Original Research paper

Niche-proxies of hydrocarbon-impacted rhizosphere soil of weeds of Bodo in Gokana, Rivers State, Nigeria

ABSTRACT

Niche-ecology and isolation studies of microbes from the environment have been described as the bedrock and driving-force for bioprocess industry. This investigation was designed to determine the microbiological quality of weeds growing on aged-crude oil polluted soil. Ten (10) weeds *Cyperus esculentus, Scleria pauciflora. Asystasia gangetica Harungana madagascariensis*, *Ancistoclaudus tectorius*, *Kyllinga erecta*, *Cinna arundinacea*, *Brassica chinensis*, *Cyperus difformis*, *Kyllinga bulbosa and Brachiaria mutica* and their rhizospheric soil were obtained from Bodo, Gokana LGA, Sludge farm and Botanical garden of the University of Port Harcourt, Rivers State, Nigeria. The soil was enriched in Mineral Salt Media and Bonny Light Crude Oil, prior to the spread-plating on solidified media. Result of the analysis showed pH of soil samples ranged from 5.26-7.2; Electrical conductivity was 53.4-80.31µS/cm, and phosphate 0.74-5.35 mg/kg. Levels of Vanadium in pre-impacted rhizosoil obtained from *Kyllinga erecta* and *Cinna arundinaceae* were 0.61 and 0.70 mg/kg respectively. Moisture content of soil obtained from polluted and pristine environments were 11.75% and 17.82% respectively. Permeability indices were 9.0 describing the pristine soil to have low plasticity. Total heterotrophic bacterial count was within 3.5-8.0 Log_{10} Cfu/g distributed among the weed rhizospheres. *Cyperus esculentus* rhizosphere soil was more dominated with others like *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *P. aeruginosa*, *P. fluorescens*. Fungal isolates were *Aspergillus terreus*, *Trichoderma* sp, and *Fusarium* sp. These findings further support the rhizosphere of plants as a rich bioresource for biomining of high throughput strains for biotechnological application.

Keywords: Bioresources, Microbial isolates, Niche-ecology, Rhizosphere, Pristine environment,

1.0 Introduction

Oil exploration in Nigeria has remained a mainstay for growth, sustenance and development of Nigeria. Crude oil mining activities have also left the nation with a lot of environmental challenges. Issues such as population explosion, increased industrialization and urbanization have increased the spate of the problems in modern times (Nwachukwu & Osuagwu, 2019). Oil spill is a term used in the industry to indicate the release of crude oil or its fractions into the environment. According to Nwachukwu & Osuagwu (2019) Over 1020 oil spill incidences have been reported in Nigeria, with Niger Delta taking a centre stage of the cases reported in the news media (Chinedu and Chukwuemeka, 2018). These cases have devastating effects on both fauna and flora of the soil (Orhorhoro et al., 2018: Odokuma, 2012). The effect of pollution on both aquatic and terrestrial ecosystems have different levels of severity to the biota; Presence of these pollutants above recommended threshold in the environment is deleterious to soil biota at varied proximal niches as pollutants are due to increased percolation and seepages, which have far reaching effect on non-target population (Abu and Ogigi, 1996; Ofoegbu, Momoh and Nwaogazie 2015). Elevated concentrations of pollutants in the soil affects soil fertility and bioavailability of nutrients to plants. These is reduction of porosity (Odokuma, 2012; Abu 2017) of soil to both aeration and moisture, and reduces soil microbial population (Gaskin & Bentham, 2010; Hou & Milke, 2000) in presence of pollutants.

Plants exist as complex microcosm primarily exploited by a variety of living things. The association between plants and microbes within a region have over the years remained poorly explained, vague and mirage (Santoyo, Moreno-hagelsieb, Orozco-mosqueda, & Glick, 2016). Rhizosphere is a narrow region around a plant root, controlling both physicochemical and biochemical conditions. The feasibility in the mutualistic interaction between plants and microorganisms for successive adaptation (De-la-peña & Loyola-vargas, 2014). However, plants produce organic compounds, these serve as nutrients for microbes which metabolising nutrients and ease absorption by plants. The synergies in the interrelationships between microbes and plants has served as both biocontrol and growth promotion of both plants and microorganisms (Kannan & Sureendar, 2009). According to Mendes *et al*. (2013) a varied communities of bacteria exist on the root region of plants where they improve seed germination and viability. Ahemad, (2014) reported that several bacterial genera exist at these regions of interaction and create a balance between plants and microbes. Advantage of having bacteria on the root region of the plants includes; the biogeochemical cycling and adsorption, absorption, solubilization and degradation of nutrients as growth factors to plants. A group of bacteria (rhizobacteria) that adhere to the root have been associated with crop yield and resistance to pest and diseases. Roots of plants provide anchorage systems, play conductive functions, nesting and protective function for soil organisms (Santoyo *et al*., 2016). Soil microorganisms are competent colonizers of the rhizosphere of plant (Kirkpatrick, White, Wolf, Thoma, & Reynolds, 2006). Plant exudates are secretions synthesized from plants and contain a wide array of organic substances which categorizes an exudate to be an attractant or repellent (Jackson, Bowles, Hodson, & Lazcano, 2012). Some are high and low molecular weight which have been described to influence plant reproductive health and timing of flowering and also the microbial diversity of the rhizosphere (Lu et al., 2018). This research was designed to determine the microbiological qualities of different weeds obtained from crude oil polluted soil within Bodo, Rivers State, Nigeria.

Materials and Methods

Study area

Goi is a community in Bodo, while Bodo ís a locality in the heart of Niger Delta southern Nigeria with about 49,000 inhabitants and 35 villages (Obiukwu, 2015). Bodo is community in Gokana, one of the kingdoms that make up Ogoniland Rivers state. The people of Bodo are predominantly farmers and fishermen/women. The community hosts Shell Petroleum

Development Company (SPDC) and the Trans-Niger pipelines. The area experienced two large oil spills in 2008 and 2009. The spills affected thousands of hectares of mangroves, fishing populations and also the livelihoods of occupants of the community. The study location is known as Bodo creek and situated within the geographical grid of 4^0 37’0” North, 7^0 16’0” East. Other comparative plant and rhizosphere soil samples were obtained from a pristine location in University of Port Harcourt.

