations in unplanted soils with those of the rhizospheres of *Amaranthus hybridicus* and *Hibiscus esculentus* respectively. This trend was also reported by Obire and Anyanwu [82] in an uncontaminated soil, while studying the effects of crude oil

concentrations on the population of fungi in soil. The culturable heterotrophic aerobic mesophilic bacterial and fungal populations, on the other hand, increased with time throughout the study period in the unplanted crude oil-contaminated mangrove swamp soil. Induced elevated response in the activity and total microbial counts have been reported to correlate positively with the presence and increasing quantities of crude oil in soil to certain levels in addition to producing a shift in the microbial community diversity pattern [39, 82, 83].

The culturable heterotrophic aerobic mesophilic bacterial and fungal populations were found to be consistently higher in the uncontaminated and contaminated (treated and untreated) rhizospheres of *P. australis* and *E. crassipes* than their corresponding unplanted soils. This observation is in unanimity with a similar study by Soleimani et al. [50] Although there is paucity of information on comparative studies on the

Fig. 4 **a** Culturable aerobic mesophilic populations of HUB in crude oil-contaminated rhizospheres of *P. australis* and unplanted mangrove swamp soil. **b** Culturable aerobic mesophilic populations of HUB in crude oil-contaminated rhizospheres of *E. crassipes* and unplanted mangrove swamp soil. Plotted values are means of triplicate samples. Paired treatments with different alphabet (a, b) differ significantly ($n = 15$, Student's t test, $P < 0.05$). S, soil; SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization; PS, plant + soil; PSO, plant + soil + oil; PSOF, plant + soil + oil + fertilizer; PSOFM, plant + soil + oil + fertilizer + microorganisms; PSOFMS, plant + soil + oil + fertilizer + microorganisms + solarization

microbial population dynamics in unplanted soil with that of the rhizosphere (planted) under crude oil contamination, a lot of information thus exist for uncontaminated soils. Various authors have reported higher populations of rhizosphere heterotrophs over their corresponding unplanted soils [48, 58, 74, 75, 84]. There are many justifications for the rise in rhizosphere microbial population and activity over their corresponding unplanted soil. The dynamics of the population of microorganisms in soil environments is related to the amount of plant residue [78]. Hence, the rhizosphere is the zone of highest microbial activities than the region of soil devoid of plant roots [85]. This is because roots excrete significant amounts of sugars, amino acids, hormones, and vitamins and compounds formed from the decomposition of shelling root cells [61, 85], which promotes such an extensive growth of bacteria and fungi that these organisms often form microcolonies on the root surface [85].

The highest culturable heterotrophic aerobic mesophilic bacterial and fungal populations were recorded in treatment PSOFM in the rhizospheres of *P. australis* and *E. crassipes* and in treatment SOFM in the unplanted mangrove swamp soils. Microbial population rise in plant rhizospheres has been shown to be stimulated by organic manure or fertilizer application [12]. A rise in total heterotrophic bacterial populations in crude oil-contaminated soil with the sole inclusion of NPK fertilizer [31, 39], as well as a combined addition of hydrocarbon-utilizing bacteria and NPK fertilizer [31], was earlier reported.

In this study, the percentage populations of culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi in the uncontaminated rhizospheres of *P. australis* and *E. crassipes* increased progressively throughout the study period while those of the uncontaminated unplanted soils remained fairly constant. The progressive culturable aerobic

Fig. 5 **a** Culturable mesophilic populations of HUF in crude oil-contaminated rhizospheres of *P. australis* and unplanted mangrove swamp soil. **b** Culturable mesophilic populations of HUF in crude oil-contaminated rhizospheres of *E. crassipes* and unplanted mangrove swamp soil. Plotted values are means of triplicate samples. Paired treatments with different alphabet (a, b) differ significantly ($n = 15$, Student's t test, $P < 0.05$). S, soil; SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization; PS, plant + soil; PSO, plant + soil + oil; PSOF, plant + soil + oil + fertilizer; PSOFM, plant + soil + oil + fertilizer + microorganisms; PSOFMS, plant + soil + oil + fertilizer + microorganisms + solarization

mesophilic hydrocarbon-utilizing microbial population rise with time in uncontaminated rhizospheres of *P. australis* and *E. crassipes* may be attributed to the increase in plant root exudate as the plant ages. Root exudates contain readily biodegradable organic macromolecules [86]. Exudates from plant roots contain a number of organic compounds which may act as sources of nitrogen and carbon for the sustenance and growth of microbes that have the capacity to breakdown organic pollutants [13].

