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Research Paper

Evaluation of crude oil degradation potential of *Fusarium* spp.

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The biodegradation potential of *Fusarium* spp was investigated on soil experimentally polluted with crude oil. The fungal load dynamics of *Fusarium* spp. and the physicochemical parameters of the polluted soil were determined using standard microbiological and analytical (Spectrophotometer, model APEL PD-303UV) methods. The parameters were determined at weekly intervals and at varying levels of crude oil concentration (10%, 20%, 30% and 40%) for a period of six weeks. There was a progressive increase in the fungal load dynamics ($3.0x10^4$±0.05cfu/g-$1.41x10^5$±0.06cfu/g; $2.9x10^4$±0.02cfu/g- $1.32x10^5$±0.02cfu/g; $3.2x10^4$±0.04cfu/g-$1.41x10^5$±0.05cfu/g and $2.4x10^4$±0.01cfu/g-$1.30x10^5$±0.02cfu/g) from week one to week six at 10%, 20%, 30% and 40% crude oil concentrations respectively. The highest fungal load ($1.41x10^5$±0.06cfu/g) was recorded on 10% crude oil concentration at week six while the lowest fungal load ($2.4x10^4$±0.01cfu/g) was recorded on 40% crude oil concentration at week one. The result of the total petroleum hydrocarbon (TPH) showed a progressive decrease (1410.6±0.215mg/kg-363.03±0.215mg/kg;1446.0±0.215mg/kg-422.77±0.215mg/kg;1589.6±0.215mg/kg-853.47±0.215mg/kg and 1948.4±0.215mg/kg-858.28±0.215mg/kg) from week one to week six at 10%, 20%, 30% and 40% crude oil concentrations respectively. Other physicochemical parameters such as pH (5.4±0.005-6.3±0.005), percentage nitrogen (0.14±0.029%-0.364±0.029%) and electrical conductivity (12.25±0.015µs - 22.2±0.015µs) showed progressive increase as observed on the 10% crude oil concentration from week one to week six respectively. The result indicates the potential of *Fusarium* spp. in the degradation of crude oil as evidenced in the progressive reduction in the total petroleum hydrocarbon. The use of *Fusarium* spp. in the bioremediation of crude oil polluted sites is recommendable.

Key words: Bioremediation, Potential, *Fusarium* spp, Hydrocarbon.

INTRODUCTION

Crude oil is a naturally occurring complex mixture of hydrocarbon and non-hydrocarbon compounds which possesses a measurable toxicity towards living systems (Nelson-Smith, 1973). The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment (Gutnick and Rosenberg, 1977). The single largest source of petroleum pollution is routine, low-level discharge such as urban runoff, cleaning operations, and oil treatment of roads for dust control. These sources, together, account

for 90% of total anthropogenic petroleum pollution (Bartha, 1986). Oil pollution also comes from oil-well blowouts, seepage, and deballasting operations, sale and use of petroleum products, pipeline overflow and breakage, and storage tank spills (Plice, 1948; Obire and Wemedo, 1996). Addition of oil to the soil as a deliberate policy of waste disposal also leads to contamination (Flowers *et al*., 1974). Obire and Amusan, (2003) reported deliberate discharge of oilfield wastewater or effluent as a source of environmental contaminant. Non-anthropogenic sources of hydrocarbons in the environment include natural seepage, and synthesis of hydrocarbons and hydrocarbon-like compounds by fungi (Alexander, 1977). The toxicity of crude oil or petroleum products varies widely, depending on their composition and concentration, on environmental factors and on the biological state of the organisms at the time of the contamination. In heavily polluted areas, there are immediate detrimental effects on plant and animal life, including agriculture (Baker, 1970; Steinhart and Steinhart, 1972; Rowell, 1977; Fagbami *et al*., 1988; NDWC., 1995). Nevertheless, different species and different life stages of organisms have different susceptibilities to pollution (Nelson-Smith, 1973).

