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Research Article

Impact of Oilfield Wastewater on Microbial Population of Cawthorne Channel in Rivers State, Nigeria

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Abstract

Oilfield wastewater contains organic and inorganic constituents which affect the aquatic environment and are hazardous to aquatic microorganisms. The impact of oilfield wastewater from Cawthorne channel rig in the Niger Delta on Cawthorne channel was investigated bi-weekly for four (4) months (January to April, 2008). A total of sixty-four (64) water samples collected from the drilling point, upstream, downstream and from a control point were analyzed for counts of bacteria and fungi using standard microbiological methods. Total heterotrophic bacteria (THB) counts ranged from 1.7×10^{4}cfu/ml to 4.8×10^{4}cfu/ml, the total fungal (TF) count ranged from 1.0×10^{1}cfu/ml to 1.9×10^{2}cfu/ml, while the total hydrocarbon utilizing bacterial (THUB) count ranged from 2.0×10^{3}cfu/ml to 9.0×10^{3}cfu/ml and the total hydrocarbon utilizing fungal (THUF) count ranged from 1.0×10^{1}cfu/ml to 1.0×10^{2}cfu/ml. Statistical analysis showed that there was no significant difference in the THB between the control and the other sampling stations; calculated F value (0.884065) < F-critical value (10.12796). The decreasing order of both total heterotrophic bacteria and fungi counts in the stations for the months of January, February and April was; Control > Downstream > Upstream > Drilling point while decreasing order of Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi was Drilling point > Upstream > Downstream > Control. Generally, the highest count for both total heterotrophic bacteria and fungi were recorded in the Control while the lowest were observed in the Drilling point. On the other hand, the highest count for both Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest counts were recorded in the Control. The types of bacteria isolated in the study included *Pseudomonas, Bacillus, Kurthia, Alcaligenes, Staphylococcus, Pediococcus, Micrococcus* and *Escherichia.* Although statistical analysis showed that there was no significant difference in the THB between the control and the sampling stations, lowest counts in the Drilling point showed that the oilfield wastewater does have effect on the microbial population and diversity. The high bacteria counts in the study revealed the impact of oilfield wastewater on aquatic microbes and diversity. The high prevalence of hydrocarbon utilizing bacteria and fungi revealed that the water body studied contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

Keywords: Cawthorne Channel; Swamp; Oilfield Wastewater; Hydrocarbon Utilizing Bacteria

Introduction

Produced waters or oilfield wastewater are water produced alongside with crude oil. They are subsurface waters associated with the reservoir rock [1,2]. They have a variation in their chemical composition and behavior since they are constrained within an aquifer, its vast amount of formation on subsurface water has been compiled by Petroleum industry [1,2] which has been a major concern to the Petroleum Production operations in the disposal of these oilfield wastewater which is a concentration of salts, hydrocarbon and heavy metals [1,3]. Produced water

contains dispersed or free dissolved oil and other dissolved organic compounds which are water soluble; Naphthenic acids, fatty acids and low molecular weight hydrocarbon and other compounds that have not been well defined [1,2].

Oilfield production activities releases large amount of hydrocarbon into the aquatic and terrestrial environment. The level of aquatic pollution by petroleum products and oil sludge has approached millions of cubic meters [4,5]. In addition, oil field production activities discharges an aqueous solution referred to as oilfield wastewater which occurs in association with oil and gas deposited in reservoirs. Oilfield wastewaters are dense and lies under the hydrocarbons in the reservoirs or in storage tanks [2]. The injection water is water usually injected into the reservoir to help force oil to the surface in order to achieve maximum oil recovery; they are both eventually produced as oilfield water along with the hydrocarbons as the oil becomes depleted. The amount of oil field wastewater increases as the reservoir is filled with injected seawater [2]. The oilfield wastewater is separated from the hydrocarbon at the surface, treated to remove much oil and then discharged into the aquatic environment [2,6].

Indiscriminate discharge of oilfield wastewater on aquatic and terrestrial environments have been a great issue or decried by the Federal Environmental Protection Agency (FEPA) [7] and Department of Petroleum Resources (DPR) [8] which its statutory responsibility is overall protection of the environment [3]. The current oil and grease limit for continental shelves region is 48mgL^{-1} monthly average and 72mgL^{-1} maximum. The oilfield wastewater discharged into the aquatic environment causes contamination of the aquatic environment since they are not well treated thereby leading to ecotoxicological problem that could create artificial food Scarcity due to damage of aquatic life [1]. During treatments, chemicals such as water clarifier and biocides are added to reduce microbial population thus affecting aquatic life [9]. The aim of the study was to cultivate, enumerate and isolate total heterotrophic bacteria and fungi total hydrocarbon utilizing bacteria and fungi, highlighting the environmental significance of these bacteria and fungi found in the oilfield wastewater of Cawthorne channel.

