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Preliminary Investigation into the Ability of Tropical Rhizobia to Degrade Petroleum Products as Environmental Pollutants and Chemical Bases for Some Syhnthetic Pesticides

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Abstract: ***This study involves the investigation of the ability of some Tropical Cowpea Rhizobia to degrade crude oil in oil spilled soil as well as crude oil products which includes diesel, kerosene and petrol. This was carried out by introducing the rhizobial strains Rhizobium species CWP A17, R. species CWP G34A, R. species CWP G34B, R. species C, Bradyrhizobium species R. 3IB, Br. species FA3, Br. species T5BL 442 , Br. speciesUSAD 4675 (400) and Br. species 1495 MAR into various fuel media. The population and absorbance of the various bacteria were taken in each case at 24 hours interval for 10 days. Each of the various Rhizobia and Bradyrhizobia degraded hydrocarbon. The physicochemical parameters of the oil polluted soil were determined alongside with those of the control. The particle size analysis of the polluted soil sample indicated that on the average the soil is made up of 10.69% sand, 84.86% silt and 4.5% clay. This corresponds to silt loam soil. Other parameters indicated the utilization of the various hydrocarbon contents by the plant. The effects of oil polluted soil on nodulating and nitrogen fixing abilities of the tropical rhzobia were examined by inoculating each of the bacterial strain on the cowpea (Vignaunguiculata) grown on the oil polluted soil in a green house. These were observed at two weeks interval for 12 weeks. The various plant indices (i.e. height, number of leaves, and leaf area) showed retarded germination of the plant of oil polluted soil when compared to the control. However, there was slight difference in the case of stem girth. The stem girth of the plants grown on oil populated soil is wider. The biodegradation experiment showed that for the various rhizobia grown in crude oil, diesel, petrol and kerosene media, the degradation of the hydrocarbon occurred, in which the organisms were able to utilize the fuels as carbon source.***

Keywords: Preliminary, Tropical, Rhizobia, Degrade, Petroleum, Products, Environmental, Pollutants, Chemical bases, Synthetic, Pesticides

1. Introduction

The term pesticide is used to describe any device or toxic chemical that kills plants or animals (i.e. pest organisms) that compete for humanity's food supply, or are otherwise undesirable. Pesticides include herbicides, insecticides, fungicides, nematicides (used to kill nematodesthat is elongated cylindrical microscopic worms) and rodenticides (Adenekan*et. al*., 2008). A pesticide consists of active and inert ingredients. The active ingredients are lethal to the pest while the inert ingredients facilitate spraying and coating the target plant.

Petroleum comprises of crude oil and petroleum products such as gasoline, fuel oils and diesel fuels. It is a complex mixture of organic compounds (Adedokun and Ataga, 2007).

The soil is a key component of natural ecosystems becauseenvironmental sustainability depends largely on sustainable soil ecosystem. Whensoil is polluted, the ecosystem is altered and agriculturalactivities are affected.Oil pollution prevents normal oxygen exchangebetween soil and atmosphere due to hydrophobic properties of oil (Atlas, 1977). It also inhibits seed germinationand plant growth (Odjegba and Sadiq, 2002). Unrefined and refined oils have been shown to be toxic to plants (Adedokun and Ataga, 2007).

In Nigeria, most of the terrestrial ecosystem and shorelinesin oil producing communities are important agriculturallands under continuous cultivation. Any contact of these lands with crude oil results to damage of soil condition, microorganisms and plants. Crude oil beyond 3% concentration in an environment becomes increasingly deleterious to soil biota and crop growth (Onuoha *et al*., 2003).

Specific Advantages of Cowpea

A lot of health benefits are associated with the consumption of cowpea leaves especially the important supply of vitamins, which strengthens human immunity and the improvement of vision and blood are of notable importance. Many farmers in East Africa are aware of the fact that cowpea is very beneficial both to human beings and animals so they tend to cultivate it more (Hallensleben *et al*., 2009). Cowpea contains about 25% protein and is the poor people's choice of affordable protein complementing diets with either cereal grains or starchy foods such as cassava, sweet potato and plantains that have very little protein. When three parts by dry weight of cereal grains are combined with one part of cowpea grains they provide a food with an appropriate balance and quality of protein for an adult and significant vitamins and minerals (Hall, 2007).

Pollution from Oil Spillages and Bioremediation

Most oils are mixtures of many different compounds, most of which are hydrocarbons. There are four main hydrocarbon groups in petroleum. The saturated hydrocarbons consist of straight chains of carbon atoms. Aromatics are hydrocarbons consisting of rings of carbon. Asphlatenes are complex polycylic hydrocarbons that contain many complicated carbon rings, and NSO compounds are mostly nitrogen, sulfur, and oxygen (Clark, 2011).

An oil spill counter measure within the marine environment, bioremediation has been defined as ''the act of adding materials (nutrients like nitrogen, phosphorus, potassium, organic matter and microbial inocula (mixed culture of oil-degrading bacteria) to contaminated environments to cause an acceleration of the natural biodegradation processes'' (U.S.C.O.T.A., 1991). Biodegradation is known to be the principal natural process for the removal of the non-volatile fraction of oil from the environment (Prince, 1993).

Bioremediation is a popular approach of cleaning up petroleum hydrocarbons from the environment because it is simple to maintain, leads to the destruction of contaminants, applicable over large areas and cost effective when compared with other methods such as physical and chemical means of hydrocarbon cleaning (Vila *et al*., 2001). Clean up and recovery from an oil spill environment is difficult and depends upon many factors, including the type of oil spilled, the temperature of the area (water or land) (affecting evaporation and biodegradation), and the types of shorelines and beaches involved.

Agrochemicals with Hydrocarbon or Petroleum Bases

Glyphosate (***N*-(phosphonomethyl) glycine**), 2-4D etc. are examples of agrochemicals with hydrocarbons bases which have adverse effects on animals and soil (United States EPA, 2007).

Some Nigerian Farmers Stories of Woes

Nenwe is one of the five towns that make up Aninri Local Government Area of Enugu State with a population of about 10,000 inhabitants and majority of the residents are subsistent farmers. Their cash crops are: okra, cassava and rice. Farmers in the community have been using ROUNDUP – the world's most widely used herbicide whose active ingredient, glyphosate is contentious for weed control for more than a decade. After which the use of the herbicides resulted in the stunted growth of theirokra. Most of the Okra did not germinate, while the few that germinated did not grow up to two inches before they started flowering making their yield meaningless. This brought about the realization that care must be taken, to avert serious scarcity of okra and other vegetables generally (Onyeji, 2019). This in a way agrees with what Odjegba and Sadiq, (2002) said about crude oil pollution in the soil.

Aim of the Study

This preliminary study is aimed at testing the potential ability of *Rhizobia* to degrade petroleum products which are made of hydrocarbons in contaminated soil. The concluding part of the study will be on the ability of the Rhizobia strains to degrade petroleum based herbicides or pesticides as pollutants to agricultural soils as encountered in Nenwe,Aninri Local Government Area of Enugu State of Nigeria.

Justification of this Study

Cowpeas play a key role in the agriculture and food supply of Nigeria. Nigeria is the largest producer and consumer of cowpeas, accounting for about 45 percent of the world's cowpea production. As of 2004, it was also the world's largest cowpea importer. Kano State is in the heart of the Nigerian "cowpea belt," and cowpeas are grown on almost every farm in Kano State and are eaten in some forms by almost every Kano consumer (Lowenberg-DeBoer and Germaine, 2008).

The major consumption center for cowpeas is the densely populated area of southern Nigeria. The grain shed includes all the countries bordering Nigeria, as well as Togo, Ghana, and Mali. In some cases, cowpeas may be shipped to Nigeria from far countries including Senegal and Sudan. Cowpea is a key crop in the traditional farming systems of the Kano area. Cowpea grain is a cash crop and also consumed by the farm families. Cowpea leaves and stems are valued as fodder for livestock. Nitrogen fixation by cowpea production and manure generated by livestock fed with cowpea residue have been identified as central to maintaining soil fertility in the Kano Closed Settled Zone (Fulton, 2006).