In addition to its effects on visible plants and animals, petroleum contamination impacts microbial populations (Ahearn and Meyers, 1976). The effect of oil on microbial populations depends upon the chemical composition of the oil and on the species of microorganisms present. Populations of some microbes increase; typically, such microbes use the petroleum hydrocarbons as nutrients. The same crude oil can favor different genera at different temperatures (Westlake *et al*., 1974). However, some crude oils contain volatile bacteriostatic compounds that must degrade before microbial populations can grow (Atlas and Bartha, 1972; Atlas, 1975). On the other hand, some microbial populations decrease or show a neutral response to petroleum hydrocarbons. The overall effects of petroleum hydrocarbons on total microbial diversity remain unclear. Hydrocarbon-degrading bacteria and fungi are widely distributed in marine, freshwater and soil habitats. Similarly, hydrocarbon-degrading *Cyanobacteria* have been reported to be wide-spread in many habitats (Chaillan *et al.,* 2004; Lliros *et al.,* 2003). Typical bacterial groups already known for their capacity to degrade hydrocarbons include *Pseudomonas, Marinobacter, Alcanivorax, Microbulifer, Micrococcus, Cellulomonas, Dietzia* and *Gordonia* groups (Brito *et al.,* 2006). Molds belonging to the genera *Aspergillus, Penicillium, Fusarium, Amorphoteca, Neosartoria, Paecilomyces, Talaromyces, Graphium* and the yeasts *(Candida, Yarrowia* and *Pichia)* have been implicated in hydrocarbon degradation (Chaillan *et al.,* 2004). It is now widely accepted that many fungi can degrade petroleum hydrocarbons, such as the white rot fungus *Phanerochaete chrysosporium* (Huynth *et al.,* 1985; Gold *et al.,* 1994), *Candida* and *sporobolomyces* (Bartha and Atlas, 1977; Yateem *et al.,* 1998). Davis and Westlake, (1979) examined 60 fungal isolates for their ability to grow on N-tetradecane, toluene, naphthalene and seven crude oils of various compositions. Forty cultures including 25 soil isolates could grow on one or more of the crude oils. The genera most frequently isolated from soils were those producing large quantities of small conidia, e.g., *Penicillium* and *Verticillium* spp., together with oil-degrading strains of *Beauvaria bassianae, Mortierilla* spp., *Phoma* spp., *Sclerobasidium oboratum* and *Tolypocladium infiatum.* Hashem and AI-Harbi, (2000) isolated *A.flavus, A.niger, Curvuiaria lunata* and *Trichodenna* spp. from sandy soil contaminated with crude oil incubated for five weeks. *Trichodenna* spp. exhibited an increasing mycelium dry weight with increasing crude oil concentrations, while *Rhizopus* spp. exhibited the lowest degradation capability of crude oil. The most widely studied fungus is *Trichodenna harzianum,* which grows best in the presence of petroleum hydrocarbon, followed by *A. flavus* and *Chaetomium botrychoid (*Bokhary and Parvaz, 1993). Also, *Penicillium* spp., *Trichodenna viride, Alternaria tenuis* and *Aspergillus terreus* can degrade petroleum oil as reported by Gomez *et al.* (2003). However, reports in the literature on the actual numbers of hydrocarbon utilizers are at variance with one another because of the methodological differences used to enumerate petroleum-degrading microorganisms. The most common method to isolate hydrocarbon degraders involves the use of hydrocarbons incorporated into an agar based medium (Horowitz *et al.,* 1978). Biodegradation of both crude and refined oils involves a consortium of organisms including both eukaryotic and prokaryotic forms. Despite the fact that many hydrocarbons are toxic to many organisms, there are many microorganisms, including bacteria and fungi that use hydrocarbons as a carbon and energy source (Da Silva *et al.,* 2003). The experiment was setup to determine the degradation potential of *Fusarium* spp on crude oil.

MATERIALS AND METHODS

Sample collection

Soil samples were collected with a soil auger at surface depth (0-15 cm) from a virgin fallow Land in the forest area of Imo State Polytechnic, Umuagwo in Ohaji Egbema Area of Imo State in South Eastern Nigeria, having no pollution history and devoid of crude oil contamination. The soil sample for physicochemical analysis was collected using calico soil bag. All samples were labeled with a permanent waterproof marker, while the soil samples for microbiological analyses were collected aseptically using 200 ml capacity sterile glass sampling container. Crude oil was obtained from Adax Petroleum Company. All samples were transported to the Laboratory immediately after collection.

Isolation, characterization and identification of fungi

The method described by Fawole and Oso, (1988) was

used. One gram of the soil for microbiological analysis was mixed with 9 ml sterile distilled water. Ten-fold serial dilution of the mixture was prepared using sterile distilled water to dilution 10^{-3}. However, 0.1ml of each diluted sample was placed on Potato Dextrose Agar PDA medium by spread plate method and incubated for 2 to 5 days at a temperature of 25°C for the enumeration of fungi. Fungal colonies formed on PDA were sub-cultured on PDA plates and incubated at 25°C and observed daily for growth. On establishment of growth, the cultures were observed for distinct colonies. These distinct colonies were made on fresh sterile PDA plates. Subcultures with uniform growth were considered to be pure. The pure fungal isolates were examined macroscopically and microscopically using the needle mounts technique as described by Fawole and Oso (1988). Their identification was performed according to the scheme of Barnette and Hunter, (1987).

