Impact of Various Concentrations of Oilfield Wastewater on Soil Microorganisms

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ABSTRACT

Oilfield wastewater contains toxic and hazardous substances that are detrimental to soil quality and soil microorganisms. It is estimated that large quantities of wastewater from operations of oil industries in Nigeria is still discharged to soil without adequate treatment. Accelerated soil quality change due to oilfield wastewater discharging into soil has long become an issue problem in Niger Delta. The impact of various concentrations (0% - control, 10%, 25%, 50%, and 75%) of oilfield wastewater on the microbial population and diversity of soil microorganisms was investigated for a period of 28 days. The oilfield wastewater sample was collected from an onshore oil production platform while the soil samples were collected 80m away from the pond where the oilfield wastewater is being discharged. Microbiological analyses were determined using standard methods. Total heterotrophic bacteria (THB) counts ranged from 7.31 to 8.70 (log_{10}CFU/g soil), total fungal (TF) count ranged from 3.95 to 4.61 (log_{10}CFU/g soil). The total hydrocarbon utilizing bacterial (THUB) count ranged from 3.01 to 5.54 (log_{10}CFU/g soil), while the total hydrocarbon utilizing fungal (THUF) count ranged from 3.00 to 4.73 (log_{10}CFU/g soil). Statistical analysis showed that there was no significant difference in the THB between the control and the treatment options, but there was significant difference in the THF, HUB and HUF between the control and the treatment options. The types of bacteria isolated in the study included *Actinomyces* sp, *Bacillus* sp, *Enterobacter* sp, *Escherichia* sp, *Klebsiella* sp, *Micrococcus* sp, *Proteus* sp, *Pseudomonas* sp, and *Rhodococcus* sp and *Staphylococcus* sp. While the fungi isolated included *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium*, *Geotricum*, *Penicillium* sp, *Saccharomyces cerevisiae*, and *Trichoderma* species. *Bacillus* sp, and *Pseudomonas* sp, *Aspergillus niger* and *Penicillium* sp, were isolated from all the treated soils including the control. *Klebsiella* sp. occurred in only 50% treatment, *Micrococcus* sp were isolated in all treatment option except control. *Enterobacter* sp occurred in 10% and 25% treatment while *Rhodococcus* sp occurred in 25%, 50% and 75%. In addition, three unidentified bacteria were isolated from the control soil. The bacterial and fungal counts in the study revealed the impact of oilfield wastewater on soil microbes. The high prevalence of hydrocarbon utilizing microorganisms revealed that the soil studied contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

Keywords: Oilfield wastewater, hydrocarbon, soil bacteria, fungi, microbial diversity.

INTRODUCTION

Crude oil exploration and exploitation activities in Nigeria are major contributors of environmental pollution in the Niger Delta. Oilfield wastewater is the associated

wastewater of crude oil production activities. Oilfield wastewater may contain hydraulic fracturing (HF) fluids, naturally occurring salts, radioactive materials, heavy metals, and other compounds from the formation such as polycyclic aromatic hydrocarbons, alkenes, alkanes, and other volatile and semi-volatile organics (Warner *et al*., 2012; Kassotis *et al*., 2015).

Increased Petroleum activities, particularly in the Niger Delta has led to pollution stress on soil and surface water, due to the discharging of large quantities of oilfield wastewater without adequate treatment techniques (Obire and Amusan, 2003; Wemedo and Obire, 2012). Oilfield wastewater containing high organic and inorganic chemicals poses environmental problems. The main crucial environmental issues of the oilfield wastewater are total petroleum hydrocarbon, total solids (TS), and inorganic chemicals including heavy metals and polycyclic aromatic hydrocarbons (PAHs), biochemical and chemical oxygen demand (BOD and COD), and pathogens (Pichtel, 2016).

In Nigeria, Oil exploitation companies are known to discharge oilfield wastewater into Streams or ponds which are also a threat to the surrounding soil and groundwater (Obire and Wemedo, 1996). Accelerated soil quality change due to oilfield wastewater discharging with large quantities of nutrients and toxic substances into the environment has long become an issue problem in Niger Delta. It is estimated that over 90% of wastewater from operations of oil industries in Nigeria is still discharged to soil, rivers and streams without adequate treatment. This is largely due to the fact that most of the oil companies have no wastewater treatment plants or where they exist the facilities are inadequate (Human Rights Watch, 1998; Van Dessel, 1996).