Materials and Methods

Description of study area

Cawthorne Channel also known as OML 18 is located at South of Deadman Island and Southwest of Adamakiri Creek which is a mangrove swamp and a distributary found in Akuku Toru Local Government Area of Rivers State. Cawthorne Channel OML 18 produces crude oil which is transported to nearby Shell Petroleum Development Company (SPDC) operated Bonny Terminal in Rivers State. Its geographical coordinates are Latitude 4.499^0,4^0 26$^{'}$ 57" North and Longitude 7.0838^0, 7^0 5$^{'}$2" East.

Collection of samples

Oilfield wastewater samples were collected from Cawthorne Channel flow Station (OML, 18). Water samples were collected from Drilling point, upstream, downstream and from a control point. The drilling point sample was collected from the drilling point of the oil rig, while the upstream and downstream samples were collected from a point 500m to the left and to the right from the drilling point. On the other hand, the control sample was collected from 2 km 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) [10]. At the end of each analysis, sample containers were thoroughly washed and rinsed with distilled water. Water samples were collected bi-weekly for four (4) months of January to April. A total of sixty-four (64) water samples collected from the drilling point, upstream, downstream 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.* [11] 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 1Litre of distilled water. The mixture was thoroughly mixed and autoclaved at 15psi at 121°C for 15 mins 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

[12] to sterile MSM, which has been cooled to 45ºC 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

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.* [13]. 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 [3].

Determination of total fungi count of samples of oilfield wastewater

Tenfold serial dilution method of Ofunne [14] was used, decimal dilutions of the samples were made by adding 1 ml of the wastewater sample to 9 ml of sterile normal saline (diluent) to give an initial dilution of 1:10, further tenfold serial dilution was made 10^{-2} dilution. 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 2-5 days and the discrete colonies that developed were enumerated as the viable counts (CFU) total heterotrophic fungi count.

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 [3]. 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 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 was supplemented with streptomycin (0.1 ml) to suppress bacterial growth [3]. 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 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 (cfu/ml) of water sample was calculated.

Characterization and identification of bacterial isolates from samples

The cultural, morphological, microscopic characteristics of 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 [15] for identification of probable bacteria.

Statistical analysis

Statistical analysis was also conducted using Duncan Multiple Range test and Analysis of variance to determine whether there is significant difference in the microbial counts between the control location and the other sampling locations.

Results

In the month of January, the total Heterotrophic bacteria count ranged from 2.95×10^{4}cfu/ml to 6.5×10^{4}cfu/ml while total fungal counts (TFC) ranged from 1.2×10^{2}cfu/ml to 1.9×10^{2}cfu/ml. The highest count for both total heterotrophic bacteria and fungi were recorded in the Control while the lowest were observed in

the Drilling point. Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 9.0×10^{2}cfu/ml to 2.7×10^{3}cfu/ml while total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 0.25×10^{1}cfu/ml to 1.6×10^{1}cfu/ml. The highest count for both Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest counts were recorded in the Control.

In the month of February, the total Heterotrophic bacteria count ranged from 1.9×10^{4}cfu/ml to 5.0×10^{4}cfu/ml while the total fungal counts (TFC) ranged from 1.0×10^{2}cfu/ml to 1.8×10^{2}cfu/ml. The highest count for both total heterotrophic bacteria and fungi were recorded in the Control while the lowest were observed in the Drilling point. The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 5.5×10^{1}cfu/ml to 1.55×10^{3}cfu/ml while the total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 0.15×10^{1}cfu/ml to 1.0×10^{1}cfu/ml. The highest count for both Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest counts were recorded in the Control.

In the month of March, the total Heterotrophic bacteria count ranged from 1.4×10^{4}cfu/ml to 3.6×10^{4}cfu/ml and the total fungal counts (TFC) ranged from 4.0×10^{1}cfu/ml to 1.5×10^{2}cfu/ml. The highest count for both total Heterotrophic bacteria and fungi were recorded in the Control, while the lowest was observed in the Drilling point. The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 1.0×10^{2}cfu/ml to 1.5×10^{2}cfu/ml. The total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 0.01×10^{1}cfu/ml to 0.75×10^{1}cfu/ml. The highest counts of both Hydrocarbon Utilizing Bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest were recorded in the Control.

