Niche-proxies of soil from rhizosphere of Weeds from 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. Ten (10) *Cyperus esculentus, Scleria pauciflora.,Asystasia gangetica Harungana madagascariensis, Ancistoclaudus tectorius, Kyllinga erecta Cinna arundinacea, Brassica chinensis, Cyperus difformis, Kyllinga bulbosa and Brachiaria mutica* weeds and 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* was 0.61 and 0.70 mg/kg respectively. Moisture content of soil obtained from polluted and pristine environments were 11.75% and 17.82%. Permeability indices were 9.0 describing the soil to have low plasticity. Total heterotrophic bacterial count was within 7.5-7.77 Log_{10} Cfu/g, with associated microbial isolates such as with *Cyperus esculentus* rhizosphere soil was more dominated with others like *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *Ps. aeruginosa*, *Ps. Florescens* present, while fungal isolates were *Aspergillus terreus*, *Trichoderma* sp, and *Fusarium* sp. These findings further supports 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,.

Introduction

Oil exploration in Nigeria has remained a live-wire for growth, sustenance and development of Nigeria, these events have also left the nation with a lot of environmental challenges. Issues such as population exploration, increased industrialization and urbanization have increased the spate of the problems in modern times (Nwachukwu & Osuagwu, 2019). Oilspill is a term used in the industry to mean the release of crude oil or its fractions into the environment. Over 1020 oil spill incidence have been reported in Nigeria, with Niger Delta taking a centre stage of the cases reported in the news media. These cases have caused devastating effects on both fauna and flora of the soil (REF). The effect of pollution on both aquatic and terrestrial ecosystems have different levels of severity to the biota.~~,~~ these Presence of pollutants above recommended threshold in the environment is deleterious to soil biota at varied proximal niches as pollutants ~~can be seen due to the increase~~ are prone to percolation and seepages, ~~the effect of the spill can~~ which have far reaching effect to non-target population (Ofoegbu, Momoh and Nwaogazie 2015). These activities Presence of elevated concentrations of pollutants in the soil affects ~~the~~ soil fertility and

Comment [PW1]: Topic does not clearly reflect what is in manuscript. Rephrase it to reflect the aim.

Comment [PW2]: Rewrite after all corrections. Check for significant findings to appear here. Mention methodologies.

Comment [PW3]: Avoid such terms in writing scientific paper. The first sentence is hanging as the word remained is past tense and refers to a previous explanation which does not exist in this manuscript. Rephrase the sentence.

Comment [PW4]: Which events are you referring t? growth, sustenance and development or Oil exploration. Rephrase the two sentences to flow

Comment [PW5]: Consider using "population explosion".

Comment [PW6]: Which industry do you refer to? Be general "Oil spill refers to the intentional or nonintentional release of crude oil or its fractions to the environment".

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bioavailability of nutrients to plants. These ~~arises from~~ are linked to reduced ~~reduction of~~ porosity (REF) of ~~the~~ soil to both aeration and moisture, ~~severe effect leads to reduction in~~ and reduces soil microbial population (REF) 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 ~~to a number of researchers~~ (Santoyo, Moreno-hagelsieb, Orozco-mosqueda, & Glick, 2016). Some peer review articles have identified a feasibility in the mutualistic interaction between plants and MO for successive adaptation (REF) and other participating niches (REF). However, plants produce carbohydrates, few of nitrate free compounds, these serves as nutrient for microbes which metabolising nutrients and ease absorption by plants. The synergyies in the interrelationships between ~~of~~ microbes and plants ~~have been reported to~~ serve as both ~~as~~ biocontrol and growth promotion of plant (Kannan & Sureendar, 2009). According to Mendes et al. (2013), ~~reported that a number~~ varied communities of bacteria exist on ~~at~~ the root region of plants ~~and~~ where they improve seed germination and viability. Ahemad, (2014) agreed to the earlier opinion that several bacterial genera exist at these regions of interaction and create a balance between plants and microbes. Advantages of having bacteria on the root region of the plants includes; the biogeochemical cycling and adsorption, solubilization and, degradation of nutrients as growth factors to plants. Group of bacteria (rhizobacteria) that adhere to the root ~~(rhizobacteria)~~ have been associated with crop yield and resistance to pest and diseases. Rhizosphere is a narrow region around a plant root, controlling both physicochemical and biochemical conditions. ~~They~~ Roots of plants ~~serve as both~~ provide anchorage systems, play conductive functions, nesting and protective function for soil organisms (Santoyo et al., 2016). This parlance is used to refer to organisms that existed and tolerated exudates from plants and played key role to the plant is were described as rhizospheric organisms also known as rhizobacteria. ~~They~~ Soil microorganisms are competent ~~in colonizing~~ colonizers of the rhizosphere of plant (REF). ~~This is because the~~Plant exuduates ~~which~~ are secretions synthesized from plants and contain~~s~~ a wide array of organic substances which categorizes an exudate to be an attractant or repellent (REF). Some are high and low molecular weight which have been described to influence plant reproductive health and timing of flowering in plants (Lu et al., 2018). This research was designed to assess the microbiological and physicochemical 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 community is situated in Gokana, one of the kingdoms that make up Ogoniland and a LGA in Rivers state. The people of Bodo are predominantly farmers and fishermen/women. The community hosts Shell Petroleum Development Company (SPDC) Trans-Niger pipelines which devastated in 2008 and 2009 by two large oil spills. The spills affected thousands of hectares of mangroves, fishing populations and also the livelihoods of occupants of the community. The study