Soil contaminated by industrial effluents has affected adversely both soil health and crop productivity. Heavy metals are one of the major pollutants of interest in the environment because of its toxicity, persistence and bioaccumulation problems (Zouboules *et al.,* 2004). Excessive accumulation of micronutrients and other heavy metals like cadmium, lead, and nickel in the plants operates as stress factors causing physiological constraints leading to decrease vigour and plant growth (Zouboules *et al.,* 2004) and therefore crop yield (Jaja and Obire, 2015). The effects of petroleum activities on the environment in the Niger Delta are evident through the pollution of soil and water bodies and human habitat in the major cities. The oilfield wastewater contains toxic and hazardous substances that are detrimental to human health if they enter the food chain (Rajaram and Ashutost, 2008).

The objective of this present study therefore was to investigate the impact of various concentrations of oilfield wastewater on soil microbes as to determine the effect of oilfield wastewater on the microbial population and diversity after treatment of soil with oilfield wastewater. This investigation was conducted for a period of 28days.

MATERIALS AND METHODS

Collection of Oilfield wastewater and soil samples

Oilfield wastewater was collected from Ogbogu Flow Station; an onshore oil production platform located in Ogba Egbema Ndoni local government Area (ONELGA) of Rivers State, Nigeria. The Oilfield wastewater samples were collected using 4 Litre capacity plastic bottles and stored in an ice packed cooler.

On the other hand, the soil samples were collected 80 meters away from the pond at a depth of 0-15cm with a sterile spatula into sterile polythene bags and stored in an ice packed cooler. The collected and appropriately labeled oilfield wastewater and soil

samples were immediately transported to the laboratory for analysis within 24 hours for processing and analyses.

Media Preparation

Nutrient Agar was used for Total Heterotrophic bacterial count; Potato dextrose agar was used for total fungal count while Mineral salt agar medium prepared according to the modified minimal salts medium (MSM) composition of Mills *et al.* (1978) was used for the isolation of total hydrocarbon utilizing bacteria. Minimal salts medium (MSM) composition is – [$MgSO_4.7H_2O$ (0.42g), KCl (0.29g), KH_2PO_4 (0.83g), Na_2HP0_4 (1.25g) $NaNO_3$ (0.42), agar (20g)] in 1Litre of distilled water. The mixture was thoroughly mixed and autoclaved at 15psi at 121°C for 15mins and was allowed to cool to 45°C. The medium was prepared by the addition of 1% (v/v) crude oil sterilized with 0.22µm pore size Millipore filter paper Moslein France (Obire, 1988) to sterile MSM, which has been cooled to 45^{o}C under aseptic condition. The MSM and crude oil were then mixed thoroughly and aseptically dispensed into sterile Petri dishes to set.

Microbiological Analysis of the Oilfield Wastewater and Soil Samples

Determination of Total heterotrophic bacterial (THB) count of oilfield wastewater and soils

The total heterotrophic bacterial (THB) count was determined using the nutrient agar and spread plate technique as described by Prescott *et al.* (2005). An aliquot (0.1ml) of each serially diluted sample using dilution factors of 10^{-5} for Raw wastewater, 10^{-2} for wastewater in the pond, and $10^{-4}$for all the soil samples was separately inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated at 37°C in an inverted position for 24 hours. After incubation, colonies that developed on the plates were counted and only counts of between 30 and 300 were recorded. The average values of replicate plates was calculated and expressed as colony forming unit - CFU/ml for oilfield wastewater and CFU/g for soil samples.

Determination of total fungi count of samples of oilfield wastewater and soils

The total count of fungi in the samples was also determined by the spread plate technique. An aliquot (0.1ml) of serial dilution (10^{-2}) of each of the various samples was plated onto separate Potato dextrose agar plates to which 0.1 ml of streptomycin solution was incorporated to suppress bacterial growth. The plates were incubated at 28°C for 5-7 days and the discrete colonies that developed were enumerated as the viable counts (CFU) of fungi in the oilfield wastewater and soil samples (Obire and Wemedo, 1996).

