Original Research Article

Response of Soil Microorganisms to Oilfield Wastewater

ABSTRACT

Environmental pollution resulting from oil exploitation and exploration has impacted negatively on the biodiversity of the affected areas. Therefore this study investigated the response of soil microorganisms to oilfield wastewater. The oilfield wastewater and soil samples were collected from an onshore oil producing platform fortnightly for a period of three months and microbiological analysis were determined using standard methods. The average range of total heterotrophic bacteria and hydrocarbon utilizing bacteria (Log_{10} CFU/ml) for the oil field wastewaters were: 8.26 to 8.30 and 3.15 to 4.11 respectively. For wastewaters in pond the THB and HUB ranged from 5.39 to 5.44 and 5.30 to 5.32 respectively. Soil around pond average counts for THB and HUB (Log_{10} CFU/g) were 7.32 to 7.35 and 4.16 to 4.22 respectively. Soil 80m away from pond average range for THB and HUB were 7.38 to 7.40 and 3.32 to 3.34respectively. Average range of the total fungi (TF) and the hydrocarbon utilizing fungi count (Log_{10} CFU/ml) for the oilfield wastewaters were 4.23 to 4.28 and 3.38 to 3.45 respectively. The average range of the TF and HUF of wastewater in pond were: January 4.49 to 4.58 and 3.63 to 3.76 respectively. Soil around the pond mean recorded 4.65 to 4.85 and 4.12 to 4.16 (Log_{10} CFU/g) respectively. Mean monthly counts for soil 80m away TF and HUF (Log_{10} CFU/g) were 5.03 to 5.05 and 3.26 to 3.34 respectively.

There was significant difference ($P<0.05$) between the THB and HUB in the various samples. For the fungi count there was no significant difference ($P>0.05$) between TF and HUF in all the samples except in sample obtained from soil 80 m away from pond.

Bacteria species isolated from the study include: *Bacillus, Aeromonas, Micrococcus, Staphylococcus, Chryseomonas, Proteus, Pseudomonas, Klebsiella.* Apart from *Aeromonas* and *Chryseomonas* the rest of the isolate were identified also as hydrocarbon utilizing bacteria. While fungi species isolated includes: *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae, Geotricum, Trichoderma, Fusarium* and *Penicillium.* Hydrocarbon utilizing fungi that occurred includes: *Aspergillus niger, Aspergillus flavus, Fusarium, Penicillium* and *Saccharomyces cerevisiae*.The response of these microorganisms in the oil polluted environment suggests that the isolated bacteria and fungi could utilize the oil as energy and carbon source and could be effective in the cleanup of the polluted sites.

Key words: Response, Oilfield wastewater, Pond wastewater, Soil, Bacteria, Fungi

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 response of soil microorganisms to oilfield wastewater by enumerating microorganisms in the oilfield wastewater, wastewater in pond, soil around the pond and in soil 80m away from the pond. This investigation was conducted for a period of three months (January–March, 2018) regarded as part of the dry season in the Niger Delta.

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 from around the pond and 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. Samples were collected twice in a month (1^{st} and 3^{rd} week) for a period of three months (January, 2018 to March, 2018).

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.

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 the total counts and the hydrocarbon utilizers.

RESULTS

The results of the total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) (log_{10}CFU/ml) of the oil field wastewaters were: January 8.26 and 3.15; February 8.30 and 4.11; March 8.26 and 4.09 respectively. For wastewaters in pond the THB and HUB were: January 5.44 and 5.32; February 5.43 and 5.31; March 5.39 and 5.30 respectively. Soil around pond mean monthly counts for THB and HUB (log_{10}CFU/g)were: January 7.35 and 4.22; February 7.33 and 4.16; March 7.32 and 4.16respectively.Soil 80m away from pond counts for THB and HUB were: January7.38 and 3.32; February 7.40 and 3.34; March 7.40 and 7.32 respectively.