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Comment [PW12]: Rephrase this sentence

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Comment [PW14]: Let definition of a term appear before you use it. "Rhizosphere"

Comment [PW15]: Which parlance do you refer to? There is no flow in the work

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Comment [PW17]: *Why are these weeds a choice? Cyperus esculentus, Scleria pauciflora.,Asystasia gangetica Harungana madagascariensis, Ancistoclaudus tectorius, Kyllinga erecta Cinna arundinacea, Brassica chinensis, Cyperus difformis, Kyllinga bulbosa and Brachiaria mutica*

Comment [PW18]: At this point explain what interests you to the selected weeds. How important are the selected weeds? Explain earlier research done on the weeds that your results will be analysed against to show case.

Comment [PW19]: Is community the objective? Consider to mention only locality and activities in the locality. Community is mobile unlike the locality.

Comment [PW20]: Are you investigating a community? Why use community? Instead of human community, I suggest that you mention distribution of the weeds in the different localities as the weeds are of interest to the research rather that human community or inhabitants

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 and Tank sludge treatment farm site at NNPC, Alesa, Eleme, Rivers State.

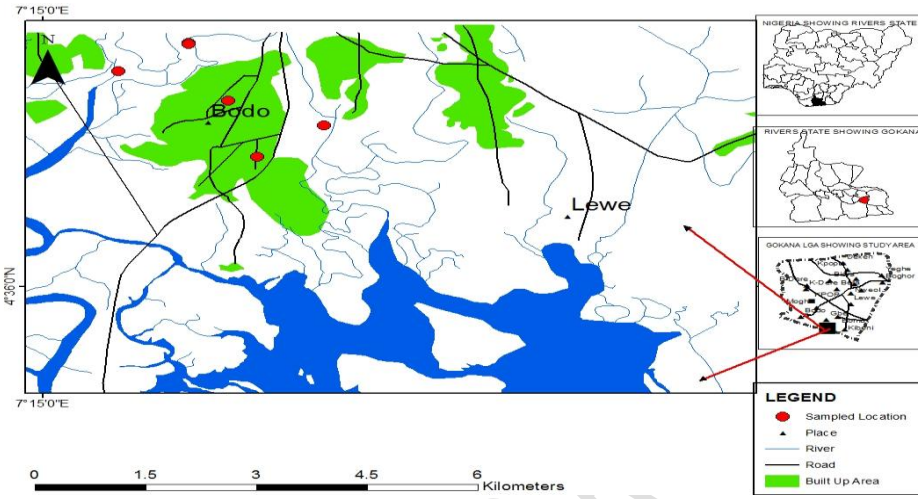

Figure 1: Geo-map of the sample collection points in Bodo, Gokana-Ogoni, Rivers State

