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REGULAR ARTICLE

EFFECT OF REFINED PETROLEUM PRODUCTS CONTAMINATION ON BACTERIAL POPULATION AND PHYSICOCHEMICAL CHARACTERISTICS OF CULTIVATED AGRICULTURAL SOIL

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ABSTRACT

An investigation into the effect of refined petroleum products contamination on bacterial population and physicochemical characteristics of cultivated agricultural soil was carried out. The soil samples obtained from the Teaching and Research Farm, Obakekere, Federal University of Technology, Akure, Ondo State were contaminated with varying volumes of petrol, diesel and kerosene. The results revealed higher bacterial populations in uncontaminated soils than contaminated soils. The counts of bacteria ranged from 3.0×10^5 to 5.0×10^5 cfu/g in uncontaminated soils and 1.0×10^5 to 3.0×10^5 cfu/g in contaminated soils. The isolated bacteria were identified as *Bacillus subtilis, Flavobacterium lutescens, Micrococcus luteus, Corynebacterium variabilis*, *Pseudomonas fluorescens*. The contamination had no significant effect on pH, potassium, sodium, organic carbon and nitrogen content of the soils, while the moisture, calcium, phosphorus and magnesium content of the contaminated soils were significantly different ($P < 0.05$) compared with the uncontaminated soils. The ability of *Bacillus subtilis, Flavobacterium lutescens, Micrococcus luteus,* and *Pseudomonas fluorescens* to utilize the refined petroleum products suggest that these bacteria had potential to bioremediate petroleum contaminated soils.

Keywords: Refined petroleum, contamination, clean-up, bacteria, bioremediation

INTRODUCTION

Soil is a natural body consisting of layers of mineral constituents of variable thickness, which differ from the parent materials on their morphological, physical, chemical, and mineralogical characteristics **(Birkeland, 1999)**. Fertile soil is rich in nutrients necessary for basic plant nutrition, including nitrogen, phosphorus and potassium. It also contain soil organic matter that improve soil structure and soil moisture retention as well as a range of microorganisms that support plant growth **(Kotke, 1993)**.

Petroleum is a fossil fuel formed under the earth crust from sedimentation and decomposition of dead plants and animals at a high temperature and pressure over a long period of time. Therefore, compounds such as petrol, diesel, kerosene, naphthalene, bitumen, natural gas are derived from fractional distillation of crude oil **(Collins, 2007)**. The hydrocarbon in crude oil is made up of alkanes, cycloalkanes, phenolics, aromatics, aliphatic **(Hyne, 2001)**.

Refined petroleum products contaminate soils through spills from tankers transporting the product or accident of the tanker **(Ijah, 2002)** as well as through leakages of underground storage tanks. Certain naturally occurring bacteria, such as *Micrococcus, Arthrobacter* and *Rhodococcus* have been shown to degrade these contaminants **(Sims *et al*., 1989)**.

Agriculture is the major occupation of an average Nigerian who depends solely on the produce from farm for food and little income from sale of harvested crops. Also, petroleum contamination of agricultural soils are unavoidable circumstances during the course of transporting petroleum and petroleum products to different parts of the country. Therefore, the need to identify microorganisms particularly bacteria capable of surviving agricultural petroleum contaminated soils become very important.

MATERIAL AND METHODS

Collection of soil samples

Soil samples were collected from the Teaching and Research Farm Obakekere, Federal University of Technology, Akure, Nigeria with the aid of soil auger at depths 15-20cm into sterile black cellophane bags. They were taken to Microbiology Research

laboratory for analysis according to **Tanee *et al.* (2008)**. Microbial properties of the collected soil sample were determined.

Collection, labelling and filling of black cellophane nylon bags with soil samples

Black cellophane nylon bags of sizes 12×16cm obtained from the Ministry of Agriculture and Development, Alagbaka, Akure, Nigeria were labelled appropriately. Eight-hundred grams of the collected soil samples were weighed with weighing balance (Triple beam 700/800 series, 2610g-5lb 2oz capacity) and poured into each labelled bag.

Contamination of soil samples with refined petroleum products

Five portions of the soil samples were contaminated with 0.15%, 0.30%, 0.60%, 1.20% and 0.00% (w/v) of petrol and designated as 'Soil-Petrol A (SPA), Soil-Petrol B (SPB), Soil-Petrol C (SPC), Soil-Petrol D (SPD) and Soil-Petrol E (SPE)' respectively with three replications each. Also, 0.05%, 0.10%, 0.20%, 0.40% and 0.00% (w/v) of diesel were used to contaminate another set of soils designated as 'Soil-Diesel A (SDA), Soil-Diesel B (SDB), Soil-Diesel C (SDC), Soil-Diesel D (SDD) and Soil-Diesel E (SDE)' respectively. Similarly, another set of soil samples were contaminated with kerosene just as diesel and designated as 'Soil-Kerosene A (SKA), Soil-Kerosene B (SKB), Soil-Kerosene C (SKC), Soil-Kerosene D (SKD) and Soil-Kerosene E (SKE)' respectively. Each of the experimental setup were in triplicate. The soil samples were then arranged in a randomised complete block design.

Planting of cowpea (*Vigna unguiculata*) seeds on contaminated and uncontaminated soils

Three - four cowpea seeds obtained from a neighbourhood market in Akure were planted into each planting bag and watered every other day. The seedlings were thinned to one seedling per planting bag to avoid overcrowding. The viability of the cowpea seeds were initially tested according to the methods of **Patil and Dadlani (2009)** before planting.