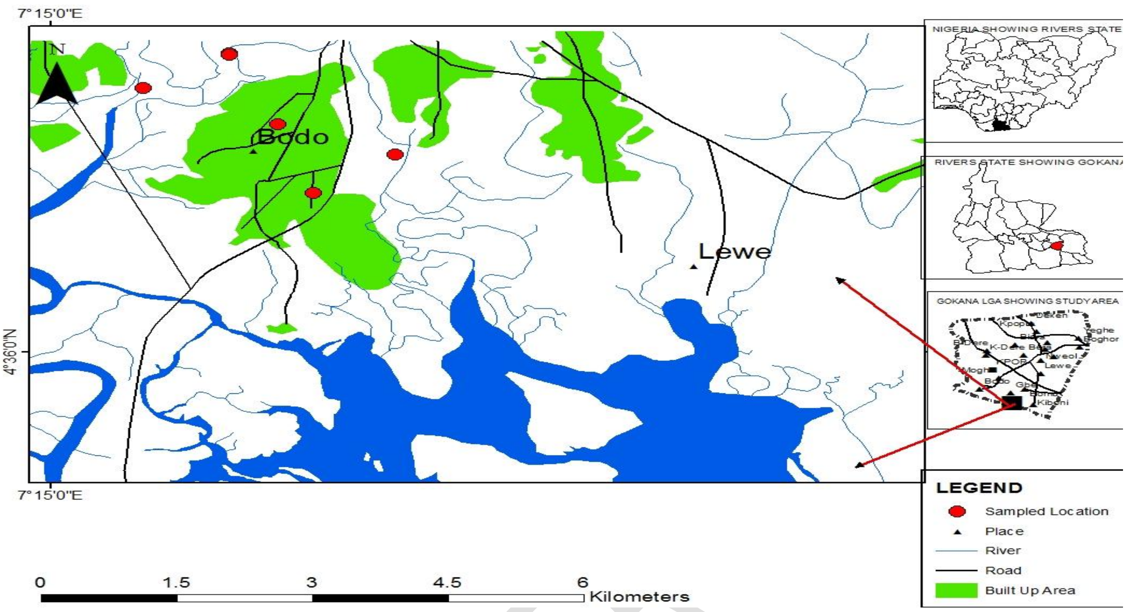

Figure 1: Geo-map of the sample collection points in Bodo, Gokana-Ogoni, Rivers State

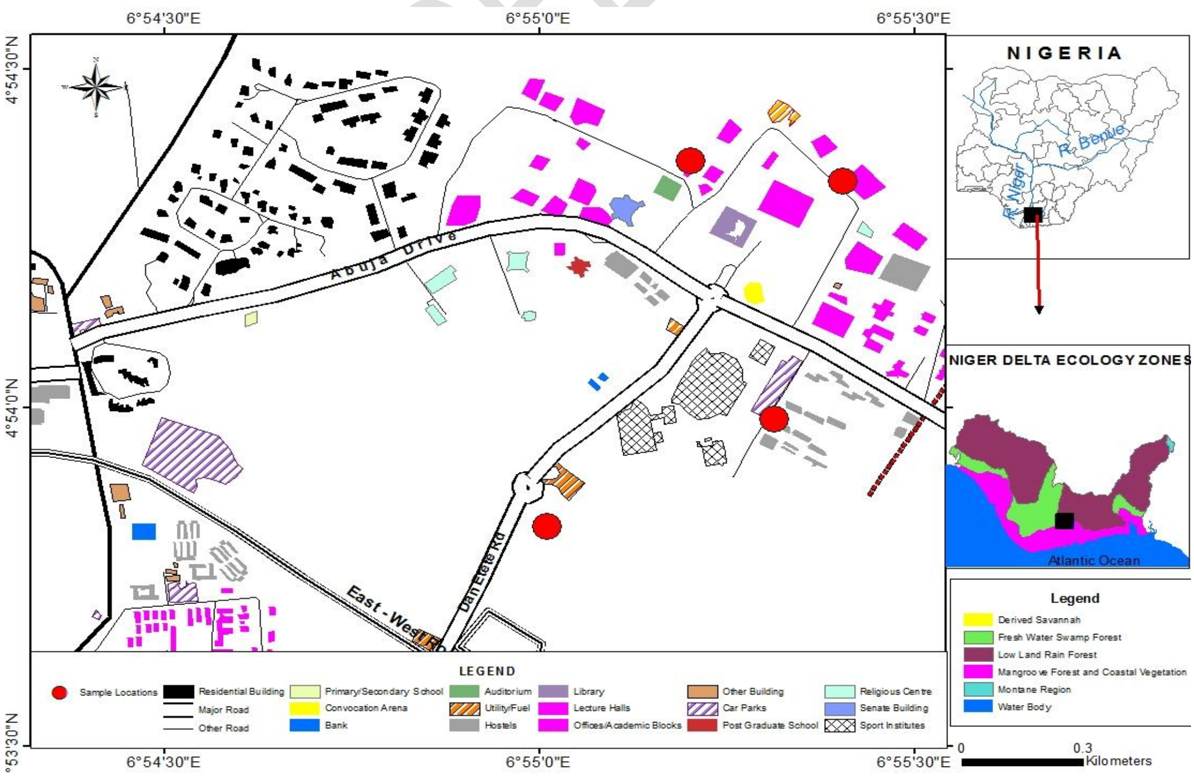

Figure 2: Geo-map of sample collection points in University of Port Harcourt, Rivers State Nigeria.

2.0 Collection of samples

Plant samples were harvested from the polluted soil, wrapped in a sterile container, sealed and labelled. Soil samples were labelled with tally on the plants. The soil samples were transported in ice-cooled chest. The plants were deposited at the University of Port Harcourt Herbarium for identification.