In the month of April, the total heterotrophic bacteria count ranged from 1.0×10^{4}cfu/ml to 2.8×10^{4}cfu/ml while the total fungal counts (TFC) ranged from 0.2×10^{2}cfu/ml to 1.4×10^{2}cfu/ml. The highest count for both total heterotrophic bacteria and fungi was recorded in the Control while the lowest was observed in the Drilling point. The Total Hydrocarbon Utilizing Bacteria (HUB) count ranged from 0.01×10^{3}cfu/ml to 0.15×10^{3}cfu/ml and the total Hydrocarbon Utilizing Fungi (HUF) counts ranged from 0.01×10^{1}cfu/ml to 0.7×10^{1}cfu/ml. The highest count for both Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest counts were recorded in the Control.

The results of the microbial counts (Log10cfu/ml) obtained in the various sampling points during the months of January, February, March and April are as shown in figures 1, 2, 3 and 4 respectively.

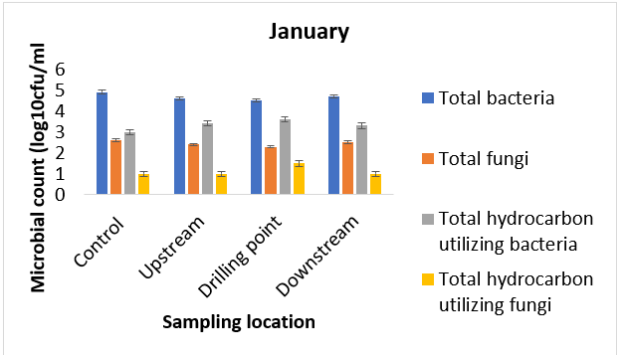

Figure 1: Microbial Counts of Cawthorne Channel in January.

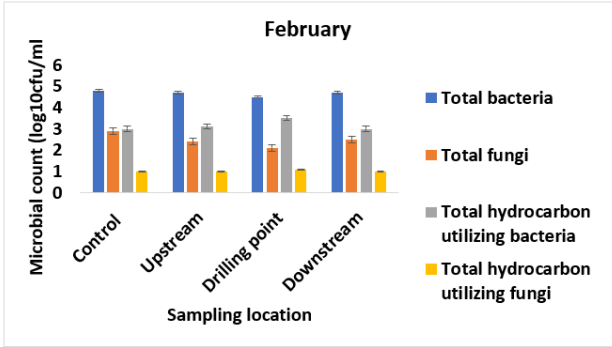

Figure 2: Microbial Counts of Cawthorne Channel in February.

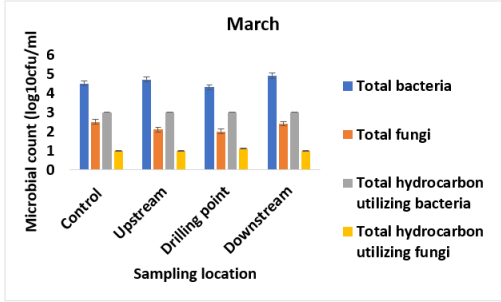

Figure 3: Microbial Counts of Cawthorne Channel in March.

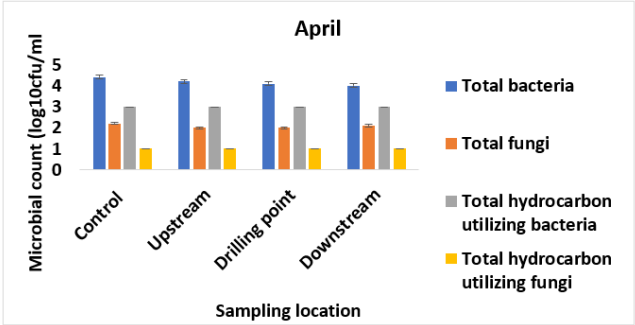

Figure 4: Microbial Counts of Cawthorne Channel in April.

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

F values in both rows (groups of microorganisms) and columns (different locations in Cawthorne Channel) are less than the F critical values; therefore, Null Hypothesis is accepted. That is, there is no significant difference between the groups of microorganisms in the Control location and the other locations of Cawthorne Channel.

Source of variation	SS	df	MS	F	P-value	F crit
Rows	1162858	3	387619.2	4.595393	0.12115	9.276619
Columns	74570.48	1	74570.48	0.884065	0.416455	10.12796
Error	253048.6	3	84349.53			
Total	1490477	7				

Table 1: ANOVA for microbial counts of Cawthorne Channel.

The bacterial isolates obtained from Cawthorne Channel during the study period are shown in table 2 below. The predominant bacteria are of the genera; *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Staphylococcus*, *Kurthia*, *Micrococcus*, *Enterococcus*, *Pediococcus* and *Escherichia*.

