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Full Length Research Paper

Nitrogen fixing capacity of legumes and their Rhizospheral microflora in diesel oil polluted soil in the tropics

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The nitrogen fixing capacity of legumes cowpea *(Vigna unguiculata)* and groundnut *(Arachis hypogea)* and their micrflora grown in diesel oil simulated utisol was investigated. Result revealed that concentration as low as 1% v/w of diesel oil significantly affected the densities of nitrogen fixing bacteria, bacteriods, actinomycetes and fungi associated with the legumes. The heterotrophic bacteria count in the rhizosphere of cowpea reduced from $2.46 \pm 0.72 \times 10^7$ to $1.5 \pm 0.37 \times 10^7$ cfu/g after a growth duration of 12 weeks while it reduced from $3.4 \pm 1.25 \times 10^7$ to $1.52 \pm 0.36 \times 10^7$ cfu/g for groundnut in the same growth period. Nitrifying bacteria count reduced from $3.25 \pm 1.19 \times 10^4$ to $4.5 \pm 0.18 \times 10^3$ cfu/g for cowpea and $3.43 \pm 1.23 \times 10^4$ to $2.7 \pm 0.21 \times 10^3$ cfu/g for groundnut. Bacteriods count also significantly ($P > 0.05$) reduced from $3.85 \pm 2.30 \times 10^5$ cfu/g for the control treatment to $1.25 \pm 2.23 \times 10^5$ cfu/g in 1% level of pollution with no bacteriod formed in both 4 and 8% pollution due to inhibition of nodule formation by the diesel oil. Significant reduction ($P > 0.05$) was also observed in fungal and actinomycetes counts. Generally, organisms in the rhizosphere of groundnut exhibited more tolerance to diesel oil pollution than those found in the rhizosphere of cowpea. This study revealed that diesel oil adversely affected nitrogen fixing bacteria and bacteriods and consequently the nitrogen fixation in the soil.

Key words: *Vigna unguiculata*, *Arachis hypogea*, bacteriods, rhizosphere, actinomycetes.

INTRODUCTION

Legumes are plants that produces nitrogen fixing root or stem nodules which forms symbiotic association with Rhizobium. They include beans, peas, clovers and soybean (Harrison, 2003). The emergence of crude oil industries has contributed immensely to changing the state of Nigeria economy and the environment. The oil industry is a major source of environmental pollution and its adverse ecological impacts have been reported (Ibia et al., 2002; Ekpo and Thomas, 2007).

This is widely spread with specifically more serious damage on the oil producing areas. The most obvious area which has generated a lot of concern is spillage resulting from oil well blowout or pipeline leakages with each major spill incident increasing the vulnerability of our fragile environment (Ibe, 2000; Ekpo and Nwankpa, 2005). The impact of petroleum exploration could alter essential microbial biogeochemical cycling processes, resulting in altered productivity of affected ecosystem (Caravaca and Rodan, 2003).

Pollution generally, can lead to a succession or total extinction of species in the affected habitat (Budny et al., 2002; Delille and Pelletier, 2002). This is because most spills are often toxic and generally cause deficiency in essential plant nutrients. Apart from phytotoxicity, nitrogen is the major element limiting plant growth in most spills and hydrocarbon contaminated sites (Wyszkowski et al., 2004).

This investigation was to assess the effects of diesel oil pollution on the rhizospheral microflora and the nitrogen fixing capacity of legumes in the tropics.