Testing the ability of the fungi species to degrade crude oil

A modified method of Adenipekun and Fasidi, (2005) was employed. Two hundred grams of sterilized moistened soil was weighed into 350 cm^3 sterilized jam bottles. Varying concentrations (10, 20, 30 and 40% w/w) of crude oil was added and mixed thoroughly with the soil. These bottles were autoclaved at 25°C for 15 minutes. After cooling, each bottle was inoculated with the isolated fungi in an inoculating chamber. The bottles were incubated at 25°C for 6 weeks. Samples were collected at weekly intervals to determine the fungal population dynamics and to check the changes in the physicochemical parameters of the samples for the six weeks. The control samples were contaminated with crude oil at varying concentrations but were not inoculated with the fungal isolates.

Soil Physicochemical Analysis

Soil physicochemical characteristics such as soil pH, total organic carbon, total nitrogen, Organic matter, soil conductivity and heavy metals (Ni, Cr) were determined before fungal inoculation and at weekly intervals after inoculation for six weeks. Aseptic sampling techniques were used to avoid contamination. The soil pH and conductivity were obtained by direct reading using pH and conductivity meter respectively.

Organic carbon determination

The organic carbon was determined using the chromic wet acid oxidation method. 0.1 g of the sample was weighed into a 250 ml conical flask, 5 ml of 1N Potassium Dichromate solution was added to the sample, followed by 10 ml of Conc. H_2SO_4. This was allowed to stand for about 30 min. The sample was diluted by adding 50 ml of distilled water, again left for another 30min. 5ml of Ortho-phosphoric acid and 3 drops of Barium diphynl-alamine indicator was added and titrated with 0.5N Ferrous Ammonium Sulphate solution.

Total nitrogen determination

Total Nitrogen was determined by semi-micro Kjeldahl method. 0.1 g of the sample was weighed into the digestion flask; One tablet of Selenium catalyst was added and moistened with a little quantity of distilled water. 5 ml of Conc. H_2SO_4 was added and placed on the digestion block. The sample was heated over a fume cupboard until the sample is digested. The digest was made up in a 50ml volumetric flask for semi-micro distillation. The Markham distillation apparatus was switched on and 10ml of the digest was introduced into the distillation chamber. 10ml of 45% NaOH was added gently and the sample allowed distilling into a 10ml of 4% Boric acid. About 50ml distillate was collected and titrated with 0.02N H_2SO_4 to get back a pinkish-red end point. Organic carbon, organic matters, % Nitrogen, Phosphorus, Potassium were determined using official methods of the Association of Analytical Chemists (AOAC, 2003).

Determination of total petroleum hydrocarbon (TPH)

The total hydrocarbon was determined by Spectrophotometer method. 1.0g of the sample was weighed into a test tube and 10ml of Toluene was added. This was placed on a water bath in the fume cupboard and digested for about 30minutes. 5ml of the digest was pipette into a 50ml flask and made up to volume. The absorbance was measured in a Spectrophotometer (model APEL PD-303UV) at 570 nm wavelength.

RESULTS AND DISCUSSION

Fungal load dynamics of crude oil polluted soil after treatment at weekly intervals

The result of the Analysis of Variance (ANOVA) of the fungal load dynamics of crude oil polluted soil after treatment at weekly intervals is presented in and (Table 1). The results in (Table1) reveals that the mean values of *Fusarium* spp increased from $3.0x10^4$±0.05cfu/g, $2.9x10^4$±0.02cfu/g, $3.2x10^4$±0.04cfu/g and $2.4x10^4$±0.01cfu/g at week one of soil treatment with crude oil at 10%, 20%, 30% and 40% levels of concentration respectively to $1.41x10^5$±0.06cfu/g, $1.32x10^5$±0.02cfu/g,$1.41x10^5$±0.05cfu/g and $1.30x10^5$±0.02cfu/g at the sixth week of soil treatment with crude oil at 10%, 20%, 30% and 40% levels of concentration respectively (Figure 1). There were significant differences at (P≤0.05) by Duncan Multiple

Table 1. Fungal load dynamics of crude oil polluted soil after treatment at weekly intervals.

Percentage of crude oil	Control cfu/g	Week 1cfu/g	Week 2 cfu/g	Week 3 cfu/g	Week 4 cfu/g	Week 5 cfu/g	Week 6 cfu/g	ANOVA F-statistic
10	$1.8x10^4 \pm 0.02^g$	$3.0x10^4 \pm 0.05^f$	$4.5x10^4 \pm 0.08^e$	$7.3x10^4 \pm 0.03^d$	$1.13x10^5 \pm 0.02^c$	$1.30x10^5 \pm 0.03^b$	$1.41x10^5 \pm 0.06^a$	33.186***
20	$1.8x10^4 \pm 0.02^g$	$2.9x10^4 \pm 0.02^f$	$4.3x10^4 \pm 0.03^e$	$7.1x10^4 \pm 0.05^d$	$1.12x10^5 \pm 0.01^c$	$1.21x10^5 \pm 0.08^b$	$1.32x10^5 \pm 0.02^a$	30.314***
30	$1.8x10^4 \pm 0.02^g$	$3.2x10^4 \pm 0.04^f$	$4.7x10^4 \pm 0.08^e$	$7.0x10^4 \pm 0.03^d$	$1.10x10^5 \pm 0.54^c$	$1.30x10^5 \pm 0.01^b$	$1.41x10^5 \pm 0.05^a$	27.37***
40	1.8x104±0.02g	$2.4x104 \pm 0.01^t$	$3.9x104 \pm 0.03^e$	$5.9x104 \pm 0.03^d$	$9.9x104 \pm 0.01^c$	$1.19x105 \pm 0.04^b$	$1.30x105 \pm 0.02^a$	10.715***

