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Evaluation of the Microbial Population of Soil around Oilfield Wastewater Pond

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The microbial quality of soil around the oilfield wastewater discharge pond was investigated to determine the microbial dynamics in the soil. Soil samples were randomly collected at four different parts around the pond and 80 meters away from the pond (control) at a depth of 0-15cm with a clean auger into sterile polythene bags from Ogbogu flow Station. The total heterotrophic bacteria, total heterotrophic fungi, hydrocarbon utilizing bacteria, and fungi were determined using standard microbiological methods. The bacterial isolates were identified using standard biochemical tests while fungal isolates were identified based on appearance on plates and microscopy. The mean counts for the total heterotrophic bacteria, fungi, hydrocarbon utilizing bacteria and fungi in the rainy season for the soil within the pond was 2.10×10^{7}, 4.63×10^{4}, 1.38×10^{4}, and 2.93×10^{4} CFU/g, respectively. The mean counts for the total heterotrophic bacteria, fungi, hydrocarbon utilizing bacteria and fungi in the dry season for the soil within the pond was 5.72×10^{6}, 1.87×10^{4}, 2.80×10^{3}, and 1.37×10^{3} CFU/g, respectively. The mean counts for the total heterotrophic bacteria, fungi, hydrocarbon utilizing bacteria and fungi in the rainy season for soil 80 m away from the pond was 2.50×10^{7}, 1.07×10^{5}, 2.4×10^{3}, and 1.9×10^{3} CFU/g, respectively. The mean counts for the total heterotrophic bacteria, fungi, hydrocarbon utilizing bacteria and fungi in the dry season for soil 80 m away from the pond was 6.17×10^{6}, 2.0×10^{4}, 1.83×10^{3}, and 1.23×10^{3} CFU/g, respectively. Statistical analysis showed a significant difference ($P\leq0.05$) in the total heterotrophic bacterial count from the pond and that of soil around 80 m away from the pond. The heterotrophic counts during the dry season were significantly lower ($P\leq0.05$) from that of the rainy season in all the samples analyzed. There was a significant difference ($P\leq0.05$) between the fungi count of soil 80

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m away in the dry season from that of soil around the pond. There was no significant difference (P≥0.05) in the total fungal counts recorded for the dry and rainy season in the various samples except that of the soil 80 m away. Hydrocarbon utilizing fungi was higher in the soil around the pond and was significantly different (P≤0.05) from that of soil 80 m away from the pond during the dry season. *Bacillus* spp., *Aeromonas* spp., *Micrococcus* spp., *Staphylococcus* spp., *Chryseomonas* spp., *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Actinomyces* spp., *Enterobacter* spp., *Rhodococcus* spp., and *E. coli* were identified from the soil. While *Bacillus* spp, *Micrococcus* spp, *Staphylococcus* spp, *Proteus* spp, and *Pseudomonas* spp were the hydrocarbon utilizing bacteria. Eight fungal genera isolated from the samples include *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Saccharomyces cerevisiae, Geotricum, Trichoderma, Fusarium,* and *Penicillium* spp*.* Hydrocarbon utilizing fungi isolated includes *Aspergillus niger, Aspergillus flavus, Fusarium* spp*., Penicillium* spp., and *Saccharomyces cerevisiae*. This investigation revealed high microbial population in the soil 80m away from the pond than those within the soil around the wastewater pond. The microbial population was affected by the season with the rainy season having a higher microbial population than the dry season.

Keywords: Oilfield wastewater; microbial dynamics; soil; pond.

