Effect of Petroleum Hydrocarbons Contamination on Soil Microorganisms and Biodegradation

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ABSTRACT

The experiment under lab condition was conducted to investigate the effect of petroleum hydrocarbon contamination on soil microorganisms and biodegradation. The experiment was conducted in 12 treatment and three replication. The treatments include soil contaminated with Kerosene, Diesel, lubricate oil and used lubricate oil in three concentration 0%, 5%, and 10%. contamination with hydrocarbons significantly decreased the count of heterotrophic bacteria and there was reversal relationship between the contaminant concentration and the count of heterotrophic bacteria. The results indicated soil contamination with hydrocarbons significantly increased the count of Hydrocarbon Utilizing Bacteria (HUB). The effect of petroleum hydrocarbons on fungi count differed according to the type of hydrocarbons, concentration of contaminant and the periods of incubation. Our results show that soil contamination by hydrocarbons caused significant increase in actinomycetes count at all hydrocarbon types and concentrations except 5% used and non-used lubrication oil. The higher percentage of biodegradation in hydrocarbons contaminated soil 49.6% was recorded in soil contaminated with 5% kerosene while the lower percentag10.8% was recorded with soil contaminated 10% lubricate oil.

Keywords: Hydrocarbons, soil microorganisms, HUB.

12

.%10 %5 %

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(%5)   (%49.6)   12   .

.   (%10)   (%10.8)

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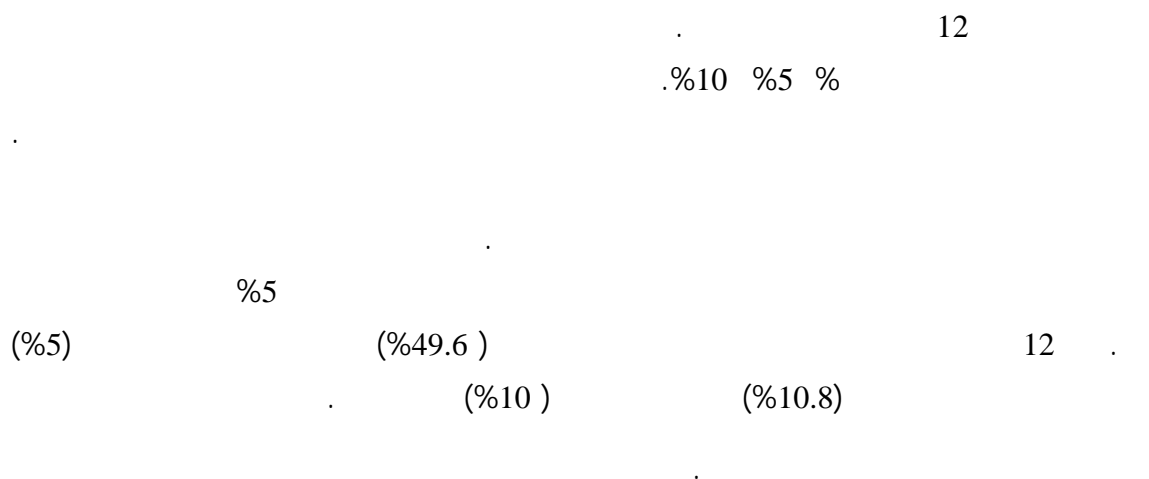
INTRODUCTION