Notes: Results are means± standard error of means of three replicates. Values in each row followed by different superscript within each row showed statistical significant difference. *** = significant at P = 0.01(1%).Values in each row followed by different superscript within each row show difference in the rate of degradation for the fungal specie.

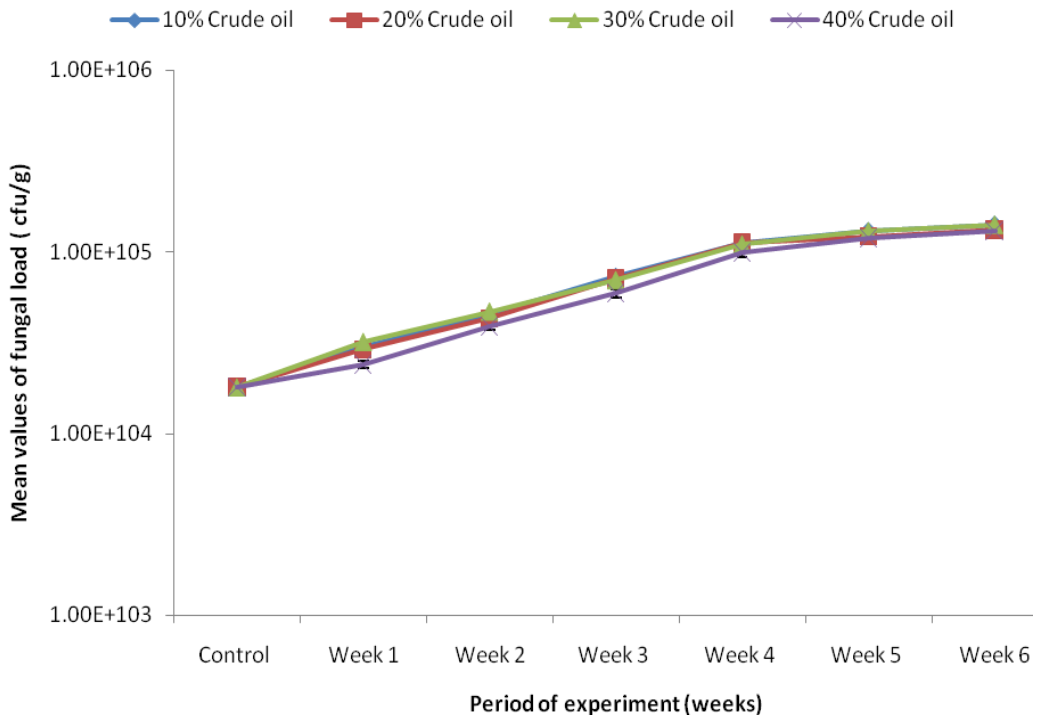

Figure 1. Fungal load dynamics of crude oil polluted soil after treatment at weekly intervals.

Range Test (DMRT) in the mean value of *Fusarium spp* found in soil polluted with crude oil at both period of degradation (weeks) and levels of crude oil concentration. The result shows that more *Fusarium spp* was found in the soil polluted with crude oil at the sixth week of crude oil degradation for all the levels of crude oil concentration and was least found in the soil at the zero weeks (control). This implies that *Fusarium spp* increased in growth

Table 2.TPH property of soil contaminated with crude oil after treatment with *Fusarium* spp.

Percentage of crude oil	Control mg/kg	Week 1 mg/kg	Week 2 mg/kg	Week 3 mg/kg	Week 4 mg/kg	Week 5 mg/kg	Week 6 mg/kg
10	386.2±0.039^{b}	1410.6±0.215^{d}	968.5±0.215^{d}	832.9±0.215^{d}	574.7±0.215^{d}	558.5±0.215^{d}	363.03±0.215^{c}
20	374.5±0.038^{c}	1446.0±0.215^{c}	1164.6±0.215^{c}	873.45±0.215^{c}	602.72±0.215^{c}	631.0±0.215^{c}	422.77±0.215^{b}
30	416.6±0.042^{a}	1589.6±0.215^{b}	1746.3±0.215^{b}	1274.5±0.215^{b}	1223.5±0.215^{a}	1108.4±0.215^{a}	853.47±0.215^{a}
40	196.5±0.020^{d}	1948.4±0.215^{a}	2148.4±0.215^{a}	1482.1±0.215^{a}	1111.5±0.215^{b}	1059.6±0.215^{b}	858.28±0.215^{a}
Anova F-statistic	9.085***	659.085***	9.085***	549.085***	436.609***	246.609***	313.574***

Notes: Results are means± standard error of means of three replicates. Values in each column within each week followed by different superscript showed statistical significant difference. *** = significant at P = 0.01(1%).