1. INTRODUCTION

Despite recent advancements in the use of renewable energy, fossil fuels (particularly oil and gas) still fulfill the majority of global energy demand and will continue to be the primary source of energy for the next several decades [1]. During the exploration of fossil fuels, fluids and other substances which are not needed (wastes) are usually produced and these substances are evacuated to enhance the collection of the target fuel. The water produced from this process is known as oilfield wastewater. According to Dai and Zhao [2], oilfield wastewater is the water removed from the liquid generated by an oil well, and this wastewater contains suspended particles and crude oil droplets including microorganisms. Aleruchi and Obire [3] reported that produced water (PW) is a complex mixture of dissolved particulate organic and inorganic chemicals that are frequently generated during the production of crude oil and gas from onshore and offshore wells, and it represents the largest volume of the waste stream in oil and gas production operations on most oil production platforms. This also agreed with Olajire [4] who reported that PW which is a mixture of formation water, sea, or freshwater that has been trapped for millions of years beneath hydrocarbons in porous reservoir medium, injection water; small volumes of condensed water from gas production and residues of treatment chemicals that have been added to ensure effective hydro-fracture operations is the largest wastewater stream (brine) that is brought up from the hydrocarbon-bearing formation strata during oil and gas exploration and production activities. During the process of oil drill or evacuation of PW to obtain the fossil fuels, disposal of PW on the environment could lead to the contamination of that environment, especially the soil. According to Pichtel [5], soil contamination can occur as a result of fluid spills during the drilling and fracturing procedures, as well as during transportation by truck or through wastewater pipelines, well casing failures, and equipment failures, as well as corrosion of pipes and tanks. Some produced waters are managed on-site or within the oil and gas field using evaporation ponds or seepage pits. Recycling of produced waters for exploration and production operations within the oil and gas field is another primary means of produced water management. Some treatment may be required to render the water suitable for reuse in drilling or hydraulic fracturing. Another management strategy is the use of produced water for dust suppression and deicing, though some states are looking more closely at this practice and restricting or removing this as an option. These management approaches are not subject to clean water act (CWA), national pollutant discharge elimination system (NPDES) permitting requirements if they do not involve discharge to surface waters (U.S. EPA. [6].

Microorganisms are ubiquitous, could tolerate varying environmental conditions and as such, they are found in every nook and cranny of our environments. They are responsible for nutrient cycling in the environment and their activities in the petroleum industry are well documented both in the recovery of crude oil and in bioremediation of polluted environments [7]. Tuccar et al. [8] reported that many microorganisms are capable

of living in the oil and water phases of oil wells, despite harsh environmental conditions such as anoxic, high temperature, and high salt in oil-bearing rocks. Youssef et al. [9] reported that due to the low redox potential in the reservoirs, oil fields have mostly facultative aerobic and strictly anaerobic bacteria. The introduction of untreated or improperly treated PW on soil surfaces could alter the microbial communities on the receiving soil [10]. Thus, this study was carried out to evaluate the microbial composition of soil around oilfield wastewater.

2. MATERIALS AND METHODS

2.1 The Study Area

The study was carried out in Ogbogu Flow Station; an onshore oil production platform located in Ogba/Egbema/Ndoni Local government Area (ONELGA) of Rivers State, Nigeria. It lies on Latitude 5.34167ºN and Longitude 6.65556ºE (Global positioning system (GPS) coordinates).

2.2 Sample Collection

Soil samples (500g each) were randomly collected at four different parts around the pond and 80 meters away from the pond (control) at a depth of 0-15cm with a clean auger into sterile polythene bags. The samples were collected bi-monthly for nine months in 2018 from Ogbogu flow Station; an onshore oil production platform located in Ogba/Egbema/Ndoni local government Area of Rivers State, Nigeria. The samples were labeled and transported to the laboratory in a cooler packed with an ice block for analysis.

2.3 Processing of Samples

The soil samples were processed using the method of Adesemoye et al. [11]. Ten grams of the soil samples were weighed and added to 90ml of sterile normal saline and this served as the stock. One milliliter of the stock was diluted serially using the ten-fold serial dilution method as described by Prescott et al. [7].

2.4 Enumeration and Characterization of Total Heterotrophic Bacteria

This was determined with the nutrient agar using the spread plate technique as described by Prescott et al. [7]. An aliquot (0.1ml) of 10^{-4} dilution of the serially diluted soil sample, was each inoculated onto different sterile nutrient agar plates in triplicates. The plates were incubated for 24 hours at 37 ℃. After incubation, colonies that appeared on the plates were counted and the mean was expressed as CFU/g discrete colonies on the plates after incubation were sub-cultured on pre-dried nutrient agar plates using a sterile wire loop which was sterilized intermittently by heating the loop to red hot for every streak carried out [7].

