Journal of Advances in Microbiology

9(1): 1-17, 2018; Article no.JAMB.38708
ISSN: 2456-7116

Laboratory-Scale Bioremediation of Crude Oil Polluted Soil Using a Consortia of Rhizobacteria Obtained from Plants in Gokana-Ogoni, Rivers State

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2018/38708
Editor(s):
(1) Naim Deniz Ayaz, Professor, Department of Food Hygiene and Technology, Kirikkale University, Kirikkale, Turkey.
Reviewers:
(1) Mohamed M. El Nady, Egyptian Petroleum Research Institute, Egypt.
(2) Chis Timur Vasile, Ovidius University, Romania.
(3) Hanan E.-S. Ali, Egyptian Petroleum Research Institute, Egypt.
(4) Selma Gomes Ferreira Leite, Federal University of Rio de Janeiro, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/23426

Original Research Article

Received 8th November 2017
Accepted 15th January 2018
Published 5th March 2018

ABSTRACT

Background: The use of consortia of rhizobacterial flora in bioaugmentation of autochthonous flora cannot be over-emphasized.
Aim: This study sought to assess the potentials of rhizobacterial obtained from pre-exposed plants to crude oil pollution at Bodo creeks, Gokana-Ogoni, Nigeria.
Methods: In this 4-weeks study, polluted soil was spiked using a soil auger while three different plants were randomly obtained from the study area and aseptically transported to the environmental microbiology laboratory, University of Port Harcourt. Baseline physicochemical and microbiological evaluation was performed on the soil samples. Biochemical and morphological features were employed in the characterization of the isolates. Bioremediation monitoring was performed on spiked soil of total petroleum hydrocarbon concentration of 17,000 mg/kg within 14 days of the study. Percentage degradation of the crude oil was evaluated.
Results: The three plants, *Schoenoplectus senegalensis, Fuirena umbellata* and *Cyperus tuberosus* from whose rhizobacteria were randomly obtained at different points at a depth of 10-15

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cm. pH in the untreated soil was observed to increase from 6.38-6.48 after 14 days exposure time. The electrical conductivity in the treated increased from 186.2-188 µs/cm. The Nitrates in the soil for the bacterial treatments alone fell from 3.42-2.51 mg/kg. Phosphates for the bacteria consortium decreased from 23.8-22.57 mg/kg. Hydrocarbon Utilizing Bacterial Count increased from 4.3 x 10^5 cfu/g at day 0 to 2.7 x 10^6 cfu/g at day 7 and 1.7 x 10^6 cfu/g at day 14. Total Heterotrophic Bacterial Count increased in the control from 2.0 x10^5 cfu/g at day 7 to 3 x 10^6 cfu/g at day 14. Bacterial isolates were *Kingella* sp, *Bacillus* sp and *Pseudomonas* sp., *Corynebacterium* sp, *Klebsiella* sp, *Staphylococcus* sp, and *Bacterioides* sp. *Bacillus* sp and *Pseudomonas* sp are most represented with 32% and 23% respectively. TPH concentration decreased from the baseline value to 13647 mg/kg in 7 days and further decreased to 9034 mg/kg at day 14. The PAHs concentration decreased from 2998.71 at day 0 to 1986.43 and 1352 mg/kg at day 14.

Conclusion: Biomining potential of rhizobacterial as observed in this study suggest a limitless application.

Keywords: Rhizobacteria; autochthonous; bioaugmentation; consortia and baseline.

1. INTRODUCTION

Bioremediation refers to a technological approach that uses the science of biodegradation in ridding contaminated site of pollutants. It is an economical and ecofriendly strategy for removal of contaminants. It is very appropriate for the remediation of the tender and biodiverse Niger Delta environment. Biodegradation is a process whereby microorganisms break down organic substances into simpler compounds [1]. Crude oil polluted matrices could be remediated by naturally occurring hydrocarbon degrading microbes through natural attenuation. Hence, processes like biostimulation and bioaugmention can increase the efficacy of remedial processes. It also offers a cost-effective clean up alternative compared to usual techniques as hydrocarbon degrading bacteria are ever-present in the environment [2]. In the Niger Delta region of Nigeria, since its major spill incident; about 1.35 million tons of crude have been recorded a yearly average spill of 240,000 barrels [3]. These multitudes of spills bear on the fragile Niger Delta environment which is a very rich biodiverse region on the planet. Oil spill in third world countries often have devastating effect on the biophysical proxies this is due to lack of sustainable technology to tackle these challenges, illiteracy, lack of awareness especially on impacts and environmental laws. Shell petroleum spill of 2009 released several million barrels of crude oil which led to pollution of farmlands and aquifer of the Bodo community [4]. Gap in the characterization of microorganisms that are associated with particular polluted environment may be due to unceasing adaptation of microbes to their environments [5]. Hydrocarbon degradation is improved in the rhizosphere because root exudates stimulate hydrocarbon degraders [6]. Such movement of gene is high in plant rhizosphere because it is stimulated by the rhizodeposites [7].

Rhizosphere was described as plant region where root is covered with a microbial flora known for outstanding response and resistance to plant exudates and vast pollutants [8]. The rhizosphere is one region that is in constant interaction with harsh bioactive substances both indigenous and exogenous to the plant. Hence, the rhizosphere has no defined size or shape [8]. Plant rhizosphere harbor more microbes than soil without rhizosphere [9]. Rhizodeposites, compounds released by plant root into surrounding soil, have been classified to include sloughed-off root cap and border cells, mucilage and exudates. It was opined that the many functions of the exudates include acquisition of nutrient, agents of invasiveness, either exist as attractants or retardants [10]. A surge in the abundance of hydrocarbon-degrading microorganisms occurs immediately after contamination of site by crude oil [11]. These hydrocarbon-degrading microorganisms prevail in plant rhizospheres; this is because plant roots and microbes are symbiotic. Different microorganisms are affiliated with the root region of some plants and they degrade different pollutants; *Pseudomonas flourescens* associated with sugar beet degrades polychlorinated biphenyls (PCBs)" and "Trichloroethane (TCE)" respectively [12].