2.1 Baseline physicochemical and geotechnical characterization of soil samples obtained from rhizosphere of plants.

The physicochemical parameters of the rhizosphere soil analysed were analysis pH, alkalinity (APHA2320B), Electrical conductivity (APHA 2510B), Salinity (APHA 2520B), Phosphate (APHA 4500PC), Nitrate (ASTM D-3867), Ammonia (APHA 4500), Moisture content (ASTM D2216), Phenol (EPA 604), heavy metals (Ni, Zn, V, Fe and Cr). Ten grams (10g) of samples was used for geotechnical analysis, included soil texture (ASTM D422), Specific Gravity (ASTM D854), Atterberg's limits (liquid, plastic and plasticity limits) (D4318). Plasticity description (ASTM, D2434) while particulate size description was determined using the sieve method.

2.2 Enrichment of samples

The soil samples were enriched in Bushnell Haas Media (Lab M) by measuring 3.2 g of the salt was dissolved in one (1) Litre of distilled water, pH of the media was adjusted using 1.0 M HCl to pH 7.2. About 98 ml of BHM was dispensed in a 250 ml conical flask to create room for adequate headspace, 1 % Bonny Light crude oil was introduced into the media and sterilization was performed at $121^{o}C$ for 15 minutes and 15 psi. Upon cooling, the sterile set up was agitated with the aid of an orbital shaker incubator (Stuart, Germany S150) the samples were shaken at 170 r.p.m at $37^{o}C$ (Ekwuabu, Chikere, & Akaranta, 2016).

2.3 Total heterotrophic bacterial and fungal counts.

Twenty-eight grams of nutrient agar was dissolved in one (1) litre of distilled water, pre-heated and sterilized at $121^{o}C$ for 15 minutes and 15 psi, the medium was allowed to cool to room temperature, 62.5 g/100 ml of nystatin was seeded to the media to inhibit the growth of fungal contaminants. The samples were dissolved in pre-sterilized normal saline. Colony count range of 30-300 Cfu/ml were and adjudged good. Fungal counts were determined by plating 0.1 ml of the diluted sample on Saboraud Dextrose Agar fortified with 0.1% lactic acid. The spread plate technique was employed and plates were incubated at room 27°C for 24 h. Result for bacterial and fungal counts were determined after 24 hours and 72 hours of incubation (Peekate & Abu, 2017).

2.4 Hydrocarbon utilizing bacterial count

Bushnell Haas agar was prepared by dissolving the powder 3.2g/L, fortified with 15 g of agar, the media was preheated and allowed to cool. One percent of lactic acid was added into the media to inhibit fungal contaminants, the prepared media was autoclaved along with other materials. Vapour phase culturing technique was adopted, pre-sterilized Whattman filter paper was placed in the lid of the petri dishes. The plates were incubated at 37^{o}C for 48 hours. Hydrocarbon utilizing fungal count was determined by seeding 100 µg/100ml chloramphenicol for inhibition of bacterial contaminants. Of the sample was spread plated after dilution, crude oil impregnated filter papers were placed on the lid of the plates and were incubated at 25^{o}C (Orhorhoro *et al*., 2018: Ekwuabu, Chikere, & Akaranta, 2016).

2.5 Identification of microbial isolates obtained from the study

Bacterial isolates were identified using methods described by Cheesbrough (2000). These battery procedures were used to ascertain the tentative identity. Isolates of fungi were identified using the method described by Frazier and West Hoff (2000) macroscopic and microscopic Atlas and reference to standard identification keys.

2.6 Statistical analysis

The data obtained was analysed using statistical package for Social Sciences (SPSS) version 23.0 physicochemical components were analysed using One-way ANOVA. Output data was compared using homogenous subset at p-value < 0.05.

3.1 RESULT

Table 1 describes the baseline physicochemical composition of rhizosphere soil obtained from weeds. The pH of the soil ranged between 5.26-7.2. The pH of the rhizosphere soil of the plant were, *Ancistoclaudus tectorius* was 5.26, *Brassica chinensis* 5.4, *C. esculentus* was 6.9, *Kyllinga erecta* was 5.9. The highest pH 7.2 was observed for *Asystasia gangetica*. Temperature of the samples were within 26.3 to 31.6 ^{o}C; Brassica *chinensis* was 31.6 ^{o}C while *Cinna arundinacea* was 30 ^{o}C. The lowest temperature recorded for 26.3 ^{o}C was with *C. esculentus, Scleria pauciflora* and *Harungana madagascariensis* had a temperature of 27 ^{o}C. *Brassica chinensis* was 400.5 µs/cm. Rhizosphere soil obtained from Bodo, Gokana had conductivity values of 80.31 µs/cm and 53.4 µs/cm for *Cyperus esculentus* and *Kyllinga erecta* respectively. Phosphate was lowest with soil obtained from *Brassica chinensis* which was 0.74 mg/kg and 5.4 mg/kg reported for *Cyperus esculentus*. The heavy metal Nickel was below detectable level for most rhizosphere soil around the obtained from Bodo, while *Brassica chinensis* had nickel concentration of 1.11 mg/kg. The level of vanadium was 0.71 and 0.61 for *Kyllinga erecta* and *Cinna arundinacea* respectively. The heavy metals Nickel, Zinc, Vanadium, Lead, Iron and Chromium were not detected in the control samples and pristine soil samples (Table 1.0)

Table 1.0: Baseline Physicochemical composition of rhizosphere soil (in 10g) obtained from plants in Ogoni, Rivers State

