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Research Article

Impact of Oilfield Wastewater from Santa Barbara Oil Rig Location on the Microbial Population of Santa Barbara River in Bayelsa State, Nigeria

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Abstract

Oilfield wastewater contains toxic substances that are detrimental to aquatic microflora. Its continuous disposal into aquatic environment has long become a menace in the Niger Delta due to oil exploration activities. The effect of oilfield wastewater from Santa Barbara oil rig was investigated bi-weekly for a period of two (2) months. A total of forty (40) water samples collected from the vicinity of the oil rig and from a control point were analyzed for counts of bacteria and fungi using standard microbiological methods. Results showed that counts of total heterotrophic bacteria (THB) ranged from 3.8×10^4cfu/ml to 9.2×10^4cfu/ml; total fungi ranged from 1.2×10^2cfu/ml to 3.2×10^2cfu/ml; total hydrocarbon utilizing bacteria (THUB) ranged from 2.6×10^3cfu/ml to 4.0×10^3cfu/ml while total hydrocarbon utilizing fungi (THUF) ranged from 0.8×10^1cfu/ml to 1.7×10^1cfu/ml. The decreasing order of both THB and fungi counts in the stations was; Control > Downstream > Upstream > Deck drainage > Drilling point. The decreasing order of THUB was Deck drainage > Drilling point > Upstream > Downstream > Control. The decreasing order of THUF was Deck drainage > Drilling point > Downstream > Upstream > Control. The bacteria isolated from the study area included *Pseudomonas, Bacillus, Aeromonas, Alcaligenes, Staphylococcus, Enterococcus, Salmonella* and *Escherichia*. Statistical analysis (ANOVA) showed that there is significant difference in THB between the control and other stations; calculated F value (34.73952) > F-critical value (6.388234). The lowest counts in the rig locations are attributed to the impact of oilfield wastewater on aquatic microbes and diversity. While the highest counts of hydrocarbon utilizing microbes is attributed to the selective enrichment of hydrocarbon utilizers. Their high prevalence revealed that Santa Barbara River contained active indigenous hydrocarbon utilizers that can be harnessed for bioremediation process.

Keywords: Swamp; Santa Barbara Oil Rig; Oilfield Wastewater; Hydrocarbon Utilizing Bacteria

Introduction

Swamp is a forested wetland which is considered to be a transition zone because both land and water play a major role in creating this environment [1]. They vary in size; water from the swamp can be Freshwater, Salt-water (Brackish) or Sea-water [2]. The freshwater swamp form along large rivers or lakes where they depend on rainwater and seasonal flooding to maintain natural water level fluctuation [3], whereas Salt water (brackish) are found along tropical and subtropical waistlines [4].

Produced water or oilfield wastewater are water that comes out of a well with crude oil during production, they contain soluble and non-soluble oil/organic substances, suspended solids, dissolved solids and various chemicals used in the production process [5].

Produced water (also called formation water, brine or saltwater) is water from underground formations that is brought to the surface during oil or gas production. The water is discharged in an oily form after its separation from the real crude oil. During oil and gas exploration other forms of wastewater are produced, these include injected water, little quantity of water that is condensed and traces of some chemicals used among which produced water is the highest generated by-product [6]. The physical and chemical properties of produced water vary considerably depending on the geographic location of the field, the geologic formation, and the type of hydrocarbon product being produced [6-8]. Dispersed oil, aromatic hydrocarbons and alkylphenols (AP), heavy metals, and naturally occurring radioactive material (NORM) are of particular environmental concern because the water has been in contact with hydrocarbon-bearing formations [8,9]. It contains some of the chemical characteristics of the formations and of the hydrocarbons. It may include water from the reservoir, water previously injected into the formation, and any chemicals added during the production processes. The composition and characteristics of naturally occurring chemical substances in produced water are closely associated to the geological characteristics of each reservoir [8]. Produced water is mostly discharged to the immediate aquatic environment during crude oil production. The organic and inorganic compounds in produced water have higher toxicity to the environment than crude oil itself.