Considering the rapid growth in the world's population, crude oil continues to be the main source of energy and raw material for industries worldwide (Abbasian *et al*., 2016). The increasing demand on petroleum products drives a surge in production, transportation and refinery – resulting in an inevitable toll on the environment (Gallego *et al*., 2001). Indeed, increased oil production and distribution is associated with higher incidents of pollution due to oil spillage (Hussain and Gondal, 2008). Furthermore, natural causes such as superficial waters and floods can spread this contamination to other areas (Zakaria, 2002). Crude oils typically consist of various types of hydrocarbons (aliphatic and aromatic). While these hydrocarbons contain plenty of carbon and hydrogen atoms, they are very nutritionally poor and insufficient to sustain many microorganism species (Abbasian *et al*., 2016; Sathishkumar *et al*., 2008; Viñas *et al*., 2005). The toxicity of these hydrocarbons to microorganisms means that contaminated soil may experience drastic changes to the population and abundances of various microbial species (Abbasian *et al*., 2015; Megharaj *et al*., 2011). The extent of these changes to the microbial populations is dependent on several factors including: the composition of the microbial community prior to the contamination, the chemical composition of the contaminant (crude oil), and the physiochemical factors of the contamination site (Abbasian *et al*., 2016; Rahman *et al*., 2002a). Certain microbial species face a challenge when grown in the presence of hydrocarbons due to their hydrophobicity and insolubility – leading to restriction of absorption by these cells, ultimately leading toxicity (Abbasian *et al*., 2015). Moreover, these hydrocarbons affect the microorganisms membrane fluidity; thus, microorganism exposed to petroleum products must adapt their membrane structure to survive (Jin *et al*., 2014). The extent of these changes are depending on the type of hydrocarbon contaminant and their carbon chain length (Abbasian *et al*., 2015). Some hydrocarbon-degrading microorganisms learn to adapt by producing and secreting a surfactant compounds to emulsify the hydrocarbon and form micelles – to be taken up later in various ways (Abbasian *et al*., 2015; Bustamante *et al*., 2012). Microorganisms are a key player in maintaining sustainable environment and ecosystem (Varjani, 2017). Microorganisms are known to be the most effective tools to naturally degrade crude oil after a spillage episode. Bioremediation is a process in which microorganism are utilized to convert dangerous organic pollutants from crude oil into environmentally friendly compounds such as CO_2 and H_2O (Ron and Rosenberg, 2014; Varjani, 2017). In the process of biodegradation, oleophilic microbes are utilized and administrated to degrade the hydrocarbons and remediate the site of spillage (Varjani and Upasani, 2013). since microorganisms are naturally effective to degrade petroleum products, it is important to understand the changes in microbial diversity post soil contamination with various petroleum products (Abbasian *et al*., 2016). In this study, we aimed to understand the changes in indigenous soil microbial diversity and abundances upon the addition of kerosene, diesel, and non-used and used lubricate oil. Our study will also evaluate the ability of the various soil indigenous microorganism species to biodegrade of these petroleum products.

MATERIALS AND METHODS

Soil Sampling and Analysis

The soil was collected from Basheka area 12 km Northeast of Mosul. The soil collected from surface area (0-20)cm. The soil air died sieved with 2 mm sieve to remove debris. The physical and chemical properties of the soil were determined as following. Soil texture by hydrometer method, Soil Reaction (pH) by glass electrode method (1:2.5 soil water suspension) (CON 720), soluble salts by Electrical Conductivity (conductivity bridge method) (Inolab pH 720), Organic Content by rapid titration method (Black *et al.,* 1965), available phosphorus by Olsen's method (Olsen *et al*., 1954) Ca and Mg by titration with EDTA (Graham *et al*., 1962), Potassium and sodium by flame photometer (PFP 7) (Jackson, 1973). Some of soil physical and chemical properties were recorded in (Table 1).

Experimental Design

200 gm of sieved soil was placed in 250 ml volumetric flask. The petroleum hydrocarbon treatments comprised of four different petroleum fractions Kerosene(K), Diesel (D), lubricate oil (O) and used lubricate oil(UO)). Treatment were applied at 0%, 5% and 10% levels. The petroleum hydrocarbon contamination involved twelve treatments in a completely randomized design replicated three times. All components were thoroughly mixed with the soil. The moisture content of soil was brought to 65% water holding capacity. Soil was turned weekly and distilled water was added to maintain them 65% of WHC. The beakers were incubated at a temperature of 25C. Soil sampling was carried out at one month and subsequently biweekly intervals for 12 weeks (Alrumman *et al*., 2015).

Microbial Count

In estimating microbial population, standard plate count methods were used to prepare nutrient agar (NA) for estimation of the heterotrophic bacteria, potato dextrose agar (PDA) for estimation of fungi, Starch casein nitrate (SCN) agar for estimation of actinomycetes, and Luria bertani agar (LBA) (in LBA yeast extract was replaced with kerosene, diesel or lubricate oil as carbon source) for estimation of hydrocarbon utilizing bacteria (HUB). One gram each of the soil samples were measured into the test tube containing 9 ml sterile distilled water and serially diluted to dilution factor 10^5 and 1 ml of the last dilutions was pipette into sterile plate with appropriate medium which were incubated at 28˚C - 30˚C. All plated were incubated inverted wise. Microbial counted were done at 48 hours for NA and 72 hours for PDA and 72 hours for LBA and 6 days for(SCN) (Stanley *et al.*, 2015; Desai, 2015; Adesina and Adelasoye, 2014).