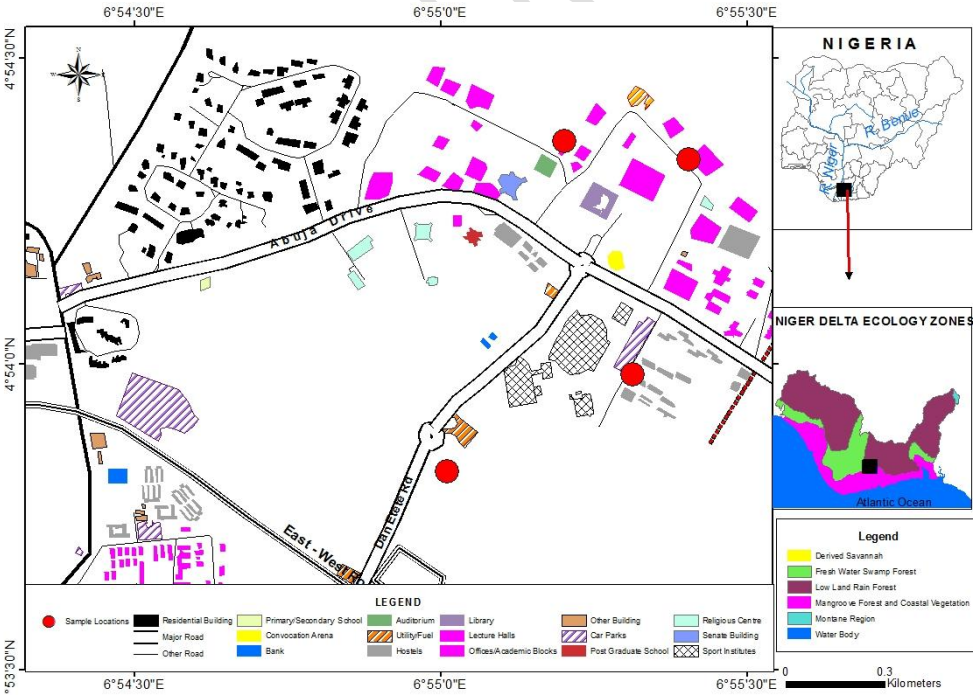

Figure 2: Geo-map of sample collection points in University of Port Harcourt, Rivers State Nigeria

~~**Sample collection:**~~ Collection of samples

Plant samples were harvested from the polluted soil, wrapped in a sterile container, sealed and labelled. The soil from the point of collection of plants were obtained using a sterile ~~hand~~ soil auger to a depth of 0 to 15 cm. Soil samples were labelled with tally on the plants. The samples were transported ~~using a freezing chest~~ to the laboratory in a freezing chest. The plants were deposited at the University of Port Harcourt Herbarium for identification.

Baseline physicochemical and geotechnical characterization of rhizosphere samples

The physicochemical analysis of rhizosphere soil samples were carried out to, the parameters 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). Geotechnical analysis includes 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 sieve method

Enrichment of samples

The soil samples were enriched with Bushnell Haas Media (Lab M) was prepared according to manufacturer's instruction. A measure of 3.2 g of the salt was dissolved in one (1) ~~a~~ 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 (BLCO) was introduced to the media and sterilization was ~~monitored~~ done at 121^{o}C for 15 minutes at 15 psi. Upon cooling, the 1 % crude polluted pond water samples was added to the sterile set up and 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).

Total fungal and heterotrophic bacterial count.

Twenty-eight grams of nutrient agar was dissolved in one (1) litre, pre-heated and sterilized at 121^{o}C for 15 minutes and 15 psi, the media 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 a pre-sterilized normal saline. Colonies range of 3-300 cfu/ml were counted and adjudged good and results lower than the benchmark were repeated for accuracy. Fungal counts were determined by plating 0.1 ml of the diluted sample on Ssaboraud Ddextrose Aagar fortified with 0.1% lactic acid. Spread plate technique was employed and plates were incubated at room temperature (28°C) for 7 days. Result for fungal count were determined after 3 days of incubation (Peekate & Abu, 2017; Abu & Ogiji, 1996).

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Hydrocarbon utilizing bacterial count

Bushnell Haas Mmedium was prepared by dissolving 3.2g/L, fortified with 15 grams of agar, the media was preheated and allowed to cool. About 1.0% One percent of lactic acid was introduced 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°C for 48 hours. Hydrocarbon utilizing fungal count was determined by seeding 100 µg/100ml chloramphenicol for inhibition of bacterial contaminants. About 0.1ml 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 room temperature (Orhorhoro et al., 2018: Ekwuabu, Chikere, & Akaranta, 2016).

Identification of microbial isolates obtained from the study

Bacterial isolates were identified using method 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.