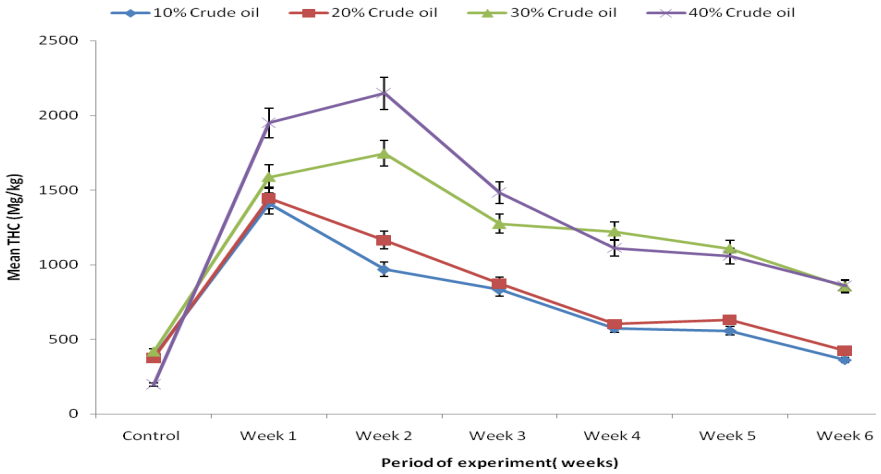

Figure 2. Mean value of total hydrocarbon property of soil contaminated with crude oil after treatment with *Fusarium* spp.

as the period of crude oil degradation increased. *Fusarium spp* was found to be highest at 10% and 30% level of crude oil concentration and least at 40% level of crude oil concentration.

Physicochemical properties of soil contaminated with crude oil after treatment with *Fusarium Spp.*

The results of the Analyses of Variance (ANOVA) of the physicochemical parameters of soil contaminated with crude oil after incubation with *Fusarium* spp. are presented in (Tables 2, 3, 4 and 5). The result of the physicochemical properties of soil contaminated with crude oil after treatment with *Fusarium* spp. indicates that the percentage Nitrogen in the soil increased in both weeks and percentage level of oil concentration (Table 5). At 10% level of oil concentration, the percentage Nitrogen in the soil degraded from 0.14±0.029% at week one to 0.364±0.029% at week six. At 20% level of oil concentration, the percentage Nitrogen in the soil degraded from 0.112±0.029% at week one to 0.48±0.029% at week six. At 30% level of oil concentration, percentage Nitrogen degraded from 0.182±0.029% at week one to 0.336±0.029% at week six and at 40% level of oil concentration, percentage Nitrogen degraded from 0.18±0.029% at week one to 0.308±0.029% at week six.

Table 3: Electrical conductivity property of soil contaminated with crude oil after treatment with *Fusarium* spp.

Percentage of crude oil	Control (µs)	Week 1(µs)	Week 2 (µs)	Week 3 (µs)	Week 4 (µs)	Week 5 (µs)	Week 6 (µs)
10	14.4±0.016^{c}	12.25±0.015^{c}	11.69±0.015^{a}	12.24±0.015^{c}	19.24±0.015^{b}	21.16±0.015^{b}	22.2±0.015^{d}
20	17.95±0.020^{a}	23.8±0.015^{a}	11.2±0.015^{b}	11.01±0.015^{d}	20.1±0.015a	22.1±0.015^{a}	34.1±0.015^{c}
30	17.18±0.020^{b}	11.59±0.015^{d}	11.21±0.015^{b}	12.44±0.015^{b}	14.6±0.015^{d}	18.26±0.015^{d}	38.28±0.015^{b}
40	12.41±0.014^{d}	21.8±0.015^{b}	9.24±0.015^{c}	14.73±0.015^{a}	17.7±0.015^{c}	20.00±0.015^{c}	44.1±0.015^{a}
Anova F-statistic	12.412***	22.412***	12.412***	12.412***	23.574***	13.574***	20.873***

Notes: Results are means± standard error of means of three replicates. Values in each column within each week followed by different superscript showed statistical significant difference. *** = significant at P = 0.01(1%).