Hydrocarbon utilizing bacterial count (HUB) of samples

The population of the hydrocarbon utilizing bacterial of oilfield wastewater and soil samples was determined by inoculating 0.1ml aliquot of the serially diluted (10^{-1} and 10^{-2}) samples of oilfield wastewater and 10^{-1} of soil samples onto mineral salt agar media using the spread plate technique described by Odokuma (2003). The Vapour Phase Transfer method will be adopted by the use of sterile filter paper discs that will be soaked in filter sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were aseptically transferred

to the inside cover of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted, average of duplicate colonies calculated colony forming units per ml of wastewater or per gram soil calculated.

Hydrocarbon utilizing fungal count (HUF) of samples

Total hydrocarbon utilizing fungal count of oilfield wastewater and soil samples was determined by inoculating 0.1ml of the serially diluted samples -1 on mineral salt agar. The mineral salt medium will be supplemented with streptomycin (0.1ml) to suppress bacterial growth (Obire and Wemedo, 1996). The Vapour Phase Transfer method was adopted by the use of sterile filter paper discs that were soaked in filter sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were aseptically transferred to the inside cover of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted, average of duplicate colonies calculated colony forming units per ml of wastewater or per gram soil calculated.

Oilfield Wastewater Impact Studies on Soil Microorganisms

Studies on the impact of oilfield wastewater on soil microorganisms was carried out by inoculating various concentrations (0% - control, 10%, 25%, 50% and 75%) of oilfield wastewater into separate soil samples and incubated in a rotary shaker. Samples were withdrawn at different time intervals or incubation periods and analyzed for microbiological properties such as bacterial and fungal count, total heterotrophic bacteria, and total fungi and for both hydrocarbon-utilizing bacteria and hydrocarbon-utilizing fungi as described above.

Characterization and identification of bacterial and fungal isolates from samples

The cultural, morphological, microscopic characteristics of the isolates from the study were observed and recorded. The morphological and biochemical tests conducted using the isolates included Gram staining, motility, catalase, oxidase, citrate utilization, sugar fermentation, hydrogen sulphide production, indole production methyl red and Voges Proskauer test. Results of the morphological and biochemical characteristics of the isolates were compared with those of known Taxa using Bergey's manual of determinative bacteriology (1994).
For the presumptive identification of fungal isolates, pure fungal cultures were observed while still on plates and after wet mount in lacto-phenol on slides under the compound microscope. Observed characteristics such as vegetative hyphae and reproductive structures were recorded and compared with the established identification key of Barnett and Hunter (1972) and Malloch (1997).

Statistical analysis

Statistical analysis was also conducted using Duncan Multiple Range test and Analysis of variance to determine whether there is significant difference between various concentration of oil field wastewater and period of incubation.

RESULTS

The result of the total heterotrophic bacterial count (log_{10}CFU/g soil) in soils treated with various concentrations of oilfield wastewater is as shown in Figure 1 below.

The counts of total heterotrophic bacteria at 0% treatment ranged from 7.31 to 7.53 with an average count of 7.81; 7.36 to 7.46 with an average count of 7.42 for 10%; 7.38 to 8.56 with an average count of 7.87 for 25% treatment, 7.31 to 8.57 with an average count of 7.89 for 50% treatment; 7.29 to 8.70 with an average count of 8.13 for 75% treatment.