A number of authors have reported biological degradation of crude oil by consortia of bacteria, Adebusoye et al. [13] observed degradation of

crude oil in water samples from polluted streams in Lagos. The define consortium involved in the degradation include: *Pseudomonas fluorescens, Corynebacterium* sp*., Acinetobacter iwoffi, Micrococcus roseus, P.aeruginosa, Alcaligenes* sp*., Bacillus* sp*., Flavobacterium sp.* and *Bacillus subtilis.* Daugulis and McCracken [14] reported the involvement of *Sphingomonas* in degrading Polyaromatic hydrocarbons (PAHs). Throne-Holst et al. [15] reported the capacity of *Acinetobacter* to utilize C_{10}-C_{40} n-alkanes as the only carbon source. Biodegradability of the pollutant is of great significance when considering appropriate remedial approach [16]. A correlation exist between the degradability of PH and its properties [17]. Studies have shown that saturated hydrocarbon is more readily degraded while polyaromatic hydrocarbon is recalcitrant [18]. It is therefore expedient that the hydrocarbon composition be evaluated first before other factors [19]. The main advantage of biostimulation is that indigenous microbes that are well-suited to the environment and well distributed are been used [20]. Biostimulation natural and synthetic nutrients have been portrayed in some research reports over the years. Abioye et al. [21] showed potency of using Brewery Spent Grain, Banana Skin and Spent Mushroom Compost as organic nutrient in bioremediation. The study lasted 84 days and 92% degradation was recorded in soil contaminated with 5% used lubricating oil amended with Brewery Spent Grains. While 55% degradation was recorded in the soil polluted with 15% used lubricating oil. Agarry et al. [22] also showed biostimulation using inorganic supply of nutrients.

2. MATERIALS AND METHODS

2.1 Source of Sample

The Shell Petroleum spill site of 2008/2009 in Bodo community, Bodo creek was place of choice for collection of soil and plants. The site which was impacted with crude oil in 2008/2009 has undergone weathering over the years. Bodo community is domiciled on longitude 4°30N and Latitude 7°15E. It is situated in Gokana Local Government Area which is also regarded as one of the six kingdoms of Ogoniland. The community has a population of approximately 62,000 dispersed across the 35 villages and their main occupation is fishing and farming. Fig. 1 and 2 depict the area where samples were collected for the study.

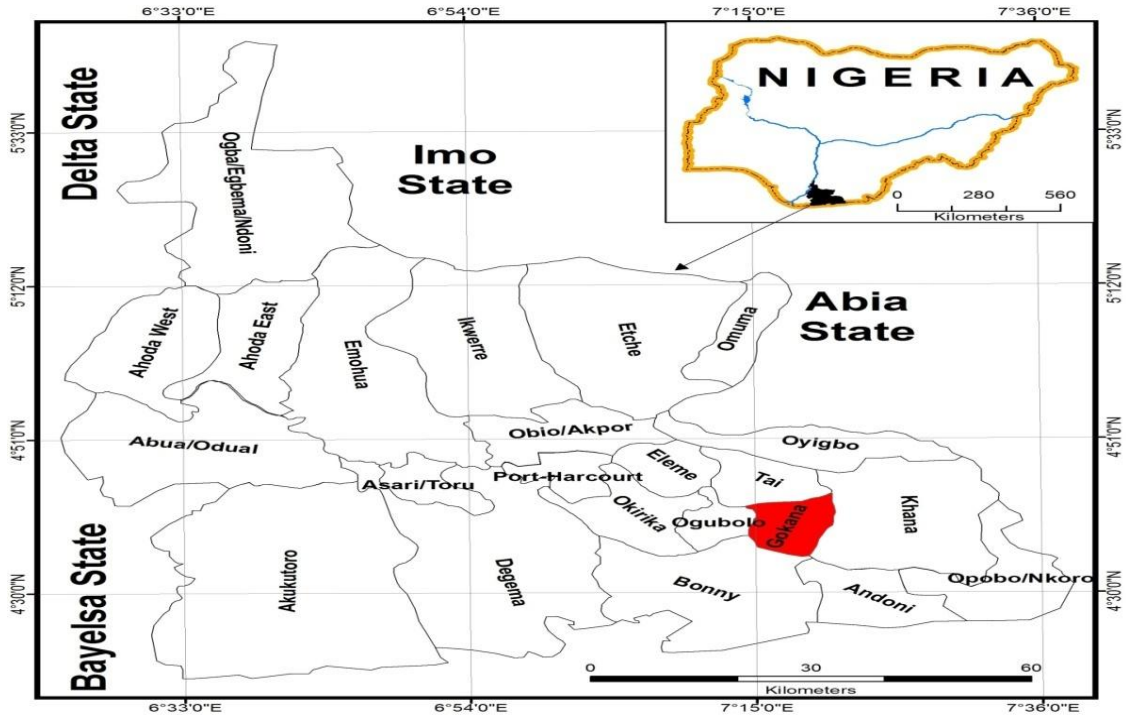

Fig. 1. Rivers state map showing Gokana LGA

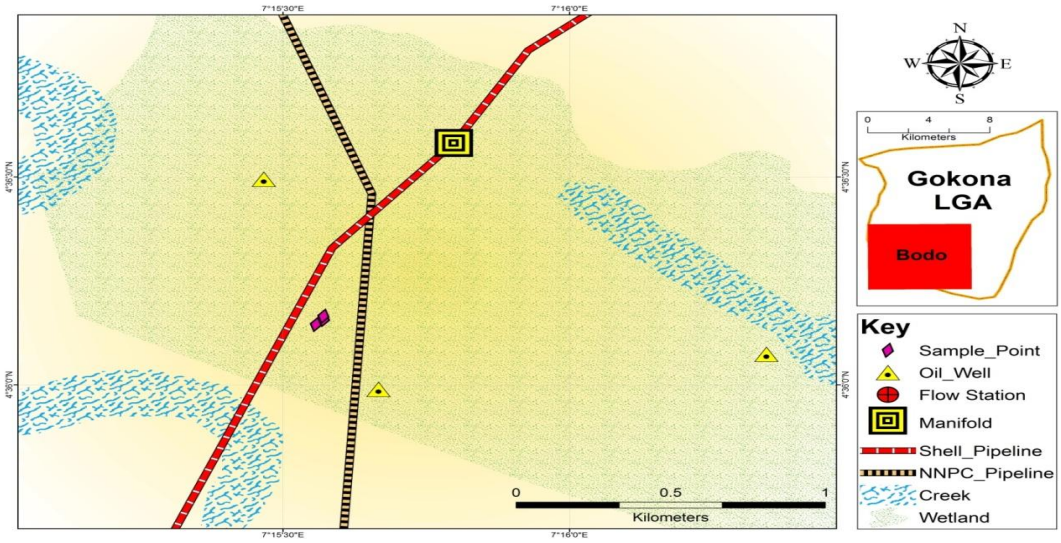

Fig. 2. Source of samples

2.2 Sample Collection (Soil and Plant)

Soil and plant samples were randomly spiked using a soil auger and obtained from the area highlighted in red in Fig. 1 with soil auger at a depth of 10-15 cm into specimen bottle and transported aseptically using a freeze-chest. The three plants, *Schoenoplectus senegalensis, Fuirena umbellata* and *Cyperus tuberosis*from whose rhizosphere bacteria and fungi were isolated for this study, were obtained from the study area at different points. The points of collection are shown in Fig. 2. The plants were replanted at the herbarium of the University of Port Harcourt for identification.