2.5 Enumeration and Characterization of Total Fungal

This was determined using the Potato Dextrose Agar (PDA) onto which sterile streptomycin (50 mg/ml) had been added to suppress bacterial growth [12]. The spread plate technique as described by Prescott et al. [7] was adopted. An aliquot (0.1 ml) of 10^{-2} dilution of all samples were inoculated in triplicates onto sterile pre-dried PDA plates and then spread evenly with a sterile glass spreader. The plates were incubated at room temperature for about 5 days after which the spores were counted and the mean of the count recorded accordingly.

2.6 Hydrocarbon Utilizing Bacteria and Fungi

The population of hydrocarbon utilizing bacteria was determined using spread plate techniques, by inoculating 0.1 ml aliquot of 10^{-1} dilution of all the samples onto mineral salt agar media while the fungi population was determined by inoculating 0.1 ml aliquot of 10^{-1} dilution of all the samples onto mineral salt agar media supplemented with streptomycin (50 mg/ml) to suppress bacterial growth [12]. The vapour Phase Transfer method of Mills et al. [13] was adopted. It employed the use of sterile filter paper discs 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 then aseptically transferred to the inside covers of the incubated Petri dishes and incubated for 5 days at 37℃ for bacteria and 5-10 at 25℃ for fungi. After the incubation period, means of the colonies were recorded and discrete colonies were sub-cultured onto pre-dried respective media.

2.7 Identification of Isolates

Representative colonies of bacteria were picked from different plates after the incubation period. They were streaked on sterile agar plates for

purification followed by characterization using their colonial morphology, cellular morphology, and biochemical tests. Biochemical tests carried out were; oxidase test, catalase test, indole test, methyl red test, Voges-Proskauer test, starch hydrolysis test, urease test, citrate test, coagulase test, sugar fermentation test, and Triple Sugar Iron agar test. Reference was made to Bergey's Manual of Determinative Bacteriology by Holt et al. [14].

While isolates of fungi were identified using their morphological features followed by microscopic examination of their wet mounts prepared with lactophenol cotton blue and reference made to fungal identification atlas by Barnett and Hunter [15].

2.8 Statistical Analysis

The mean and standard deviations of the microbial populations were calculated using Microsoft excel while ANOVA was used to check for a significant difference. The Duncan test was used in separating the mean in areas there was a significant difference. The SPSS (version 25) was used.

3. RESULTS

The mean of the heterotrophic and hydrocarbon utilizing bacterial counts of the soil samples obtained during the dry and rainy seasons are presented graphically in Figures 1 and 2, respectively. The microbial load of pond soil and soil 80m away from the pond showed that the total heterotrophic bacterial load and a total heterotrophic fungal load of the soil samples 80m away from the pond were higher than counts recorded for soil samples collected within the pond. This was not in conformity with counts recorded in hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) for the rainy season as counts recorded for HUB and HUF were higher in samples collected within the ponds than those collected 80m away from the pond. Statistical analysis showed a significant difference ($P \leq 0.05$) in the total heterotrophic bacterial count from the pond and that of soil around 80 m away from the pond. Heterotrophic counts in the soil around the pond also showed a difference ($P \leq 0.05$) from that of 80 m away. The heterotrophic counts during the dry season were significantly lower ($P \leq 0.05$) from that of the rainy season in all the samples analyzed. Also, the total heterotrophic bacterial counts recorded in the soil around the ponds were lower than the values recorded for those 80m away from the pond for both seasons (Fig 3). The hydrocarbon utilizing bacterial counts for the soil obtained within the ponds were higher than those recorded 80m away from the pond in the rainy and dry seasons. There was a significant difference ($P \leq 0.05$) in the hydrocarbon utilizing bacterial count obtained from the soil around and 80 m away from the pond. The highest fungal count of $1.07 \times 10^5 \pm 3.920$ CFU/g was recorded in the soil 80 m away from the pond during the dry season. There was a significant difference ($P \leq 0.05$) between the fungi count of soil 80 m away in the dry season from that of soil around the pond. There was no significant difference ($P \geq 0.05$) in the total fungal counts recorded for the dry and rainy season in the various samples except that of the soil 80 m away from the pond. Hydrocarbon utilizing fungi was higher in the soil around the pond ($2.9 \times 10^4 \pm 9.438$ CFU/g) and was significantly different ($P \leq 0.05$) from that of soil 80 m away from the pond $1.9 \times 10^3 \pm 3.578$ CFU/g during the dry season.