The significance of oil and natural gas in modern civilization is well known. Nevertheless, oil and gas exploration and production activities produce solid, liquid, and gas waste with 80% liquid waste composition, even it reaches 95% in the old oil fields. Produced water is the largest liquid waste generated by the activity [10]. Oilfield wastewater or produced water contains various organic and inorganic components. Discharging produced water can pollute surface and underground water and soil [11]. Global produced water production is estimated at around 250 million barrels per day compared with around 80 million barrels per day of oil. As a result, water to oil ratio is around 3:1 that is to say water cut is 70%. Oilfields are responsible for more than 60% of produced water generated worldwide [11,12]. Continuation of large produced water discharges is expected because the water cut (the relative amount of water to oil) increases with the age of the production wells [11,13]. Thus, the question of biological effects of produced water discharges is a matter of continued relevance. Usually, produced water is treated to remove dispersed oil by either flotation or gravity in separation tank batteries, and then discharged to the environment. Alternatively, it may be injected into a nearby non-producing well [14]. Produced water need treatment to meet the quality requirement of wastewater set by the Government before being disposed to the environment [15,16]. The utilization of the produced water and the disposal of untreated produced water containing hazardous materials can interfere with the environmental sustainability. The aim of this study was to evaluate the impact of oilfield wastewater from the vicinity of Santa Barbara oil rig on the Santa Barbara River in Bayelsa state, Nigeria.

Materials and Methods

Description of study area

Santa Barbara oil rig also known as OML 29 or Santa Barbara flow Station (OML 29) is an onshore oil production platform located in Santa Barbara River, South of Brass Creek and East of Odiama Creek in Nembe local government Area of Bayelsa State, Nigeria. It is owned by Nigerian National Petroleum Corporation (NNPC) and AIETO Energy Resources. Its geographical coordinates are Latitude 4.3358,4^0 20. 89" North and Longitude 6.6022, 6^0 36. 81" East.

Collection of samples

Oilfield wastewater samples were collected from Santa Barbara flow Station (OML 29) using sterilized glass bottles. Water samples were collected from Drilling point, Deck drainage, upstream, downstream and from a control point on the Santa Barbara River. The drilling point sample was collected from the drilling point of the oil rig, while the deck drainage sample was collected from the deck on the point of drilling. On the other hand, the upstream and downstream samples were collected from a point 500m to the left and to the right from the drilling point. While the Control sample was collected from 2km upstream from the point of drilling. Immediately after collection of each sample, sample bottles were appropriately labeled and immediately stored in an ice packed cooler box. The samples were thereafter transported to the laboratory within 24 hours for processing and microbiological analyses. Sample storage was done according to standard laboratory practices as recommended by the American Public Health Association (APHA) [17]. At the end of each analysis, sample containers were thoroughly washed and rinsed with distilled water. Water samples were collected bi-weekly for two (2) months of November and December to April. A total of forty (40) water samples collected from the drilling point, upstream, downstream (vicinity of the Santa Barbara oil rig) and from a control point were analyzed for counts of bacteria and fungi using standard microbiological methods.

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.*[18] was used for the isolation of total hydrocarbon utilizing bacteria and fungi. 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 1 Litre 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 0.22 µm pore size Millipore filter paper to sterile MSM, which has been cooled to 45ºC under aseptic condition [19]. The MSM and crude oil were then mixed thoroughly and aseptically dispensed into sterile Petri dishes to set.

Microbiological analysis of the oilfield wastewater samples

Determination of total heterotrophic bacterial (THB) count of oilfield wastewater

The total heterotrophic bacterial (THB) count was determined using the nutrient agar and spread plate technique as described by Prescott., *et al.* [20]. An aliquot (0.1 ml) of each serially diluted sample using dilution factors of 10^{-4} for oilfield wastewater and of 10^{-5} for the other river water samples were 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 were calculated and expressed as colony forming unit - cfu/ml for oilfield wastewater or river water [21].