The percentage populations of culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi in the contaminated rhizospheres of *P. australis* and *E. crassipes* and contaminated unplanted soils increased progressively up to day 90 before declining in all the applied treatments. Similar findings were reported by Odokuma and Dickson [31], and Obayori et al. [83]. The observed growth trend in

the populations of culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi observed in the present study may be related to oil depletion, nutrients exhaustion [83], and possible selective microbial inhibition resulting from toxic components of crude oil and metabolic byproducts (Ikueasn [87] 2017).

The percentage populations of culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi were consistently higher in uncontaminated and contaminated (treated and untreated) rhizospheres of *P. australis* and *E. crassipes* in comparison with their corresponding unplanted soils. Likewise, Moreira et al. [54] reported higher population of hydrocarbon-degrading bacteria in oil-contaminated rhizosphere of *Rizophora mangle* in mangrove swamp. Similarly, significant higher populations of atrazine-, toluene-, benzene-, and xylene-degrading microorganisms

Fig. 6 **a** Residual TPH in crude oil-contaminated rhizosphere of *P. australis*. **b** Residual TPH in crude oil-contaminated rhizosphere of *E. crassipes*. Plotted values are means of triplicate samples. Values with same alphabet at the respective time intervals did not differ significantly ($n = 12$, ANOVA, $P < 0.05$). Linear forecast trend line indicates probable complete remediation time of soil for the most efficient treatment in planted soil (PSOFM). PSO, plant + soil + oil; PSOF, plant + soil + oil + fertilizer; PSOFM, plant + soil + oil + fertilizer + microorganisms; PSOFMS, plant + soil + oil + fertilizer + microorganisms + solarization. **c** Residual TPH in unplanted crude oil-contaminated mangrove swamp soil. Plotted values are means of triplicate samples. Values with same alphabet at the respective time intervals did not differ significantly ($n = 12$, ANOVA, $P < 0.05$). Linear forecast trend line indicates probable complete remediation time of soil for the most efficient treatment in unplanted (SOFM) soil. SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization

were reported to occur in poplar tree rhizosphere comparing with the corresponding non-rhizosphere soil [88]. The highest percentage populations of culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi were also recorded in rhizosphere of treatment PSOFM and treatment SOFM in unplanted soils. This may be attributed to the addition of NPK fertilizer and indigenous hydrocarbon utilizers. The addition of fertilizers and hydrocarbon degraders to crude oil-contaminated soils have been reported to increase the population of hydrocarbon-utilizing microorganisms [31, 39, 43].

A progressive decrease in residual TPH occurred in both contaminated (treated and untreated) rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove swamp soil with time. This progressive decrease in soil residual TPH could be attributed to a combination of natural attenuation process and the effect of the applied treatments. Decrease in residual TPH with time in treated and untreated crude oil-contaminated soils have been reported [31, 39, 40, 51, 53, 83].

Consistently, there were higher TPH losses in the rhizospheres of both plants than their unplanted soils receiving

Table 2 Average daily crude oil degradation rate in contaminated rhizospheres of *P. australis* and *E. crassipes*, and unplanted mangrove swamp soil

Average daily crude oil degradation rate (mg/kg/day)			
Treatment	<i>P. australis</i>	<i>E. crassipes</i>	Unplanted soil
SO	26.64 ± 1.2*a	30.24 ± 1.0*a	12.0 ± 1.0**a
SOF	42.96 ± 1.5*b	42.96 ± 1.2*b	37.44 ± 1.0**b
SOFM	44.64 ± 1.5*c	49.20 ± 1.7**c	40.32 ± 1.2***c
SOFMS	40.32 ± 2.0*d	42.96 ± 1.0*b	40.32 ± 1.8*c