2.3 Experimental Design

The earthen pots design was simulated using plastic plates as described by Riskuwa-Shehu and Ijah [23], about 1.2 kg of soil was put into 3.0 liters earthen pots. The soil was amended with 50 ml of Port Harcourt sourced crude to attain a total petroleum hydrocarbon content of 17000 mg/kg. The soil was homogenized by manually stirring it. About 10% of the consortium was introduced and stirred daily to augment the existing flora. The set was stirred and hydrated for the study duration.

2.4 Microbiological Analysis

2.4.1 Enumeration of total culturable heterotrophic bacteria (TCHB)

This analysis was done to ascertain the bacterial titre of the plants. The spread plating was adopted in this evaluation using a basal medium. The soil around the plant was dislodged from the plant roots and use to prepare a soil extract stock with 10 grams rhizosphere in a sterile normal saline diluent. About 1.0 ml aliquots of appropriate dilutions were injected into duplicates of plates and sterile cool nutrient agar was poured into the plates. The inoculated plate was sealed and allowed to grow for 18-24 hours at 37°C. Titre was calculated in mg/l.

2.4.2 Enumeration of total culturable fungi (TCF)

The basal medium for mycological investigation was fortified with a portion of lactic acid then a spread plate method was employed, prepared and then distributed into the sterile disposable petri plates. About 0.1 milliliter of sample was introduced into plates spread on already solidified and cooled media. Monitored at 3-7 days, observable growth was expressed as cfu/gram [24].

2.4.3 Enumeration of total culturable hydrocarbon utilizing bacteria (HUB)

The vapour-phase method by Abu and Chikere, [25] was employed. Appropriate dilution of the samples withdrawn from the rhizosphere soil of the 3 different plants was introduced into a solidified plate of compounded Bushnell Hass medium fortified with 1.0% nystatin. The medium components were 0.2 g of $MgSO_4$. $7H_2O$, 0.022 g of $CaCl_2$, 1.0 g of KH_2PO_4, 1.0 g of K_2HPO_4, 1.0 g of NH_4NO_3 and 0.05 g of $FeCl_3$ and 15 grams

of bacteriological agar. All these components were weighed out and dissolved and autoclaved, Nystatin (100 µg/100 ml) was used to inhibit fungal growth. The media was sterilized under standard conditions before they were distributed into sterile petri dishes. Filter paper (whatman No 1) crude oil soaked and slightly drained papers were placed in the cover of the petri dishes, then inverted to provide the vapour saturation to the inoculums [26].

2.5 Biochemical Characterization of Bacterial Isolates

The biochemical reactions carried out are as stated below. The identities of the isolates were confirmed using standard biochemical tests [27,28].

2.6 Development of Consortia

The method of Nwadinigwe and Onyeizu [29], was modified for consortium production. The rhizobacteria were picked from the purification plates and a loopful was inoculated into the sterilized nutrient broths. The organisms were incubated for 28 h at 37°C. The cell count of the 18 h culture was obtained using a heamocytometer and standardized using 0.5 Macfarland standard prior to application to the experimental set up. A consortia concentration of $3.0x10^5$ cells/ml.

2.7 Physicochemical Composition of Soil Content

pH (APHA 4500 H^+), Temperature (APHA 2550B), Electrical Conductivity (APHA 2510B) was done colorimetrically according to (APHA, 1999) Determination of Phosphorus, Nitrate and Sulphuric Acid were determined by spectrophotometric approach. Organic Carbon was determined by Walkey-Black Method [30] was adopted.

2.7.1 Determination of trace/heavy metals (APHA 3030E)

The method of APHA 3030 (Nitric Acid Digestion) was employed for the analysis. About 5mL HNO_3 and few beads were placed in to the well mixed volume of acid and sample appropriate for the expected metal concentration to a flask or beaker. Mixture was gently heated and evaporated on a heating mantle, the final volume was made up to ten mililiters (10 ml) before precipitation. The heating was continuous with addition of conc. HNO_3 heating is completed as indicated by a colour change. The sample was not allowed to dry during digestion. The flask or beaker walls were washed with water and filtrate was transferred to volumetric flask, adding rinsing to the volumetric flask. The flask was allowed to cool and dilute to mark.

2.7.2 Total petroleum hydrocarbons and polycyclic aromatic hydrocarbons

The soil sample was extracted for total petroleum hydrocarbons with Analar grade hexane and acetone (1:1, v/v) in an extraction bottle equipped with Teflon cover. Each sample was sonicated for 1 h and the two phases were separated by decanting. Extracted organic phase was concentrated or reduced to a volume of 1 ml using vacuum rotary evaporator. One micro litre of the final extracting solution was injected and eluted in already calibrated Gas Chromatograph, (HP 5890). The calibration was carried out by using commercially available TPH concentration in the sediment samples. The concentration of TPH present was quantified every 7-day interval using GC [31].

2.7.3 Calculation of percentage degradability (%) of TPH

Percentage degradation of TPH was worked out using the equation:

$$TPH\ Degradation\ (\%) = \frac{CTPH_{to} - CTPH_{tn}}{CTPH_{to}} \text{ x } 100\%$$

Where,

$CTPH_{t0}$= Concentration of TPH at day 0 and
$CTPH_{tn}$= Concentration at a particular day n [32]

3. RESULT AND DISCUSSION

3.1 Results

3.1.1 Physicochemical composition of soil, baseline and experimental set up

Table 1 shows the physicochemical components of the soil used in the study, referred to as the baseline of the remedial work. Firstly, pH of the baseline of the soil was observed to be 6.38, after treatment, it increased to 7.18 pH in the untreated soil was observed to increase from 6.38-6.48 for a 14days exposure time. The

electrical conductivity in the treated increased from 186.2-188. The Nitrates in the soil for the bacterial treatments alone fell from 3.42-2.51 mg/kg. Phosphates for the bacteria consortium decreased from 23.8-22.57 mg/kg. Total organic carbon decreased from 8.05 to 5.03% and the control also decreased from 8.05 to 6.51%. The concentration of cadmium decreased from 2.529-1.57 mg/kg. Lead concentration decrease from 4.382 to 2.61 while that of the control decreased from 4.38-3.06. Iron concentration decreased from 984 to 896.96 mg/kg in the bacterial consortium. Zinc concentrations decreased from 135.18539 to 126.73 mg/kg.