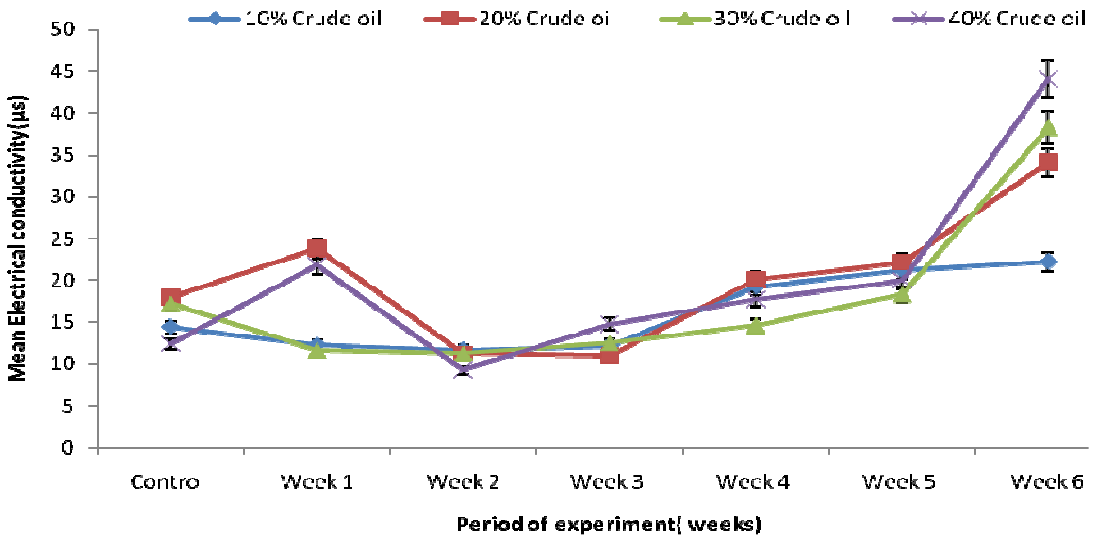

Figure 3. Mean value of Electrical conductivity property of soil contaminated with crude oil after treatment with *Fusarium* spp.

There was significant difference in the mean percentage Nitrogen in the soil between each level of oil concentration at various weeks. The percentage Nitrogen content of the soil inoculated with *Fusarium* spp was highest for 10% level of concentration at week five and 20% level of concentration at week six and least a week three at 20% level of oil concentration and week four at 40% level of oil concentration. The pH in the soil increased in both weeks and percentage level of oil concentration (Table 4). At 10% level of oil concentration, the pH in the soil ranged from 5.4±0.005 at week one to 6.3±0.005 at week six. At 20% level of oil concentration, the pH in the soil ranged from 5.31±0.005 at week one to 5.9±0.005 at week six. At 30% level of oil concentration, pH ranged from 5.28±0.005 at week one to 5.78±0.005 at week six and at 40% level of oil concentration,

Table 4. pH level of soil contaminated with crude oil after treatment with *Fusarium* spp.

Percentage of crude oil	Control	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
10	6.33±0.083^{a}	5.4±0.005^{a}	6.02±0.005^{a}	5.81±0.005^{c}	5.75±0.005^{c}	5.9±0.005^{b}	6.3±0.005^{a}
20	5. 95±0.080^{b}	5.31±0.005^{b}	5.97±0.005^{b}	5.88±0.005^{b}	6.11±0.005^{a}	6.26±0.005^{a}	5.9±0.005^{b}
30	5.88±0.079^{b}	5.28±0.005^{c}	5.99±0.005^{b}	5.82±0.005^{c}	5.94±0.005^{b}	5.88±0.005^{c}	5.78±0.005^{c}
40	5.99±0.079^{b}	5.42±0.005^{a}	6.00±0.005^{a}	5.94±0.005^{a}	5.66±0.005^{d}	5.92±0.005^{b}	5.00±0.005^{d}
Anova F-statistic	7.909***	7.299***	7.99***	17.996***	10.873***	12.873***	8.562***

Notes: Results are means± standard error of means of three replicates. Values in each column within each week followed by different superscript showed statistical significant difference. *** = significant at P = 0.01(1%).