Enumeration of bacterial population

Nutrient agar (NA) medium was prepared according to manufacturer's instruction, sterilized and poured into Petri dishes. One gram of each contaminated and uncontaminated soil sample was diluted serially until fifth dilutions and 0.1ml aliquot from the fifth dilution was inoculated on the freshly prepared media, incubated at 37^0C for 24 hours and observed for growth. Colonies were counted and recorded as colony forming units per gram of soil (cfu/g). Isolates were subcultured repeatedly to obtain pure isolates and characterized according to the methods described by **Holt *et al.* (1994)**.

Bacterial characterization

The bacterial species that were isolated from the contaminated and uncontaminated soil samples were characterized further using morphological, physiological and biochemical properties that included Gram reaction, indole production test, nitrate reduction, catalase test, motility test, oxygen relation, carbohydrates (glucose, lactose and sucrose) utilization test and starch hydrolysis were determined according to standard methods of Bergey's Manual of Determinative Bacteriology **(Holt *et al*., 1994)**.

Determination of rates of utilization of refined petroleum products by isolated bacteria

Minimal salts medium (MSM) of Zajic and Supplisson (1972) containing; 0.27g K_2HPO_4, 0.6g NH_4Cl, 0.03g $MgSO_4.7H_2O$, 0.015g NaCl, 0.0015g $NaSO_4.7H_2O$ and 150ml distilled water with 1% refined petroleum product (petrol, diesel, kerosene) as the only source of carbon. The MSM was inoculated with 0.1ml nutrient broth of 24 hours old cultures of isolated bacteria. The setup was incubated at 30^0C for six (6) days. Turbidity produced as a result of microbial growth was monitored visually at the end of incubation period and their absorbance reading at 540nm on UNICO 1100RS spectrophotometer were determined.

Measurement of physicochemical parameters

The physicochemical parameters of petrol, diesel and kerosene contaminated soils and uncontaminated soils (control) were determined every four week interval for a total of twelve weeks. The pH was measured using pH meter (PHH-65A) following standardization with

appropriate buffers. The moisture content of each soil sample was determined by drying 10grams of the soil in an oven at 80^0C until a constant weight was reached and the percentage moisture content was calculated. Organic carbon content was measured by heating air-dried soils at 350^0C and 440^0C overnight in a muffle furnace and using a conversion factor of 1.724 to convert derived organic matter to organic carbon **(Nelson and Sommers, 1996)**. Available phosphorus, exchangeable magnesium and calcium were determined using standard method **(AOAC, 1990)**. Sodium and potassium ion concentration were determined using the method of **Harris (1995)**. Total nitrogen was measured using the Macrokjeldahl digestion method **(Heads, 1992)**.

Data obtained were subjected to a single factor analysis of variance (ANOVA) while the significant means were separated with the Duncan's multiple range test (DMRT) at 5% confidence level (P = 0.05) using Statistical Package for Social Sciences (SPSS).

RESULTS

Total plate counts of bacteria in uncontaminated and contaminated soil samples

Table 1 showed the total plate counts of bacteria in petrol and diesel contaminated soils which ranged from 1.0×10^5 to 3.0×10^5 cfu/g and 2.0×10^5 to 3.0×10^5 cfu/g in kerosene contaminated soils, while in uncontaminated soils, the counts ranged from 3.0×10^5 to 5.0×10^5 cfu/g.

Table 1 Total plate count of bacteria (cfu/g)$\times 10^5$ in uncontaminated and contaminated soil samples

Weeks	Petrol contaminated soils					Diesel contaminated soils					Kerosene contaminated soils				
	SPA	SPB	SPC	SPD	SPE(control)	SDA	SDB	SDC	SDD	SDE(control)	SKA	SKB	SKC	SKD	SKE(control)
	counts $\times 10^5$					counts $\times 10^5$					counts $\times 10^5$				
3	3	2	2	2	4	2	2	1	2	4	3	2	3	3	4
6	3	2	3	2	3	2	3	2	3	5	2	3	2	3	4
9	3	2	2	1	4	2	2	2	3	4	3	2	2	3	4
12	3	2	3	2	3	3	2	2	3	4	3	2	2	3	4

Legend: Cfu/g – colony forming unit per gram; SPA, SPB, SPC, SPD, SPE – treatments containing 0.15%, 0.30%, 0.60%, 1.20%, 0.00% concentrations of petrol respectively; SDA, SDB, SDC, SDD, SDE – treatments containing 0.05%, 0.10%, 0.20%, 0.40%, 0.00% concentrations of diesel respectively; SKA, SKB, SKC, SKD, SKE – treatments containing 0.05%, 0.10%, 0.20%, 0.40%, 0.00% concentrations of kerosene respectively.

Bacterial characterization

The details of the bacterial isolates are shown in Table 2. Isolate A was found to be gram positive, motile, aerobic, spore former, catalase positive and hydrolyzed starch. Isolate B was gram positive, non-motile, facultative aerobe, catalase positive and non-spore former. Isolate C was gram negative, non-motile, aerobic, non-spore former, catalase positive and negative to nitrate reaction. Isolate D was gram positive, non-motile, aerobe, non-spore former and catalase positive. Isolate E was gram negative, motile, aerobe, non-spore former and catalase positive. On the basis of these features, isolates A, B, C, D and E were tentatively identified as *Bacillus subtilis, Corynebacterium variabilis, Flavobacterium lutescens, Micrococcus luteus* and *Pseudomonas fluorescens* respectively.