The cowpea (*Vignaunguiculata*var. sampea-1) locally known as "EwaOloyin" is main source of plant protein, which an average citizen can get at affordable price. Planting of this cowpea is usually encouraged but, some farm lands around us are usually polluted with pesticides especially herbicides(with petrochemicals as chemical bases), crude oil and its products. This pollution disallows good growth of cowpea so, ability of certain microorganism involved in symbiosis with this legume root to allow its growth on oil contaminants is an important investigation, to encourage the cultivation and growth of this legume on polluted lands.

The bacterium *Rhizobium* has symbiotic relationship with legumes. It is good at fixing natural atmospheric nitrogen into the root of the legume. How much rhizobia could withstand petroleum and break it down as a main carbon source for growth and normal activity was of interest.

Rhizobium species CWP G34A is known for its nitrogen fixing and nodulating ability for cowpea over a period of time. It was generated from the Department of Microbiology at The Federal University of Technology, Akure, Nigeria and various researches have been carried out on it which established this.

The specific objectives of the research are to:

(a) Determine the physicochemical parameters of oil spilled soil samples from Okochiri, Rivers State;
(b) Investigate the abilities of some tropical cowpea rhizobia to degrade crude oil and its refined products (i.e.diesel, petrol and kerosene);if biodegradation of hydrocarbons of petroleum products are possible using

rhizobia, then petroleum based pesticides which are harmful to soil and plants too may be bio-degradable. .

(c) Determine the effects of oil polluted soil on the nodulating and nitrogen fixing abilities of the tropical rhizobia;

2. Materials and Methods

2.1 Materials

Legume

The legume used in this study is a Nigerian cowpea commonly called "EwaOloyin" (*Vignaunguiculata* var. sampea-1). The cowpea was obtained from Bodija market in Ibadan and authenticated by the Institute of Agricultural Research and Training (I. A. R. and T.), Ibadan. Soil samples were collected from a crude oil polluted soil site at Okochiri in Okricha Local Government Area of Rivers State while the control soil was collected from a non-polluted site in FUTA(Federal University of Technology, Akure).

Test Microorganisms

The rhizobial species, bacteria and fungi used in this research ss were *Rhizobium* species CWP AI7, *R.* spp. CWP G34A, *R.* spp. CWP G34B, *R.*spp C, *Bradyrhizobium* species R.3IB, *Br.* spp. FA3, *Br.* spp. T5BL 442, *Br.* spp.USAD 4675 (400) and *Br.* spp. 1495 MAR. The bacteria were *Aerococcus viridians, Bacillus megaterium, Bacillus pumilus, Bacillus subtilis, Cellulomonasflavigena, Corynebacterium* spp.and*Micrococcus varians* while the fungi were *Aspergillusflavus* and *Aspergillusrapens.*

Preparation of Culture media

Nutrient Agar (NA)

The Nutrient Agar (Lab M. Topley House, England) medium was prepared by dissolving 2.8 g powder of the NA in 100ml of distilled water. The dissolved powder was sterilized at 121^{o}C for 15min.

Sabouraud Dextrose Agar (SDA)

Sabouraud Dextrose Agar (Lab M. Topley House, England) was also prepared by dissolving 6.5 g powder into 100ml of distilled water. The medium was sterilized at 121^{o}C for 15min.

Yeast Mannitol Broth and Agar (YMBA)

Yeast mannitol broth was prepared by mixing 10 gmannitol, 0.59 g dipotassiumhydrophosphate, 0.2 g magnesium sulphate, 0.2 g sodium chloride, 0.5 g yeast extract with a litre of distilled water. The pH was adjusted to 6.8. Agar (15 g) was then added. The medium was sterilized at 121^{o}C for 15min.

The fuel medium used for bacteria (FMB) and fungi (FMF)

The fuel medium used for bacteria (FMB) contained K_2HPO_4 (1.8 g), KH_2PO_4 (1.2 g), NH_4Cl (4.0 g), $MgSO_4.7H_2O$ (0.2 g) and $FeSO_4.7H_2O$ (0.01 g) while the fuel medium for fungi (FMF) include $NaHPO_4$ (2.0 g), K_2SO_4 (0.17 g), NH_4NO_3 (4.0 g), KH_2PO_4 (0.53 g), and $MgSO_4.7H_2O$ (0.1 g) (Ijah and Abioye, 2003). These were prepared and 50ml lot of each medium was dispensed into separate bottles. Even fuel (20% v/v) was transferred to the medium seperately. The medium was sterilized at 121^{o}C for 15min.

Determination of Physicochemical Parameters of the Soils

Measurement of pH: The pH values of the soil samples were determined using pH meter (HANNA instrument pH 2000) standardized at pH 4.0 and 7.0 with standard buffer solutions.

Determination of Moisture Content

Moisture contents of soil samples were determined by pre-weighing and drying the soils at 80^{o}C in an oven for 24 h and re-weighing to obtain dry weight (Odjegba and Sadiq, 2002).

Determination of Organic Matter and Organic Carbon Content

The amount of soil organic matter and organic carbon were determined by Walkley-Black oxidation method. One gram soil sample ground to fine powder was weighed to 250 ml flask and 10 ml of 0.2M potassium chromate was added. Twenty milliliter of H_2SO_4 was added and mixed vigorously. This mixture was left for 30 min then 100 ml distilled water was added to it. Eight drops of diphenylamine indicator was added and it was titrated with 0.4 N Ferrous Ammonium Sulphate till there is colour change from violet to green. The amount of potassium chromate used up is amount of organic carbon present. The amount of potassium chromate used up is equivalent to amount of carbon present. Organic matter was calculated in proportion to the organic carbon.

Determination of Phosphorus, Sodium, Calcium and Magnesium

Phosphorous was quantified spectrophotometrically using Bray 1 method. This was done by weighing 5.0 g of dried soil in a dry cup and 25 ml of bray reagent was added. This was shaken for a minute and filtered. From the filtrate 8 ml was taken and 5 drops of F5 indicator was added with ammonium molybdate. This was read in the spectrophotometer at wavelength 560 nm.

Sodium, calcium and magnesium were determined by weighing 5.0 g of dried soil in a dry cup. Twenty-five milliliter of 1N ammonium acetate (pH 7.0) was added and shaken for 30 min. The mixture was filtered and the filtrate read in the colourimeter at the different wavelengths of each element.

Total Hydrocarbon Content

Total hydrocarbon content was determined spectrophotometrically using xylene. Five grams of soil sample was poured into 250 ml flask and 50 ml xylene added to it. The mixture was shaken vigorously in a shaker for 30 min and filtered. The residue was rinsed with xylene and filtered. The mixture was left at room temperature to evaporate the xylene. The absorbance of the extract was determined at 410 nm.

Copper, Cadmium, Lead, Manganese, Zinc and Iron
Copper, Cadmium, Lead, Manganese, Zinc and Iron were determined by wet digestion method. The following acid mixtures were used as digestion medium: perchloric acid, Hydrochloric acid and sulphuric acid.

Determination of Nitrogen
Nitrogen contents of soils were measured using macro-kjeldahl digestion method. Two grams of the soil were weighed into macro- kjeldahl flask and 20 ml of concentrated H_2SO_4 was added to it with kjeldahl catalyst tablet. The flask was heated until the solution becomes clear and the soil residue white. This was allowed to cool and 50 ml de-ionized water was added to 100 ml mark. This was thoroughly mixed and allowed to settle so a clear solution was taken for the nitrogen determination.

Test for Cowpea Nodulation and Nitrogen Fixation by the Various Rhizobia

Cultivation of rhizobia
Fifteen milliliters of Yeast mannitol broth (YMBC) prepared as explained above was inoculated with each of the rhizobia in separate bottles and was incubated at 30^{o}C 24 hours. The grown cells were spun at 3600 rpm for 20 min in a centrifuge (MSE MINOR 35). The supernatant was decanted and the centrifuged cells were washed with sterile distilled water and re-suspended in sterile distilled water.