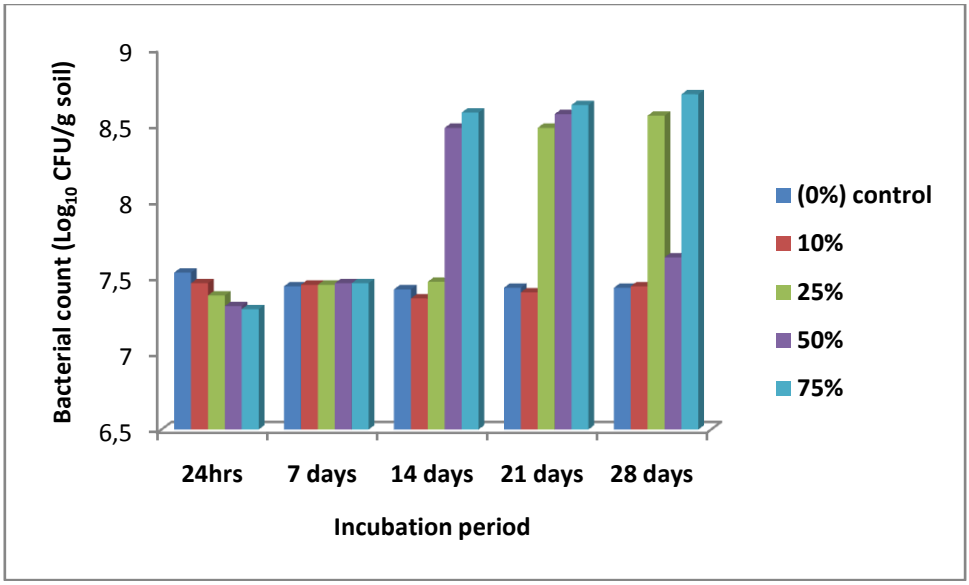

Fig.1: Total heterotrophic bacterial count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater

The result of the total fungal count (log_{10}CFU/g soil) in soils treated with various concentrations of oilfield wastewater is as shown in Figure 2 below. The counts of total fungi at 0% treatment ranged from 3.95 to 4.32 with an average count of 4.05; 4.16 to 4.25 with an average count of 4.20 for 10%; 4.18 to 4.51 with an average count of 4.34 for 25%; 4.14 to 4.61 with an average count of 4.38 for 50%; 4.06 to 4.62 with an average count of 4.39 for 75% treatment.

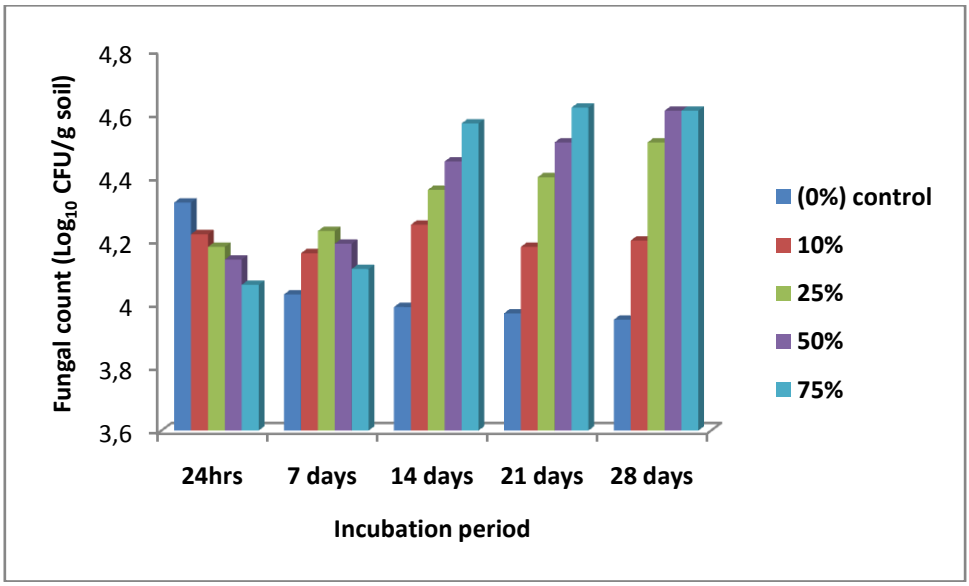

Fig.2: Total fungal count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater

The result of the total hydrocarbon utilizing bacterial count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater is as shown in Figure 3 below. The counts of total hydrocarbon utilizing bacteria at 0% treatment ranged from 3.01 to 3.18 with an average count of 3.1; 4.29 to 4.58 with an average count of 4.44 for 10% treatment, 4.33 to 4.47 with an average count of 4.42 for 25% treatment, 4.27 to 4.43 with an average count of 4.36 for 50% treatment, 4.25 to 5.54 with an average count of 4.77 for 75 treatment.