Hydrocarbons Determination

To extract the hydrocarbons from soil.10 g of soil was placed in a dry baker and add 10 ml of organic solvent (hexane). The solvent filtered and placed in a weighted baker and dried in the 80 C. The precipitate was weighted. the percentage of remaining hydrocarbons in soil were calculated (Mishra *et al*., 2001)

Statistical Analysis

ANOVA and correlation analyses were carried out. The means were compared using Duncan's test at $p < 0.05$ after, ANOVA. The relationships between HUB and percentage of hydrocarbon biodegradation were determined using Pearson's correlation analysis.

RESULTS AND DISCUSSION

Soil Properties

Table (1) shows some physical and chemical properties for soil under study.

Table 1: some soil physical and chemical properties

Characteristics	Results	Characteristics	Results
pH	7.1	K(meq/L	108
Ec	433	So_4(meq/L)	26.47
Organic matter	1.03	Clay (%)	22.7
Available Phosphorus (mg\L)	1.5	Silt (%)	49.25
Ca (meq/L)	108	Sand(%)	28.05
Mg(meq/L	13	Soil Texture	Silt Loam

Heterotrophic Bacteria Count

The Fig. (1) shows the total aerobic heterotrophic bacterial counts for the control (uncontaminated) soil ranged from $0.2.18 \times 10^5$ colony forming unit (CFUg^{-1}) soil to 2.83×10^5 CFUg^{-1} soil while counts for 5%, 10% kerosene, 5%, 10% diesel oil, 5%, 10% non-used lubrication oil and 5%,10% used lubrication oil contaminated soils ranged from 1.49×10^5 to 1.87×10^5 CFU^{-1} soil, 