Germination of Cowpea Seeds and Planting
Apparently healthy seeds of the cowpea were surface sterilized with 3.85% m/v sodium hypochlorite for five minutes. The hypochlorite solution was removed and the seeds were properly rinsed with sterile tap water for ten times. The seeds were transferred to sterile agar-agar plate. They were kept at 28^{o}C for 4 days.

Inoculation of Seedlings
Three of the cowpea seedlings were transferred into sterile sand in a plant jar set up containing plant nutrient. Each seedling was inoculated with 0.5ml of the 24 hours culture of each rhizobial strain. A plant jar was used for separate species of microbe. 1.5ml of respective microbe was separately inoculated in each cowpea seedling in crude oil polluted soil. The control was not inoculated with the rhizobial cells. These were left for 6 weeks and observed for nodulation.

Test for nodulation and other growth factors in the various Rhizobium species
The above stated experiment was repeated and plants were left for 12 weeks during which nodulation and other growth parameters (number of leaves, stem girth and plant height) were determined. Numbers of leaves, stem girth and the height of each plant were measured at forthnight interval. Also at harvest, wet weight of each plant and the number of effective nodules were determined thus: plants were removed from the soil and the roots were washed in tap water to detach soils that adhere to them. The plants were gently blotted with soft paper to remove any free surface moisture. They were weighed immediately. The dry weight of each plant was also determined by oven drying the plant for 12 hours at 100^{o}C until a stable weight was attained (Akyeampong, 1989). The nodules were counted, external and internal colours were examined. The rhizobial strains that nodulated the legumes were used for further investigations.

3. Results

3.1 Physicochemical Properties of Oil Spilled and Agricultural Soils

Physicochemical characteristics of the oil spilled and unpolluted agricultural soils are as shown in Table 1. The total hydrocarbon (THC) contents (the crude oil polluted soil and agricultural soil) before and after planting were 21.41% and 15.97% for crude oil polluted soil and 0.76% and 0.65% for unpolluted agricultural soil. This data showed that after planting the legume, the amounts of THC measured reduced significantly in crude oil polluted soil but the reduction was insignificant in the control agricultural soil.

The total nitrogen, phosphorus and potassium were higher in the crude oil polluted soil compared with the agricultural soil. Except for phosphorus (P), the nitrogen (N) and potassium (K) content did not reduce considerably after planting in crude oil spilled soil relative to agricultural soil even though the reduction of potassium content in the unpolluted soil is insignificant (Table 1). It was observed that for virtually all (90.48%) the parameters the contents measured were higher in the contaminated soil than in the unpolluted agricultural soil. The composition of the macro-elements (nitrogen, potassium, phosphorus, calcium, magnesium and sodium) and trace elements (copper, manganese, zinc, iron and cadmium) were 1.69%, 0.86 cmolkg^{-1}, 14.62 mgkg^{-1}, 0.48 cmolkg^{-1}, 2.43 cmolkg^{-1}, 0.64 cmolkg^{-1}, 5.59 mgkg^{-1}, 1.59 mgkg^{-1}, 1.84 mgkg^{-1}, 1.61 mgkg^{-1} and 2.83 mgkg^{-1} respectively in crude oil spilled soil. Apart from the pH and silt in agricultural soil, sand in polluted soil and H^+ in both soils the values of all the components analysed in both soils decreased after planting.

Table 1: Physicochemical characteristics of oil spilled and agricultural soils before and after planting

Parameters		Crude-oil soil		Agricultural soil	
		B P	A P	B P	A P
pH		6.20^{a}	6.00^{a}	6.10^{b}	6.40^{a}
Na	cmolkg^{-1}	0.64^{a}	0.51^{a}	0.50^{a}	0.31^{a}
K	cmolkg^{-1}	0.86^{a}	0.63^{a}	0.70^{a}	0.43^{a}
Ca	cmolkg^{-1}	0.48^{a}	0.40^{a}	0.56^{a}	0.43^{a}
P	(mgkg^{-1})	14.62^{a}	12.62^{b}	8.49^{a}	6.43^{b}
OC	%	16.49^{a}	12.30^{b}	0.59^{a}	0.50^{a}
OM	%	28.42^{a}	21.21^{b}	1.01^{a}	0.86^{a}
TN	%	1.69^{a}	1.45^{a}	0.06^{a}	0.04^{b}
THC	(%)	21.41^{a}	15.97^{b}	0.76^{a}	0.65^{a}
H^+	(%)	0.037^{a}	0.110^{b}	0.033^{b}	0.090^{a}
MC (%)		24.89^{a}	21.85^{b}	12.70^{a}	10.22^{b}
Mg (cmolkg^{-1})		2.43^{a}	1.47^{b}	1.14^{a}	0.18^{b}
Zn	mgkg^{-1}	1.84^{a}	1.64^{b}	0.19^{a}	0.12^{a}
Mn	mgkg^{-1}	1.59^{a}	1.39^{b}	0.13^{a}	0.10^{a}
Cu	mgkg^{-1}	5.59^{a}	4.31^{b}	0.29^{a}	0.15^{a}
Pb	mgkg^{-1}	2.66^{a}	1.47^{b}	0.11^{a}	0.06^{b}
Cd	mgkg^{-1}	2.83^{a}	1.25^{b}	0.13^{a}	0.10^{a}
Fe	mgkg^{-1}	1.61^{a}	1.35^{a}	1.30^{a}	1.03^{a}
Sand	%	10.69^{a}	15.72^{a}	19.95^{a}	19.50^{a}
Silt	%	84.86^{a}	80.40^{b}	68.81^{a}	71.55^{a}
Clay	%	4.5^{a}	3.88^{a}	11.23^{a}	8.95^{a}

Values having the same alphabet horizontally are not significantly different from one another at P ≤ 0.05
Legend: BP- Before planting, AP- After planting, OC- Organic content, OM- Organic matter, TN- Total nitrogen, THC- Total hydrocarbon content, H^+- Hydrogen ion and MC- Moisture content.

Effect of Oil Polluted Soil on the Nodulating and Nitrogen Fixing Abilities of the Tropical Rhizobia
The *Rhizobium* species strains CWP A17, CWP G34A, CWP G34B, *Bradyrhizobium* species FA3, R.3IB and USAD 4675 strain 400 nodulated and fixed nitrogen into the cowpea plants at six weeks of growth . The rhizobia were therefore chosen for use in the experiment. Despite the inoculation of the cowpea plants grown in the crudeoil polluted soil, with three times the cell load of rhizobia that was inoculated on the cowpea plants grown in agricultural soil, the cowpea cultivated in the crude oil polluted soil in the presence of the selected rhizobia did not form nodules . In contrast, the legume inoculated with the same organism nodulated the cowpea in unpolluted soil.

The heights of the plant after two weeks in the crude oil polluted soil were about half of those plants grown in agricultural soil except for the control and cowpea inoculated with *R.* species A17 (Table 2). The heights of plants inoculated with *Rhizobium* species strains CWP A17, CWP G34A, CWP G34B, *Bradyrhizobium* species FA3, R.3IB and USAD 4675 were 9.53 cm, 9.77 cm, 8.23 cm, 9.03 cm, 8.63 cm and 8.67 cm in polluted soil and 7.37 cm, 17.83 cm, 16.50 cm, 16.73 cm, 16.83 cm and 8.10 cm in unpolluted soil. This growth pattern continued for more than 90% of the planting period. There was a general increase in the heights of plants with the increase in period of planting on both agricultural and oil spilled soils. The heights of the agricultural soil grown cowpea plants at harvest were 2-3 times higher than the height of the plants grown in crude oil polluted soil (Table 2). The difference in heights of cowpea plants grown in crude oil polluted soil, was not significant in the various weeks except week 2 which showed significant difference in heights of plants inoculated with CWP G34A and CWP G34B with values 9.77 and 8.23 respectively. While in the case of agricultural soil grown plants there was no significant difference between the various plants heights inoculated with the different rhizobia.

For the first few weeks of the planting experiment, the leaves of the cowpea plants grown in crude oil polluted soil were very green, (greener than the leaves of agricultural soil grown plants) . During the early stages of growth, one of the cowpea plants grown in crude oil soil had leaf curl, but the leaves grew normally with time as the experiment went on, even though some leaves withered during this period.