3.1.2 Biochemical characteristics of isolates from the rhizosphere of plants

Tables 2 to 4 revealed bacterial isolates obtained from S. *senegalensis* (SE001), *F. umbelleta* (SE002) and *C. tuberosis* (SE003) respectively. The isolates in Table 2 include *Kingella* sp, *Bacillus* sp and *Pseudomonas* sp. In Table 3, the isolates identified were *Bacillus* sp, *Corynebacterium* sp, *Klebsiella* sp, *Staphylococcus* sp, and *Pseudomonas* sp. The isolates in Table 4 include *Pseudomonas* sp, *Corynebacterium* sp, *Bacillus* sp and *Bacterioides* sp. *Bacillus* and *Pseudomonas* sp. are common to all the tables while *Kingella* sp and *Klebsiella* sp are specific to Tables 2 and 3 respectively. *Corynebacterium* sp can be seen in both Tables 3 and 4 while *Staphylococcus* sp and *Bacterioides* sp is specific to Tables 3 and 4.

3.1.3 Frequency of occurrence of bacterial isolates obtained from the rhizosphere

The pie chart in Fig. 3 portrays the percentage occurrence of all the isolates that were obtained from the various plants. In the chart, *Bacillus* sp and *Pseudomonas* sp. are most represented with 32% and 23% respectively. All the other isolates were represented equally with 9% each.

Table 1. Physicochemical composition of soil treated with consortia of rhizobacteria at day 14

Parameters	Baseline	Control	SEBA
pH	6.38	6.48	7.18
Electrical Conductivity (µs/cm)	186.2	190.5	188.10
Nitrate (mg/kg)	3.42	2.83	2.51
Phosphates (mg/kg)	23.8	22.88	22.57
Total organic carbon (%)	8.05	6.51	5.03
Cadmium (mg/kg)	2.529	2.215	1.57
Lead (mg/kg)	4.382	3.05769	2.61
Iron (mg/kg)	984	930.17	896.96
Zinc (mg/kg)	135.18539	133.74	126.73

SEBA: Experimental set up using bacterial consortia

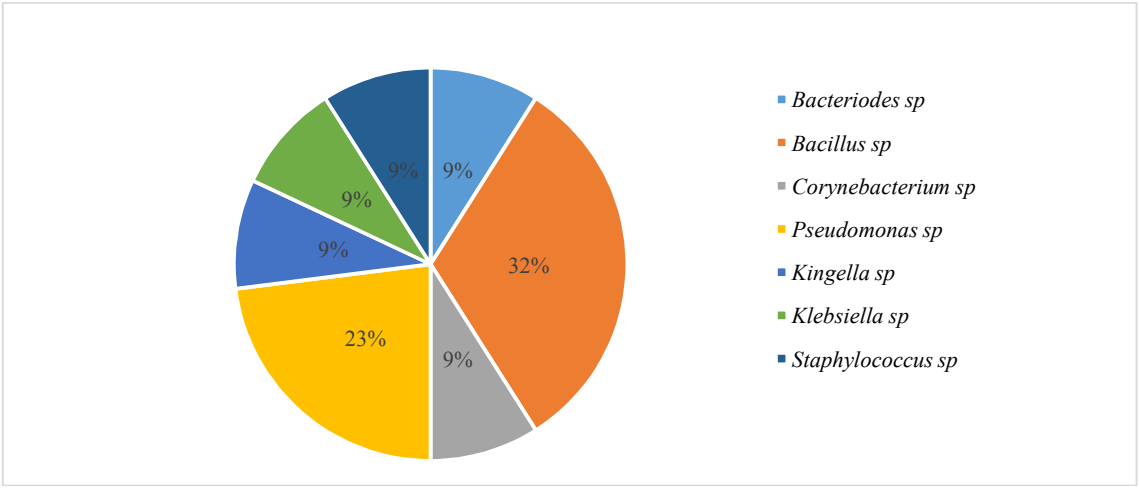

Fig. 3. Frequency of occurrence of the isolates gotten from the plants' rhizosphere

Table 2. Biochemical characteristics of isolates from rhizosphere of *S. senegalensis*

Test characteristics		Isolates					
		SE001A	**SE001B**	**SE001C**	**SE001D**	**SE001E**	**SE001F**
Shape		Rod	Rod	Rod	Chain Rod	Rod	Rod
Gram reaction		+	-	+	+	-	-
Catalase		+	-	+	-	-	-
Oxidase		-	+	-	+	+	+
Citrate							
TSI	Slant	B	B	B	B	B	B
	Butt	B	B	A	A	A	A
	H_2S	-	-	+	+	+	-
	Gas	-	-	-	-	+	+
Indole							
MR			+	-	+	-	-
VP			+	+	-	+	+
Urease		+	-		-	+	+
Fructose		-	AG	AG	AG	AG	AG
Lactose		-	-	-	-	-	-
Maltose		zG	G	AG	AG	AG	AG
Manitol		+	+	+	+	+	+
Sucrose		+	+		+		+
Starch hydrolysis		+		+	+	+	+
Tentative Identity		*Bacillus* sp	*Kingella* sp	*Bacillus* sp.	*Bacillus* sp	*Psuedomonas* sp	*Psuedomonas* sp

Table 3. Biochemical characteristics of isolates from rhizosphere of *F. umbellate*

Test characteristics		Isolates							
		SE002A	**SE002B**	**SE002C**	**SE002D**	**SE002E**	**SE002F**	**SE002G**	**SE002H**
Shape		Rod	Rod	Short Rod	Cocci	Cocci	Rod	Rod	Rod
Gram reaction		+	+	-	+	+	+	-	-
Catalase		+	+	+	+	+	+	+	+
Oxidase		+	+	-	+	+	+	+	+
Citrate		+	+	+	+	+	+	+	+
TSI	Slant	A	B	A	A	A	B	B	B
	Butt	A	A	A	A	A	A	A	A
	H_2S	-	-	-	-	-	-	-	+
	Gas	+	+	+	+	+	+	+	-
Indole									
MR		+	+	-	-	-	+	-	-
VP		+	+	+	+	+	+	+	+
Urease		+	+	+	+	+	+		+
Fructose		AG	AG	-	AG	AG	AG	AG	AG
Lactose		-	-	-	-	-	-	-	-
Maltose		AG	AG	AG	AG	AG	AG	AG	AG
Manitol		+	+	+	+	+			+
Sucrose									
Starch hydrolysis		-	-	+	+	+	-	+	-
Tentative Identity		*Bacillus* sp.	*Corynebacterium* sp.	*Klebsiella* sp	*Staphylococcus* sp	*Staphylococcus* sp	*Bacillus* sp.	*Pseudomonas* sp	*Pseudomonas* sp