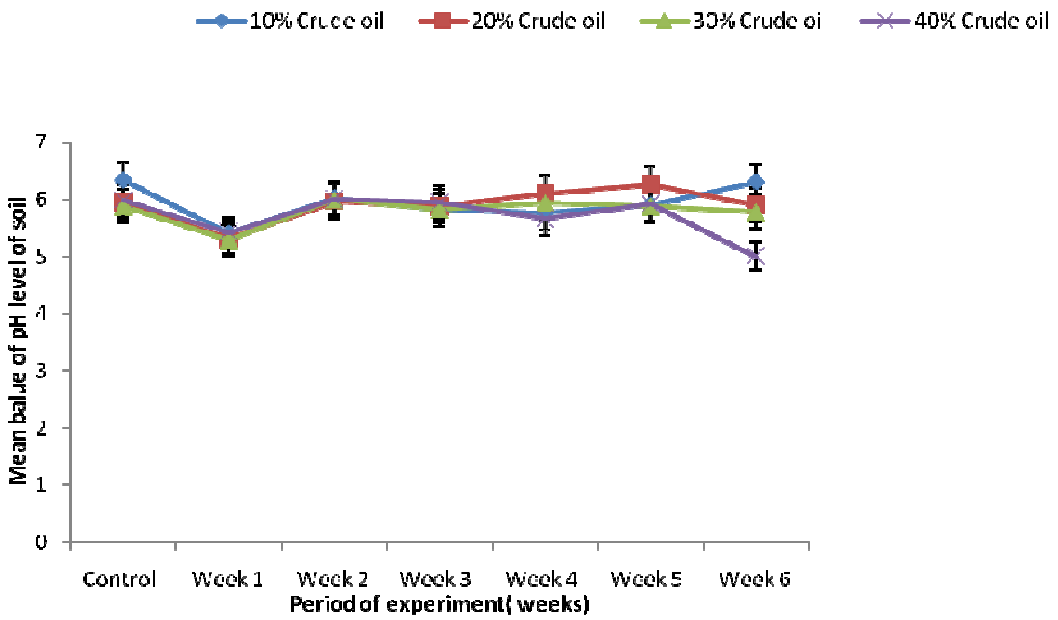

Figure 4. PH level of soil contaminated with crude oil after treatment with *Fusarium* spp.

pH ranged from 5.42±0.005 at week one to 5.00±0.005 at week six. There was significant difference in the mean pH in the soil between each level of oil concentration at various weeks. The pH content of the soil inoculated with *Fusarium* spp was highest for 10% level of concentration at week six and least at week six at 40% level of oil concentration. The electrical conductivity in the soil increased in both weeks and percentage level of oil concentration (Table 3). At 10% level of oil concentration, the electrical conductivity in the soil ranged from 12.25±0.015µs at week one to 22.2±0.015µs at week six. At 20% level of oil concentration, the electrical conductivity in the soil ranged from 23.8±0.015µs at week one to 34.1±0.015µs at week six. At 30% level of oil concentration, electrical conductivity ranged from 11.59±0.015µs at week one to 38.28±0.015µs at week six and at 40% level of oil concentration, electrical conductivity ranged from 21.8±0.015µs at week one to 44.1±0.015µs at week six. There was significant difference in the mean electrical conductivity in the soil between each level of oil concentration at various weeks. The electrical conductivity content of the soil inoculated with *Fusarium* spp was highest for 40% level of concentration at week six and least at week three at 20% level of oil concentration.

The total hydrocarbon in the soil decreased in both weeks and percentage level of oil concentration (Table 2 and Figure 2). At 10% level of oil concentration, the total hydrocarbon in the soil degraded from 1410.6±0.215mg/kg at week one to 363.03±0.215mg/kg at week six. At 20% level of oil concentration, the total hydrocarbon in the soil degraded from 1446.0±0.215mg/kg at week one to 422.77±0.215mg/kg at week six. At 30% level of oil concentration, total hydrocarbon degraded from 1589.6±0.215mg/kg at week one to 853.47±0.215mg/kg at week six and at 40% level of oil concentration, total hydrocarbon degraded from 1948.4±0.215mg/kg at week one to 858.28±0.215mg/kg at week six. There was significant difference in the mean total hydrocarbon in the soil between each level of oil concentration at various weeks. The total hydrocarbon content of the soil inoculated with *Fusarium* spp. was

Table 5. Nitrogen property of soil contaminated with crude oil after treatment with *Fusarium* spp.

Percentage of crude oil	Control %	Week 1%	Week 2 %	Week 3 %	Week 4 %	Week 5 %	Week 6 %
10	0.140±0.021^{b}	0.14±0.029^{b}	0.137±0.029^{b}	0.116±0.029^{c}	0.232±0.029^{a}	0.48±0.029^{a}	0.364±0.029^{b}
20	0.28±0.023^{a}	0.112±0.029^{c}	0.112±0.029^{d}	0.106±0.029^{d}	0.182±0.029^{b}	0.364±0.029^{b}	0.48±0.029^{a}
30	0.126±0.021^{b}	0.182±0.029^{a}	0.126±0.029^{c}	0.196±0.029^{b}	0.140±0.029^{c}	0.35±0.029^{c}	0.336±0.029^{c}
40	0.28±0.023^{a}	0.18±0.029^{a}	0.14±0.029^{a}	0.280±0.029^{a}	0.106±0.029^{d}	0.118±0.029^{d}	0.308±0.029^{d}
Anova F-statistic	8.258***	8.328***	8.28***	18.288***	12.562***	15.562***	15.589***

Notes: Results are means± standard error of means of three replicates. Values in each column within each week followed by different superscript showed statistical significant difference. *** = significant at P = 0.01(1%).