Statistical analysis

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

RESULT

Table 1 describe baseline physicochemical composition of rhizosphere soil obtained from weeds. pH of the soil ranged between 5.26-7.2. The pH of the soil rhizosphere soil obtained 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 °C; *Brassica chinensis* was 31.6 °C while *Cinna arundinacea* was 30 °C While the lowest temperatures was recorded for 26.3 °C for C. *esculentus*, But *Scleria pauciflora* and *Harungana madagascariensis* had a temperature of 27 °C. Refinery effluent had a conductivity of 5520.9 µs/cm and *Brassica chinensis* was 400.5 µs/cm. Rhizosphere soil obtained from Bodo, Gokana were 80.31 µs/cm and 53.4 µs/cm was reported for *Cyperus esculentus* and *Kyllinga erecta*. Phosphate was lowest with soil obtained from *Brassica chinensis* which was 0.74 mg/kg and 5.4 mg/kg phosphate was reported for *Cyperus esculentus*. Heavy metals like Nickel was below detectable level for most weeds in 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*

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164 *arundinacea*. The heavy metal (Nickel, Zinc, Vanadium, Lead, Iron and Chromium) were
165 not detected in the control samples (Table 2).

166

Table 1: Baseline Physicochemical composition of rhizosphere soil obtained from plants in Ogoni, Rivers State

	Rhizosoil (polluted soil Bodo)								
Baseline Parameters	*Cyperus esculentus*	*Scleria pauciflora.*	*Asystasia gangetica*	*Harungana madagascariensis*	*Ancistoclaudus tectorius*	*Kyllinga erecta*	*Cinna arundinacea*	Refinery Effluent	*Brassica chinensis*
pH	6.9	6.9	7.20	6.50	5.26	5.90	6.3	6.98	5.4
Temperature (^{o}C)	26.3	27.0	28.0	27.0	29.0	28.7	30.0	27.2	31.6
Conductivity (µS/cm)	53.4	40.2	48.6	32.1	11.5	80.3	11.3	5520.9	400.5
Oil and Grease (mg/kg)	90.3	74.6	61.4	113.2	100.5	67.9	73.4	237.1	111.6
Salinity (ppt)	68.8	70.7	60.0	50.6	42.7	52.4	88.7	229.80	61.5
Alkalinity	18.8	16.1	22.1	12.1	11.17	15.43	67.5	19.2	12.7
Phosphate(mg/kg)	5.6	3.10	1.8	2.1	3.0	4.1	5.3	3.69	0.74
Ammonia (mg/kg)	2.14	1.30	1.0	0.50	1.10	2.0	1.95	1.98	1.32
Phenol (mg/kg)	90.3	74.6	61.4	50.4	34.5	26.5	110.8	76.9	15.3
Hydrogen sulphide(mg/kg)	12.4	11.5	11.7	20.7	31.6	19.9	43.3	51.3	20.2
Nickel (mg/kg)	BDL	BDL	BDL	BDL	BDL	BDL	0.02	0.08	1.1
Zinc (mg/kg)	0.14	0.10	0.32	0.6	1.5	1.7	3.34	0.12	5.8
Vanadium (mg/kg)	0.04	0.01	0.07	0.01	0.31	0.71	0.7	0.18	0.01
Lead (mg/kg)	BDL	0.1	0.18	0.2	0.01	0.05	0.02	0.08	0.09
Iron (mg/kg)	0.26	0.14	0.09	0.2	0.20	0.33	0.01	0.01	0.63
Chromium (mg/kg)	0.08	0.04	0.02	0.01	0.05	0.021	0.04	0.08	0.02
Sulphates(mg/kg)	4.8	5.8	9.4	3.3	1.9	10.8	15.4	3.0	6.45

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

		Rhizosphere soil (Uniport)	
Baseline Parameters	*Cyperus difformis*	*Kyllinga bulbosa*	*Brachiaria mutica*
pH	6.9	5.90	7.8
Temperature (^{o}C)	29.3	31.7	28.0
Conductivity (µS/cm)	103.4	80.31	212.32
Oil and Grease (mg/kg)	40.3	17.9	8.4
Salinity (ppt)	21.8	12.4	15.7
Alkalinity	11.8	4.7	3.8
Phosphate(mg/kg)	9.6	11.1	7.7
Ammonia (mg/kg)	2.14	2.0	1.95
Phenol (mg/kg)	4.5	3.11	5.2
Hydrogen sulphide(mg/kg)	1.4	1.0	7.3
Nickel (mg/kg)	BDL	BDL	BDL
Zinc (mg/kg)	BDL	BDL	BDL
Vanadium (mg/kg)	BDL	BDL	0.01
Lead (mg/kg)	BDL	0.05	BDL
Iron (mg/kg)	1.26	1.91	1.5
Chromium (mg/kg)	BDL	0.006	BDL
Sulphates(mg/kg)	1.06	1.8	3.1
TPH (mg/kg)			