Table 4. Biochemical characteristics of isolates from the rhizosphere of *Cyperus tuberosis*

Test characteristics		Isolates				
		SE003A	**SE003B**	**SE003C**	**SE003D**	**SE003E**
Shape		Rod	Rod	Rod	Rod	Rod
Gram reaction		-	+	+	+	-
Catalase		+	-	-	+	-
Oxidase		+	+	+	+	-
Citrate						
TSI	Slant	B	B	B	B	A
	Butt	A	A	A	A	B
	H_2S	+	+	-	+	-
	Gas	-	-	-	-	-
Indole						
MR		-		+	+	+
VP		-	-	+	-	-
Urease		+	+			
Fructose		-	-	-	AG	AG
Lactose		-	-	-	-	-
Maltose		AG	-	-	AG	AG
Manitol			+			
Sucrose						
Starch hydrolysis		-	+	-		
Tentative Identity		*Pseudomonas*sp	*Corynebacterium* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacterioides* sp

3.1.4 Change in hydrocarbon utilizing bacterial count (HUBC), total heterotrophic bacterial count (THBC)

Figs. 4 to 6 show the change that occurred in HUBC and THBC in the samples treated with bacterial consortium (SEBA) and the untreated (CONTROL). It was observed that HUBC increased constantly for the untreated soil from initial value of 2.2 x 10^4 cfu/g to 1.4 x 10^6 at day 7 and 3 x 10^6 cfu/g at day 14. In the soil treated with bacterial consortium only, HUBC increased from 4.3 x 10^5 cfu/g at day 0 to 2.7 x 10^6 cfu/g at day 7 and 1.7 x 10^6 cfu/g at day 14. In Fig. 5, TFC increased constantly in the soil treated with bacterial consortium only from 2 x 10^4 cfu/g at day 0 to 3 x 10^5 cfu/g at day 7 and 4 x 10^5 at day 14. TFC in the control increased from 2.2 x 10^4 cfu/g to 1.4 x 10^6 cfu/g and decreased to 6 x 10^5 cfu/g at day 14. THBC increased in the control from 2 x 10^5 cfu/g at day 7 to 3 x 10^6 cfu/g at day 14. While for the bacterial consortium alone there was a decrease from 9 x 10^6 to 2.8 x 10^6 at day 7 and 2 x 10^5 cfu/g at day 14.

3.1.5 The effects of treatments on TPH and PAHs

Figs. 7 and 8 shows the concentration in mg/kg of TPH and PAHs in the treated soil and the control at day 0, 7 and 14. In Fig. 7, the TPH concentration decreased from the baseline value to 13647 mg/kg in 7 days and further decreased to 9034 mg/kg at day 14. The PAHs