Baseline Parameters	*Cyperus esculentus*	*Scleria pauciflora.*	*Asystasia gangetica*	*Harungana madagascariensis*	*Ancistocladus tectorius*	*Kyllinga erecta*	*Cinna arundinacea*
pH	6.93±0.05[d]	6.93±0.07[c]	7.35±0.15[b]	6.53±0.03[d]	5.63±0.4[d]	5.93±0.03[c]	6.3
Temperature (°C)	26.92±0.62[h]	27.5±0.50[e]	27.5±0.5[e]	27.45±0.5[f]	27.4±1.6[f]	28.85±0.15[g]	30.0
Conductivity (µ s/cm)	53.95±0.55[g]	41.1±0.90[f]	47.57±1.04[f]	32.54±0.45[g]	11.7±0.27[g]	81.26±0.95[i]	11.32
Salinity (ppt)	69.25±0.45[i]	70.8±0.10[i]	60.25±0.25[g]	50.8±0.20[h]	42.8±0.10[h]	53.7±1.3[h]	88.7
Alkalinity (ppm)	19.3±0.50[f]	16.21±0.11[d]	23.61±1.5[d]	7.1±0.1[a]	11.34±0.17[a]	15.87±0.44[e]	67.5
Phosphate (mg/kg)	5.64±0.04[cd]	3.12±0.01[b]	1.84±0.02[a]	2.15±0.05[b]	3.05±0.05	4.16±0.06[cd]	5.3
Ammonia (mg/kg)	2.17±0.03[b]	1.35±0.10[a]	0.96±0.03[a]	0.60±0.07[a]	1.14±0.04[a]	2.10±0.14[ab]	1.95
Phenol (mg/kg)	90.8±0.53[i]	74.8±0.20[i]	61.7±0.3[g]	50.7±0.30[h]	33.85±0.65	26.9±0.6[g]	110.8
H_2S (mg/kg)	12.66±0.26[f]	11.7±0.20[d]	12.06±0.38[c]	21.10±0.41[e]	31.8±0.2	20.5±0.4[f]	43.3
Nickel(mg/kg)							
Zinc(mg/kg)	0.18±0.04[a]	0.11±0.01[a]	0.38±0.06[a]	0.61±0.08[a]	1.49±0.04[a]	1.71±0.04[ab]	3.34
Vanadium(mg/kg)	0.04±0.001[a]	0.02±0.01[a]	0.08±0.01[a]	0.02±0.01[a]	0.34±0.03[a]	0.78±0.09[a]	0.67
Lead(mg/kg)	-a	0.12±0.02[a]	0.20±0.02[a]	0.23±0.01[a]	0.02±0.01[a]	0.06±0.05[a]	0.02
Iron(mg/kg)	0.28±0.02[a]	0.16±0.02[a]	0.095±0.01[a]	0.21±0.01	0.21±0.01[a]	0.36±0.03[a]	0.01
Chromium(mg/kg)	0.08±0.001[a]	0.05±0.01[a]	0.03±0.01[a]	0.06±0.01	1.84±0.07[a]	0.31±0.001[a]	0.039
Sulphates(mg/kg)	4.89±0.01[c]	5.96±0.15[c]	9.45±0.05[bc]	1.84±0.07	10.1 ±0.1[b]	11.0±0.20[g]	15.4

Data presented as Mean ± Standard Error; Similar superscripts in a column imply there was no significant difference, those with different superscripts are significant at p-value <0.05; ppt= parts per thousand;

Table 2: Physicochemical composition of rhizosphere soil obtained from a pristine location University of Port Harcourt

	Rhizosphere soil		
Baseline Parameters	*Cyperus difformis*	*Kyllinga bulbosa*	*Brachiaria mutica*
pH	6.93±0.03^{d}	6.2±0.3^{d}	7.8±0.001^{b}
Temperature (^{o}C)	28.1±1.2^{g}	33.4±1.65^{f}	28.5±0.5^{d}
Conductivity (µS/cm)	101.8±1.6^{h}	81.41±1.1^{g}	216.87± 17^{e}
Salinity (ppt)	21.1±0.7^{f}	11.87±0.54^{e}	15.95±0.25
Alkalinity (ppm)	12.15±0.35^{e}	4.81±0.11^{cd}	3.89±0.1^{ab}
Phosphate (mg/kg)	9.75±0.15^{de}	11.16±0.06^{e}	7.81±0.11^{b}
Ammonia (mg/kg)	2.18±0.04^{ab}	2.06±0.06^{abc}	1.97±0.02^{a}
Phenol (mg/kg)	4.58±0.08^{bc}	3.17±0.06^{bc}	5.21±0.005^{ab}
Hydrogen sulphide(H_2S) (mg/kg)	1.43±0.03^{a}	1.06±0.06^{ab}	7.32±0.02
Nickel (mg/kg)	-	-	-
Zinc (mg/kg)	-	-	-
Vanadium (mg/kg)	-	-	0.02±0.01^{a}
Lead (mg/kg)	-	-	-
Iron (mg/kg)	1.28±0.02^{abc}	1.92±0.01^{abc}	1.57±0.07^{a}
Chromium(mg/kg)	-	0.013±0.01^{a}	-
Sulphates (mg/kg)	1.07±0.01^{a}	1.86±0.06^{ab}	3.13±0.03^{ab}

Data presented as Mean ± Standard Error; Similar superscripts in a column imply there was no significant difference, those with different superscripts are significant at p-value <0.05; ppt= parts per thousand; - =Below Detectable Level

Table 3 describes the geotechnical evaluation of the soil samples obtained from the pristine soil from the herbarium in University of Port Harcourt. The result showed that the soil had 82.43 wt % sand, 14.19 wt% clay and 2.48% silt while polluted soil had 87.72% silt, 9.01% sand and 1.98% clay for the Bodo polluted soil Bodo-Ogoni. Moisture content for the pristine soil obtained from Uniport was 17.82% while polluted soil was 11.75%. Permeability description of both pristine and rhizosphere polluted soil were both low and had permeabilities of 6.3e^{-6} and 4.73e^{-6} cm/sec respectively. Organic carbon was high with the polluted soil with 31.85%. Plasticity index of the soil samples (rhizosoil and their control) was observed to be 7.1 and 8.9 and were reported to have low plasticity.

Table 3: Geotechnical qualities of soil samples obtained during the study.