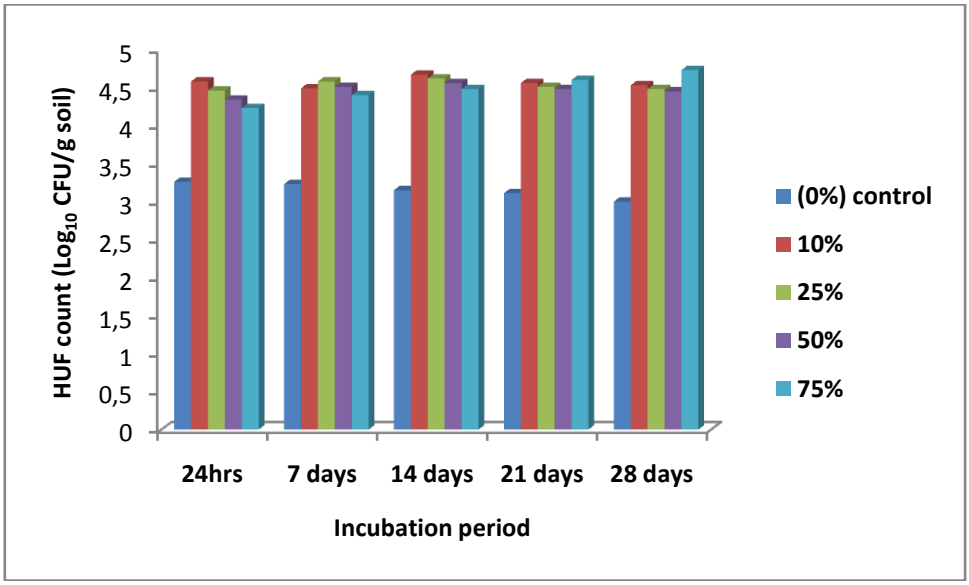

Fig.3: Total hydrocarbon utilizing bacterial count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater

The result of the total hydrocarbon utilizing fungal count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater is as shown in Figure 4 below. The counts of total hydrocarbon utilizing fungal at 0% treatment ranged from 3.00 to 3.26 with an average count of 3.15; 4.49 to 4.67 with an average count of 4.57 for 10% treatment, 4.46 to 4.62 with an average count of 4.53 for 25% treatment, 4.34 to 4.56 with an average count of 4.47 for 50% treatment, 4.23 to 4.73 with an average count of 4.49 for 75 treatment.

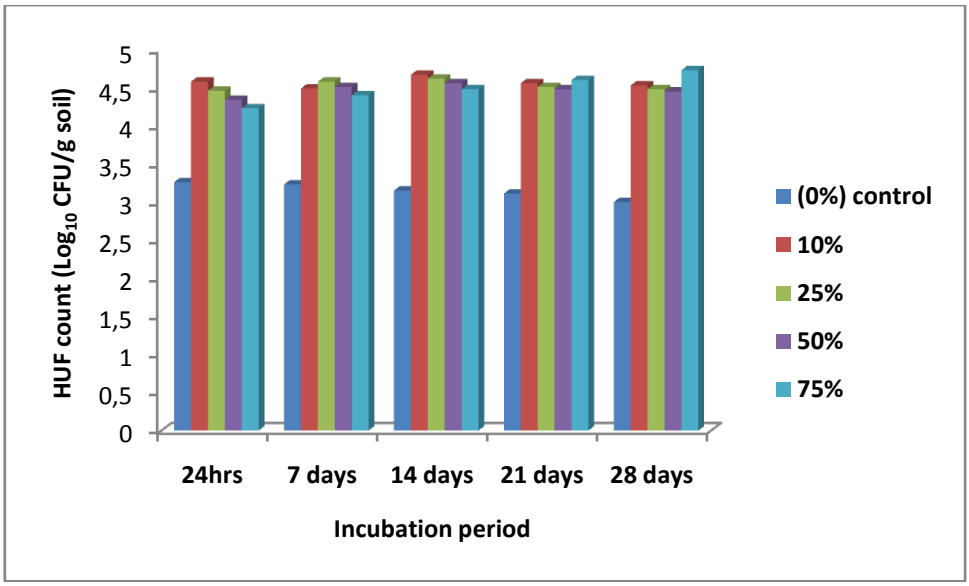

Fig.4: Total hydrocarbon utilizing fungal count (log_{10}CFU/g) in soils treated with various concentrations of oilfield wastewater

The predominant bacteria and fungi that were isolated from soils treated with various concentrations of oilfield wastewater are as shown in Table 1 and Table 2 respectively. The negative (-) sign indicates absence of indicated organism in the row in the concentration.