Table 2: Heights (cm) of Cowpea Plants Grown in Crude Oil Polluted and Agricultural Soils

Strain	Weeks After Planting											
	2		4		6		8		10		12	
	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS
*R.*spp. CWP A17	9.53^{ab}	7.37^{a}	10.20^{a}	25.50^{a}	10.97^{a}	42.97^{a}	11.30^{a}	52.43^{a}	11.80^{a}	29.53^{a}	8.60^{a}	29.57^{a}
*R.*spp. CWP G34A	9.77^{a}	17.83^{a}	11.33^{a}	24.67^{a}	12.13^{a}	18.53^{a}	12.93^{a}	19.10^{a}	13.00^{a}	19.40^{a}	13.00^{a}	20.30^{a}
*R.*spp. CWP G34B	8.23^{b}	16.50^{a}	9.83^{a}	22.80^{a}	12.07^{a}	27.53^{a}	13.20^{a}	28.70^{ab}	13.27^{a}	19.93^{a}	13.27^{a}	19.97^{a}
*Br.*spp. R.3IB	9.03^{ab}	16.73^{a}	11.53^{a}	23.67^{a}	12.10^{a}	26.20^{a}	12.00^{a}	26.00^{a}	8.33^{a}	18.03^{a}	4.93^{a}	18.13^{a}
*Br.*spp. FA3	8.63^{ab}	16.83^{a}	11.007^{a}	26.07^{a}	12.23^{a}	36.33^{a}	12.77^{a}	36.33^{a}	12.97^{a}	34.33^{a}	12.67^{a}	33.10^{a}
CONTROL	8.67^{ab}	8.10^{a}	11.27^{a}	19.07^{a}	12.40^{a}	23.30^{a}	13.20^{a}	23.30^{a}	13.33^{a}	27.00^{a}	13.00^{a}	27.10^{a}
LSD (0.05)%	1.47	10.88	2.31	11.26	2.82	26.66	3.84	25.34	6.62	35.47	9.15	35.70

Values having the same alphabet vertically are not significantly different from one another at P ≤ 0.05
Legend:
AS: Agricultural Soil
COS: Crude Oil Soil
Control: Soil without rhizobium
LSD: Least Significant Difference

In case of the number of leaves of the plants grown in crude oil polluted soil, there was no significant difference in week 2, 8 and 10 while there was significance between the plants inoculated with CWP G34A with value 5.33 compared with the remaining plants at week 4. Also, plants inoculated with and CWP A17 had the least significance with value 4.00 at the same week. Plants inoculated with *Bradyrhizobium* species FA3 with value 8.33 had significant difference in number of leaves at week 6 when compared to *Br.* species R.3IB, *R.* species CWP G34B and *R.* species CWP A17 with values 6.00, 5.67 and 5.33 respectively. Also, plants inoculated with *R.* species CWP G34B, *R.* species CWP R.3IB and *R.* species CWP A17 are significantly different in week 12 with the following values: 7.67, 2.33 and 1.00 (Table 3). While in case of the agricultural soil grown plant there was no significant difference in number of leaves for weeks 10 and 12, but there was significance between control and plants inoculated with the various rhizobia at week 2, control and others apart from *Br.* species FA3 at week 4. Also, plants inoculated with *R.* species CWP A17 showed significant difference when compared with that inoculated with *R.* species CWP G34A and control at week 6 and *R.* species CWP A17 and *R.* species CWP G34A at week 8 (Table 3).

Table 3: Number of Leaves of Cowpea Plants Grown in Crude Oil Polluted and Agricultural Soils

Strain	Weeks After Planting											
	2		4		6		8		10		12	
	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS
R.spp. CWP A17	3.67^{a}	5.00^{a}	4.00^{c}	7.67^{a}	5.33^{b}	11.33^{a}	1.27^{a}	11.33^{a}	1.23^{a}	7.00^{a}	1.00^{b}	4.47^{a}
R.spp. CWP G34A	3.67^{a}	5.00^{a}	5.33^{a}	7.67^{a}	7.00^{ab}	6.00^{ab}	1.20^{a}	5.33^{b}	1.20^{a}	4.00^{a}	5.33^{ab}	6.00^{a}
R.spp. CWP G34B	3.33^{a}	4.00^{a}	4.67^{b}	8.00^{a}	5.67^{b}	10.67^{ab}	1.23^{a}	10.33^{ab}	1.23^{a}	7.67^{a}	7.67^{a}	7.33^{a}
Br.spp. R.3IB	3.67^{a}	5.00^{a}	4.67^{b}	8.00^{a}	6.00^{b}	9.00^{ab}	1.23^{a}	8.00^{ab}	1.23^{a}	7.67^{a}	2.33^{b}	3.67^{a}
Br.spp. FA3	3.67^{a}	4.67^{a}	4.67^{b}	6.67^{ab}	8.33^{a}	11.33^{a}	1.27^{a}	10.67^{ab}	1.20^{a}	11.67^{a}	5.00^{ab}	11.67^{a}
CONTROL	3.33^{a}	1.33^{b}	4.67^{b}	4.67^{b}	6.67^{ab}	6.00^{b}	1.30^{a}	8.67^{ab}	1.30^{a}	10.67^{a}	6.00^{ab}	12.67^{a}
LSD (0.05)%	1.40	1.91	0.66	2.61	2.33	4.82	3.16	5.96	4.69	9.52	5.16	10.59

Values having the same alphabet vertically are not significantly different from one another at P ≤ 0.05%
Legend
AS: Agricultural Soil
COS: Crude Oil Soil
Control: Soil without rhizobium
LSD: Least Significant Difference

For the stem girth, in the crude oil grown plants, only weeks 4 and 12 showed significant difference in which there was difference between plants inoculated with *R*. species CWP A17 and the control which was not inoculated. There was difference between plant inoculated with rhizobia *Br*. species R.3IB, *R*. species G34A, *R*. species G34B and *Br*. species FA3 (Table 4). In case of the agricultural soil grown plants only week 2 showed significant difference between the control and the remaining plants inoculated with the different rhizobia (Table 4).

At harvest the wet weight of the various plants grown on crude oil polluted soil and inoculated with the various rhizobia had no significant difference between them and even with the control. Similarly, the dry weight showed no significant difference (Table 5). Also, for the agricultural soil grown plants inoculated with the various rhizobia there was no significant difference in the wet and dry weights (Table 5). Cowpea plants grown on crude oil polluted soil produced small pods despite their inability to form nodules.

Table 4: Stem Girth (cm) of Plants Grown on Crude Oil Polluted and Agricultural Soils

Strain	WEEKS AFTER PLANTING											
	2		4		6		8		10		12	
	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS	COS	AS
R.spp. CWP A17	1.23^{a}	1.20^{a}	1.27^{a}	1.20^{a}	1.27^{a}	1.27^{a}	1.23^{a}	1.27^{a}	1.23^{a}	0.80^{a}	0.80^{ab}	0.80^{a}
R.spp. CWP G34A	0.90^{a}	1.13^{a}	1.20^{ab}	1.27^{a}	1.20^{a}	0.87^{a}	1.20^{a}	0.90^{a}	1.20^{a}	0.90^{a}	1.20^{a}	0.93^{a}
R.spp. CWP G34B	1.13^{a}	1.13^{a}	1.20^{ab}	1.17^{a}	1.23^{a}	1.33^{a}	1.23^{a}	1.40^{a}	1.23^{a}	0.87^{a}	1.23^{a}	0.93^{a}
Br.spp. R.3IB	1.10^{a}	1.10^{a}	1.23^{ab}	1.17^{a}	1.23^{a}	1.17^{a}	1.23^{a}	1.17^{a}	0.80^{a}	0.80^{a}	0.40^{b}	0.80^{a}
Br.spp. FA3	1.10^{a}	1.20^{a}	1.27^{a}	1.33^{a}	1.27^{a}	1.30^{a}	$1.2O^{a}$	1.33^{a}	1.20^{a}	1.33^{a}	1.27^{a}	1.33^{a}
CONTROL	1.07^{a}	0.47^{b}	1.17^{b}	1.20^{a}	1.30^{a}	1.23^{a}	1.30^{a}	1.23^{a}	1.30^{a}	1.23^{a}	1.30^{a}	1.23^{a}
LSD (0.05)%	0.48	0.60	0.09	0.19	0.14	0.59	0.17	0.65	0.53	1.20	0.68	1.23