Parameters	Pristine soil (Uniport)	Pristine soil (Goi, Bodo)	Rhizosphere Polluted soil
Sand (wt %)	83.22 ± 0.79^{c}	27.0 ± 0.7^{b}	9.23 ± 0.22^{a}
Clay (wt %)	15.34 ± 1.16^{b}	41.17 ± 0.47^{c}	1.99 ± 0.01^{a}
Silt (wt %)	3.91 ± 0.43^{a}	39.06 ± 0.73^{b}	87.19 ± 0.53^{c}
Soil type	17.87 ± 0.05^{b}	Clay Loam	Silt loam
Moisture (%)	17.87 ± 0.05^{b}	11.28 ± 0.24^{a}	11.88 ± 0.13^{a}
Permeability (cm/sec)	0.0024 ± 0.00015^{b}	0.0001 ± 0.01^{a}	0.00001 ± 0.0002^{a}
Permeability description	Medium	Low	Low
Organic carbon (%C)	31.85	3.81	12.96
Total Organic Carbon (mg/kg	11.58 ± 0.10^{b}	23.70 ± 0.90^{c}	31 ± 0.01^{a}
Total Hydrocarbon content (mg/kg)	5.84 ± 0.04^{b}	9.22 ± 0.12^{c}	103 ± 0.08^{a}
Liquid limit	27.71 ± 0.3^{c}	20.27 ± 0.27^{b}	18.62 ± 0.12^{a}
Plastic limit (%)	19.03 ± 0.53^{b}	17.62 ± 0.32^{b}	13.28 ± 0.07^{a}
Plasticity index	8.95 ± 0.04^{b}	9.85 ± 0.05^{c}	7.3 ± 0.2^{a}
Plasticity description	Low	Low	Low

Figure 3 describes the microbial population monitoring of the soil samples obtained from the rhizosphere of weeds. The study revealed that Total heterotrophic count for the control samples were significantly ($p< 0.05$) different from the soil obtained from the rhizosphere of plants. The results revealed that 7.5 Log_{10}Cfu/g to 7.77 Log_{10}Cfu/g, for Rz4, A. *tectorius* had a 5.11 Log_{10}Cfu/g while *A. gangetica* had the highest total Heterotrophic Bacterial Count (THC) of 6.38 Log_{10}Cfu/g. *Kyllinga bulbosa* had a Total fungal count of 6.7 Log_{10}Cfu/g and a hydrocarbon utilizing fungal and bacterial counts of 4.76 Log_{10}Cfu/g and 5.16 Log_{10}Cfu/g respectively. Soil samples obtained from *S. pauciflora*, had HUB and THBC of 5.79 Log_{10}Cfu/g and 6.04 Log_{10}Cfu/g. Table 5.0 shows microbial characterization and identification from the rhizosoil samples obtained from weeds in Bodo polluted soil, *Cyperus esculentus* rhizosphere soil was dominated with *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *P. aeruginosa*, *P. fluorescens*, Fungal isolates obtained from the study. Table 5. Organisms such as *Aspergillus terreus*, *Trichoderma* sp, and *Fusarium* sp. *S. pauciflora* had *Micrococcus* sp, *B. cereus*, *B. subtilis* and *Pseudomonas* sp while *A. niger*, *Mucor* sp, *Fusarium* sp and *Penicillium* sp are the fungi isolated.

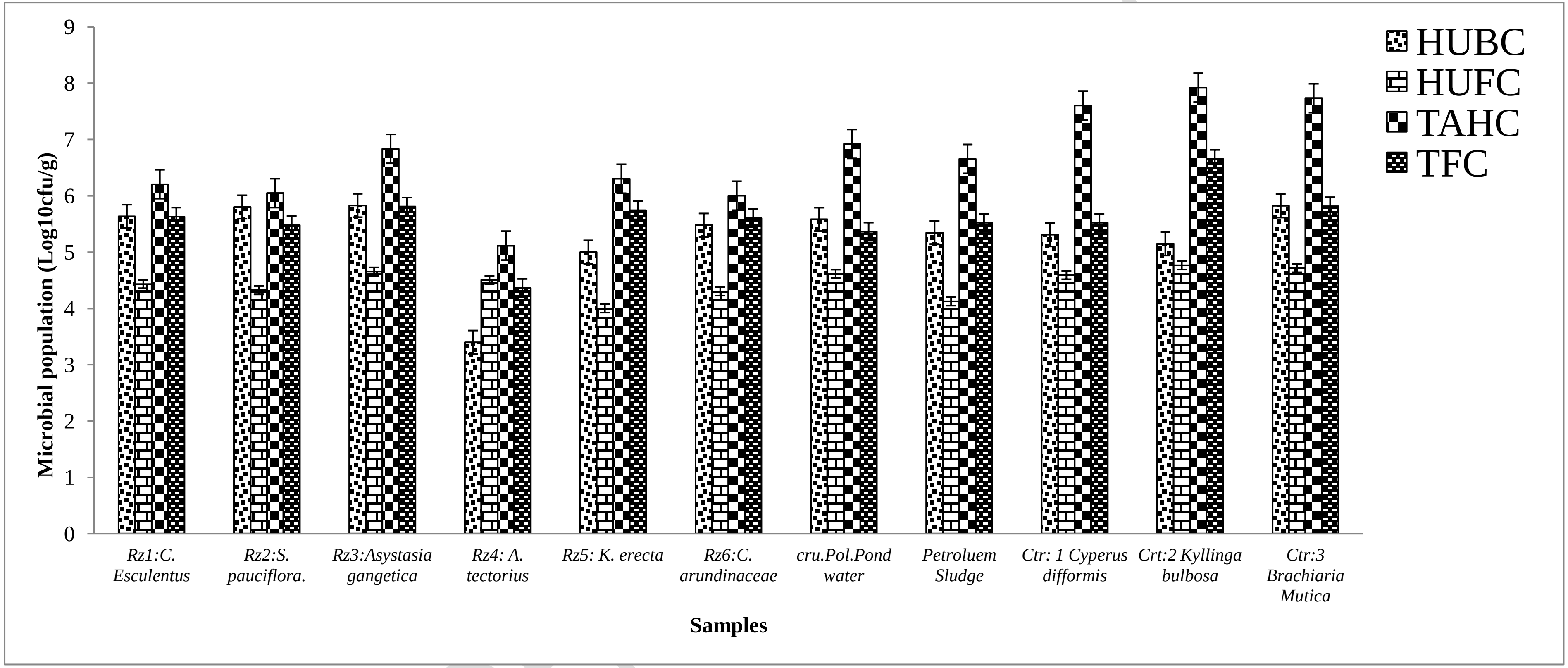

Figure 3: Average microbial population of Rhizosphere soil, pond water, petroleum sludge obtained from plants pre-exposed to crude oil and pristine environment **.Legend: HUBC= Hydrocarbon utilizing bacterial count; HUFC= Hydrocarbon Utilizing Fungal count, THBC= Total heterotrophic Bacterial count; TFC= Total Fungal Count**