Table 2 Morphological and biochemical characteristics of bacterial isolates from contaminated and uncontaminated soil samples

Characteristics	**Isolates**				
	A	**B**	**C**	**D**	**E**
Morphological					
Gram reaction	+	+	-	+	-
Colour	Creamy	Whitish	Creamy	Yellow	Greenish
Edges	Rough	Smooth	Smooth	Smooth	Smooth
Surface	Rough	Rough	Smooth	Rough	Rough
Cell shape	Rod	Rod	Rod	Cocci	Rod
Colony morphology	Opaque, flat rhizoid	transparent, flat, spherical	Opaque, flat, spherical	Opaque, flat, spherical	Opaque, flat spherical
Biochemical					
Catalase	+	+	+	+	+
Motility	+	-	-	-	+
Spore	+	-	-	-	-
Oxygen relation	aerobe	facultative aerobe	aerobe	aerobe	aerobe
Nitrate reaction	-	-	-	-	+
Indole	-	-	-	-	-
Carbohydrate utilization					
Starch hydrolysis	+	-	-	-	-
Glucose	-	-	-	-	-
Lactose	-	-	-	-	-

Legend: + = Positive; - =Negative; Probable organisms: A- *Bacillus subtilis;* B- *Corynebacterium variabilis;* C- *Flavobacterium lutescens;* D- *Micrococcus luteus;* E- *Pseudomonas fluorescens.*

Occurrence of isolated bacteria in uncontaminated and contaminated soil samples

The bacterial isolates and their percentage of occurrence in petrol-contaminated soils and uncontaminated soils are illustrated in Table 3a. The isolated bacteria were *Bacillus subtilis, Corynebacterium variabilis, Flavobacterium lutescens, Micrococcus luteus* and *Pseudomonas fluorescens.* The order of occurrence was *B. subtilis* (30.77%), *P. fluorescens* (25%), *F. lutescens* (21.15%), *M. luteus* (19.23%) and *C. variabilis* (3.85%). However, *B. subtilis*, *P. fluorescens*, *F. lutescens*, and *M. luteus* with the exception of *C. variabilis* were isolated from soils contaminated with various concentrations of petrol.

Table 3a Occurrence of bacterial isolates from petrol - contaminated and uncontaminated soils over a period of 12 weeks

Isolated bacteria	SPA	SPB	SPC	SPD	SPE	PO(%)
	3 6 9 12	3 6 9 12	3 6 9 12	3 6 9 12	3 6 9 12(Weeks)	
Bacillus subtilis	+ + + +	+ - - -	+ + + +	- + + +	+ + + +	30.77
Corynebacterium variabilis	- - - -	- - - -	- - - -	- - - -	- + - +	3.85
Flavobacterium lutescens	- - + -	- + - +	+ + + +	+ - + -	+ - + -	21.15
Micrococcus luteus	+ + - +	+ - + -	- + - +	+ - - -	+ - + -	19.23
Pseudomonas fluorescens	+ + + +	- + + +	- - - -	- + - +	+ + + +	25

Legend: SPA, SPB, SPC, SPD, SPE – treatments containing 0.15%, 0.30%, 0.60%, 1.20% and 0.00% concentrations of petrol respectively; + = Present; - = Absent; PO = Percentage Occurrence (%)

Similar bacterial isolates were observed in diesel and kerosene contaminated soils and uncontaminated soil samples (Table 3b and 3c). The percentage of occurrence of the bacteria in diesel-contaminated soils and uncontaminated soils were *B. subtilis* (30.88%), *P. fluorescens* (28.30%), *M. luteus* (28.30%) *F. lutescens* (5.66%), and *C. variabilis* (5.66%). In the same vein *B. subtilis*, *P. fluorescens* and *M. luteus* with the exception of *F. lutescens* and *C. variabilis* were isolated from soils contaminated with various concentrations of diesel.

Table 3b Occurrence of bacterial isolates from diesel - contaminated and uncontaminated soil over a period of 12 weeks

Isolated bacteria	SDA				SDB				SDC				SDD				SDE				PO(%)
	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12(Weeks)	
Bacillus subtilis	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	30.88
Corynebacterium variabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	5.66
Flavobacterium lutescens	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	5.66
Micrococcus luteus	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	28.30
Pseudomonas fluorescens	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	28.30

Legend: SDA, SDB, SDC, SDD, SDE – treatments containing 0.05%, 0.10%, 0.20%, 0.40% and 0.00% concentrations of diesel respectively; + = Present; - = Absent; PO = Percentage Occurrence (%)

In the case of kerosene-contaminated soils and uncontaminated soils the order of occurrence was *B. subtilis* (25.86%), *P. fluorescens* (25.86%), *M. luteus* (24.13%) *F. lutescens* (22.41%), and *C. variabilis* (1.72%). Also, *B. subtilis*, *P. fluorescens, M. luteus* and *F. lutescens* with the exception of *C. variabilis* were isolated from soils contaminated with various concentrations of kerosene.

Table 3c Occurrence of bacterial isolates from kerosene - contaminated and uncontaminated soil over a period of 12 weeks

Isolated bacteria	SKA				SKB				SKC				SKD				SKE				PO(%)
	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12	3	6	9	12(Weeks)	
Bacillus subtilis	+	-	+	+	+	+	+	-	-	-	+	+	+	+	-	+	+	+	+	+	25.86
Corynebacterium variabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1.72
Flavobacterium lutescens	+	+	+	+	-	-	-	-	+	+	-	+	+	-	+	-	+	+	+	+	22.41
Micrococcus luteus	-	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	24.13
Pseudomonas fluorescens	+	+	+	+	-	+	-	+	+	-	+	-	-	+	+	+	+	+	+	+	*25.86*

Legend: SKA, SKB, SKC, SKD, SKE – treatments containing 0.05%, 0.10%, 0.20%, 0.40%, 0.00% concentrations of kerosene respectively; + = Present; - = Absent; PO = Percentage Occurrence (%)

Utilization of refined petroleum products by bacterial isolates

Bacillus subtilis grew heavily in diesel supplemented medium and moderately on both petrol and kerosene medium. This was followed by *Micrococcus luteus* which grew heavily in petrol medium and minimally in diesel and kerosene medium. *Pseudomonas fluorescens* exhibited moderate growth in diesel and kerosene medium and minimal growth in petrol medium. Also, *Flavobacterium lutescens* grew minimally in both petrol and kerosene medium whereas it had infinitesimal growth in diesel medium (Table 4).