0.96×10^5 CFUg^{-1} soil to 1.63×10^5 CFUg^{-1} soil and 1.29×10^5 CFUg^{-1} to 1.79×10^5 CFUg^{-1} soil 1.03×105 to 1.32×10^5 CFUg^{-1}, 1.05×10^5 to 1.32×10^5 CFUg^{-1} soil and 0.9×10^5 to1.13×10^5 CFUg^{-1} soil, 1.19×105 to 1.51×10^5 CFUg^{1-} soil and 0.88×105 to 1.16×105

$CFUg^{-1}$ soil respectively. The results indicated soil contamination with hydrocarbons significantly decreased the count of heterotrophic bacteria and there was reversal relationship between the contaminant concentration and the count of heterotrophic bacteria. After twelve weeks incubation. Soil contamination with 5% and 10% of each hydrocarbon kerosene, 5 and10% diesel, 5 and 10% non-used lubricate oil and 5 and 10% used lubricate oil decreased heterotrophic bacteria 23%, 39%, 24%, 44%, 44%,52%, 42% and 50% respectively and these results are consistent with (Kucharski *et al*., 2004; Wyszkowska and Kucharski, 2001; Adesina and Adelasoye, 2014) who concluded the hydrocarbon contamination soil cause decrease of heterotrophic bacteria count and increase heavy metals concentration and they suggested decreases in both count and diversity of bacteria communities in hydrocarbon contaminated soils are caused by presence of toxin effect for heavy metals. Our results show that there were no significant differences in count of heterotrophic bacteria between soil contaminated with non-used and used lubricate oil
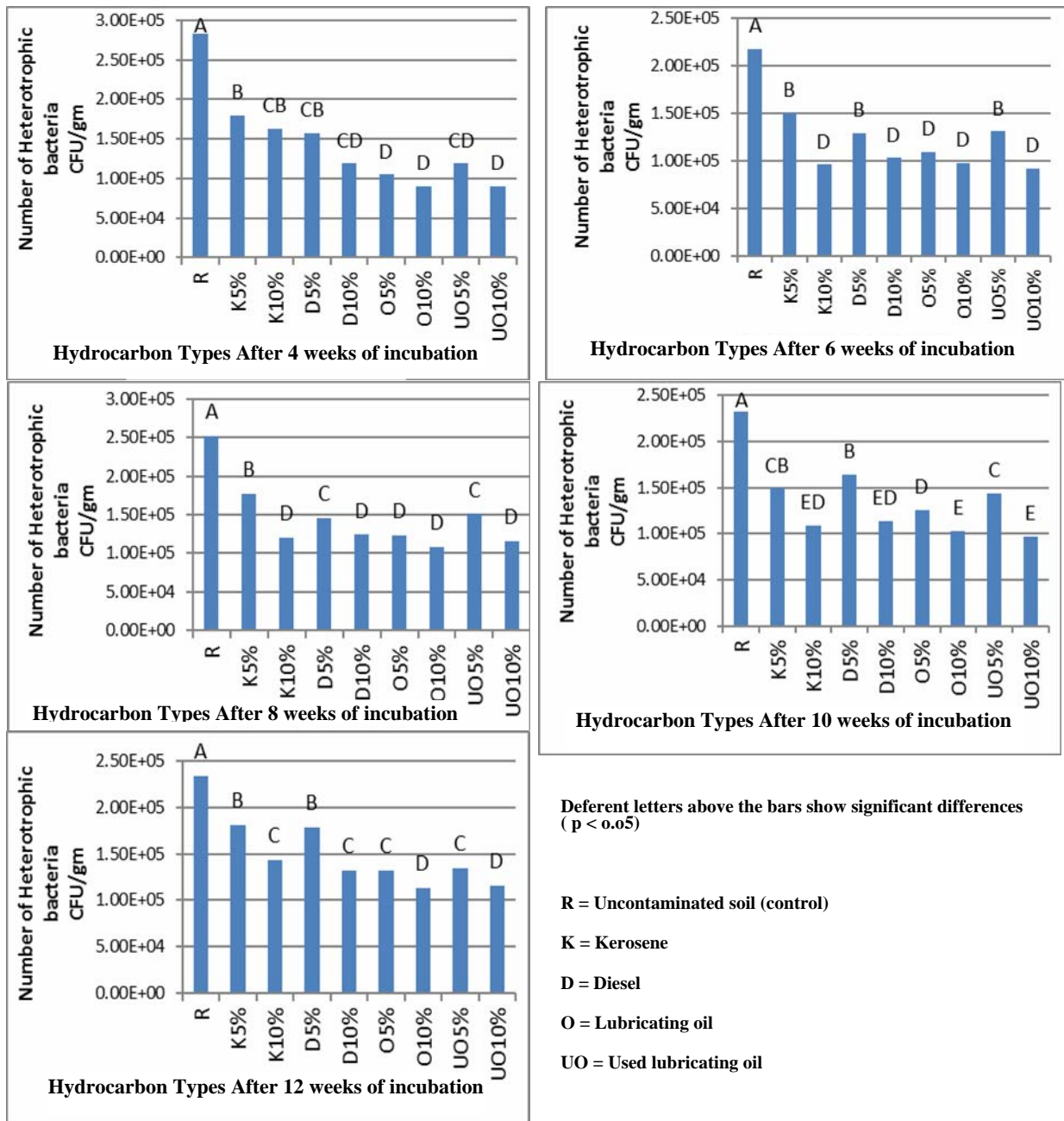