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Table 3 describes the geotechnical evaluation of the soil sample obtained ~~from the~~from the pristine soil from ~~uniport~~ the herbarium in UNIPORT. The result showed that the ~~soil had~~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 6.3e~~permeabilities of 6.3e^{-6} and 4.73e^{-6} cm/sec respectively. Organic carbon was high with the polluted soil with 31.85%. Plasticity of the soil 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 %)	82.43	26	9.01
Clay (wt %)	14.19	40.7	1.98
Silt (wt %)	2.48	39.79	87.72
Soil type	Loamy sand	Clay Loam	Silt loam
Moisture content (%)	17.82	11.52	11.75
Permeability (cm/sec)	2.23E-03	6.30E-06	4.73E-06
Permeability description	Moderate	Low	Low
Organic carbon (%C)	31.85	3.81	129.63
Total Organic Carbon (mg/kg	11.48	22.79	300.09
Total Hydrocarbon content (mg/kg)	5.8	9.1	95
Liquid limit (%)	27.41	20.52	18.73
Plastic limit (%)	18.51	17.93	13.35
Plasticity index	8.91	9.0	7.1
Plasticity description	Low	Low	Low
Particulate size distribution	Silt(0.02 mm) Clay (0.002mm) Sand (2.00mm)	Silt(0.02 mm) Clay (0.002mm) Sand (2.00mm)	Silt(0.02 mm) Clay (0.002mm) Sand (2.00mm)

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~~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 a TAHC of 6.38 Log_{10}Cfu/g. *Kyllinga bulbosa* 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. Soil samples obtained from *S. pauciflora*, had HUB and THBC of 5.79 Log_{10}Cfu/g and 6.04 Log_{10}Cfu/g. 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

Table 4 represents microbial evaluation of the soil samples obtained from weeds of Bodo polluted soil, *Cyperus esculentus* rhizosphere soil was dominated with *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *Ps. aeruginosa*, *Ps. florescens*, Fungal isolates associated with plant samples were presented in Table 5. Organisms revealed includes *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. Refinery wastewater was reported to harbour *Salinococcus* sp. *Staphylococcus* sp. and *Micrococcus* sp. while fungal isolates are *Monilia* sp. and *Prunius* sp. (Tables 5, 6 and 7).

Comment [PW40]: These soils are definitely of different types which has significant influence on rhizosphere biota. What was the objective of testing the weeds in different kinds of soils? If they were similar weeds subjected to different kinds of soils it would be better. This compromises the whole results as is already known to be different.