Table 1: Bacteria isolated from soils treated with various concentrations of oilfield wastewater

Concentration of oilfield wastewater				
0% (control)	10%	25%	50%	75%
-	-	*Actinomyces* sp	-	-
Bacillus sp	*Bacillus* sp	*Bacillus* sp	*Bacillus* sp	*Bacillus* sp
-	*Enterobacter* sp	*Enterobacter* sp	-	-
-	-	*Escherichia* sp	*Escherichia* sp	-
-	-	-	*Klebsiella* sp	-
-	*Micrococcus* sp	*Micrococcus* sp	*Micrococcus* sp	*Micrococcus* sp
Proteus sp	*Proteus* sp	-	-	*Proteus* sp
Pseudomonas sp	*Pseudomonas* sp	*Pseudomonas* sp	*Pseudomonas* sp	*Pseudomonas* sp
-	-	*Rhodococcus* sp	*Rhodococcus* sp	*Rhodococcus* sp
-	*Staphylococcus* sp	-	*Staphylococcus* sp	*Staphylococcus* sp

Table 2: Fungi isolated from soils treated with various concentrations of oilfield wastewater

Concentration of oilfield wastewater				
0% (control)	10%	25%	50%	75%
-	-	*Aspergillus flavus*	*Aspergillus flavus*	*Aspergillus flavus*
A. fumigatus	*A. fumigatus*	*A. fumigatus*	*A. fumigatus*	_

A. niger	*A. niger*	*A. niger*	*A. niger*	*A. niger*
Fusarium	*Fusarium*	-	*Fusarium*	*Fusarium*
Geotricum sp	-	-	-	-
Penicillium sp	*Penicillium* sp	*Penicillium* sp	*Penicillium* sp	*Penicillium* sp
-	*Saccharomyces* sp	*Saccharomyces* sp	*Saccharomyces* sp	*Saccharomyces* sp
Trichoderma sp	-	-	-	-

DISCUSSION

The present study had revealed the microbial population and diversity of bacteria and fungi in soils treated with various concentrations of oilfield wastewater. The total heterotrophic bacteria count on 24hrs was more than the treated soils. The 10% treatment decreased on day 7 and day 21 and gradually increased as the study progressed. It could be that the oilfield wastewater introduced into the soil sample was toxic which was responsible for the decreased growth phase of the bacteria. An initial gradual increase in bacterial population following the application of petroleum hydrocarbon but a decline as the biodegradation progressed was also reported by Amadi and Odu (2003). The 25% treatment option increased from 24hrs to day 28 and more notable increase on day 21 and day 28. There was significant difference on day14 and day 21 in the 50% treatment. The noticeable increase could be attributed to the hydrocarbon utilizers that are part of the total heterotrophs present in the sample. The 50% treatment reduced significantly on day 28. There was static growth on all the treatment on day 7. The toxicity of petroleum constituents varies depending on their composition and concentration. According to Colwell and Walker (1977) the scale of pollution depends on the quality of oil and damage done to the environment. Steinhart and Steinhart (1972) also noted immediate detrimental effect on biological forms in heavy polluted areas. Growth in 75% treatment progressively increased from the 24hrs to day 28 without reducing. The highest count was observed on day 28 on 75% treatment option.

The total fungi count on 24hrs was more in the control compared to the other treatment options. The fungi count in the treatment options were more than the control apart from 24hrs. This result agrees with that of Obire and Anyanwu (2009).

The hydrocarbon utilizing bacteria decreased from 24hrs to day28 in the control, and other treatment options (10%, 25%, 50%, 75%) were higher than the control from 24hrs to day 28. There was significant increase on day 14, 21 and 28 in 25%, 50% and 75% treatment options.