Deferent letters above the bars show significant differences (p < o.o5)

R = Uncontaminated soil (control)

K = Kerosene

D = Diesel

O = Lubricating oil

UO = Used lubricating oil

Fig. 1: Effect of soil contaminated with hydrocarbons on heterotrophic bacteria count Hydrocarbon Utilizing Bacteria (HUB) Count

The Fig. (2) shows the HUB counts for the control (uncontaminated) soil ranged from 0.45 x 10^5 soil to 0. 94 x 10^5 CFUg^{-1} soil, while counts for 5%, 10% kerosene and 5%, 10% diesel oil., 5%,10% non-used lubrication oil and 5%,10% used lubrication oil contaminated soils ranged from 1.49 x 10^5 to 1.85 x 10^5 CFU^{-1} soil, 2.14 x 10^5 to 2.52 x 10^5 CFUg^{-1} soil, 1.12 x 10^5 to 1.44 x 10^5 CFUg^{-1} soil, 1.30 x 10^5 to 2.02 x 10^5 CFUg^{-1}, 1.16 x 10^5 to 1.36 x 10^5 CFUg^{-1}g soil, 0.85 x 10^5 to1.44 x 10^5 CFUg^{-1} , 1.12 x 105 to 1.38 x 10^5 CFUg^{-1}g and 0.90 x 10^5 CFUg^{-1} to 1.16 x 10^5 CFUg^{-1} respectively. The results indicated soil contamination with hydrocarbons significantly increased the count of HUB. After 12 weeks incubation. Addition of 5%, 10% Kerosene, 5%, 10% Diesel, 5%, 10 % lubricate oil and 10% used lubricate oil, 5% used lubricate oil and 10% used lubricate oil increased HUB 294%, 436%, 206%, 330%, 206%, 168%, 174% and 147%. Respectively. Our results show during all periods of incubation the addition of 10% kerosene was record the higher increased percentage in HUB count however the addition 10% of non-used lubricate oil recorded the lower increase percentage in HUB count. This result agreed with (Lawlor *et al*., 1997; Das and Chandran, 2011; Amrazaitiene *et al.,* 2013; Markowicz *et al*., 2016; Cueva *et al.*, 2016) who reported higher population of HUB in hydrocarbon polluted soil due to the UBH can utilize hydrocarbon as carbon and energy sources this favored condition for the rapid replication led to higher population of HUB.
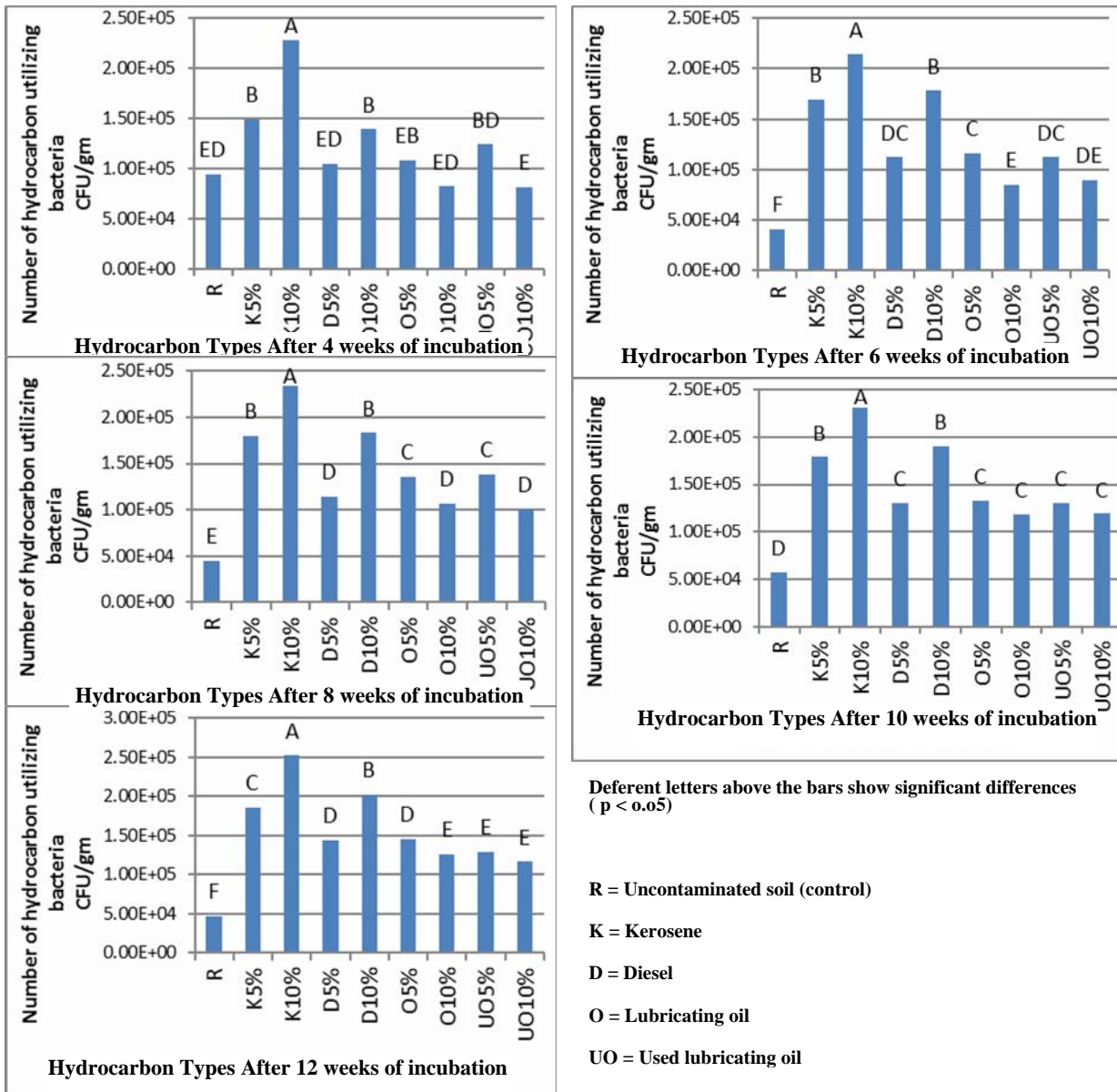

Deferent letters above the bars show significant differences (p < o.o5)

R = Uncontaminated soil (control)

K = Kerosene

D = Diesel

O = Lubricating oil

UO = Used lubricating oil

Fig. 2: Effect of soil contaminated with hydrocarbons on hydrocarbon utilizing bacteria (HUB) count