Table 4 Biochemical characteristics of bacterial Isolates from both pristine and impacted rhizosphere soil

ISOLATE CODE	GRAM MORPHOLOGY	GRAM MORPHOLO	CATALASE	OXIDASE	CITRATE	MOTILITY	Glycerol	GAS	SLANT	BUTT	STARCH HYDROLYSI	IINDOLE	MR	VP	GluCOSE	LACTOSE	SUCROSE	Maltose	Arabinose	xylose	Mannitol	Salicin	Trehalose	Sorbitol	Galactose	**Probable isolates**
1	Rod	+	+	+	-	+	+	-	K	A	+	-	-	-	Ag	-	-	+	A/g	-	A/g	-	A	A/g		*Arthrobacter* sp.
2	Rod	+	+	+	+	-	+	-	K	K	+	+	+	-	A/g	-	-	-	A/g	A	-	A	-	-	A	*B. anthracis*
3	Rod	+	+	+	+	-	-	-	A	A	+	-	+	-	A/g	-	A	A/g	-	A/g	-	-	A	-	A	*B. subtilis*
4	Rod	-	-	-	+	+	A/g	-	A	A	+	-	+	-	Ag	Ag	Ag	A/g	-	A	A	A/g	-	-	A	*Pseudomonas* sp.
5	Cocci	+	+	+	+	-	-	-	K	A	+	-	+	-	A/g	A/g	A/g	-	-	-	-	A/g	-	-	-	*Salinococcus* sp.
6	Rod	+	+	-	+	+	-	+	K	A	-	-	-	-	A/g	-	-		-	-	A	-	-	-	A	*Achromobacter* sp
7	Rod	-	+	+	+	+	-	+	A	A	-	-	-	+	A/g	A/g	A/g	A	A	A/g	A/g	A	A/g	A/g	A/g	*P. fluorescence*
8	Rod	-	+	-	-	+	-	-	A	K	+	-	+	-	A/g	Ag	A/g	-	-	A	-	-	A/g	-	A	*Pseudomonas* sp
9	Rod	+	+	-	-	+	-	-	A	A	+	-	-	-	A/g	A/g	A/g	A	A	A/g	A	A	-	A	-	*Bacillus.* sp.
10	Rod	+	+	+	-	-	-	+	A	A	+	+	+	-	A/g	-	Ag	-	A	A	-	A/g	A	-	A	*Bacillus cereus*
11	Rod	+	-	+	+	-	-	-	K	A	-	-	+	-	A/g	A/g	A/g	-	-	A	-	-	A	-	A	*Clostridium* sp
12	Rod	-	+	+	-	+	-	+	A	A	+	+	+	-	A/g	A/g	Ag	A/g	A/g	A/g	A	A	A/g	A	A/g	*E. coli*
13	Rod	+	+	-	-	+	-	+	K	A	-	-	+	+	A	-	A	A	-	-	-	A	A	-	A	*Bacillus thuringiensis*
14	Cocci	+	+	+	-	-	-	-	A	K	-	-	-	-	A/g	-	A/g	A	A	-	-	A	-	A	A	*Staphylococcus* sp.
15	Cocci	+	+	+	-	-	-	-	K	A	+	+	-	-	A	-	A	-	-	-	-	A		A	-	*Micrococcus sp*
16	Rod	+	+	+	-	-	A	-	K	K	-	-	-	-	A	-	A	-	A	A	-	A	-	-	A	*Paenibacillus* sp
17	Rod	+	-	-	+	-	-	+	K	K	-	-	-	+	A	-	A	-	A/g	A	-	A	A	-	A/g	*B. lugardi*
18	Rod	-		-	+	+	+	+	A	A	+	+	-	-	A/g	A	A/g	-	-	-	-	A	-	-	-	*Klebsiella* sp.

Key:+=positive; -=Negative, A=Acid formation; K= Alkaline; A/g= Acid formation and gas production; A= Acid formation alone
MR=Methyl Red, VP= Vogues Poskauer test

Table 5: Bacterial isolates associated with rhizosphere of weeds obtained during the study

Rz1:Cyperus esculentus	***Rz2:Scleria pauciflora.***	***Rz3:Asystasia gangetica***	***Rz4:Harungana madagascariensis.***	***Rz5:Ancistoclaudus erectus.***	***Rz6: Cinna arundinaceae***	***Rz7: Kyllinga erecta***
Achromobacter sp. *B. lichenformis* *B. anthracis* *B. subtilis* *B. fumari* *Arthrobacter* sp. *Pseudomonas* sp. *P. florescence* *P. aeruginosa* *Salinococcus* sp.	*Micrococcus* sp. *B. cereus.* *B. subtilis* *Pseudomonas* sp.	*Acinetobacter* sp. *B. thuringiensis* *Paenibacillus* sp. *Micrococcus* sp.	*B. lugardi* *B. subtilis* *B. thuringiensis* *Achromobacter* sp. *Pseudomonas* sp.	*Klebsiella* sp. *Pseudomonas* sp. *Achromobacter* sp.	*Pseudomonas* sp. *P. aeruginosa.* *Klebsiella* sp.	*B. thuringiensis* *B. subtilis*
Rz8:Cyperus esculentus	***Rz9:Kyllinga bulbosa***	***Rz10:Brachiaria mutica***		**Petroleum Sludge**	**Petroleum polluted pond water**	**Produce water**
B. thuringiensis *P. florescence* *P. aeruginosa* *Arthrobacter* sp. *Klebsiella* sp.	*Staphylococcus* sp. *Micrococcus* sp. *Alcaligenes* sp. *Flavobacterium* sp.	*B. cereus.* *B. subtilis* *Citrobacter* sp. *Alcaligenes* sp. *Pseudomonas* sp. *Klebsiella* sp. *Escherichia* sp.		*B. subtilis* *B. cereus.* *Paenibacillus* sp.	*P. florescence* *P. aeruginosa* *Achromobacter* sp. *Flavobacterium* sp.	*Salinococcus* sp. *Staphylococcus* sp. *Micrococcus* sp. *Klebsiella* sp.