Values having the same alphabet vertically are not significantly different from one another at P ≤ 0.05
Legend
AS: Agricultural Soil
COS: Crude Oil Soil
Control: Soil without rhizobium
LSD: Least Significant Difference

Table 5: Wet and Dry Weights (g) of Cowpea Plants Grown in Crude Oil Polluted and Agricultural Soils

Strain	Wet Weight		Dry Weight	
	COS	AS	COS	AS
A17	1.50^{a}	3.85^{a}	0.23^{a}	0.53^{a}
G34A	1.49^{a}	3.59^{a}	0.19^{a}	0.28^{a}
G34B	2.27^{a}	3.18^{a}	0.32^{a}	0.31^{a}
R.3IB	1.80^{a}	3.70^{a}	0.21^{a}	0.51^{a}
FA3	1.61^{a}	4.49^{a}	0.19^{a}	0.61^{a}
CONTROL	1.35^{a}	3.81^{a}	0.27^{a}	0.24^{a}
LSD (0.05)%	1.29	1.61	0.203	0.35

Values having the same alphabet vertically are not significantly different from one another at P ≤ 0.05%
Legend
AS: Agricultural Soil
COS: Crude Oil Soil
Control: Soil without rhizobium
LSD: Least Significant Difference

Biodegradation of Oil by Rhizobia

Biodegradation of Crude Oil

In minimal medium supplemented with crude oil, the optical density of each rhizobium reduced between 0 and 24 hours of incubation after which there was an increase in the optical density of the rhizobia. At 48 hr*R*. spp. CWP A17 and *Br*. spp. R.3IB had the highest O.D. (1.061 and 0.940). The lowest O.D. (0.318) at this time was observed in *Br*. spp. 1495 (Fig. 2). All the strains increased growth at 216 hr except *Br*. spp. T5BL and USAD 4675 which had their peak growth at 240 hr. In comparison, some oil degrading bacteria and fungi showed similar growth pattern except *Corynebacterium* species which had an increase in optical density between 0 and 48 hours of incubation (Fig. 3). Maximum growth was observed for *Bacillus megaterium*

(BM) with O.D. (1.979) at 216 hr followed by *Aerococcus viridian* (AV) with O.D. (1.343) and *Corynebacterium* species (C SPP) with O.D. (1.140). *Bacillus megaterium*(BM) with O.D. (1.848) experienced a decline in growth at 240 hr. Optical density also decreased in fungi (Fig. 4) *Aspergillusflavus*(AF) (O.D. 0.043) at 24 hr and later increased with highest O.D. (1.952) read at 240 hr. *Aspergillusrapens*(AR) had more steady growth but experienced a notable decline at 96 hr (O.D. 0.676)(Fig.1, 2 and 3).

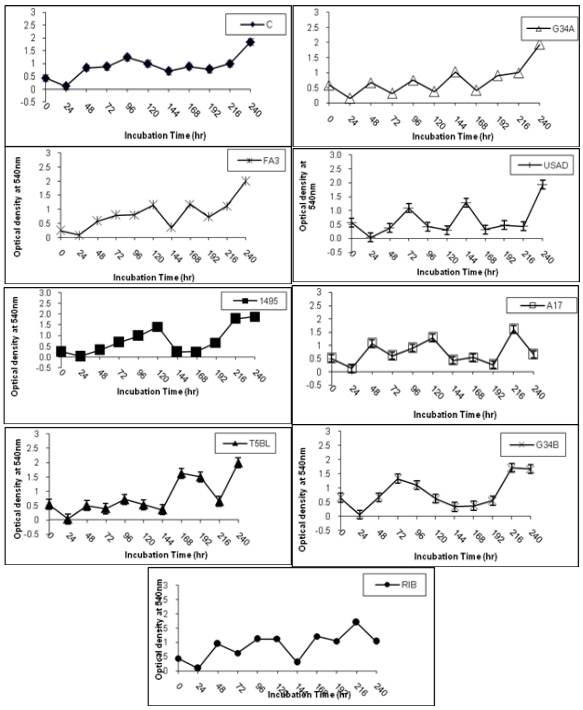
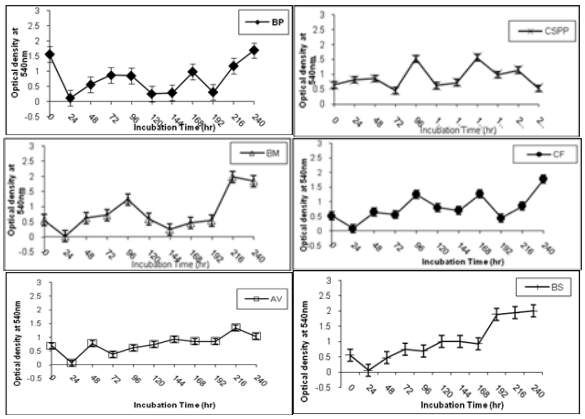


Figure 1: Degradation of crude oil by rhizobia

Legend:
C – *R.* species C
G34A – *R.* species CWP G34A
FA3 – *Br.* species FA3
USAD – *Br* . species USAD 4675 (400)
1495 – *Br.* species 1495
A17 – *R.* species CWP A17
G34B – *R.* species CWP G34B
R.3IB – *Br.* species R.3IB
T5BL – *Br.* species T5BL



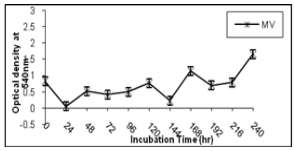


Figure 2: Degradation of crude oil by bacteria

Legend:
BP – *Bacillus pumilus*
C spp – *Corynebacterium*species (C SPP)
CF – *Cellulomonasflavigena*
AV – *Aerococcus viridian*
BM – *Bacillus megaterium*
MV – *Micrococcus varians*
BS – *Bacillus subtilis*

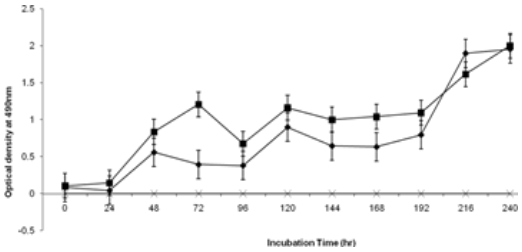
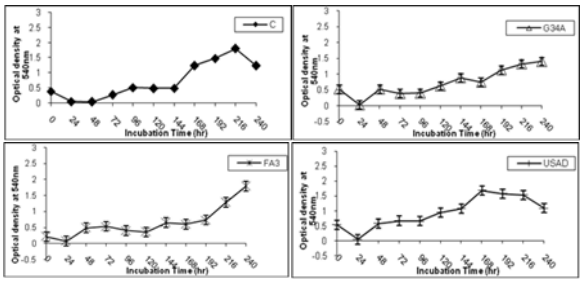


Figure 3: Degradation of crude oil by fungi

Legend:
♦ – Aspergillusflavus(AF)
▪ – Aspergillusrapens(AR)

Biodegradation of Diesel

Figure 4,5 and 6 show the performance of the microorganisms In the presence of diesel. All rhizobia had an initial decrease in their respective O.D. after 0 hour of incubation. These O.D. values increased after 24 hours of incubation except for *R.*species C which had its O.D. increased after 48 hours of cultivation. All rhizobia increased in O.D. with increased incubation period until peak O.D. values of 1.806 and 1.959 were obtained for *R.* spp. C and *Br.* spp. 1495 . In diesel O.D. values ranged between 0.152 and 0.740 at 48 hours of incubation for all the rhizobia. When compared with the oil degrading bacteria and fungi as mentioned above, it was observed that all the bacteria had similar trend of a decrease at 24 hr and an increase at 48 hr in O.D. At 240 hr*Bacillus subtilis* (BS) had the highest O.D. (1.998) followed by *Bacillus megaterium* (BM) while other bacteria had decrease in growth at same period. C SPP, BP, CF, MV and AV degraded the diesel optimally at 216 hours of cultivation with O.D. values of 1.890, 1.629, 1.592, 1.459 and 1.350 compared to the degradation of diesel between all the microbes. Both fungi *Aspergillusflavus*(AF) and *Aspergillusrapens*(AR) showed a steady increase in growth throughout the incubation period. The O.D. was lowest at 0.832 and highest at 1.989 at 240 hr.