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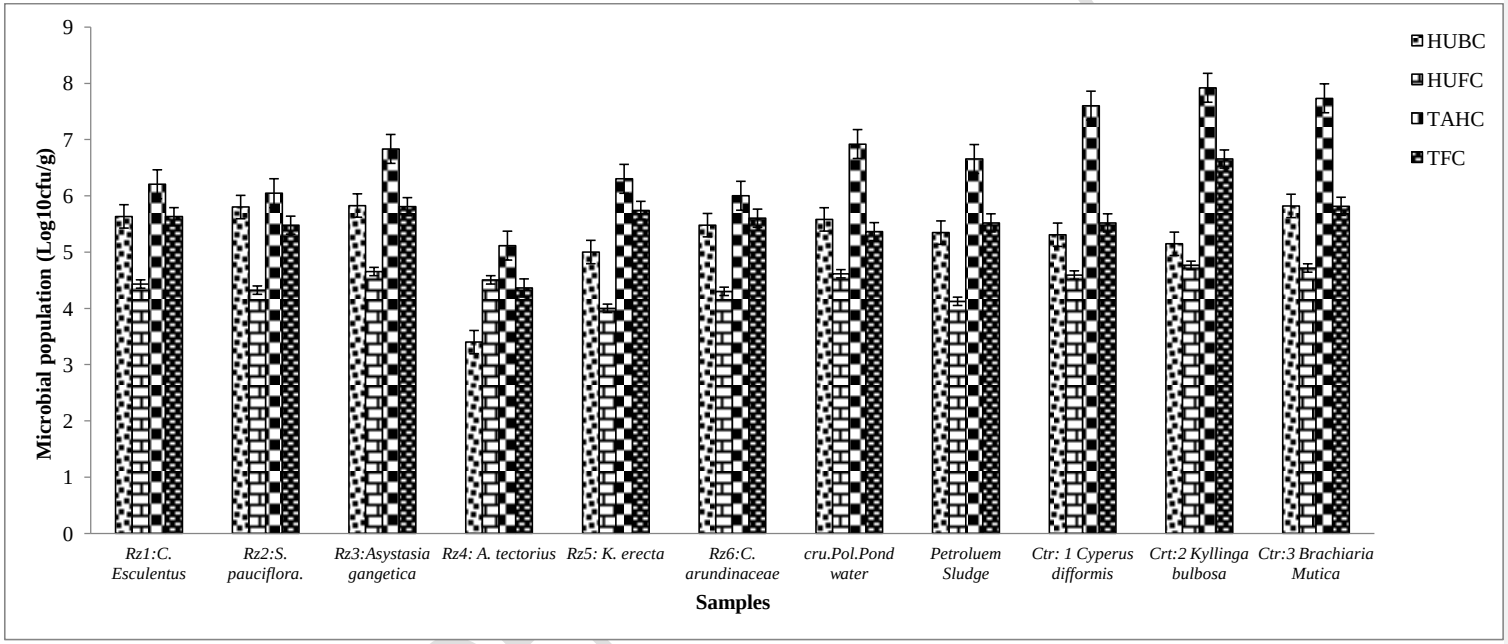

Legend: HUBC= Hydrocarbon utilizing bacterial count; HUFC= Hydrocarbon Utilizing Fungal count, TAHC= Total heterotrophic Bacterial count; TFC= Total Fungal Count

Figure 3: Average microbial population of Rhizosphere soil, pond water, petroleum sludge obtained from plants pre-exposed to crude oil and pristine environment

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	*Ps. 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 thuriiensis*
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

220 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.	*Micrococcus* sp.	*Acinetobacter* sp.	*B. lugardi*	*Klebsiella* sp.	*Pseudomonas* sp.	*B. thuringiensis*
B. lichenformis	*B. cereus.*	*B. thuringiensis*	*B. subtilis*	*Pseudomonas* sp.	*Ps. aeruginosa.*	*B. subtilis*
B. anthracis	*B. subtilis*	*Paenibacillus* sp.	*B. thuringiensis*	*Achromobacter* sp.	*Klebsiella* sp.	
B. subtilis	*Pseudomonas* sp.	*Micrococcus* sp.	*Achromobacter* sp.			
B. fumari			*Pseudomonas* sp.			
Arthrobacter sp.						
Pseudomonas sp.						
Ps. florescence						
Ps. aeruginosa						
Salinococcus sp.						
Rz8:Cyperus esculentus	***Rz9:Kyllinga bulbosa***	***Rz10:Brachiaria Mutica***		**Petroleum Sludge**	**Petroleum polluted pond water**	**Produce water**
B. thuringiensis	*Staphylococcus* sp.	*B. cereus.*		*B. subtilis*	*Ps. florescence*	*Salinococcus* sp.
Ps. florescence	*Micrococcus* sp.	*B. subtilis*		*B. cereus.*	*Ps. aeruginosa*	*Staphylococcus* sp.
Ps. aeruginosa	*Alcaligenes* sp.	*Citrobacter* sp.		*Paenibacillus* sp.	*Achromobacter* sp.	*Micrococcus* sp.
Arthrobacter sp.	*Flavobacterium* sp.	*Alcaligenes* sp.			*Flavobacterium* sp.	*Klebsiella* sp.
Klebsiella sp.		*Pseudomonas* sp.				
		Klebsiella sp.				
		Escherichia sp.				

221 Table 6: Fungal microflora obtained from rhizosphere region of plants pre-exposed to
222 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.