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MATERIALS AND METHODS

Soil analysis

The soil used was an acidic sandy loamy soil classified as ferralitic sandy loam utisol (D'Hoore, 1964) collected from the botanical garden of University of Uyo, Nigeria. The soil had no previous exposure to petroleum hydrocarbons. The physico- chemical characterization of the soil samples were carried out using standard methods. The initial soil sampling was carried out for routine analysis. The soil samples were collected at a depth of 0 – 15 cm, air dried and sieved through a 2 mm sieve. The particles size distribution was determine by the pipette method of (Gee and Bauder, 1986). Soil pH was done using 1:2 soil/water volume ratio on a digital pH meter (model EQ-610.). Organic carbon in the soil was determine by the dichromate oxidation method of Walkey-Black wet oxidation as describe by Nelson and Sommers (1982). Total nitrogen was estimated by macro-Kjeldhal procedure (Jacson, 1962). Available phosphorus was estimated using the method of Bray and Kurtz (1945). Exchangeable bases Ca, Mg, Na and K were extracted using Batch method with 1 M NH_4OH. Ca and Mg were determined using Ethylene Diamine Tetra Acetic Acid (EDTA) and measured using a flame photometer. Exchangeable acidity in the soil was extracted with 1 M KCl and determined for Aluminum by titration method with 0.05 M NaOH. Fe, Cu, Zn and Mn were extracted with sodium bicarbonate and their concentrations read from Atomic Absorption Spectrophotometer (AAS). The total hydrocarbon content (THC) of the soil was estimated calorimetrically at 400 nm using Fisher's Electro-photometer after extraction with Carbon tetrachloride.

Soil treatment

The soil collected from botanical garden of University of Uyo, Nigeria, was polluted with diesel oil. Each experimental unit had 3 kg of soil weighed into pots. The diesel oil was applied evenly using a fine hose and properly worked into the soil in each of the treatments (0, 30.0, 120 and 240 ml) and replicated six times. These gave a percentage pollution of 0, 1, 4 and 8%v/w. The pots were then labelled and exposed to ambient conditions for one week before planting the test crops. Pots containing garden soil with no oil supplements served as control.

Cultivation of test crops

The legumes *Vigna unguiculata* (cowpea) of the variety IT 97 400-3 and *Arachis hypogea (*groundnut) of the variety Rm P12 belonging to the families papilioceae were obtained form the University of Agriculture, Umudike, Nigeria. Apparently, healthy seeds were sorted out and 4 seeds of each test crops were planted in the oil simulated soils. The seeds on germination were thinned to 2 seedlings per pot to create space for vigorous growth.

Enumeration of microorganisms from rhizosphere and nodules

The rhizosphere microorganisms of legumes were enumerated by the viable plate count method of Collins and Lyne (2004). Two samples of the rhizosphere soil (plant root plus adhering soil) were carefully obtained per plant for each treatment (oil contamination and uncontaminated (control) utisols. 10 g of the soil sample was uniformly mixed in 100 ml of sterile water. Ten-fold serial dilutions ranging from 10^{-1} to 10^{-7} of the oil contaminated and uncontaminated soil samples were prepared. From each dilution, 0.1 ml was plated out on appropriate microbial media. Bacto- plate count agar (Difco) plates, into which three drops of fungizone (50 gml^{-1}) had been incorporated to inhibit fungal growth, were used for the estimation of heterotrophic bacteria. Duplicates plates from dilution 10^{-6} were prepared and incubated at 28 ± 2°C for 48 h before enumeration.

Bacto–Actinomycete agar (Difco) was used for the enumeration of actinomycetes in the rhizosphere of the crops. The pH of the medium was adjusted to 5.5 by incorporation of lactic acid to suppress the growth of non- filamentous bacteria. The plates were incubated at 28 ± 2°C for 48 h before enumeration. The number of diazotrophic bacteria was estimated on Bacto- nitrate agar (Difco). Inoculated nitrate agar plates were incubated at 37°C for 4 days before enumeration. Uninoculated nitrate agar plates were also incubated to serve as control. The rhizosphere fungal flora of the legumes were enumerated on Sabouraud dextrose agar (Difco) plates into which three drops of streptomycin (50 gml^{-1}) was incorporated to suppress bacterial growth. The fungal plates were incubated at ambient temperature for 5 - 7 days before enumeration.

The bacteriods were enumerated using the method of Agboola (1986). Nodules were carefully obtained per plant for each treatment (oil contaminated and uncontaminated soil). The nodules were held in a Gooch crucible and washed thoroughly. The crucible containing the nodules was then dipped into a Petri dish containing diluted mercuric chloride solution (ratio 1:1000) for 5 min. These were then immersed into successive dishes of sterile water, using sterile forceps and were crushed in a sterile moter. 1 ml of sterile water was then put into the crushed nodule to obtain 10^{-1} dilution. From this a serial dilution to a dilution of 10^{-4} for the oil contaminated and uncontaminated nodule sample were prepared. 0.1 ml of the forth dilution was plated on yeast mannitol agar. Bacteriods plates were incubated at ambient temperature for 5 - 7 days before enumeration.