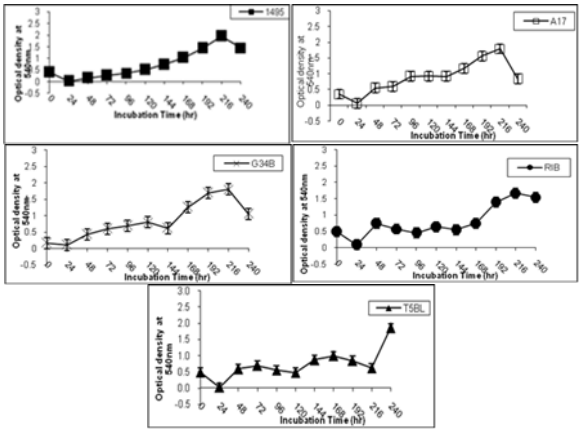


Figure 4: Degradation of diesel by rhizobia

Legend:
C – *R.* species C
G34A – *R.* species CWP G34A
FA3 – *Br.* species FA3
USAD – *Br* . species USAD 4675 (400)
1495 – *Br.* species 1495
A17 – *R.* species CWP A17
G34B – *R.* species CWP G34B
R.3IB – *Br.* species R.3IB
T5BL – *Br.* species T5BL

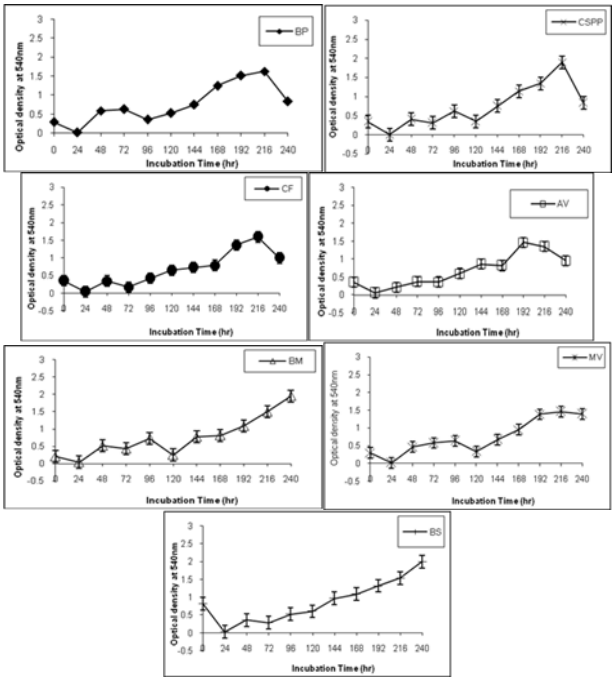


Figure 5: Degradation of diesel by bacteria

Legend:
BP – *Bacillus pumilus*
C spp – *Corynebacterium*species (C SPP)
CF – *Cellulomonasflavigena*
AV – *Aerococcus viridian*
BM – *Bacillus megaterium*
MV – *Micrococcus varians*
BS – *Bacillus subtilis*

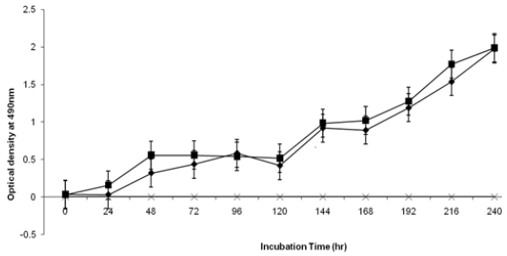


Figure 6: Degradation of diesel by fungi

Legend:
♦ – Aspergillusflavus(AF)
▪ – Aspergillusrapens(AR)

Biodegradation of Keresene

In the minimal medium containing kerosene, the O.D. of each rhizobium reduced at 24 hr except for *R.* species CWP G34A and *R.* species CWP G34B which cell loads increased. Besides *Br*. spp. R.3IB and *Br*. spp. USAD (400), all the rhizobial strains grew optimally at 216hr. At 240 hr*Br*. speciesR.3IB degraded the kerosene most (O.D. 1.998) followed by *Br.* speciesUSAD 4675 with highest O.D. of 1.962 recorded for 240 hr. There was a general decline in growth of the rhizobia at 240 hr of incubation (Fig. 7). The optical density of each bacterial strain used decreased at 24 hr but increased at 48 hr (Fig. 8). At this increase the lowest O.D. of 0.035 was recorded for *Corynebacterium* spp. The increase in O.D. continued during the degradation experiment until optimum O.D. values of 1.954 and 1.979 were obtained ranging at 168 to 216 hours. The highest O.D. of 1.954 was observed for *Corynebacterium* spp. (C spp.) at 216 hours of the degradation experiment. The fungal O.D. increased without an initial decrease (Fig. 9). *Aspergillusrapens*(AR) had its highest O.D. 0.572 earlier during 96 hr of the experiment in contrast to *Aspergillusflavus* (AF) that had its optimum O.D. 1.994 at 192 hr. Both experienced decline at 240 hr.

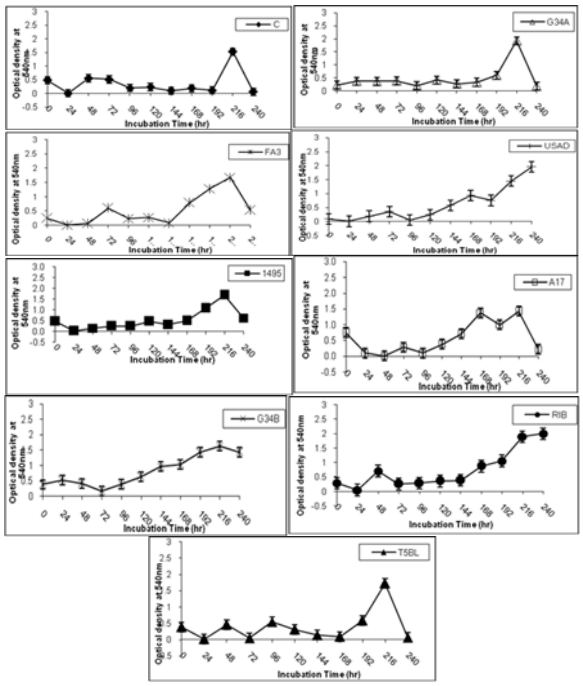


Figure 7: Degradation of kerosene by rhizobia

Legend:
C – *R.* species C
G34A – *R.* species CWP G34A

FA3 – *Br.* species FA3
USAD – *Br* . species USAD 4675 (400)
1495 – *Br.* species 1495
A17 – *R.* species CWP A17
G34B – *R.* species CWP G34B
R.3IB – *Br.* species R.3IB
T5BL – *Br.* species T5BL