Determination of total fungi count of samples of oilfield wastewater

The total count of fungi in the samples was also determined by the spread plate technique. An aliquot (0.1 ml) 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 [21].

Hydrocarbon utilizing bacterial count (HUB) of samples

Total hydrocarbon utilizing bacteria count of oilfield wastewater samples was determined by inoculating 0.1 ml of the serially diluted samples (10^{-4}) on mineral salt agar. The Vapor Phase Transfer method was adopted by the use of sterile filter paper discs that were impregnated with filter sterilized crude oil which served as the only carbon source in the mineral salt agar [21]. The sterile crude oil-soaked filter papers were aseptically transferred to the inside covers of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted and the average of duplicate colonies were calculated and recorded as colony forming units per ml (cfu/ml) of sample.

Hydrocarbon utilizing fungal count (HUF) of samples

Total hydrocarbon utilizing fungal count of oilfield wastewater was determined by inoculating 0.1 ml of the serially diluted samples (10^{-2}) on mineral salt agar. The mineral salt medium will be supplemented with streptomycin (0.1 ml) to suppress bacterial growth [21]. The Vapor 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 covers of the inoculated Petri dishes and incubated for 5 days at room temperature. Colonies that develop were counted and average of duplicate colonies were calculated and recorded as colony forming units per ml (cfu/ml) of water sample.

Characterization and identification of bacterial isolates from samples

The cultural, morphological, microscopic characteristics of the pure 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 supplied 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 [22].

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

In the month of November, the total Heterotrophic bacteria count ranged from 4.5×10^4cfu/ml to 9.2×10^4cfu/ml, the highest count was obtained in the Control while the lowest was observed in the Drilling point. The total fungal counts (TFC) ranged from 1.5 $\times 10^2$cfu/ml to 2.6×10^2cfu/ml. The highest count was recorded in the Control while the lowest was observed in the Drilling point. The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 4.5 $\times 10^4$cfu/ml to 3.0×10^4cfu/ml. The highest count was observed in the Drilling point while the lowest was recorded in Control. The total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 1.8 $\times$ 10^2cfu/ml to 1.2×10^4cfu/ml. The highest was recorded in the Deck Drainage while the lowest was recorded in the Control.

The month of December, the total Heterotrophic bacteria count ranged from 3.8×10^4cfu/ml to 8.2×10^4cfu/ml, the highest count was obtained in the Control while the lowest was observed in the Deck Drainage and Drilling Point. The total fungal counts (TFC) ranged from 1.3×10^2cfu/ml to 2.2×10^2cfu/ml. The highest count was recorded in the Control while the lowest was observed in the Drilling point. On the other hand, total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 3.7×10^4cfu/ml to 1.7×10^4cfu/ml. The highest count was observed in the Deck Drainage while the lowest was recorded in Control. The total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 1.7×10^2cfu/ml to 0.4×10^4cfu/ml. The highest was recorded in the Deck Drainage while the lowest was recorded in the Control.

The results of the microbial counts (log10cfu/ml) of total heterotrophic bacteria and total fungi and of hydrocarbon utilizing bacteria and fungi obtained for the various sampling points during the months of November and December are as shown in figure 1 and 2 respectively.

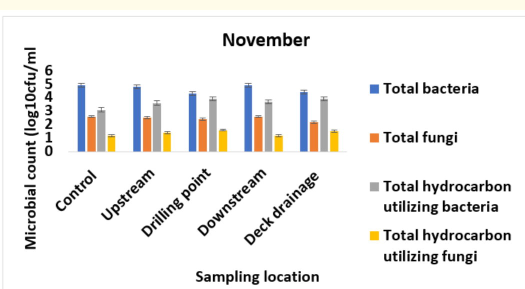

Figure 1: Microbial Counts of Santa Barbara for the month of November.