Table 6: Fungal microflora obtained from rhizosphere region of plants pre-exposed to pollution

Sample source	**Total fungal flora**	**Probable Identity**	**Hydrocarbon utilizing fungal flora**	**Probable identity**
Rz1: *C. esculentus*	a) Suded- army green, grey rough reverse side; b) Whitish-suede dense mycelia. Brown reverse side c) Fluffy-white with a ring and raised centre with salt crystals	a)*Aspergillus terreus* b) *Trichoderma* sp. c)*Fusarium* sp.	a)Wooly-white hairlike mycelia b) Green rough surface, brown reverse side	a) *Fusarium* sp. b) *Aspergillus flavus*
Rz2: *S. pauciflora*	a) White mycelia with a black tips covering at the centre b) Dull-leaf green surface with venation c) Fluffy-white with a ring and raised centre with salt crystals	a)*Aspergillus niger* b)*Penicillium* sp. b)*Fusarium* sp.	a)Smooth green surface fungi b) Fluffy-white with a ring and raised centre with salt crystals b)White fluffy, no colour at the reverse side	*Penicillium* sp. *Fusarium* sp. *Mucor* sp.
Rz3: *Asystasia gangetica*	a)Creamy smooth growth and rough depressed centre	a)*Candida* sp.	a)Creamy smooth fungi.	a)*Candida* sp.
Rz4: *A tectorius*	a)Whitish flat mycelia with a circular ring. b)white dense mycelia and spots of liquid crystals	a) *Prunius* sp. b) *Monilia* sp. .	Rough flat – bacterial-like growth	a)Rhodotorula sp.
Rz 5: *K. erecta*	a)Tiny brown-raised mycelia with a cream, rough reverse side b) white Hair-like growth	*Cladosporium* sp. *Mucor* sp.	a) Tiny brown-raised mycelia with a cream, rough reverse side	a) Mucor sp.
Rz6: *C arundinacea*	white dense mycelia and spots of liquid crystals Fluffy-white with a ring and raised centre with salt crystals	*Monilia* sp. *Fusarium* sp.	a) Fluffy-white with a ring and raised centre with salt crystals. b) Creamy smooth fungi.	b) *Fusarium* sp. b) *Candida* sp.

Table 7: Fungal microflora of rhizobacterial flora of plants on pristine soil.

Sample source	**Total fungal flora**	**Probable Identity**	**Hydrocarbon utilizing fungal flora**	**Probable identity**
Ctr1:	a)Suded- army green, grey rough reverse side; b)Tiny brown-raised mycelia with a cream, rough reverse side c)Fluffy-white with a ring and raised centre with salt crystals d)White mycelia with a blacktips covering at the centre	a)*Penicillium* sp b)*Cladosporium* sp. c)*Fusarium* sp d)*Aspergillus* niger	a)Fluffy-white with a ring and raised centre with salt crystals b)White mycelia with a black tips covering at the centre.	a)Fusarium sp b) *Aspergillus niger*
Ctr2:	Bright leaf-green round colony, with veneations Round raised white Hair-like growth	*Penicillium* sp. *Mucor* sp	Smooth, raised, mucoid growth Round raised white Hair-like growth	*Rhodotorula* sp. *Mucor* sp
Ctr3	white dense mycelia and spots of liquid crystals Whitish flat mycelia with a circular ring.	a) *Monilia* sp. b)*Prunius*	No growth	No growth

4.0 Discussion

Phytodiversity of polluted environment is reflective of the history of devastation on the ecosystem, loss in biodiversity, geotechnical and physicochemical qualities. Diversity of plants has been identified as a measure of their ability to tolerate pollutant. Orhorhoro *et al*. (2018) identified *Schoenopletus senegalensis*, *Fuirena umbellate* and *Cyperus tuberosis* in Ogoniland, Rivers State. Edwin-wosu, (2016) reported a vast number of plant species in pristine environment in Rivers State. These separate accounts agree with the report of the present study; *Cyperus esculentus, Scleria pauciflora, Asystasia gangetica, Harungana madagascariensis, Ancistoclaudus tectorius, Kyllinga erecta, Cinna arundinacea,* and *Brassica chinensis* further asserts that plant diversity in aged polluted sites in Rivers State could held fasten the process of recovery from crude oil pollution.