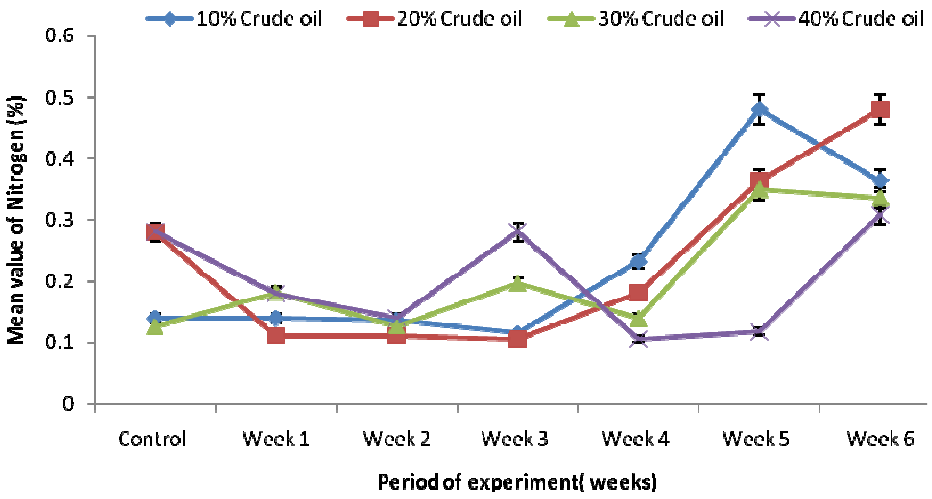

Figure 5. Nitrogen property of soil contaminated with crude oil after treatment with *Fusarium* spp

highest for 40% level of concentration at week two and least a week six at 10% level of oil concentration.

Crude oil pollution on the soil caused a reduction in pH, electrical conductivity and percentage nitrogen content of the soil (Tables 3, 4, and 5 and Figure 3, 4 and 5). The observed reduction in pH and conductivity was similar to the findings of Osuji and Nwoye, (2007). A reduction in pH implies increased acidity which is a problem for agricultural soils because many metal cations are more soluble and available in the soil solution at very low pH including Cd, Cu, Hg, Ni, Pb and Zn (McBride, 1994). The resulting increased acidity could be due to the fact that hydrocarbons contain many free cations causing them to have properties of a weak acid. Reduced conductivity could be due to the non-polar nature of the crude oil bringing about reduced ionic movement in the soil (Akpoveta *et al*., 2011). Significant increase in pH and conductivity were observed from week one to week six of the bioremediation (Tables 4 and 3, Figures 3 and 4). The observed increase on introduction of crude oil could be due to the fact that crude oil contains varying proportions of nitrogenous substances and is highly carbonaceous. An increase in such properties is deemed desirable since they are important soil parameters that are critically important in maintaining soil fertility. The observed increase in pH and conductivity was due to the bioremediation process which removed contaminant and introduced some salts and ions. The result of this study also showed an increase in the pH of the soil at different crude oil concentration and at different period. This is in line with the observations of (Onuh *et al.,* 2008a). The increase in the acidity of the soil at the beginning of the study could be as a result of increased crude oil concentration as opined by Amadi *et al.,* 2005; Osuji and Adesiyan, 2005. Strong acidic soils (pH 4 to 5) have been reported to have high concentration of soluble aluminum and manganese salts, which are toxic to plants but carbon mineralization and organic matter breakdown are rapid in neutral to slightly alkaline soils (Hunt, 1996). Higher pH range (6 to 9) provides better conditions for mineralization of hydrocarbons since most microorganisms capable of metabolizing hydrocarbons develop best at pH conditions close to neutrality (Tanee and Kinako, 2008; Manuel *et al.,* 1993; Atlas and Bartha, 1992). The total hydrocarbon in the treated samples decreased progressively during the study period. This can be assumed to be as a result of increase in the hydrocarbon utilizing fungal count which used up the

hydrocarbon in the crude oil to multiply in number. However, it could also be as a result of the breakdown of the crude oil in the crude oil polluted soil. High concentration of THC in soil is detrimental to the growth and productivity of plants and animals according to (Okolo *et al.,* 2005; Osuji *et al.,* 2004 and Salanitro *et al.,* 1997). Osuji and Udoetok, (2008) further opined that total extractible hydrocarbon content of soils is used to assess and ascertain the extent of contamination of sites.

Conclusion

The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining, which in turn has resulted in gross pollution of the environment. The potential of *Fusarium spp.* in the degradation of crude oil has however resulted to the reduction in the total petroleum hydrocarbon. Its effect on microbial populations depends upon the chemical composition of the oil and on the species of microorganisms present. The use of *Fusarium spp.* in the bioremediation of crude oil polluted sites is therefore recommendable.

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