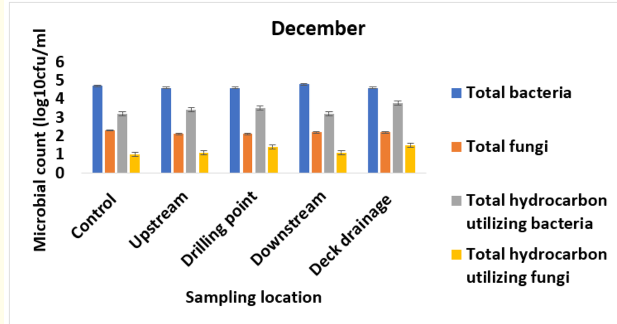

Figure 2: Microbial Counts of Santa Barbara for the month of December.

The microbiological counts (Log10cfu/ml) obtained in the various sampling points throughout the study are as shown in figure 3. The highest count of Total Heterotrophic Bacteria (THB) was recorded in the Control, while the lowest was observed in the Deck drainage. The highest count of total fungal counts (TFC) was recorded in the Control, while the lowest was observed in both the Deck drainage and Drilling point. On the other hand, the highest count of both the Total Hydrocarbon Utilizing Bacteria (HUB) and total Hydrocarbon Utilizing Fungi (HUF) was observed in the Deck Drainage, while the lowest was recorded in Control.

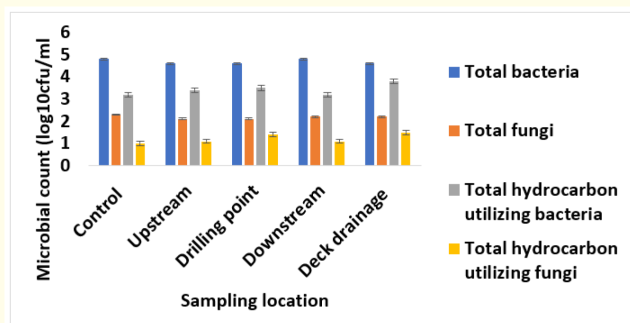

Figure 3: Microbial Counts of Santa Barbara.

The Analysis of variance (ANOVA) obtained for the microbial counts of Santa Barbara during the study period is as shown in table 1 below.

F values in both rows (groups of microorganisms) and columns (different locations in Santa Barbara) are greater than the F critical values; therefore, Null Hypothesis is rejected. That is, there is

Source of variation	SS	df	MS	F	P-value	F crit
Rows	840251.8	4	210062.9	34.73952	0.002305	6.388234
Columns	96308.93	1	96308.93	15.92725	0.016253	7.70865
Error	24187.2	4	6046.801			
Total	960747.9	9				

Table 1: ANOVA for microbial counts of Santa Barbara.

significant difference between the groups of microorganisms in the Control location and in the other locations of Santa Barbara.

The bacteria isolated from Santa Barbara rig location are shown in table 2. The predominant bacteria are of the genera; *Pseudomonas, Bacillus, Aeromonas, Alcaligenes, Staphylococcus, Enterococcus, Salmonella* and *Escherichia*.

Isolate code	Organism	Isolate code	Organism
SB1	*Pseudomonas* spp	SB10	*Escherichia coli*
SB2	*Aeromonas* spp	SB11	*Salmonella* spp
SB3	*Staphylococcus* spp	SB12	*Pseudomonas* spp
SB4	*Alcaligenes* spp	SB13	*Staphylococcus* spp
SB5	*Staphylococcus* spp	SB14	*Pseudomonas* spp
SB6	*Pseudomonas* spp	SB15	*Enterococcus* spp
SB7	*Pseudomonas* spp	SB16	*Escherichia* spp
SB8	*Enterococcus* spp	SB17	*Bacillus* spp
SB9	*Pseudomonas* spp	-	-

Table 2: Bacteria Isolates from Santa Barbara

Key: SB = Santa Barbara.