Fungi Count

Fig. (3) shows the effect of petroleum hydrocarbons on fungi count differed according to the type of hydrocarbons, concentration of contaminant and the periods of incubation. Soil contamination with 10% deasil, 10% lubricate oil and 10% used lubricate oil increased fungi count after four, six and ten weeks incubation. while soil contaminated with 5% kerosene, 5% diesel. 5% non-used oil and 5% used oil decreased fungi count after periods 4, 6 and 10 weeks incubation while after 12 weeks incubation. Soil contamination at all studied hydrocarbons and concentration increased fungi count. Soil contamination with 5%, 10% kerosene, 5%, 10% diesel oil., 5%, 10% non-used lubrication oil and 5%,10% used lubrication increased fungi count 29%, 49%, 73%, 139%, 37%, 80%, 54%, and 122% respectively. The increase of fungi count at later periods may be due to the fungi need a time to adapt to new circumstances (hydrocarbon polluted). These results are consistent with Lawlor *et al*., 1997; Xenia and Refugio, 2016 who reported the increased in fungi count due to the ability of some of the fungi to utilize hydrocarbons as carbon and energy sources.
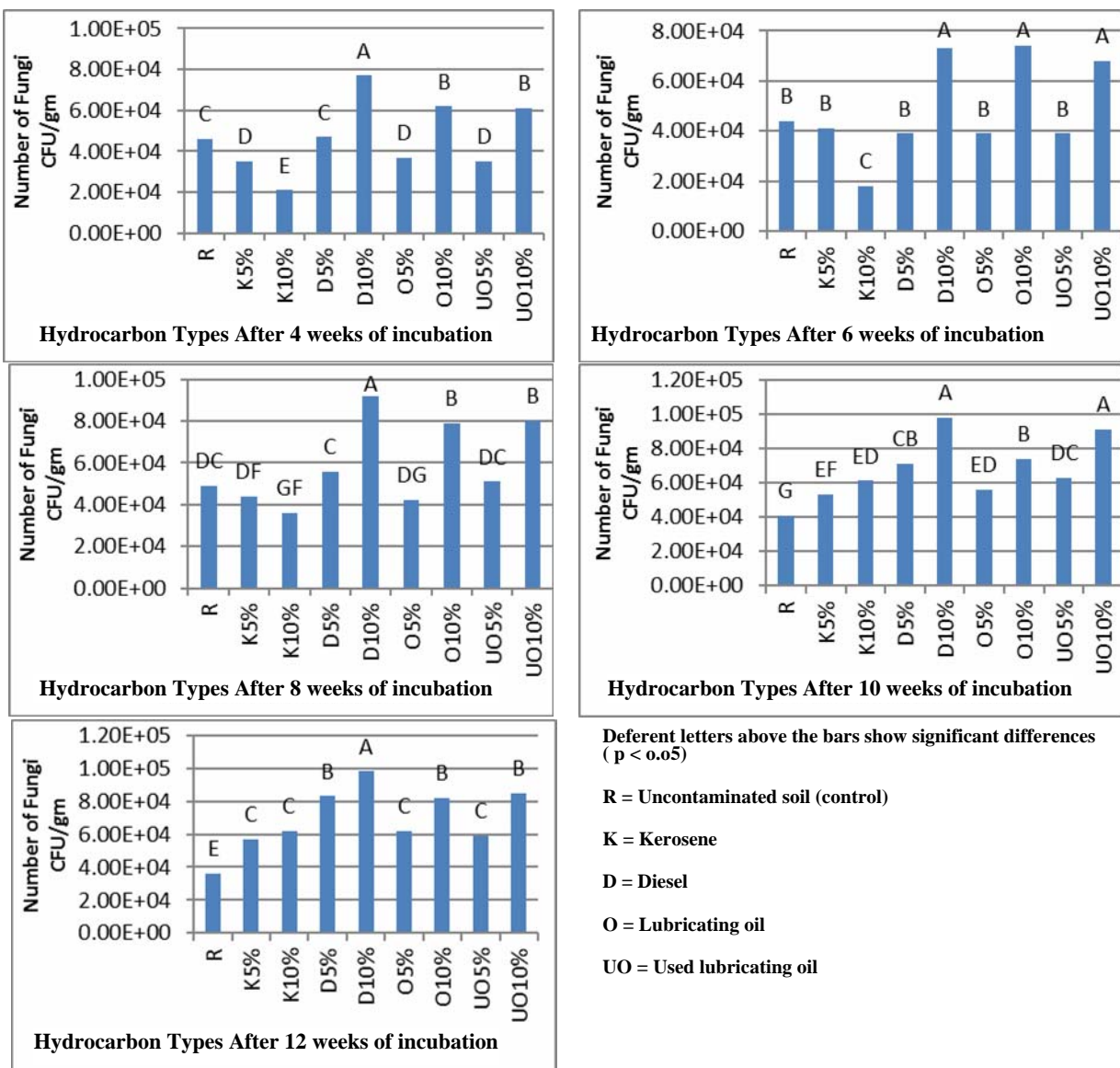