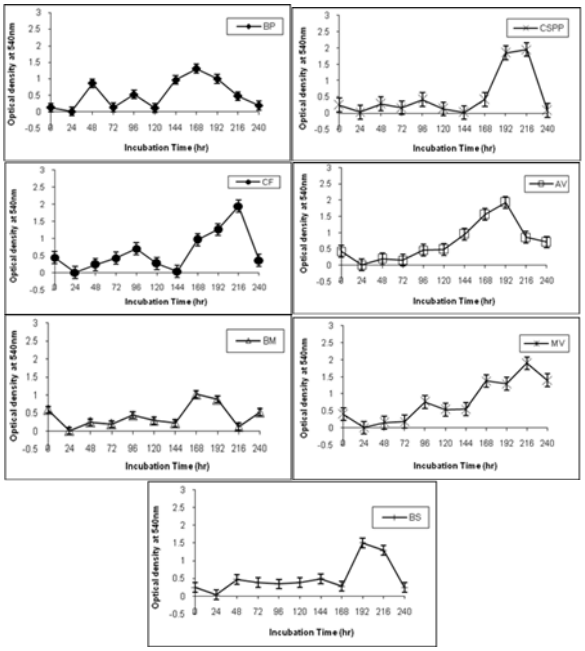


Figure 8: Degradation of kerosene by bacteria

Legend:
BP – *Bacillus pumilus*
C spp – *Corynebacterium*species (C SPP)
CF – *Cellulomonasflavigena*
AV – *Aerococcus viridian*
BM – *Bacillus megaterium*
MV – *Micrococcus varians*
BS – *Bacillus subtilis*

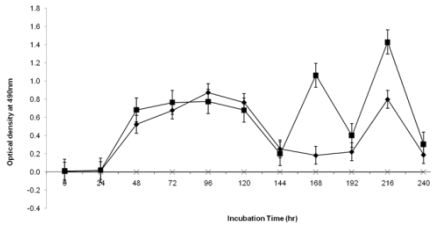


Figure 9: Degradation of kerosene by fungi

Legend:
♦ – Aspergillus flavus(AF)
▪ – Aspergillus rapens(AR)

Biodegradation of Petrol

In the minimal medium supplemented with petrol all the rhizobia showed similar growth pattern compared to when they were cultured in other fuels (Fig. 10). Between 48 and 120 hours of incubation the values were 0.055 to 1.153. The highest optimum O.D. values were generally lower than in diesel (1.998) and kerosene (1.998). *Rhizobium* spp. CWP G34B grew best in the petrol attaining peak with O.D. of 1.153 at 96 hr. In the case of the bacterial strains used the highest O.D. values were obtained at 48 – 120 hours of growth in petrol, after which there was a decline (Fig. 11).

Petrol was best degraded at 96 hr by *Micrococcus varians* (MV) having an O.D. of 1.049. This was followed by *Aerococcus viridians* (O.D. 0.853) at the same incubation time. The optical density of each fungus tested increased to 72 hr. *A. rapens* had best O.D. (1.429) at 216 hr of incubation although *Aspergilliusflavus* showed maximum growth at 96 hours but with lower O.D. (0.875 (Fig.12)

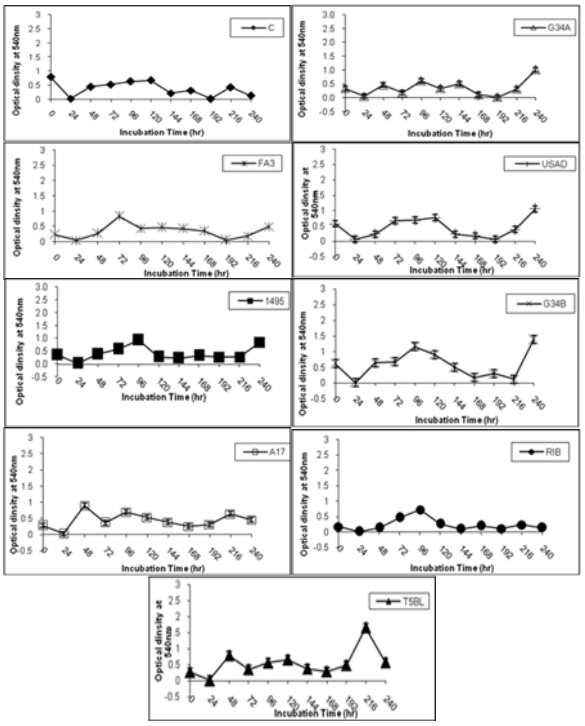
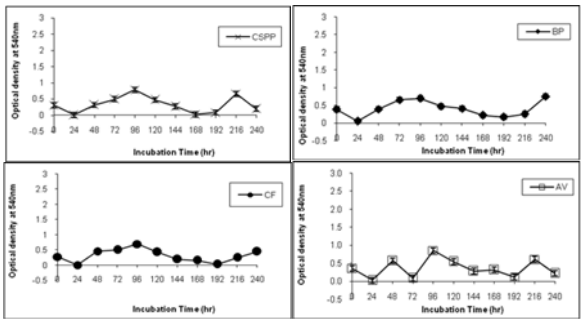


Figure 10: Degradation of petrol by rhizobia

Legend:
C – *R.* species C
G34A – *R.* species CWP G34A
FA3 – *Br.* species FA3
USAD – *Br* . species USAD 4675 (400)
1495 – *Br.* species 1495
A17 – *R.* species CWP A17
G34B – *R.* species CWP G34B
R.3IB – *Br.* species R.3IB
T5BL – *Br.* species T5BL



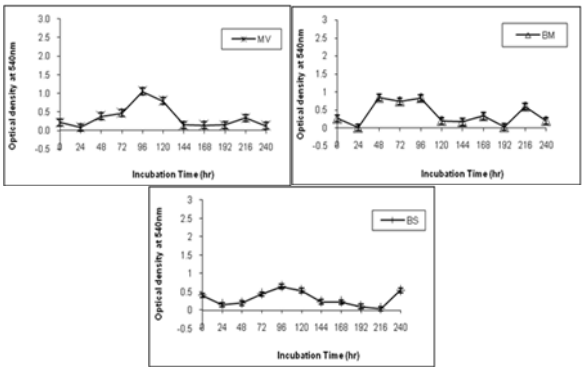


Figure 12: Degradation of petrol by bacteria

Legend:
BP – *Bacillus pumilus*
C spp – *Corynebacterium*species (C SPP)
CF – *Cellulomonasflavigena*
AV – *Aerococcus viridian*
BM – *Bacillus megaterium*
MV – *Micrococcus varians*
BS – *Bacillus subtilis*

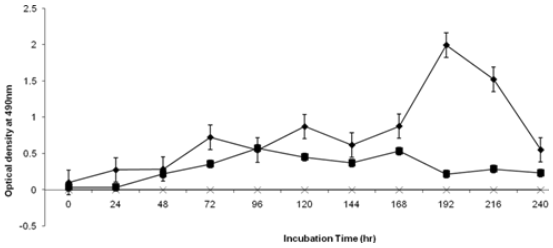


Figure 12: Degradation of petrol by fungi

Legend:
♦ – Aspergillus flavus(AF)
▪ – Aspergillus rapens(AR)

4. Discussion

Based on the soil textural triangle classification, the data obtained for particle size of the soil samples from the oil polluted and agricultural sites showed that each soil corresponds to silt clay loam and silt loam types of soil (Ayotamuno and Kogbara, 2007) soil types. The measured lower contents of the physicochemical parameters (total hydrocarbon, phosphorus, nitrogen, potassium, moisture content, calcium, magnesium, sodium, zinc iron, organic content, organic matter, manganese, cadmium, copper and clay) in the crude oil and agricultural soils after planting indicate that the plants absorbed immobilized some amount of the various components of the soil. These minerals are ionized or solubilized from solid phase of soil to make them available for plant uptake (Epstein, 1972). Soils are known to provide the essential micro and macronutrients required for plant development and growth.

From the physicochemical result obtained, it is evident that the crude oil polluted soil was actually rich in plant nutrient, and that the polluted land would have being rich for agricultural purpose. Analysis of the soil sample from the affected area showed that the organic content of the polluted soil was high, this might be responsible for its ability to produce greener leaves and growth to the level of producing some pods.

None of the rhizobia nodulated the cowpea when grown on crude oil polluted soil. This could be due to the fact that the amount of hydrocarbon in the crude oil polluted soil was too high for the good performance of the various rhizobia following its frequent exposure to spill which would have impaired aeration (Akyeampong, 1989) and this led to their inability to degrade and utilize all the hydrocarbons for plants growth and nodule formation. Hence, the oil incapacitated the rhizobia from effective nodulation and nitrogen fixation in contrast to agricultural soil. Agricultural soil supported nodule formation, nitrogen fixation and good growth of the cowpea.