Discussion

The present study has revealed the microbial counts of heterotrophic and hydrocarbon-utilizing bacteria and fungi and types of bacteria in the vicinity of the Santa Barbara oil rig location. The results showed that counts of both heterotrophic bacteria and fungi occurred in the decreasing order for both the months of November (Figure 1) and December (Figure 2) as Control > Downstream > Upstream > Deck drainage > Drilling point. While it was almost the reverse for the counts of hydrocarbon utilizing bacteria in the decreasing order for both the months of November and December as Deck drainage > Drilling point > Upstream > Downstream > Control. And for the counts of hydrocarbon utilizing fungi as Deck drainage > Drilling point > Downstream > Upstream > Control. These were also the trend for the total counts throughout the duration of the study as shown in Figure 3. This reverse trend of the counts of hydrocarbon utilizing bacteria as compared to the counts of both heterotrophic bacteria and fungi indicated that the oil production activities and oilfield wastewater has an effect on the ecology and metabolic activities of the microorganisms. Although statistical analysis showed that there was no significant difference in the counts of total heterotrophic bacteria and fungi between the control and the sampling stations, their low counts in the rig location are attributed to the selective enrichment of hydrocarbon utilizing microorganisms and also of the impact of oilfield wastewater on aquatic microbes and diversity. The high prevalence of hydrocarbon utilizing microorganisms revealed that Santa Barbara River contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

The high count of both hydrocarbon utilizing bacteria and fungi obtained in the vicinity of the oil rig in this study signifies the impact of the oil production activities and oilfield wastewater on the Santa Barbara River. The high prevalence hydrocarbon utilizing bacteria and fungi found in this study concurs with a research carried out by Aleruchi and Obire [23]. They also reported that the high counts of hydrocarbon utilizing bacteria and fungi in wastewater samples can be attributed to inorganic and organic constituent found in the oilfield wastewater that serves as nutrient for bacteria and fungi growth. The continuous discharge of treated oilfield wastewater will have a deleterious effect on the proper functioning of the aquatic ecosystem thereby affecting aquatic and agricultural resources that are of economic importance [24].

On the other hand, bacterial and fungal populations play a role in degradation of hydrocarbon contaminations. Atlas [25] and Leahy and Colwell [26] reported that the rate of petroleum hydrocarbon biodegradation in nature is determined by the populations of indigenous hydrocarbon degrading microorganisms. Leahy and Colwell [26] concluded that hydrocarbon biodegradation depends on the composition of the microbial community and its adaptive response to the presence of hydrocarbons. This is supported by the fact that both counts of heterotrophic bacteria and fungi counts were highest in the control water samples than in the water samples from the vicinity of the Santa Barbara oil rig location. While the reverse was the case for hydrocarbon utilizing bacterial and fungal counts in the study.

The bacteria isolated during the study were; *Pseudomonas*, *Bacillus*, *Aeromonas*, *Alcaligenes*, *Staphylococcus*, *Enterococcus*, *Salmonella* spp and *Escherichia coli* which are also hydrocarbon utilizing bacteria indicated that the oilfield waste water contained high hydrocarbon contents. Similar organisms were also isolated by Aleruchi and Obire [27] indicating high hydrocarbon content contained in the oilfield wastewater that is being discharged into Santa Barbara River.

Conclusion and Recommendations

The hydrocarbon utilizing bacteria plays an important role in the bioremediation of oil polluted environment. The occurrence of these microorganisms in the waste water may be to the constant exposure of these microorganisms to hydrocarbon (oily) components of the waste water which could have made the organisms to have the ability to utilize and grow in the presence of the hydrocarbon. The high prevalence of hydrocarbon utilizers in the sampling stations suggests that the hydrocarbon utilizers were adapted to the quantity of hydrocarbons in the environment and hereby increased the number of hydrocarbon utilizers in the polluted area. The study also revealed that most of the organisms isolated as total heterotrophic bacteria and total fungi were part of the utilizers. The response of these microorganisms in the oil polluted environment suggests that the isolated bacteria and fungi could utilize the crude oil as energy and carbon source which serves as nutrient for their growth. The occurrence of these viable bacteria and fungi in the oilfield wastewater in the present investigation can be harnessed as bioremediation agent in crude oil cleanup exercise in polluted environments.

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