Table 4 Utilization of refined petroleum products by bacterial isolates

	Qualitative and quantitative growth		
Isolated bacteria	**Petrol medium**	**Diesel medium**	**Kerosene medium**
Bacillus subtilis	++ (0.523)	+++ (0.631)	++ (0.423)
Corynebacterium variabilis	- (0.028)	- (0.012)	- (0.021)
Flavobacterium lutescens	+ (0.256)	- (0.019)	+ (0.319)
Micrococcus luteus	+++ (0.616)	+ (0.360)	+ (0.249)
Pseudomonas fluorescens	+ (0.318)	++ (0.412)	++ (0.504)

Legend: **+++ :** Heavy growth, **++ :** Moderate growth, **+ :** Minimal growth, **- :** Infinitesimal growth,
In pararethesis ( ): Quantitative growth of bacteria at 540nm

Physicochemical properties

The pH values in contaminated soils (CS) ranged from 5.51 to 6.99, while those of uncontaminated soils (UCS) ranged from 5.72 to 6.98. This shows no significant difference ($P > 0.05$) between pH values in CS and UCS (Figure 1). The moisture content of CS ranged from 8.44% to 18.23%, while those of uncontaminated soils UCS ranged from 8.07% to 8.92% (Figure 2). The organic carbon content ranged from 0.11% to 6.54% in CS and 0.21% to 5.27% in UCS (Figure 3).

The mineralogical characteristics of the soils showed that available phosphorus level of CS (0.01 – 2.35mg/kg) were lower than those of UCS (0.86 – 2.27mg/kg) (Figure 4). Magnesium ion concentration ranged from 0.17mg/100g to 12.62mg/100g in CS and 0.73mg/100g to 1.13mg/100g in UCS (Figure 5). The calcium ion concentration in CS ranged from 0.94mg/100g to 8.83mg/100g while those of UCS ranged from 0.82mg/100g to 1.45mg/100g (Figure 6). The sodium ion concentration in CS and UCS ranged from 0.11mg/kg to 0.72mg/kg and 0.39mg/kg to 0.68mg/kg respectively (Figure 7). The potassium ion concentration in CS ranged from 0.12mg/100g to 0.74mg/100g, while it ranged from 0.39mg/100g to 0.68mg/100g in UCS (Figure 8). The nitrogen level in CS ranged from 0.05% to 0.43% and 0.07% to 0.37% in UCS (Figure 9).

However, the results revealed that the moisture content, calcium ion and magnesium ion concentration were higher in CS compared to those of UCS whereas there were no significant differences ($P > 0.05$) in potassium ion, sodium ion, organic carbon and nitrogen concentration in CS and UCS.