Isolation of microorganisms from rhizosphere and nodules

Discrete bacterial colonies which developed on the plates were randomly picked and purified by sub-culturing into fresh nutrient agar plates using streak plate techniques. Discrete colonies which appeared on the plates were then transferred into nutrient agar slants and stored as stock cultures for further test.

The fungi present in the rhizosphere of the legumes were isolated using the same procedure for bacterial isolation above but employing Sabauraud dextrose agar plates fortified with streptomycin. A portion of each fungal colony which developed was picked using sterile inoculating needle and aseptically sub- cultured into fresh Sabauraud dextrose agar plate. The plates were kept as stock cultures for identification tests.

Characterization and identification of isolates

The bacterial isolates were gram-stained and examined morphologically. Biochemical characterization was carried out including the isolates' ability to assimilate sugars. The probable identities of the isolates were determined using methods described by Holt et al. (1994), Buchanan and Gibbons (1974). The fungal isolates were examined macroscopically and then identified according to the method described by Domsch et al. (1980), Huntar and Benneth (1973) and Larone (1976). Bacteriods isolates were identified based on their cultural characteristics and cell morphology (Agboola, 1986).

RESULTS

The physico-chemical properties of the soil and the

Table 1. Some physicochemical properties of utisoil investigate.

Soil properties	Utisol before pollution					Utisol treated with diesel oil (12 weeks after pollution)			
		Cowpea (% v/w)				Groundnut (% v/w)			
Particle size (Depth 0 - 15 cm)		**0**	**1**	**4**	**8**	**0**	**1**	**4**	**8**
Sand	85.6	81.92	79.92	79.92	81.92	83.92	81.92	79.92	79.92
Silt	10.2	8.00	8.00	10.00	10.00	8.00	10.00	8.00	10.00
Clay	12.8	10.08	12.08	10.08	8.08	8.08	10.08	12.08	12.08
Chemical properties									
pH	6.16	6.1	6.40	6.30	6.00	6.10	6.10	6.20	6.20
Electrical Conduct. (ds/m)	0.047	0.327	0.168	0.132	0.092	0.024	0.100	0.136	0.107
Organic carbon (%)	1.84	1.79	2.31	2.69	4.11	1.10	2.89	2.02	4.86
Total Nitrogen (%)	0.134	0.05	0.10	0.12	0.18	0.06	0.13	0.11	0.21
Available P.(mg/kg)	69.93	65.33	56.66	52.99	47.33	68.66	52.66	50.66	47.99
Exchangeable									
- Ca (mol/kg)	2.27	2.56	2.40	2.60	2.00	2.34	2.40	2.70	2.48
- Mg (mol/kg)	1.20	1.10	1.10	1.20	1.00	1.13	1.07	1.20	1.30
- Na (mol/kg)	0.06	0.06	0.04	0.05	0.05	0.05	0.04	0.05	0.04
- K (mol/kg)	0.08	0.07	0.07	0.06	0.07	0.08	0.07	0.07	0.06
Exchangeable acidity									
(mol/kg)	1.56	1.44	1.60	1.76	1.40	1.50	2.64	1.60	1.90
Cation Exchange									
Capacity (cmol/kg)	5.17	5.23	5.11	5.68	4.52	5.37	6.22	5.62	5.78
Base saluation (%)	69.83	68.07	68.69	69.04	69.03	67.35	57.56	71.53	67.13
Total Hydrocarbon (mg/kg)	0.00	0.00	30.0	50.0	300.0	0.00	0.00	50.0	100.0
Heavy metals									
Fe	923.5	1313	1251	1251	1042	1396	1021	1125	1084
Cu	18.20	20.3	18.4	19.35	20.85	22.15	24.35	24.65	22.1
Zn	67.55	82.6	84.7	80.75	87.5	83.6	100.3	102.6	85.35
Mn	253.6	363.2	370.8	373.6	371.6	383.7	408.1	413.8	332.1
Cd		1.05	0.8	1.20	0.55	1.80	1.45	0.95	1.10
Cr		43.75	85.45	55.85	48.8	58.9	77.05	655.5	82.1
V		3.55	4.95	2.40	3.75	2.90	5.45	4.30	2.45