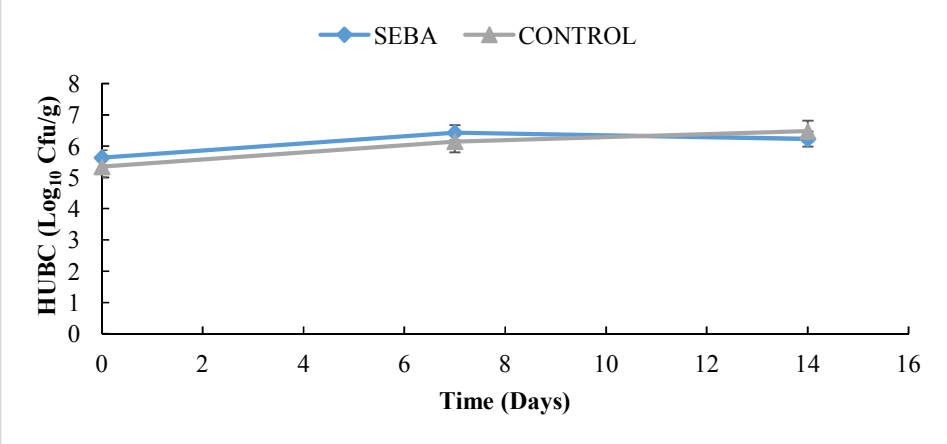

Fig. 4. Change in the population of Hydrocarbon utilizing bacteria over 14 days

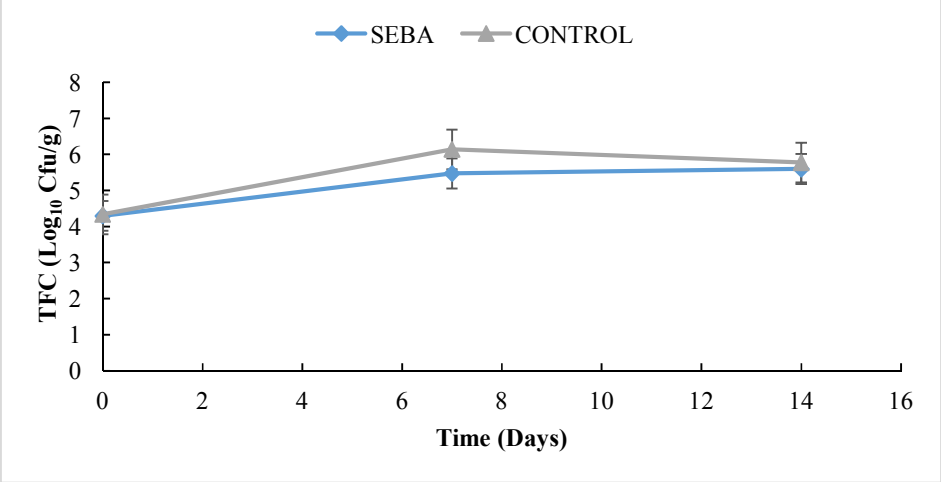

Fig. 5. Change in the population of total fungi over 14 days

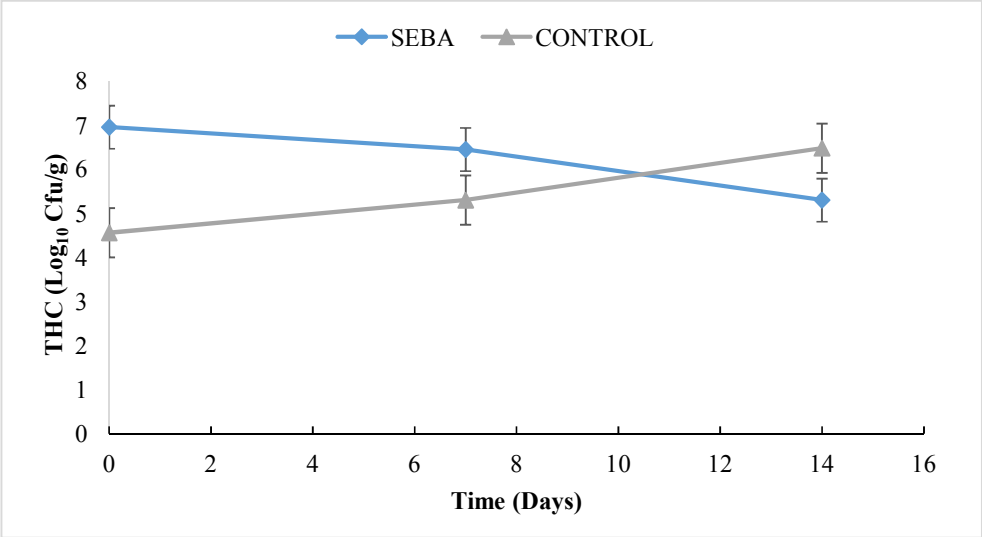

Fig. 6. Change in the population of total heterotrophic bacteria over 14 days

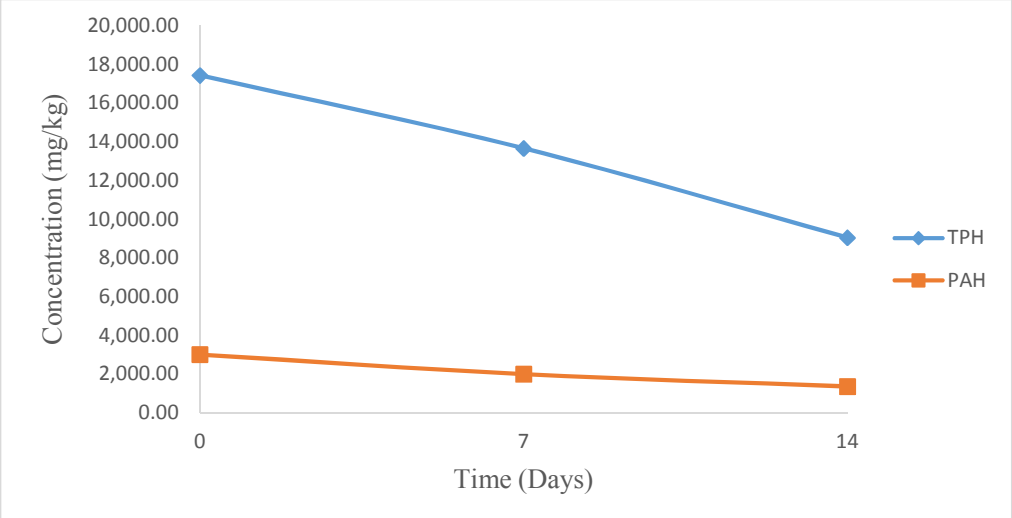

Fig. 7. The effect of treatment (SEBA) on TPH and PAHS

concentration decreased from 2998.71 at day 0 to 1986.43 and 1352 mg/kg at day 14. Fig. 8 represents the control. Here, there was a slight decrease in the TPH and PAHs concentration. The TPH concentration decreased from baseline value to 16972.45 mg/kg at day 7 and 16493.88 mg/kg at day 14, and the PAHs decreased from 2998.71 at day 0 to 2662.73 mg/kg at day 7 and 2311.45 mg/kg at day 14.

3.1.6 Gas chromatograms of treated and untreated soil

Figs. 9 – 14 are gas chromatograms showing the various peaks of TPH and PAHs components for both baseline and treated at day 7 and 14. In Figs. 9 and 11, the peaks of TPH component for the soil treated with bacteria consortium alone also reduced. No complete removal of any TPH components was observed, however, there was considerable reduction in peaks as compared to the control. For the soil sample treated with bacterial consortium, at day 14, there was a decrease in the total 16 components that were present in the baseline in Figs. 12 to 14 components; Anthracene and Benz(a) anthracene were completely removed as seen in Fig. 14.