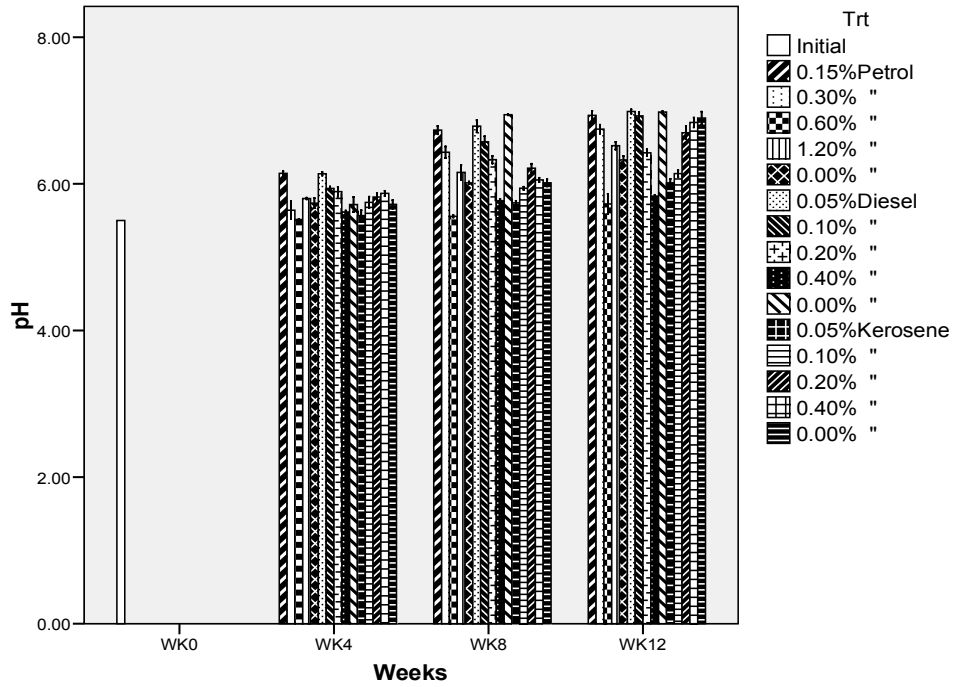

Figure 1 pH values in CS and UCS

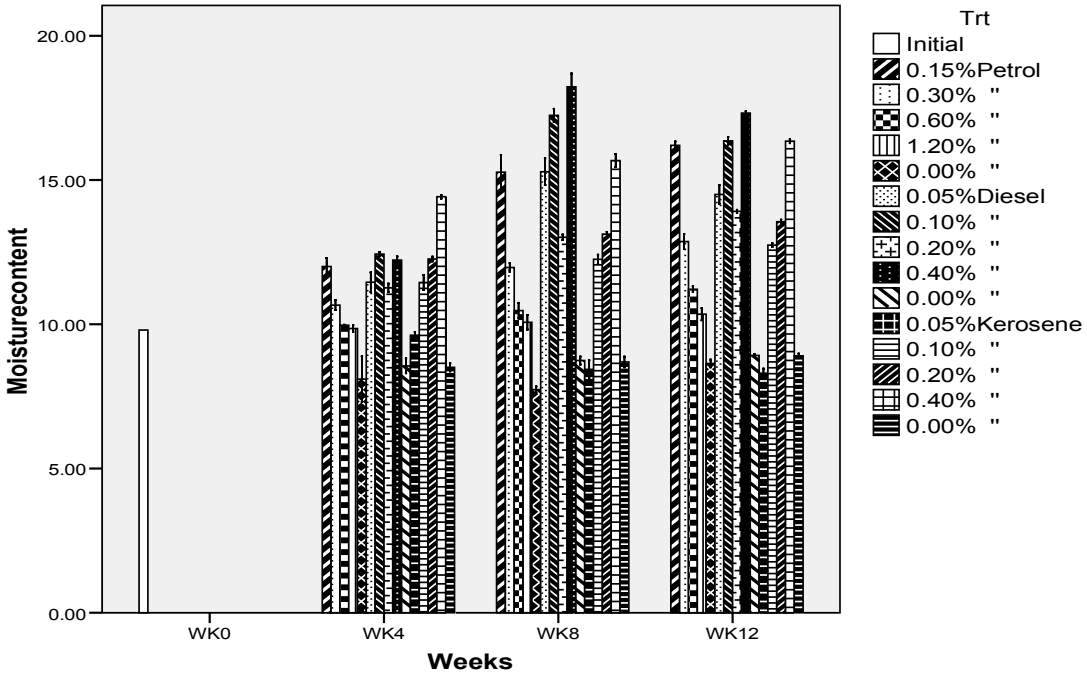

Figure 2 Moisture content (%) of CS and UCS

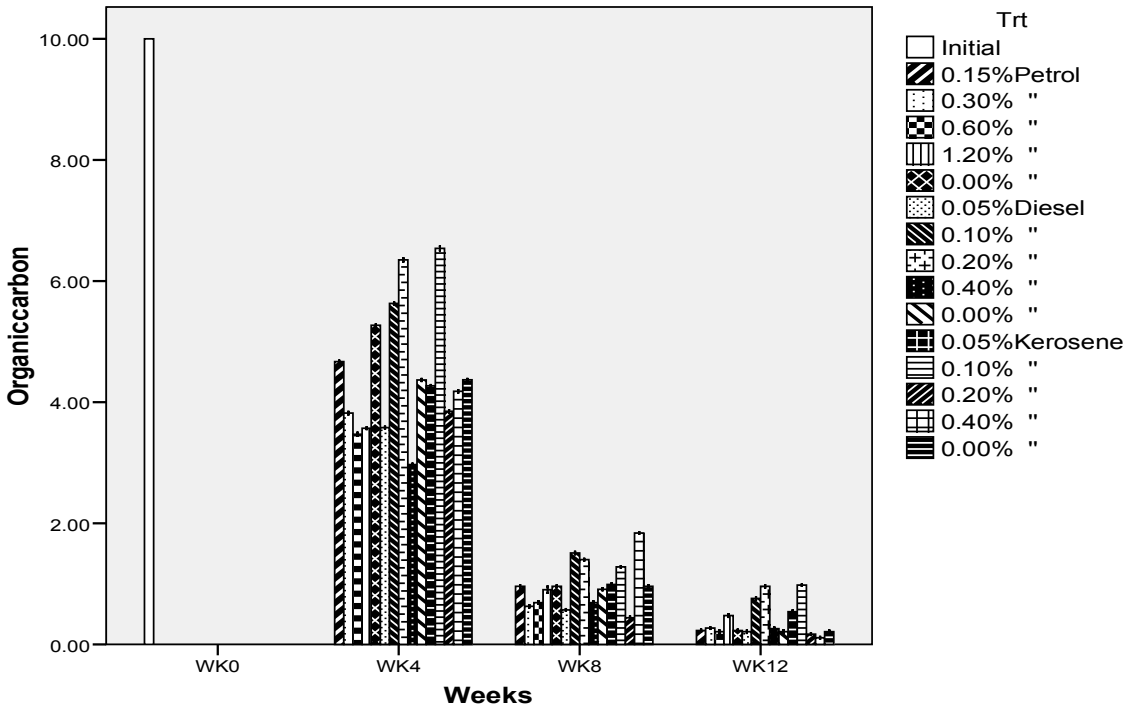

Figure 3 Organic carbon (%) in CS and UCS

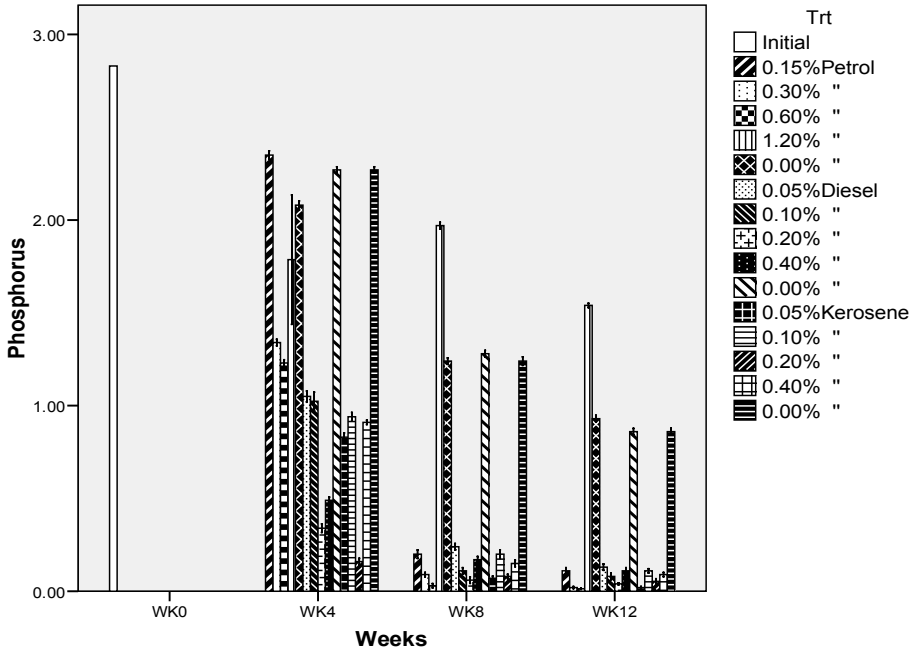

Figure 4 Available phosphorus (mg/kg) in CS and UCS

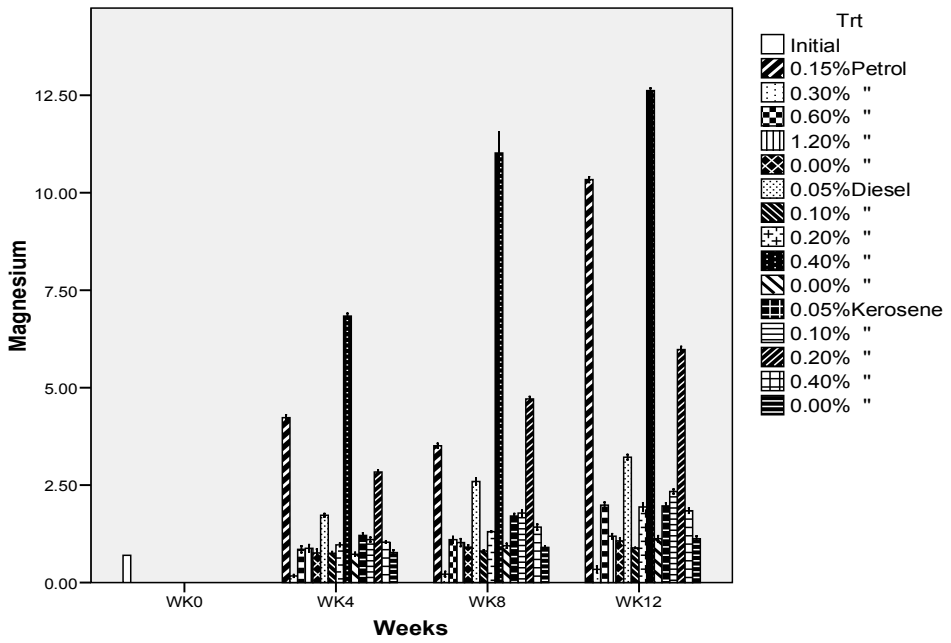

Figure 5 Magnesium ion (mg/100g) in CS and UCS

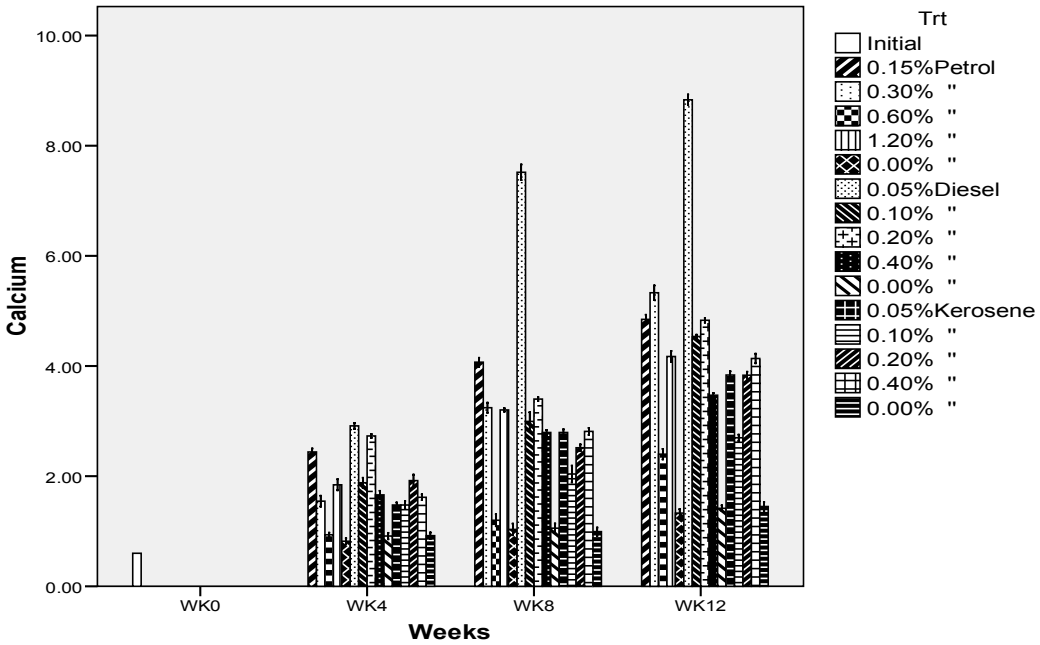

Figure 6 Calcium ion (mg/100g) in CS and UCS

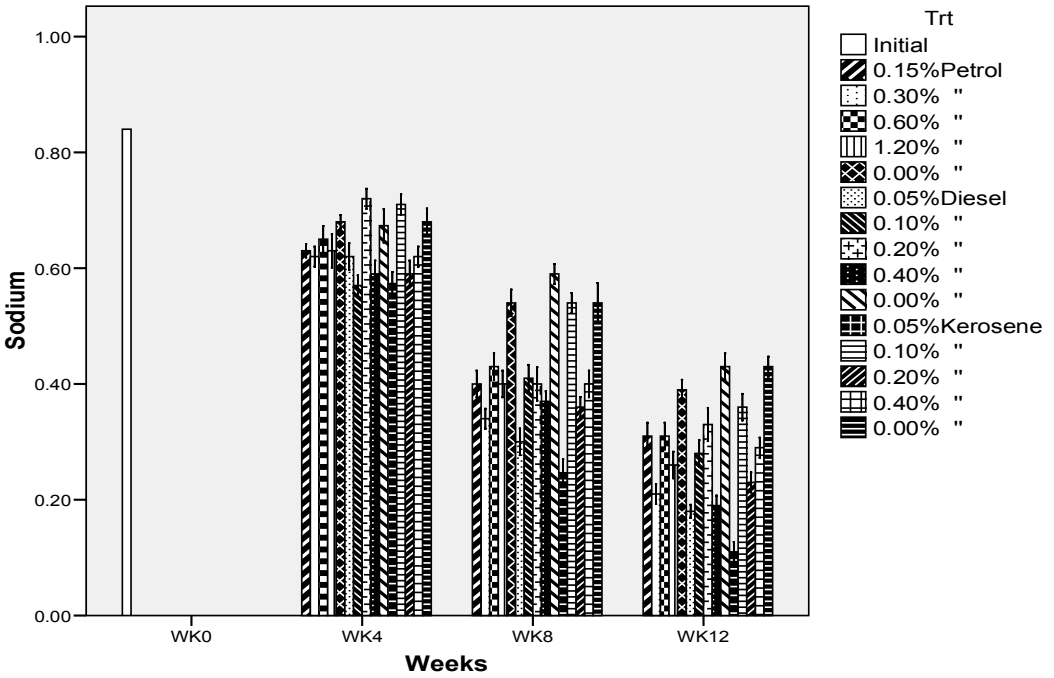

Figure 7 Sodium ion (mg/kg) in CS and UCS

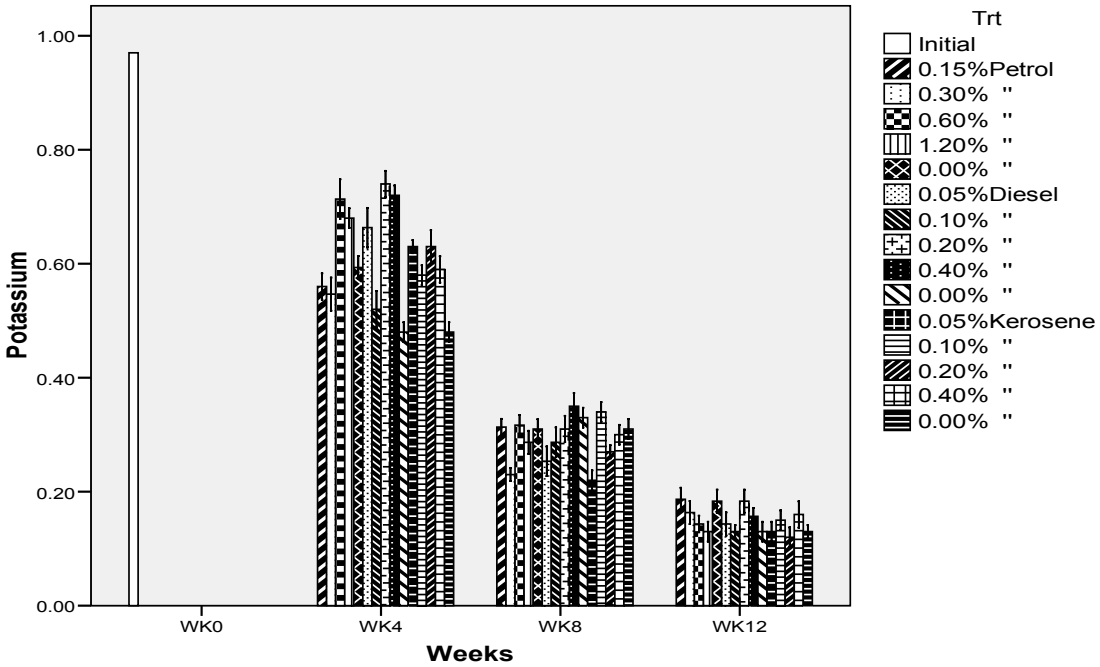

Figure 8 Potassium ion (mg/100g) in CS and UCS

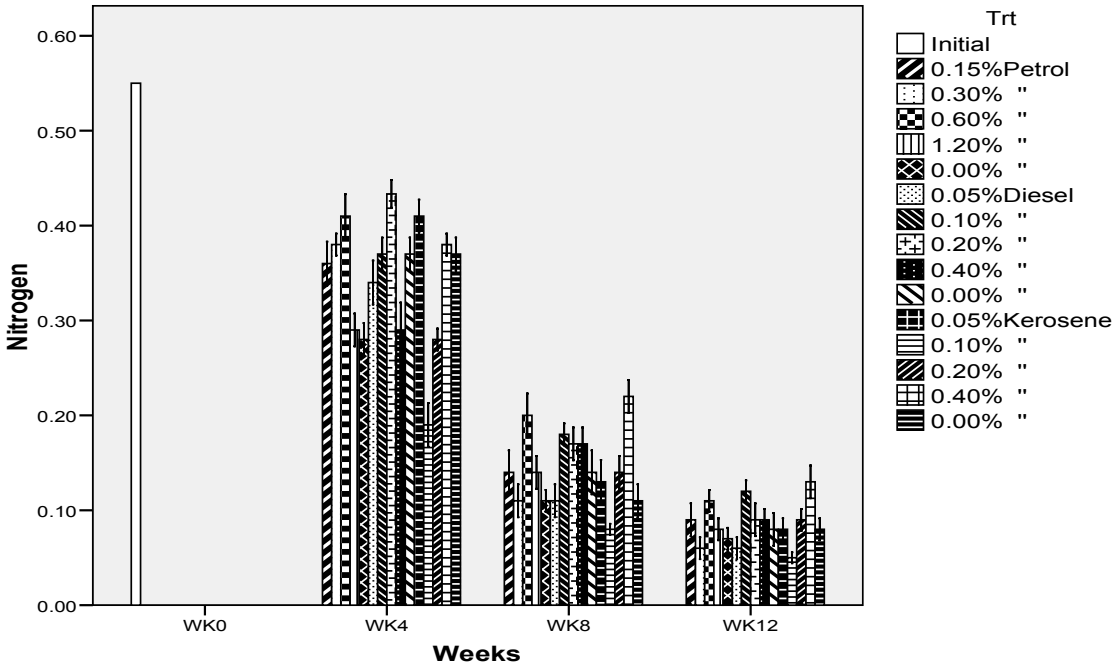

Figure 9 Nitrogen concentration (%) in CS and UCS

DISCUSSION

The total plate counts of bacteria was higher in uncontaminated soils (UCS) than contaminated soils (CS). This is not in agreement with **Ijah and Abioye (2003)** who reported higher counts of bacteria in petroleum CS compared with UCS. Although, the difference was not significant and also stated that petroleum contamination boosts carbon supply in the soil, hence favours the growth of bacteria. However, in this present study, environmental stress and toxicity caused by the hydrocarbons may be the reason for lower counts of bacteria in CS compared with UCS. In addition, it may be that the resident bacteria are adjusting to the new carbon sources in CS, thus, resulting in low counts.