experimental plot before and after pollution are presented in Table 1. The soil, an acidic sandy loamy soil (utisol) with no hydrocarbon content, had originally considerable amount of nitrogen (0.134%), available phosphorus (69.93 mg/kg) and organic carbon (1.84%). On simulation with diesel oil, organic carbon content of the utisol increased with increased level of pollution while available phosphorus reduced to 47.33 and 47.99 mg/kg in the 8% soil treatment in cowpea and groundnut respectively. Also the total nitrogen content were slightly increased in 8% polluted soil to 0.18 and 0.21% for cowpea and groundnut respectively within 12 weeks after planting. The simulated soil had THC of 30 and 50 mg/kg in cowpea and groundnut respectively at 1% pollution level.

Microbial analysis of the rhizosphere and nodules of the crop cultivated in the oil contaminated and uncontaminated utisol are presented in Tables 2, 3, 4, 5 and 6. Table 2 shows the total heterotrophic bacteria count (THBC) in the rhizosphere of the legumes. There was a gradual increase in the THBC up to the 8^{th} weeks after planting (WAP) and thereafter reduction in some of the treatments. It was however, observed that there was a significant decrease in count in the rhizosphere of both the cowpea and groundnut in the soil with higher levels of pollution.

The actinomycetes count in the rhizosphere of legumes is presented in Table 3. It was observed that the highest pollution level of diesel oil significantly reduced the mean count to $6.5 \pm 0.37 \times 10^2$ cfu/g for cowpea and $1.9 \pm 0.06 \times 10^2$ cfu/g for groundnut compared to the control with 1.6

Table 2. Heterotrophic bacteria count (x10^7 cfu/g) in the rhizosphere of legumes grown in oil contaminated and uncontaminated ultisol

Crop	Treatment	Age (weeks after planting)						
		2	4	6	8	10	12	mean
Cowpea	0	1.51	1.95	2.31	2.5	3.01	3.51	2.46±0.72
	1	1.32	1.81	1.91	2.0	2.54	2.71	2.04±0.50
	4	1.21	1.5	1.7	1.82	2.02	2.22	1.75±0.36
	8	1.03	1.21	1.41	1.56	1.81	2.03	1.50±0.37
Groundnut	0	2.0	3.0	5.02	4.7	3.53	2.2	3.40±1.25
	1	1.51	2.82	4.0	3.51	3.11	2.82	2.16±0.84
	4	1.31	1.51	3.51	3.0	2.72	2.52	2.42±0.85
	8	1.0	1.31	2.01	1.81	1.61	1.41	1.52±0.36

Cowpea, Sx = 0.69. $LSD_{0.05}$ = 0.51. *Significant at P > 0.05, Groundnut, Sx =.1.09. $LSD_{0.05}$ = 0.88. *Significant at P >0.05.

Table 3. Actinomycetes count (x10^3 cfu/g) in the rhizosphere of legumes grown in oil contaminated and uncontaminated utisol

Crop	Treatment	Age (weeks after planting)						
		2	4	6	8	10	12	mean
Cowpea	0	0.51	1.5	1.8	1.92	2.01	2.21	1.65±0.61
	1	0.43	1.3	1.51	1.72	1.8	2.0	1.46±0.55
	4	0.21	1.01	1.31	1.51	1.71	1.82	1.26±0.59
	8	0.12	0.33	0.61	0.8	0.94	1.12	0.65±0.37
Groundnut	0	0.7	1.3	2.8	3.0	3.2	3.9	2.48±1.22
	1	0.5	1.2	2.5	2.0	2.2	2.5	1.81±0.82
	4	0.4	1.0	1.5	1.0	1.2	1.5	1.10±0.41
	8	0.3	0.23	0.2	0.13	0.06	0.14	0.19±0.06

Cowpea, Sx = 0.65. $LSD_{0.05}$ = 0.58. *Significant at P > 0.05. Groundnut, Sx =.1.12. $LSD_{0.05}$ = 0.75. *Significant at P >0.05.

Table 4. Nitrifying bacteria count (x10^4cfu/g) in the rhizosphere of legumes grown in oil contaminated and uncontaminated ultisol.