Isolate code	Organism	Isolate code	Organism
CW1	*Pseudomonas* spp	CW 10	*Escherichia coli*
CW 2	*Bacillus* spp	CW 11	*Micrococcus spp*
CW 3	*Staphylococcus* spp	CW 12	*Pseudomonas* spp
CW 4	*Alcaligenes* spp	CW 13	*Staphylococcus* spp
CW 5	*Kurthia* spp	CW 14	*Pediococcus spp*
CW 6	*Micrococcus spp*	CW 15	*Enterococcus* spp
CW 7	*Pediococcus spp*	CW 16	*Escherichia coli*
CW 8	*Enterococcus* spp	CW 17	*Bacillus* spp
CW 9	*Pseudomonas* spp	-	-

Table 2: Bacteria Isolates from Cawthorne Channel.

CW: Cawthorne Channel.

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 Cawthorne Channel oil rig in Rivers State of Nigeria. The fungi and bacteria population of the water samples were low, these low microbial counts may be due to the stress to which the organisms were exposed in the wastewater [3,8]. Once the oil is separated from the wastewater, it is subjected

to various forms of treatment including removal of oil; addition of chemicals such as biocides which reduces the microbial population in the wastewater before final discharge [8]. The results showed that more heterotrophic bacteria and fungi were isolated from the control and the least from the drilling point while the reverse was the case for counts of hydrocarbon utilizing bacteria and fungi. The decreasing order of both total heterotrophic bacteria and fungi counts in the stations for the months of January, February and April was; Control > Downstream > Upstream > Drilling point while decreasing order of Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi was Drilling point > Upstream > Downstream > Control. On the other hand, the decreasing order of both total heterotrophic bacteria and fungi counts in the months of March was; Control > Downstream > Upstream > Drilling point. The decreasing order of Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi was Drilling point > Downstream > Upstream > Control.

Generally, the highest count for both total heterotrophic bacteria and fungi were recorded in the Control while the lowest were observed in the Drilling point. On the other hand, the highest count for both Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing Fungi were recorded in the Drilling point while the lowest counts were recorded in the Control.

This shows that the oilfield wastewater deposited at the drilling point acted as a source of nutrients for the indigenous Hydrocarbon utilizing bacteria and Hydrocarbon utilizing Fungi thus enriching or increasing their counts or population in water samples in the drilling point than in other stations. The presence of heterotrophic bacteria and fungi in the wastewater revealed that the chemical treatment introduced into the water did not completely eliminate the bacteria and fungi rather only reduced their number to a minimal level, thus they can survive in it. The bacteria species isolated in the study are: *Pseudomonas, Bacillus, Alcaligenes, Staphylococcus, Kurthia, Micrococcus, Enterococcus, Pediococcus* spp and *Escherichia Coli*. The occurrence of these bacteria in the oilfield wastewater (oily) suggests that the bacteria has the ability to utilize the traces of oil present in the wastewater and also has the ability to grow in an oil agar medium in connection with Okpokwasilli and Olise [16]. Obire and Wemedo [17] also reported similar organisms having the ability to utilize crude oil as a sole source of carbon thus confirming the ability of bacteria to thrive in oilfield wastewater. It was noted that after the purported treatment by oil exploration companies, the wastewater still contains dispersed or free oil, dissolved and other hydrocarbon contents utilized as nutrients by microorganisms for their growth [2,8].

The environmental consequences of the release of oilfield wastewater (oily) into the aquatic environments are dexterous (affecting microbial population and aquatic life) [1,8]. In this context Obire and Amusan [18] reported a significant effect of oilfield wastewater (formation water) on the microbial population of fresh water stream in Nigeria. Obire and Wemedo [3] concluded that oilfield wastewater had little effect on microorganisms in aquatic environment. However, the contradictory results in the present study may be due to the environmental consequences of oilfield wastewater in the Cawthorne Channel.

Although statistical analysis showed that there was no significant difference in the THB between the control and the sampling stations, lowest counts in the Drilling point showed that the oilfield wastewater does have effect on the microbial population and diversity. The high bacteria counts in the study revealed the impact of oilfield wastewater on aquatic microbes and diversity. The high prevalence of hydrocarbon utilizing bacteria and fungi revealed that the water body studied contained active indigenous hydrocarbon utilizers which can be harnessed for bioremediation process.

Conclusion and Recommendation

The significance presence of these organisms in the oilfield wastewater can be attributed to their ability to utilize hydrocarbons in the water which was confirmed by their growth on oil agar. 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 hydrocarbon utilizing isolates in the study can be used in bioremediation exercise if they are introduced into a crude oil polluted environment since the wastewater contains hydrocarbon which is a major source of nutrient. The hydrocarbon utilizing bacteria can be applied minimally to crude oil polluted/ contaminated environment to decontaminate it and also used for cleanup of crude contaminated environment.

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