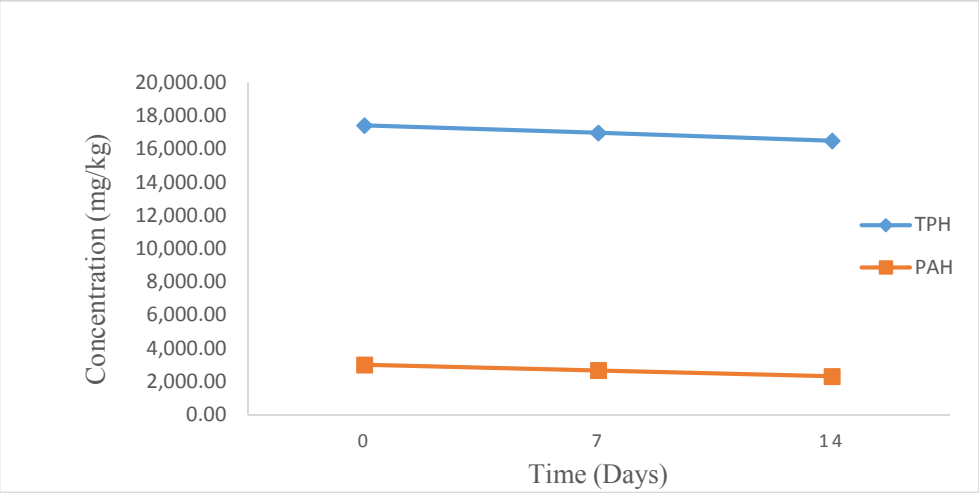

Fig. 8. The effect of control on TPH and PAHS

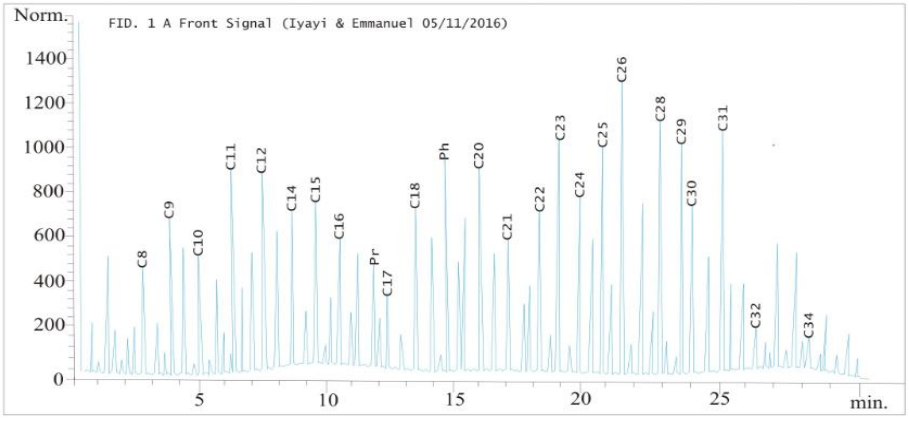

Fig. 9. TPH Gas chromatogram of untreated polluted soil

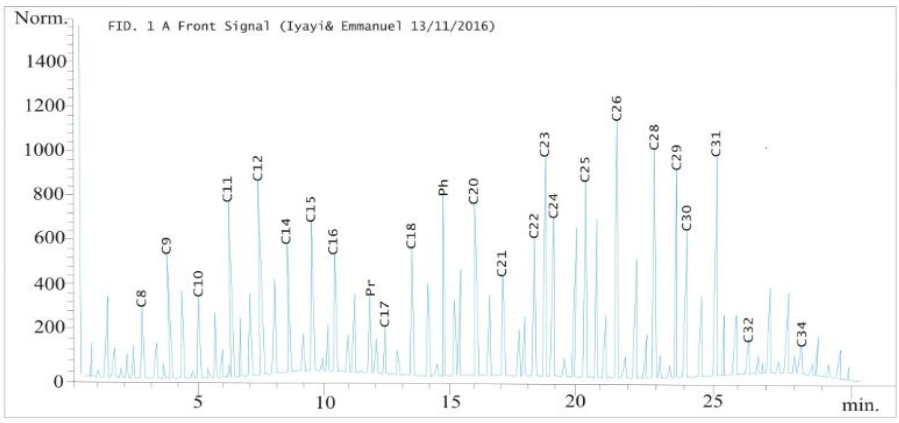

Fig. 10. TPH Gas chromatogram of soil treated with a consortium of rhizo-bacteria at day 7

3.2 Discussion

The attributes of rhizobacterial species during remediation and mineralization of pollutants and contaminants cannot be overemphasized. The report of Omotayo et al. [33], suggests that these group of microorganisms that co-exist at the rhizosphere of plants or pre-exposed plants have

been shown to participate in remediation of hydrocarbons. Furthermore, they were able to isolate *Arthrobacter, Bacillus pumilus, B. sphaericus* and *Serratia marcescens*. In our present study the presence of *Pseudomonas* sp*, Corynebacterium* sp*., Bacillus* sp*, Bacterioides* sp*, Staphylococcus*sp*, Klebsiella* sp *and Kingella* sp were comfirmed. The study carried out by Daane et al. [34] reported the presence of *Flavobacterium, Pseudomonas putida* and

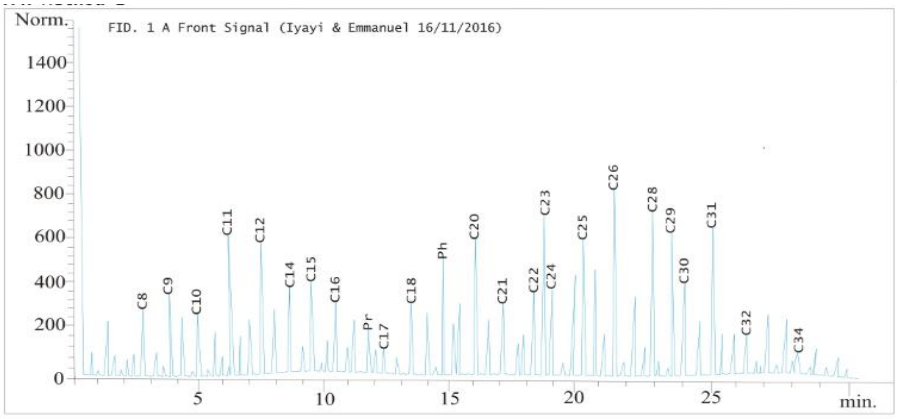

Fig. 11. TPH Gas chromatogram of soil treated with a consortium of rhizo-bacteria at day 14

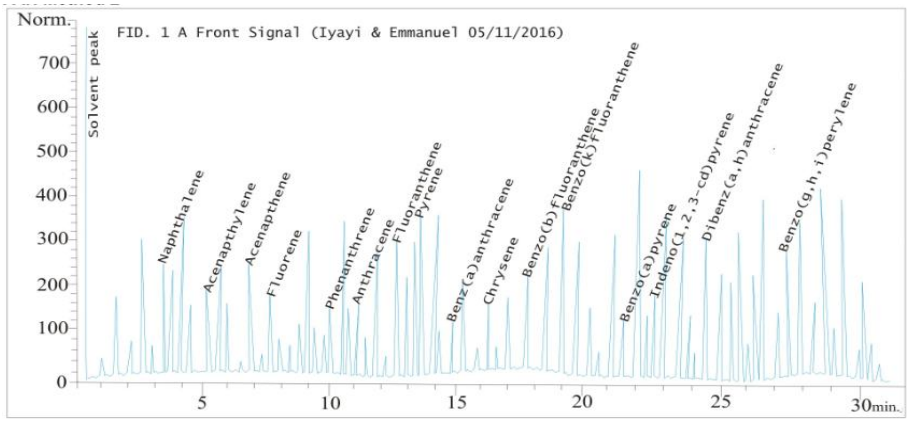

Fig. 12. Gas chromatogram of untreated polluted soil for PAH

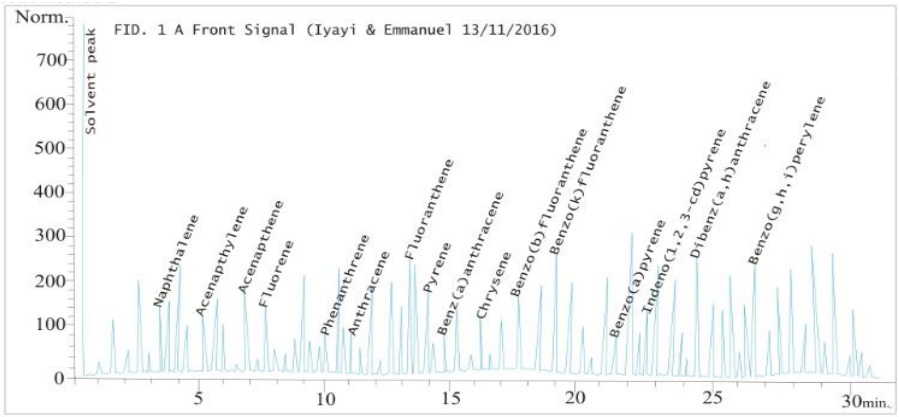

Fig. 13. Gas chromatogram of soil treated with a consortium of rhizo-bacteria at day 7 for PAH