The counts of bacteria in kerosene CS were higher than those in petrol and diesel CS. However, the bacterial count in kerosene CS was less than those obtained by **Ijah and Abioye (2003)** who studied the microbial properties of CS thirty (30) months after kerosene spill and reported higher counts of bacteria in kerosene polluted soils (KPS) than kerosene free soils (KFS). This observation may not be unconnected with the fact that the organisms had fully adapted to the carbon in soil. Therefore, the bacterial count could be period dependent.

The isolation of *Bacillus subtilis, Flavobacterium lutescens, Micrococcus luteus* and *Pseudomonas fluorescens* except *Corynebacterium variabilis* in CS is in agreement with **Antai and Mgbomo (1989)** who reported the distribution of hydrocarbon utilizing bacteria in oil spill areas. The high percentage occurrence of *Pseudomonas* and *Bacillus* in CS agrees with **Ijah and Abioye (2003)** who reported that *Pseudomonas* and *Bacillus* produce spores which may shield them from the toxic effects of the hydrocarbons. This could be a justifiable reason for the high occurrence of these two isolates.

Rates of utilization of refined petroleum products varied. However, the greater ability of *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas fluorescens* in utilizing petrol, diesel and kerosene in minerals salt medium than the rests of the isolates tested suggest that the organisms can adapt easily to the petrol, diesel and kerosene medium. There are many scientific reports **(Antai, 1990; Ijah and Abioye, 2003; Ijah and Antai, 2003)** on the utilization and degradation of petroleum hydrocarbon in soil by microorganisms most especially *Bacillus* species. The results of this investigation points to *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas fluorescens* as promising isolates in the clean-up of soils polluted with refined petroleum products.

Contamination of the soils had no effect on the pH values since no significant differences existed in pH of CS and UCS. However, the increase in pH values in both CS and UCS may be as a result of continuous addition of hydrogen and hydroxyl ions from water during wetting of cultivated soils thereby reducing the acidity of the soils and making pH to tend towards neutrality. The high moisture content in CS compared to UCS could be as a result of the contaminants viscosity which tend to increase the water holding capacity of the CS **(Collins, 2007)**.

The limitation of phosphorus in most soils because it is released very slowly from insoluble phosphate **(Norman and Hunter, 2008)** could be a reason for the amount observed in this study. However, the low phosphorus concentration in CS compared to UCS may be due to the fact that the contamination prevent the release of phosphorus, hence, unavailable to microorganisms for utilization. Calcium and magnesium ions in CS were higher than those in UCS. This may be as a result of prevention of percolation of soluble calcium and magnesium **(Epstein, 2005)** caused by the contaminants. The concentration of potassium, sodium, nitrogen and organic carbon in CS and UCS had no significant difference indicating the stability of these nutrients in CS.

CONCLUSION

This study revealed that refined petroleum products contamination had a remarkable effect on the bacterial population negatively. The moisture content, pH, calcium, magnesium content of the soils increased while potassium, sodium, organic carbon, nitrogen and phosphorus were limiting at the end of study. These limiting nutrients could be augmented by the addition of substances containing them for growth of plants and microorganisms. The predominantly occurring microorganisms in the contaminated soils namely; *Bacillus* and *Pseudomonas* could be employed for bioremediation in environments polluted with refined petroleum products.

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