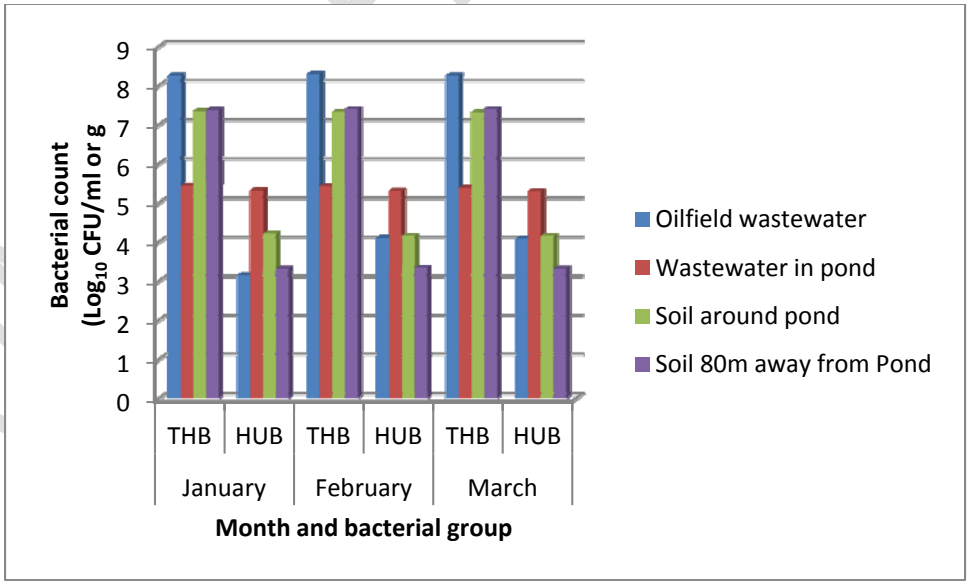

Fig. 1: Counts of total heterotrophic bacterial (THB) and hydrocarbon utilizing bacteria (HUB) of wastewater and soil.

Mean monthly counts of the total fungi (TF) and the hydrocarbon utilizing fungi (Log_{10} CFU/ml) for the oilfield wastewaters were: January 4.26 and 3.45; February 4.23 and 3.38; March 4.28 and 3.43 respectively. The mean monthly counts of the TF and HUF for wastewater in pond were: January 4.58 and 3.76; February 4.49 and 3.63; March 4.56 and 3.63 respectively. Soil around the pond mean monthly counts for TF and HUF (Log_{10} CFU/g) were: January 4.85 and 4.16, February 4.74 and 4.12; March 4.65 and 4.12 respectively. Mean monthly counts for soil 80m away TF and HUF were: January 5.05 and 3.26; February 5.03 and 3.32; March 5.05 and 3.34 respectively.

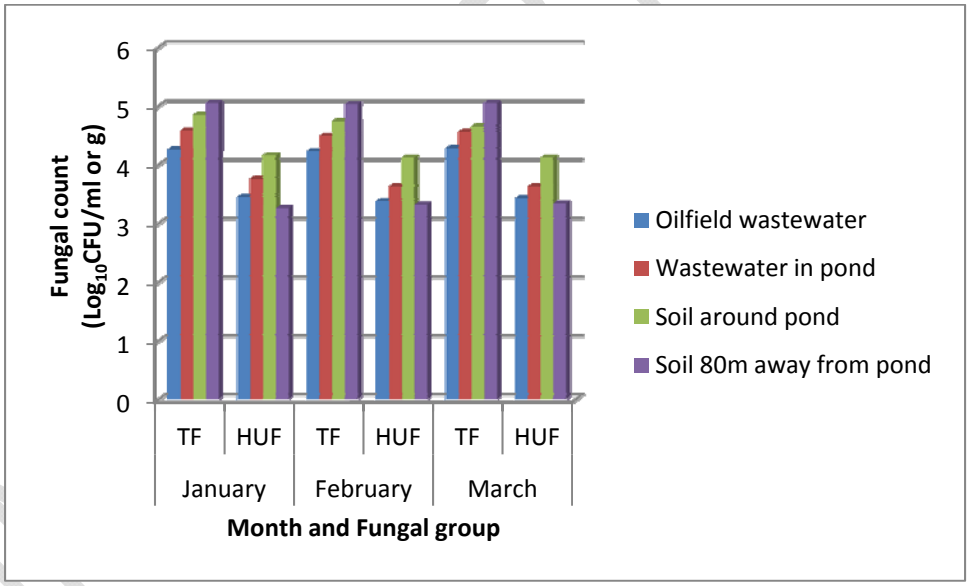

Fig. 2: Counts of total fungi (TF) and hydrocarbon utilizing fungi (HUF) of wastewater and soil.

The predominant bacteria and fungi that were isolated from the various samples are as shown in Table 1 and Table 2 respectively. The negative (-) sign indicates absence of indicated organism in the row in the various samples.