Crop	Treatment	Age (weeks after planting)						
		2	4	6	8	10	12	mean
Cowpea	0	1.52	2.5	3.01	3.73	4.02	4.91	3.28±1.19
	1	1.01	1.51	2.01	1.5	1.32	1.01	1.39±0.37
	4	0.6	1.4	1.0	0.8	0.61	0.4	0.80±0.35
	8	0.41	0.61	0.71	0.51	0.3	0.21	0.45±0.18
Groundnut	0	1.81	2.4	3.11	3.8	4.5	5.01	3.43±1.23
	1	1.22	1.0	0.71	0.51	0.3	0.2	0.65±0.39
	4	0.81	0.6	0.5	0.3	0.21	0.11	0.42±0.26
	8	0.6	0.42	0.31	0.21	0.09	0.04	0.27±0.21

Cowpea, Sx = 1.27. $LSD_{0.05}$ = 0.65. *Significant at P > 0.05. Groundnut, Sx =.1.46. $LSD_{0.05}$ = 0.65. *Significant at P >0.05.

± 0.61 × 10^3 cfu/g and 2.48 ± 1.22 × 10^3 cfu/g respectively. Table 4 shows the nitrifying bacteria count in the rhizosphere of legumes grown in diesel oil contaminated utisol. The nitrifying bacteria were found to be highly sensitive to the diesel oil that even 1% pollution was significantly different compared to the control.

Fungi count in the rhizosphere of legumes in soil contaminated with diesel oil is presented in Table 5. The rhizosphere of cowpea was observed to enhanced greater fungal growth compared to that of groundnut. There was however, a significant decrease caused by the diesel oil contamination of the soil. Table 6 shows the

Table 5. Fungi count (x10^4cfu/g) in the rhizosphere of legumes grown in oil contaminated and uncontaminated ultisol.

Crop	Treatment	Age (weeks after planting)						
		2	4	6	8	10	12	mean
Cowpea	0	7.1	9.1	10	12	13	15.1	11.5±2.88
	1	1.5	2.1	4.1	5.1	7.1	8.0	4.65±2.61
	4	1.3	3.0	5.0	6.1	6.4	7.0	4.80±2.21
	8	0.4	1.1	3.0	4.0	4.4	5.2	3.02±1.90
Groundnut	0	0.4	0.61	0.7	0.91	1.61	1.7	0.98±0.54
	1	0.12	0.2	0.2	0.32	0.41	0.81	0.34±0.25
	4	0.14	0.2	0.24	0.3	0.51	0.72	0.35±0.22
	8	0.2	0.32	0.44	0.51	0.7	0.92	0.51±0.26

Cowpea, Sx = 3.86. $LSD_{0.05}$ = 2.42. *Significant at P > 0.05. Groundnut, Sx =.0.41. $LSD_{0.05}$ = 0.34. *Significant at P > 0.05.

Table 6. Bacteroids count (x 10^5 cfulg) in the rhizosphere of legumes grown in oil contaminated and uncontaminated utisol.

Crop	Treatment	Age(weeks after planting)						
		2	4	6	8	10	12	mean
Cowpea	0	0	5.3	5.1	5.0	5.7	2.0	3.85±2.30
	1	0	5.5	2.0	0	0	0	1.25±2.23
	4	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0
Groundnut	0	0	5.1	4.8	7.7	5.3	6	4.8±2.57
	1	0	0	1.1	0.5	5.0	0.5	1.10±1.91
	4	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0

bacteriods count in legumes grown in contaminated soil.

The diesel oil greatly affected soil bacteriod counts. It was observed that there was no bacteriod formation in the 4 and 8% pollution of the diesel oil. Table 7 shows the microorganisms isolated from the rhizosphere of legumes planted in diesel oil contaminated and uncontaminated soil. Though the diesel oil affected some organisms which disappear during the course of the experiment, most of the organisms were able to survive till the end of the experiment.