Results are presented as means ± SE. Values with the same number of asterisk along the same row did not differ significantly ($n = 9$, ANOVA, $P < 0.05$). Values with the same alphabet along the same column did not differ significantly ($n = 12$, ANOVA, $P < 0.05$)

SO, soil + oil; SOF, soil + oil + fertilizer; SOFM, soil + oil + fertilizer + microorganisms; SOFMS, soil + oil + fertilizer + microorganisms + solarization

corresponding treatments. This result corroborates earlier reports that increased crude oil biodegradation exist at higher rate in planted soils than non-vegetated soils [49–51, 53]. The observed differences may be attributed to the presence and increased plant roots biomass which increased root surface area and secretion of microbial enhancing metabolites such as phenols which stimulated the activity of microorganisms in the root zone [50]. Furthermore, lipophilic compounds which are present in exudates or lysates of roots may enhance pollutant water solubility or raise the population of microorganisms capable of producing biosurfactants [13, 88]. Enzymes emanating from microorganisms and plants can influence organic pollutant solubility and bioavailability through side group modification [12]. Some organic components of root exudates may act as co-metabolites in the degradation of the pollutant [9]. In addition, enzymes such as peroxidase, dehalogenase, laccase, nitrilase, and niroreductase capable of breaking down pollutants are liberated into root zones by plants and stay active in the rhizosphere for a period of time [89]. The increased degradation recorded in the rhizosphere over the unplanted soils in this study may therefore be largely due to microbial degradation activities and partially to plant-formed degradative enzymes within the rhizosphere.

The highest rate of TPH loss in the rhizosphere was recorded in treatment PSOFM for both plants, while treatment SOFM recorded the highest loss for unplanted soil. The combined application of NPK fertilizer and the inoculation of indigenous hydrocarbon-degrading microorganisms are most likely responsible for the accelerated TPH loss in treatments PSOFM and SOFM as the inoculated hydrocarbon-utilizing bacteria and fungi were recovered from untreated crude oil-contaminated rhizosphere of both plants. The combine application of fertilizers and appropriate microbial addition to petroleum hydrocarbon-contaminated soil has been reported to increase hydrocarbon degradation and removal in vegetated

soil [51], and non-vegetated soil [31]. The selective inoculation of microbes with distinctive or wide-range degrading attributes into rhizosphere of plants (bioaugmented rhizoremediation) can speed up bioremediation of polluted soils [12], and microorganisms recovered from the polluted sites of interest are usually more suitable for the bioremediation of the pollutant itself [48].

E. crassipes recorded a higher rate of TPH loss in its contaminated rhizosphere than that of *P. australis*. TPH degradation efficiency of plants varies widely with plant species [49, 51, 90, 91]. The presence of crude oil in contaminated water has been reported to stimulate the growth of *E. crassipes* over uncontaminated water [21]. *E. crassipes* has been shown to have remarkable ability to absorb and metabolize phenols, polyaromatic hydrocarbons, and other organic compounds [92–95]. Without rhizosphere bacteria, *E. crassipes* have been reported to decrease the concentrations of naphthalene in waste water [91]. Although a higher percentage population of hydrocarbon-utilizing bacteria and fungi occurred in the rhizosphere of *E. crassipes* than *P. australis* in this study, the higher loss in soil residual TPH observed in its rhizosphere could be attributed to the combined degrading actions of its rhizosphere microorganisms as well as its ability to directly metabolize the oil.

Conclusion

The results of this study showed that, while biostimulation and bioaugmentation techniques substantially removed oil from the contaminated mangrove soils, enhanced rhizoremediation (involving the inoculation of specific well-adapted culturable aerobic mesophilic hydrocarbon-utilizing bacteria and fungi along NPK fertilization in the rhizospheres of the highly adaptive, prolific, cosmopolitan aquatic macrophytes, *P. australis* and *E. crassipes*) comparatively produced higher TPH removal rates. This finding strongly suggests that enhanced rhizoremediation is a better and efficient bioremediation technique than biostimulation and bioaugmentation for the clean-up of crude oil-contaminated mangrove swamp in the Niger Delta.

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