Table 1: Bacteria isolated from Oilfield Wastewater and Soil Samples

Types of bacteria	Oilfield wastewater	Wastewater in pond	Soil around pond	Soil 80m away from pond
Bacillus sp	+++	+++	+++	+++
Aeromonas sp	+++	+++	-+-	---
Micrococcus sp	+++	+++	+++	+++
Staphylococcus sp	+++	--+	---	---
Chryseomonas sp	+++	+-+	---	---
Proteus sp	+++	++-	+++	+++
Klebsiella sp	+++	+++	---	---
Pseudomonas sp	+++	+++	+++	+++

Table 2: Fungi isolated from Oilfield Wastewater and Soil Samples

Types of fungi	Oilfield wastewater	Wastewater in pond	Soil around pond	Soil 80m away from pond
A. .niger	+++	+++	+++	+++
A. fumigatus	+++	+++	+++	+++
A. flavus	++-	---	+++	+++
Penicillium sp	+++	+++	+++	+++
Fusarium sp	+++	+++	---	---
Saccharomyces cerevisiae	+++	+++	---	---
Geotricum sp	---	+++	-++	+++
Trichoderma sp	---	---	+++	+++

DISCUSSION

Microbial populations play a role in degradation of hydrocarbon contaminations, Atlas (1981) and Leahy and Colwell (1990) reported that the rate of petroleum hydrocarbon biodegradation in nature is determined by the populations of indigenous hydrocarbon degrading microorganisms. Leahy and Colwell (1990) concluded that hydrocarbon biodegradation depends on the composition of the microbial community and its adaptive response to the presence of hydrocarbons. Heterotrophic bacteria counts were higher in the oilfield wastewater in the various months of study compared to other samples. The occurrence of bacteria in oilfield wastewater despite the treatment with biocide during the treatment process could be attributed to organic and inorganic constituents that serve as nutrients for bacterial growth. This was also reported by Wemedo *et al.* (2012), and Marshall and Derinny (1988). The hydrocarbon utilizing bacteria count was higher in the wastewater pond than

other samples. This could be as a result of continues discharge of petroleum products into the wastewater pond. It has been reported by several researchers that continuous discharges of crude oil into the ecosystem may result in selective increase or decrease in microbial population (Okpokwasili and Nnubia, 1995; Okpokwasili and Odokuma, 1996).There was significant difference ($P<0.05$) between the THB and HUB in the various samples.

The soil 80m away from the pond had higher total fungi count than other samples. Higher HUF counts were observed in the soil around pond and could be attributed to the presence of residual crude oil in the polluted soil which boosts the carbon supply in the soil thereby favoring the growth of the hydrocarbon utilizing fungi which were adapted to the quantity of hydrocarbons in the environment. It was also observed that total fungi counts were greater than HUF counts in all the samples in the various months. There was no significance difference ($P>0.05$) between the HF and HUF in all samples except in soil 80m away from the pond. This indicates that samples were polluted with hydrocarbons. The result is in agreement with that reported by Chikere and Azubuike (2014).

Bacteria species isolated from the study include: *Bacillus, Aeromonas, Micrococcus, Staphylococcus, Chryseomonas, Proteus, Pseudomonas, Klebsiella.* Apart from Aeromonas and Chryseomonas the rest of the isolate were identified also as hydrocarbon utilizing bacteria. While fungi species isolated includes: *Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae, Geotricum, Trichoderma, Fusarium* and *Penicillium.* Hydrocarbon utilizing fungi that occurred includes: *Aspergillus niger, Aspergillus flavus, Fusarium, Penicillium* and *Saccharomyces cerevisiae*. The following organisms; *Staphylococcus sp, Fusarium, Chryseomonas sp, Klebsiella sp,* and *Saccharomyces cerevisiae* were not isolated in the soil samples while *Trichoderma sp* was only isolated in all soil samples. High numbers of certain oil-degrading microorganisms from an environment implies that those organisms are the active degraders of that oil and may have the ability to produce spores that may shield them from toxic effects of the hydrocarbon. Similar reports were published by Okerentugba and Ezeronye (2003), Onifade and Abubakar (2007), Boboye *et al.* (2010), Obire and Wemedo (1996) and Obire and Amusan, 2003.

CONCLUSION AND RECOMMENDATIONS

The diversity of the microbial community in the study area decreased in response to environmental stress. The limited numbers of the population have specific properties such that they can persist within the environment and thus can be effective in the cleanup of polluted area.

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