DISCUSSION

The investigation on the impact of diesel oil pollution on the rhizosphere of legumes revealed several adverse effects both on the legumes' capacity to mutually enhance the nitrogen fixation, the microbial community of the rhizosphere and the physico-chemical properties of the utisol. Total nitrogen increased slightly to the highest pollution level (8% treatment). This may be due to the diesel oil which is known to affect the macro elements content of the soil and modifies the soil biological activities as a result of its effect on the urease, acid and alkaline phosphatase activities in the soil (Wyszkowski and Wyszkowska, 2005; Akubugwo et al., 2009) . Similar findings had earlier been reported by Odu (1981) that soil organic matter and soil nitrogen increases after degradation of oil polluted soils by microbial biodegraders.

Available phosphorus reduced with increased concentration of diesel oil applied to cultivated soil. This may be due to the effect of the diesel oil on the microorganisms which affect the activities of the enzyme phosphatase, therefore inhibiting the release of phosphorus from organic materials in the soil. It could also be attributed to the phosphorus utilized by the plants since it has been reported that nitrogen- fixing leguminous plants utilize more phosphorus due to the high energy cost of nitrogen fixation and the maintenance of func-tional nodules (Graham, 1998). Margesin and Schinner (2001) working on diesel -oil-contaminated soil in the Alpine glacier Skiing Area, also observed a decrease in the available phosphorus content of the soil and attributed it to microbial metabolism and immobilization of apatite (Calcium phosphate). The pH was also observed to slightly increase in both the rhizosphere of

Table 7. Microorganism isolated from diesel oil contaminated and uncontaminated Utisol.

Bacteria	Fungi	Yeast	Bacteriods
Clotridium botilinum	*Aspergillus flavus**	*Sacchoromyces Cerevisa*	*Rhizobium phaseoli*
Micrococcu s luteus	*Aspergills niger**	*Candida tropocalis*	*Rhizobium*
Lystevia monocytogens	*Aspergillus Fumigatus**	*Candiad psevdotropocalis*	*leguminosarium*
Actinomyces isrealii	*Aspergillus terreus**		
Bacillus pumilus	*Botrytis aclad*		
Clostridium hastolyticum	*Penicillum citrinum**		
Bacillus spharicus	*Penicillum trequentans**		
Bacillus circus	*Fusarium roseum**		
Pseudomonas mallci	*Trichoderma horizonum**		
Actinomyces neeslundii	*Absidia* sp.*		
Enterococcus feacalis	*Scopolariopsis candida*		
Shigella dysenteriac	*Cephalosporium* sp.*		
Pseudomonas avriginosa			
Azotobater nigricns			
Nitrosomonas euroaea			
Nitrobacter vulgaris			
*Bacillus megatorium**			
Bacillus sp.*			
*Corynebacterium ovis**			
*Norcardia madurae**			
*Bacillus polymyxa**			
*Bacillus brevis**			

cowpea and groundnut. The exchangeable cations (Ca, Mg, Na, K) showed no remarkable difference from the results before pollution. The slight decrease could be due to the effect of immobilization of nutrients by the microorganisms as previously reported by Essien and Udotong (1999).

A diversed microbial community in the rhizosphere of the legumes in the simulated diesel oil polluted soil was observed. Specifically, there was a gradual increase in the THBC upto the 10_{th} week in most of the treatments. This increase is as a result of the utilization of the diesel oil by indigenous hydrocarbon biodegraders as their energy and carbon source. Similar findings have been reported by Roscoe et al. (1989) who noted increase in the microbial population in a crude oil contaminated soils. It has also been reported that plant rhizosphere are highly favourable for the proliferation and metabolism of microbial types, hence the increase in rhizosphere heterotrophic bacteria population observed corresponds with increase amount of nutrient accumulation in the site (Brown, 1995). Increase in soil nutrient as a result of biodegradation has also been known to enhance microbial growth due to availability of carbon, energy, nitrogen and sulfur, which are essential in the synthesis of amino acid in the microbial system (Okpokwasili and James, 1995; Chikere and Okpokwasili, 2003). Li et al. (2007) also noted increase growth of aerobic heterotrophic bacteria in a diesel-oil-stimulated soil with increase activities of soil dehydrogenase, hydrogen-peroxidase and polyphenol oxidase.