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225 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 black tips 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

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Discussion

Phytodiversity of polluted environment is reflective of the history of devastation on the ecosystem, loss in biodiversity, geotechnical and physicochemical qualities. Adaptation ofto plants ~~have~~ has been identified as a measure of adaptability to tolerate pollutant. Orhorhoro et al., (2018) identified *Schoenopletus senegalensis*, *Fuirena umbellate* and *Cyperus tuberosis* Edwin-wosu, (2016) reported a vast number of plant species in pristine environment in Rivers State. These separate accounts agrees with the report of the present study; *Cyperus esculentus*, *Scleria pauciflora*, *Asystasia gangetica*, *Harungana madagascariensis*, *Ancistoclaudus tectorius*, *Kyllinga erecta*, *Cinna arundinacea*, and *Brassica chinensis.*

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~~activities within the region of the soil. 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 had pH and temperature of 6.9 and 29.3 ^{o}C respectively. However Ofoegbu (2015) reported a pH 6.37 and 28 ^{o}C in their separate investigation. 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. Which 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. Rhizodeposites 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, as 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), although they reported 13.9 mg/kg, this was in agreement with the position of this study.

Incidence of oil spill in Niger Delta have caused devastating damages to arable lands in the region. Microbiological qualities of the soil revealed a steady decline from the microbial indices, the range revealed that 7.5 Log_{10}Cfu/g to 7.77 Log_{10}Cfu/g. 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. 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. 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 THB 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,

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Ogoni. The level of microbial interaction could be used as predictive component pollution monitoring and control.

Bacterial diversity in~~of~~ the soil obtained from rhizosphere region of the weeds were documented from the study. The result ~~suggest~~suggests the dominance of *Bacillus* sp and *Pseudomonas* sp from the soil with presence of *Achromobacter* sp, *B. lichenformis*, *B. anthracis*, *B. subtilis*, *B. fumari*, *Arthrobacter* sp, *Pseudomonas* sp, *Ps. aeruginosa*, *Ps. Florescens*. Fungal isolates associated with samples were *Aspergillus terreus*, *Trichoderma* sp, and *Fusarium* sp. The result corroborates with the report of Olowomofe et al. (2017) where they isolated bacterial isolates from tarsand 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 ~~opined~~ showed that the dominance of Gram negative microbes and few spore formers may be observed from crude oil polluted soil. The report of Omotayo *et al*., (2014) supports ~~the fact~~ that there is a level of sociomicrobial interaction of microbes in different environmental media, plays a key feature in the distribution of soil microbiota. Furthermore, Orhorhoro et al. (2018) corroborated the earlier studies and they were able to associate *Arthrobacter* sp., *Bacillus pumilus*, *B. sphaericus* and *Serratia marcescens*. This study revealed the presence of *Pseudomonas* sp. *Corynebacterium* sp., *Bacillus* sp. *Bacterioides* sp. *Staphylococcus* sp. *Klebsiella* sp. *and Kingella* sp. However, 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. Van Hamme and Ward, (2001) suggested that these organisms have a selective resistance to oil interfaces, thereby secreting an organic acid that aids degradation of hydrocarbon. The findings of this study also ~~corroborates~~corroborate the report of Ukaegbu-Obi and Mbakwem-Aniebo (2014) who reported the presence of *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Alcaligens* 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 petroleum hydrocarbon fractions.(Ekwuabu et al., 2016; Olowomofe et al., 2017; Yrjälä et al., 2010)

Conclusion and Recommendation

Niches within rhizosphere of plants affected by exudates and exogenous secretions from plant microbe-interaction. The quality exudates either as an attractant or repellent affects microbial indicators of niches. Rhizosphere soil from *Cyperus esculentus* had a higher species diversity. Bacterial load was observed to decline. pH of most soil samples were slightly acidic and hence encouraged a narrow range of fungal isolates. 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 microbes at the rhizosphere region of weeds.

Comment [PW44]: Opined? Is it an opinion? Did he conduct research to make it factual? Check application of word opined to support research findings

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Comment [PW45]: Ckeck application of word "However"

Comment [PW46]: Rephrase to bring meaning

Comment [PW47]: Did this study test the types of plant exudates? No. pollution was the key target. So conclude on impact of pollution but not exudates. Or link the exudates to crude oil. Is there any synergistic effect or are there suppressant effects?

Comment [PW48]: It is not in order to conclude this because the soil type effect also contributes to this difference. You need to address this anomaly.

Comment [PW49]: Where? Hanging sentence

Comment [PW50]: Too general. Give the specific sites with low pH

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. Weeds in Bodo, Ogoniland could harbour countless families of functional and degradative bacterial communities which could be veritable roles in the clean-up of the pollutants in the Niger Delta.

Comment [PW51]: Recommend on your findings but not what you think

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Comment [PW52]: Confirm all references to be in text and the reference section