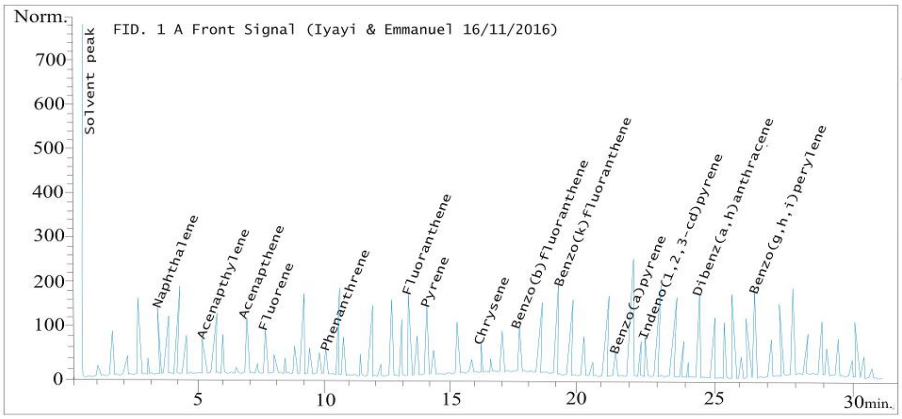

Fig. 14. Gas chromatogram of soil treated with a consortium of rhizo-bacteria at day 14 for PAH

Mycobacterium sp. Van Hamme and Ward, [35] suggested that these organisms have a selective resistance to oil interfaces, thereby secreting an organic acid that aids degradation of hydrocarbon. The results of this present study strongly agrees with the report Ukaegbu-Obi and Mbakwem-Aniebo [36] who reported the presence of *Acinetobacter, Bacillus*, *Pseudomonas*, *Alcaligens* and *Micrococcus*.

The percentage occurrence of any group of bacterial isolate describes the nature of the environment. The study revealed the predominance of *Bacillus* sp and *Pseudomonas* sp. Ukaegbu-Obi and Mbakwem-Aniebo [36], reported the dominance of *Flavobacterium* sp and *Pseudomonas* sp. This further agrees with the report of Tesar et al. [37], who opined the dominance of Gram negative microbes and few spore formers may be observed from crude oil polluted soil. This entails the microbial flora had the capacity to degrade crude oil, but the capacity of rhizobacterial species isolated from pristine soil did not perform better when compared to the ones isolated from crude oil polluted soil. Furthermore, the application of microbial population from rhizosphere of pre-exposed plants can offer huge benefits for remediation of different media.

The microbial load of the different plants varied from each other, *S. senegalensis* ($6.65x10^4$ cfu/g), *F. umbellata* ($9.55\ x10^4$ cfu/g) and *Cyperus tuberosis* ($2.5\ x10^4$ cfu/g). The study carried out by Ukaegbu-Obi and Mbakwem-Aniebo [36], had a mean count of $7.28x10^5$ cfu/g for *Elaeis guineensis* and reported that the uncontaminated soil had a lower microbial count for hydrocarbon utilizing than the polluted soil while the study reported $1.90\ x10^5$ cfu/g were similar to the ones reported by a previous study. Kuiper and Lagendijk [12] reported that the bacterial load suggest conditioning for other vital microorganisms to grow and enhance degradation. Abu and Ogigi [38] reported that the count reported for hydrocarbon degradation can be a reflection of the ease in the use of hydrocarbon components of the matrix. While Jeyanthi et al. [39] reported that the count and flora of any plant is perculiar to that plant. The Myccorhizae present at the rhizosphere regions had a higher symbiotic relationship which could be pivotal to environmental recovery process.

The changes in microbial population most cases indicate, initial contact and challenges in bio-availabilities and can be accounted for die-offs. But in this case the population of the total heterotrophic count for consortia bacteria alone (SEBA) increased from $6.0x10^4$ cfu/g at day 0 to $3.0x10^5$ cfu/g at day 14 while that of the control decreased. Similar observation was recorded by Omotayo et al. [33], who reported an increase in the population of the heterotrophic counts from $1.10x10^2$ cfu/g to $5.13x10^7$cfu/g at day 14. This is in agreement with our observation in this study; here we suggest slow growth of the bacterial isolates. While former researchers observed a pH decrease, in this study pH rose from 6.38-7.18. The loss in the nitrate and phosphate suggest not just the hydrocarbon components were used, but other nutrients served as proxies for proliferation of microbial presence.

The consortia were able to degrade a good amount of hydrocarbons. The frequency of tilling enhanced aeration of the set up and could enhance degradation of crude oil. The findings of

this study agree with Ghazali et al. [40], who recorded a 19.34% loss in TPH in 60 days. The study recorded reduction in heavy metals, nitrates, phosphates, with the consortia of bacteria and fungi. This agrees with the view of Pilon-Smith [41], who reported that the rhizobacterial consortia had the ability to remediate Chromium and Zinc, other heavy metals, phosphates and inorganic pollutants. Reed and Glick [42], reported the removal of trichloroethane and some other pesticides.

4. CONCLUSION

The results of this study show that consortium of rhizobacteria from pre-exposed plants possess significant ability to remove hydrocarbon contaminants in impacted soil. Specifically, augmenting with bacterial consortium made from different plants species, under the family cyperaceae, has proved effective in the removal of hydrocarbon components. This proposes bioaugmentation as viable and doable.

5. RECOMMENDATIONS

Further studies should be carried out on the functional groups involved in the remedial process. The microbial groups should be identified and characterized using modern genetic tools; specific representative of the consortium should be examined for their capability to degrade specific components of TPH and PAHs and their bioemulsifying and surfactant producing abilities as well. This could offer a very efficient route to recovery of the crude oil impacted Bodo creek.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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