Deferent letters above the bars show significant differences (p < o.o5)

R = Uncontaminated soil (control)

K = Kerosene

D = Diesel

O = Lubricating oil

UO = Used lubricating oil

Fig. 3 : Effect of soil contaminated with hydrocarbons on Fungi count Actinomycetes Count

The Fig. (4) shows the actinomycetes counts for the control (uncontaminated) soil ranged from 0.51×10^5 to 0.63×10^5 CFUg^{-1} soil while counts for 5%, 10% kerosene, 5%, 10% diesel oil, 5%,10% lubrication oil and 5%,10% used lubrication oil contaminated soils ranged from 0.94×10^5 soil to 1.17×10^5 CFU^{-1} soil, 1.27×10^5 to 2.03×10^5 CFUg^{-1} soil, 0.89×10^5 to 1.11×10^5 CFUg^{-1} soil, 1.27×10^5 to $2,09 \times 10^5$ CFUg^{-1}g soil, 0.48×10^5 to 0.77×10^5 CFUg^{-1} and 0.57×10^5 CFUg^{-1} to1.16×10^5 CFUg^{-1} soil, 0.45×105 to 0.81×10^5 CFUg^{-1} soil and 0.92×105 CFUg-^{1} soil to 1.16×105 CFUg^{-1} soil respectively. Our result show soil contaminated by hydrocarbons significantly increased actinomycetes count at all hydrocarbon types and concentrations except 5% used and non-used lubrication oil. These results agree with (Schippers *et al,* 2005; Hamamura *et al*., 2013) who concluded actinomycetes can utilize hydrocarbons as carbon and energy sources. (Goodfello and Williams, 1983) reported actinomycetes are the most important microorganisms that have the ability degrade and analyze organic matter because they have many enzymatic systems capable of biodegrade organic matter
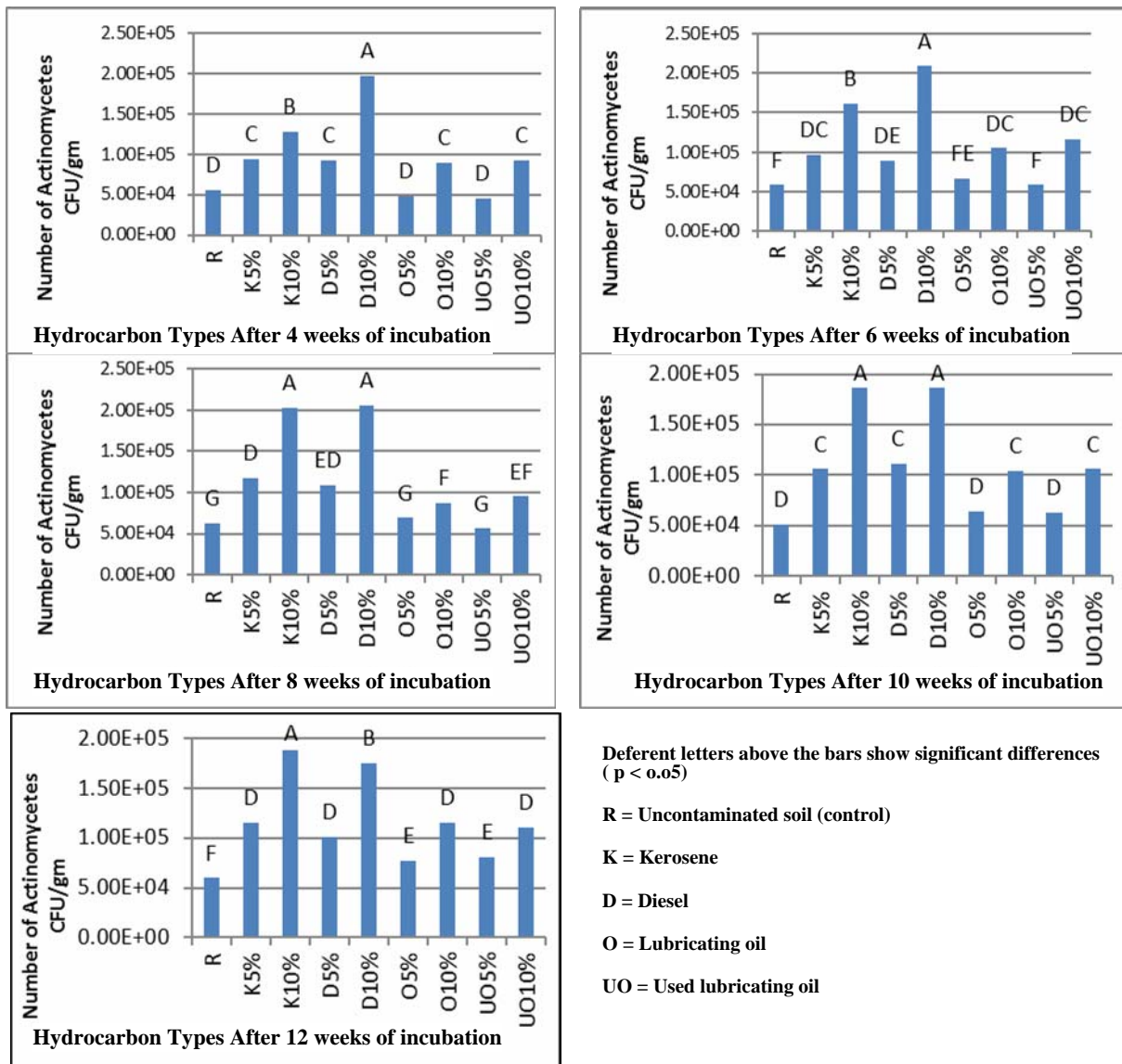