Physicochemical attributes of the soil samples obtained from rhizosphere regions of weed serves as eco-indicators of niches and could further describe the quality of bio-activities within the region of the soil. The pH of the rhizosphere soil during the study was observed to be slightly acidic and temperature mesophilic, *B. chinensis* had a pH 5.4 and a temperature of 31.6^{o}C, samples obtained from *C. esculentus* had a pH 6.9 and 26.3 ^{o}C. These findings corroborated the earlier report of Wang *et al*.(2013) that the temperature of pristine soil should be lower than that of the polluted soil. The current study observed pH and temperature of 6.9 and 29.3 ^{o}C respectively for control sites and this was in agreement with the report of Ofoegbu et al. (2015) reported a pH 6.37 and 28 ^{o}C in their separate investigation conducted in Choba Rivers State. This is because crude fraction could conduct heat and energy. The presence of long-chain and persistent hydrocarbon fraction as well could also have a low degradation process and impact on the pH of the environment. Alkalophilic and mesophilic environments could encourage the synthesis of enzymes and bioavailability of nutrients (Olowomofe, Oluyege, & Sowole, 2017). Electrical conductivity (E.C) is a measure of residual ions, radicals and polarity. In this study, it ranged from 11.32- 80.3 µS/cm. This tallied with the report of Ekwuabu *et al*. (2016) in whose report E.C was 12.0 µS/cm for a pre-impacted soil. The polarity could impact the porosity of the soil, thereby retarding the flow of nutrients and water. Rhizodeposits affects the quality of conduction and ease the passage or flow of nutrients, the variation could arise from the deposits and leaching activity caused by the pollutant. One of the limiting nutrients that retards growth is phosphorus, it ranged from 0.74-5.6 mg/kg. Phosphorus and phosphates aid absorption of nitrates in microbiome. They could be easily washed off by run-offs and seepages. It could also be affected by seasonal variations Wang *et al.* (2013), who reported values as high as 13.9 mg/kg, this was in agreement with the position of this study.

Incidences of oil spills in the Niger Delta have caused devastating damages to arable lands in the region. Bacterial load of the soil suggest a sharp decline in microbial indices, such as 7.5 Log_{10}Cfu/g to 7.77 Log_{10}Cfu/g for THBC for polluted and control respectively. The result for Rz4 *A. tectorius* had a 5.11 Log_{10}Cfu/g while samples obtained from *A. gangetica* had a TAHC of 6.38 Log_{10}Cfu/g. *Kyllinga bulbosa* rhizosphere soil had a population of 6.7 Log_{10}Cfu/g for TFC, 4.76 Log_{10}Cfu/g HUFC while HUBC was 5.16 Log_{10}Cfu/g from polluted soil obtained from Bodo Ogoni, Rivers State. while Soil samples obtained from *S. pauciflora*, had HUB and THBC of 5.79 Log_{10}Cfu/g and 6.04 Log_{10}Cfu/g respectively.

Crude oil polluted water had 6.9 Log_{10}Cfu/g, for THBC, 5.56 Log_{10}Cfu/g, while the total fungal count was 5.36 Log_{10}Cfu/g, Ekwuabu et al.(2016) reported THBC of 7.89 Log_{10}Cfu/g. Furthermore Olowomofe *et al.* (2017) reported 5.3-7.9 Log_{10}Cfu/g for polluted soil in Bodo, Ogoni. The level of microbial load could be used as a predictive component in pollution monitoring and control.

Bacterial diversity in the soil obtained from rhizosphere region of the weeds were documented from the study. The result suggests the dominance of *Bacillus* sp and *Pseudomonas* sp in the rhizosphere other genera included *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *P. aeruginosa*, *P. fluorescens*. Fungal isolates associated with rhizosphere soil were *Aspergillus terreus*, *Trichoderma* sp, and *Fusarium* sp. The result corroborates the report of Olowomofe *et al*. (2017) who isolated bacteria from tar sand with more of *Pseudomonas* sp. and *Bacillus* sp. This corroborates with the report of Yrjälä, Keskinen, Åkerman, Fortelius, & Sipilä, (2010) whose study revealed the preponderance of *Bacillus* sp. at the rhizosphere of weeds. This further agrees with the report of Tesar *et al*., (2002) who reported that Gram-negative bacteria and a few spore formers may be observed from crude oil polluted soil. The report of Omotayo *et al*., (2014) supports that there is a level of interaction of microbes in different environmental media, play may be a key feature in the distribution of soil microbiota. Furthermore, Orhorhoro *et al*. (2018) described the presence of *Arthrobacter* sp., *Bacillus pumilus*, *B. sphaericus* and *Serratia marcescens* in the rhizosphere soils of aged-polluted soil in Gokana Rivers State. *Pseudomonas* sp. *Corynebacterium* sp., *Bacillus* sp. *Bacterioides* sp. *Staphylococcus* sp. *Klebsiella* sp. *and Kingella* sp in the present study. Furthermore, Daane *et al*. (2001) reported the presence of *Flavobacterium*, *Pseudomonas putida* and *Mycobacterium* sp. Ukaegbu-Obi and Mbakwem-Aniebo, (2014) reported the dominance of *Flavobacterium* sp and *Pseudomonas* sp in Rivers State, Nigeria. Van Hamme and Ward, (2001) supported that many organisms have a selective resistance to oil interfaces, thereby secreting an organic acid that aids degradation of hydrocarbon. The findings of this study also corroborate the report of Ukaegbu-Obi and Mbakwem-Aniebo (2014) who reported the presence of *Acinetobacter, Bacillus*, *Pseudomonas*, *Alcaligenes* and Micrococcus as rhizophytes. 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. These bacterial isolates have been associated with degradation and tolerance to petroleum hydrocarbon fractions(Ekwuabu *et al*., 2016; Olowomofe *et al*., 2017; Yrjälä *et al*., 2010)

Conclusion and Recommendation

Niches within rhizosphere of plants are affected are by exudates and exogenous secretions from plant microbe-interaction. Rhizosphere soil from *Cyperus esculentus* had a higher species diversity from both polluted and pristine environments. pH of most soil samples from Goi, Ogoni were slightly acidic and hence encouraged a narrow range of fungal isolates, from the study. Geotechnical considerations suggest total organic carbon, plasticity and porosity of the soil samples were low and were affected by the pollutant. *Pseudomonas* and *Bacillus* sp were the most dominant bacterial isolates while *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. were the most dominant fungi at the rhizosphere region of the weeds.

Recommendation

Rhizobiology and niche-indices of impacted soil could represent a whole new perspective in biomining of high throughput strains for biotechnological development in Nigeria. The weeds obtained from the study and the soil obtained from their rhizosphere region in Bodo, Ogoniland. Microbial isolates obtained during the study suggest a far-reaching microbial diversity at the rhizosphere region harbour countless functional and degradative bacterial communities which could play veritable roles in the clean-up of the pollutants in the Niger Delta.

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