Remarkable decrease in plant growth was noted in crude oil polluted soil relative to control. Plant growth in polluted soil was five times and above higher in control compared to polluted soil at harvest. This result is consistent with works of previous authors (Odjegba and Sadiq, 2002; Nwoko*et al*., 2007). The high concentration of the hydrocarbon contaminant retarded the growth of the plants. The pathway of nutrient release to plant is very vital, any interruption of this pathway obviously has a negative influence on the normal development and growth of plants (Nwoko*et al*., 2007). The presence of crude oil in the soil-plant microenvironment appeared to have affected normal soil chemistry wherein nutrient uptake as well as the amount of water were reduced. Similar observations were also reported by Ayotamuno and Kogbara (2007) who discussed that the infiltration of crude oil contaminant into soil pores leads the expulsion of air thus depleting oxygen reserves in the soil and impeding its diffusion to the deeper layers of the soil.

For the first few weeks of the second planting experiment, the leaves of plants grown in crude oil polluted soil were very green (greener than the leaves of agricultural soil grown plants). This could be due to the high carbon content of the crude oil polluted soil which probably stimulated the intensity of the green colouration. The burning sensation observed on the leaves of some of the plants grown on the crude oil soil at the early stages of growth corroborates with the findings of Wiltse*et al*., (1998), who stated that remediation of crude oil contaminated soils using plants (phytoremediation) has shown that certain plants could contain, translocate and volatilize petroleum-hydrocarbons as they grow on crude oil contaminated soils although not without constrains like leaf burn, wilting and stunted growth. This is due to the adaptive mechanism of some plants to crude oil contaminated soil which include the uptake of hydrocarbons from the contaminated soil by plants (phytoaccumulation), and transfer of volatile fractions of the contaminants to the atmosphere through the leaves (phytovolatilization) (Wiltse *et al*., 1998, Schwab *et al*., 1999). Also, plants have capacity to withstand relatively high concentration of organic chemicals such as oil without toxic effect and they can uptake and convert these contaminants quickly to less toxic metabolites in some cases by rhizodegradation. This is achieved by the release of root exudates and enzyme that stimulates mineralizaton of the oil contaminant. This process builds up organic carbon in the immediate environment (Nwoko*et al*., 2007).

Leguminous plants such as *Vignaunguiculata* capable of fixing nitrogen may have had advantage in remediation of

oil contaminated soil because of their ability to provide their own nitrogen fertilizer via nitrogen fixation to correct imbalance in C: N ratio (Nwoko*et al*., 2007). However, lack of nodules formation on plants grown on crude oil polluted soil compared with the control soil was due to the fact that the amount of hydrocarbon in the crude oil polluted soil was too high for the good performance of the various rhizobia, this led to their inability to initiate nodule formation. Hence, the oil incapacitated the rhizobia from effectively degrading all the hydrocarbons and utilize it as nutrient. Also, the high level of hydrocarbon content of the crude oil in the polluted soil prevented maximum aeration for the optimum performance of the rhizobia since they are aerobic organisms. The infiltration of contaminants into soil pores might have led to the expulsion of air thus depleting oxygen reserves in soil and impeding its diffusion to the deeper layers. This could have resulted into increased microbial activities and decreased amount of available oxygen in the soil environment (Ayotamuno, 2007). Occurrence of large amount of hydrocarbon in crude oil polluted soil led to a nitrogen insufficiency and hence upsets the carbon-nitrogen ratio at the spill site thereby threatening the survival of soil biota (Jobson *et al*., 1974) but the total nitrogen (TN) was high relative to the agricultural soil. Biodegradation of petroleum and its products used as sole carbon and energy source by rhizobia is an indication that the organisms have potential for bioremediation. Rhizobia can degrade the complex components of crude petroleum pollutants that may be present in the oil spilled environment particularly agricultural lands (Nwachukwu, 1999). Microorganisms are equipped with metabolic machinery to use petroleum as a carbon and energy sources (Diaz 2003, Van Hamme *et al*., 2003).They are involved in degrading a considerable number of organic pollutants that are parts of oil. These pollutants include: aliphatic compounds, n-alkanes, diesel fuel and tetrachloroethylene, monoaromatic compounds, toluene, benzene, xylene, ethylbenzene and polycyclic aromatic hydrocarbons (Bastiaen, 2000, Al-Deeb and Malkawi, 2009).

The decrease in the optical density reading of each rhizobium between 0 and 24 hours of incubation in minimal medium supplemented with crude oil might be due to environmental changes which the organisms must adapt to. *Rhizobium* species CWP A17 and *Br*. spp. R.3IB had the highest O.D. (1.061 and 0.940) at 48 hours of incubation because they were able to utilize crude oil in the medium as their carbon source faster than others while *Br*. spp. 1495 which had the lowest O. D. (0.318). *Corynebacterium* species had an increase in optical density between 0 and 48 hours of incubation due to the fact that it adapted faster to the crude oil medium and had started utilizing it as nutrient for multiplication in contrast to other oil degrading bacteria and fungi. When the activities of the various organisms were compared, *Bacillus megaterium*(BM) with O.D. (1.979) had the best performance which means it strived well in the crude oil medium and could possibly remediate crude oil soil faster than others.

The reduction in the optical density reading of each rhizobium between 0 and 24 hours of incubation in minimal medium supplemented with diesel could also be due to changes in environmental conditions which the organisms must strive to adapt to. This reduction persisted in *R.* species C till 48 hours of cultivation in contrast to other rhizobia and it could also be due to adaptation potential of this organism to diesel. *Bacillus subtilis* with O.D. (1.998) performed best among the different organisms in the diesel medium meaning that it may bioremediate diesel contaminated soil faster than the various rhizobia, bacteria and fungi used in this study.

Generally, in the minimal medium containing kerosene the decrease in cell load of each rhizobia at 24 hr could be due to the fact that the organism could not degrade the kerosene in the medium at the beginning therefore they needed some period to utilize it as their sole carbon. Optimal growth of the rhizobia at 216 hr implied that the organism had adapted to environmental conditions of the medium at that period. *Bradyrhizobium*speciesR.3IB (O.D. 1.998). General reduction in cell load of the various rhizobia at 240 hr implies that the rhizobia were able to degrade and utilize the kerosene for their growth and the kerosene in the medium which is the sole carbon source had depleted at this hour of incubation. *Bradyrhizobium*speciesR.3IB had the best activity among all the organisms and could likely degrade kerosene faster than other organisms considered in this study under same conditions.

The degradation of the petrol in the minimal medium supplemented with petrol by the various organisms was low. The highest optimum O.D. (1.429) was obtained in *A. rapens* in comparison with the degradation activities of the various organisms in the other fuel media eventhough they had similar degradative performances at the initial stage. However, since *A. rapens* had the greatest degradative ability in petrol medium, it shows that it was able to utilize the petrol in the medium as the sole carbon source better than other organisms in study. Therefore, it could degrade petrol faster than the other organisms when under the same environmental conditions.

5. Conclusion

From the physicochemical result obtained, it is evident that the crude oil polluted soil was actually rich in plant nutrient, and that the crude oil polluted land at Okochiri, Okricha Local Government Area, River State would have being rich for agricultural purpose. Hence, the need to reclaim the soil for agricultural use.

From the planting experiments it could be concluded that the oil polluted soil had negative influence on the nodulating and nitrogen fixing abilities of the tropical rhizobia because none of the rhizobia formed nodules which is an evidence of good nitrogen fixation.

Rhizobia are not too commonly used in bioremediation, even though, some like *Rhizobia galega* can bioremediate, the ability of the chosen rhizobia to degrade was considered.

The biodegradation experiment showed that for the various rhizobia grown in crude oil, diesel, petrol and kerosene media, the degradation of the hydrocarbon occurred, in which the organisms were able to utilize the fuels as carbon source. Therefore, they can be used to reclaim oil spillage and probably agricultural soil polluted with agrochemicals

which are hydrocarbon based in the environment t(this will be the second phase and the concluding part of this investigation, in which not soil polluted with petrolueum products but agricultural soil polluted with agrochemicals which are hydrocarbons based will be used.

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