Deferent letters above the bars show significant differences (p < o.o5)

R = Uncontaminated soil (control)

K = Kerosene

D = Diesel

O = Lubricating oil

UO = Used lubricating oil

Fig. 4: Effect of soil contaminated with hydrocarbons on Actinomycetes count

Hydrocarbons Biodegradation

The percentage of hydrocarbons biodegradation in the soil contaminated with 5% and 10% of kerosene, diesel, lubricate oil and used lubricating oil is shown in (Table 2). At the end of twelve weeks the result indicate the higher percentage of biodegradation 49.6% was recorded in soil contaminated with 5% kerosene while the lower percentage of biodegradation 10.8% was recorded with soil contaminated 10% non- used lubricate. The percentage of hydrocarbons biodegradation in the soil contaminated with 5% diesel, 5% non-used lubricate oil and 5% used lubricate oil were 32%, 28% and 24% respectively. However the biodegradation of Kerosene 10%, diesel 10%, and used lubricate oil were 30%, 25.6% and 12.4% respectively. Positive correlation was found between HUB count and percentage of hydrocarbons biodegradation. Our results show there was reversal relationship between hydrocarbons biodegradation percentage and the contaminant concentration and this consistence with (Boldu-Prenafeta *et al*., 2004; Ambrazaitiene *et al*., 2013; Abioye *et al*., 2012; Rahman *et al*., 2002b). High concentration of contaminant causing decreasing in biodegradation percentage due to high concentration can be inhibitory of microorganisms by toxic effects (Abioye *et al*., 2012; Rahman *et al*., 2002b; Ijah and Antai, 2003) for this reason reported that bioremediation is a useful method of soil remediation if contaminant concentrations are moderate. Also the results show there are differences between biodegradation percent of hydrocarbons and these results agreed with (Ambrazaitiene *et al*., 2013; Boldu-Prenafeta *et al*., 2004) who concluded the rate of biodegradation depends on the microbial population, the type, structure and level of contamination. The biodegradation percentage in hydrocarbons contaminated soil was in the order of kerosene > Diesel> lubricate oil > used lubricate oil. The kerosene, diesel, and lubricate oil consisted mainly of n-alkanes with chain length of C10–C16, C10–C22 and C15 - C50 respectively. These result consistence with (Wang *et al*., 1998) who concluded the biodegradation rate of n-alkane inverse proportional with chain length and branched alkanes less ability to biodegradation. The results show there was no huge differences in biodegradation percentage between non-used and used lubricate oil.

Table 2: Biodegradation of contaminant hydrocarbons in soil after 12 weeks of incubation

Contaminated Hydrocarbons	Residual Hydrocarbon %	Hydrocarbon Biodegradation %
K 5%	50.4 %	49.6 %
K 10%	70 %	30 %
D 5%	68 %	32 %
D 10%	74.4 %	25.6 %
O 5%	72 %	28 %
O 10%	89.2%	10.8 %
UO 5%	76 %	24 %
UO 10%	87.6 %	12.4 %

K = Kerosene   O = Lubricating oil
D = Diesel   UO = Used lubricating oil

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