The relatively low counts of actinomycetes encountered in the plant rhizosphere were expected. Specifically, significant decrease ($P = 0.05$) was observed in 8% treatment in the rhizosphere of cowpea and in both 4 and 8% in the rhizosphere of groundnut. The differences in the actinomycetes count in the microflora of cowpea and groundnut could be attributed to differences in the type of exudates produced by the legumes. Li et al. (2007) working with diesel-oil-simulated soil observed a significant decrease of the colonies of soil actinomyces and filamentous fungi and noted that this can be taken as a sensitive biological indicator of petroleum contamination. The differences in microbial community of different plant species has also been reported by Muratova et al. (2003) and noted that the actinomycetes in the rhizosphere of alfalfa was less than those found in the rhizosphere of reed in a butiment polluted soil. This difference is said to be the result of interaction between plant roots, root exudates and microorganisms within the rhizosphere and they play important role in regulating rhizosphere microbial processes and thereby significantly affecting plant growth (Bonkowski et al., 2000).

Nitrifying bacteria was observed to reduce with increased concentration of diesel oil. This could be due to the fact that under the diesel oil environment, the nitrifying bacteria could not effectively compete with other

organisms that multiplied rapidly, resulting in the exhaustion of the available inorganic nitrogen. This finding agrees with the report of Odu (1981) who noticed that aerobic nitrogen fixers became relatively more abundant than other organisms while nitrifying organisms became considerably reduced in number. It has also been reported that soil polluted with bitumen recorded reduced count of nitrifying, nitrogen fixing, denitrifying and amonifying bacteria in the rhizosphere of both alfalfa and reed (Muratova et al., 2003).

Fungal population was observed to reduce with increase concentration of diesel oil. However, it was observed that in both 4 and 8% pollution, the count of fungi in groundnut rhizosphere was lower than those in cowpea. Though both rhizosphere received the same treatment, the exudates from the cowpea root must have ameliorated the effect of the diesel oil, thus the higher count of fungi in its rhizosphere compared to those of the groundnut. Yong and Crowley (2006) working on the rhizosphere microbial community structure with the use of 165 ribosomal DNA (rDNA) fingerprints, observed that the bacteria and fungi communities in the rhizosphere were substantially different in different root zones and that a rhizosphere community may be altered by changes in root exudates composition caused by changes in plant nutritional status.

The effect of the diesel oil was more pronounced on the count of the bacteriods. The 4 and 8% pollution totally inhibited the formation of nodules with only few nodules in the 1% concentration compared to the 100% nodule formation in all the control. This is expected because hydrocarbon has been known to adversely affect the nitrogen fixing bacteria within the rhizosphere (Muratova et al., 2003).

It was also observed in this study that some bacterial species. *Bacillus pumilus*, *Pseudomonas mallci*, *Enterococcus feacalis* and *Micrococcus luteus* and all fungal species except *Scopolaropsis candida* persisted after the pollution. This could be due to the fact that these microorganisms were able to degrade the diesel oil thereby increasing nutrient status of the soil for their survival. This observation was also reported by Ekpo and Thomas (2007) noting that in a crude oil impacted soil, some bacteria and most fungi possess enhanced physiological tolerance and were able to utilize the crude oil. Some microorganisms example *Clostridium botulinum, Listeria monocytogen* and *Rhizobium leguminosarium* were isolated only before pollution. They could have been eliminated because of their inability to utilize hydrocarbons as their sole carbon and energy source (Yong and Crowley, 2006).

Conclusion

This study has revealed that although there was a general decrease in the microbial biomas in the rhizosphere of the leguminous plant with increase in concentration of the diesel oil, there were variations in the plant rhizosphere. Heterothrophic bacteria and actinomycetes in the rhizosphere of groundnut (*A. hypogea*) exhibited higher resistance to the diesel oil compared to the rhizosphere of cowpea *(V. unguiculata).* On the other hand, fungi in the rhizosphere of cowpea was observed to exhibit higher resistance compared to those in the rhizosphere of groundnut. The diesel oil adversely affected the nodulation of the legumes, the nitrogen fixing bacteria and consequently the nitrogen fixing capacity of the legumes. This study has also revealed that the diesel oil affects the development of nodules and the multiplication of bacteriods inside the nodules. It is therefore important to guide against the pollution of our agricultural soils with diesel oil to enhance the fertility status of our soil.

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