The higher count of hydrocarbon degraders observed in the treatment soils as compared to the control could be attributed to their ability to survive the toxicity of the oilfield wastewater and to utilize the hydrocarbons present in it as carbon and energy source. This is in consonance with previous findings as the proportion of hydrocarbon utilizers in heterotrophic communities have generally been reported to increase as a result of exposure to petroleum (Leahy and Colwell, 1990; MacNaughton *et al.,* 1999). The highest hydrocarbon utilizing bacteria count was found on 75% treatment option on day 28. According to Michalcewic (1995), Wyszkowska and Kucharski (2001) petroleum products entering soil can produce different effects on soil microorganisms including saprophytic fungi (Hong-Gyu Song and Bartha, 1990; Michalcewicz and Lawrynowicz, 2004) bringing about changes in quantitative and qualitative composition of soil microorganisms. Many studies (Nowak *et al*. 1998; Boszczky-Maleszak *et al*., 2000; Kucharski and Jastrzebska, 2001; Baran *et al*. 2002; Wyszkowska *et al*., 2006) confirm

that petroleum substances can considerably modify the natural biological activities of soils. They are highly toxic in most cases and strongly inhibiting the development of microorganisms and consequently depressing the enzymatic activity of the soils.
The total hydrocarbon utilizing fungal count in the control decreased as the experiment progressed. The highest growth was noticed on day 28 on the 75% treatment option.
The types of bacteria isolated in the study included *Actinomyces* sp, *Bacillus* sp, *Enterobacter* sp, *Escherichia* sp, *Klebsiella* sp, *Micrococcus* sp, *Proteus* sp, *Pseudomonas* sp, and *Rhodococcus* sp and *Staphylococcus* sp. While the fungi isolated included *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium*, *Geotricum*, *Penicillium* sp, *Saccharomyces cerevisiae*, and *Trichoderma* species. *Bacillus* sp, and *Pseudomonas* sp, *Aspergillus niger* and *Penicillium* sp, were isolated from all the treated soils including the control. *Klebsiella* sp. occurred in only 50% treatment, *Micrococcus* sp were isolated in all treatment option except control, *Enterobacter* sp occurred in 10% and 25% treatment. It is worthy of note that *Rhodococcus* sp occurred in the higher concentrations of 25%, 50% and 75%. While the fungal isolates, *Penicillium* sp and *Aspergillus niger* were isolated in all the treatment option including the control (0%). *Aspergillus fumigatus* were isolated in 0%, 10%, 25% and 50% treatment option. *Trichoderma* sp and *Geotricum* sp were isolated only in the control (0%). *Fusarium* sp were isolated in all option except 25% treatment option. *Saccharomyces cerevisiae* were isolated from in all treatment option except the control (0%). *Aspergillus flavus* were isolated from all treatment except 0% and 10 %.
The microbial counts and types obtained in the study revealed the impact of oilfield wastewater on soil microbes. Table 1 showing the predominant bacteria isolated from the soil samples indicated that the control soil which is 80m away from the oilfield wastewater discharge pond is impacted by activities such as gas flaring of the oil field affected the diversity of the soil bacteria hence it recorded the lowest number in type of bacteria. In addition, three unidentified bacteria were isolated from the control soil. The reverse was the case for the number in types of fungi in Table 2 as the control soil and the 50% concentrations recorded the highest number. It was also observed that *Geotricum* and *Trichoderma* species which occurred in the control were completely eliminated by the oilfield wastewater in the treated soils which indicated that the oilfield wastewater was microbiocidal to certain microbes. All the isolates from the bacteria and fungi were also hydrocarbon utilizers isolated except *Geotricum* sp and *Trichoderma* species. This explains why both isolates were completely eliminated by the oilfield wastewater in the treated soils. These results showed that different group of microorganisms responded differently to the same toxicant which supports the previous findings of Obire and Anyanwu (2009). The prevalence of hydrocarbon utilizing microorganisms indicated that indigenous populations of soil heterotrophic microbes with particular reference to bacteria have been displaced by active indigenous hydrocabon utilizers. This is attributed to the proliferation of hydrocabon utilizers as a result of the enrichment of their growth in the presence of the oilfield wastewater. The selective enrichment of a toxicant or pollutant as is in this present study has serious deleterious effect on environmental quality and soil ecology.

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