3.1 Identification of Isolates and Percentage Occurrence from Samples

Twelve genera of bacteria (465 isolates) isolated from the samples include; *Bacillus* spp., *Aeromonas* spp., *Micrococcus* spp., *Staphylococcus* spp., *Chryseomonas* spp., *Proteus* spp., *Pseudomonas* spp., *Klebsiella* spp., *Actinomyces* spp., *Enterobacter* spp., *Rhodococcus* spp., and *E. coli.* Except for *Aeromonas* spp., *Klebsiella* spp., *Actinomyces* spp., *Enterobacter* and *Chryseomonas* the rest of the isolates were identified also as hydrocarbon utilizing bacteria. Eight fungi (387 isolates) genera isolated from the samples include *Aspergillus fumigatus, A. niger, A. flavus, Saccharomyces cerevisiae, Geotricum, Trichoderma, Fusarium,* and *Penicillium* spp. Hydrocarbon utilizing fungi that were isolated includes *Aspergillus niger, A. flavus, Fusarium* spp., *Penicillium* spp., and *Saccharomyces cerevisiae*.

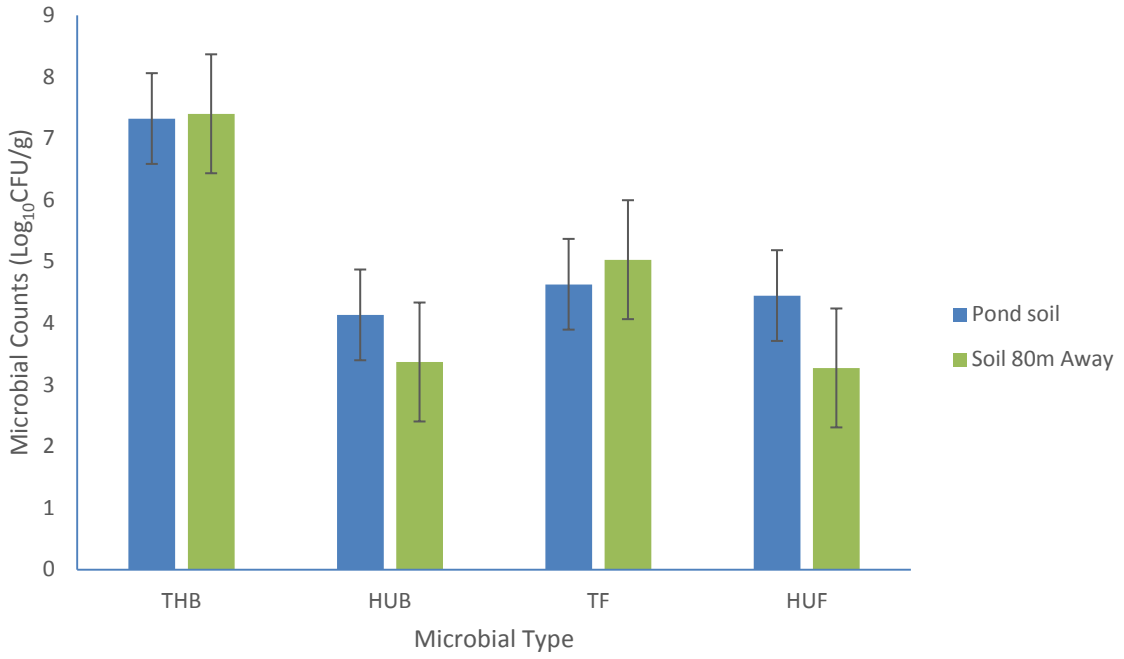

Fig. 1. Microbial counts of the Pond soil and Soil 80m away from the pond during the Rainy Season

Total heterotrophic bacteria (THB), Hydrocarbon utilizing bacteria (HUB), total fungi (TF), and Hydrocarbon utilizing fungi (HUF)

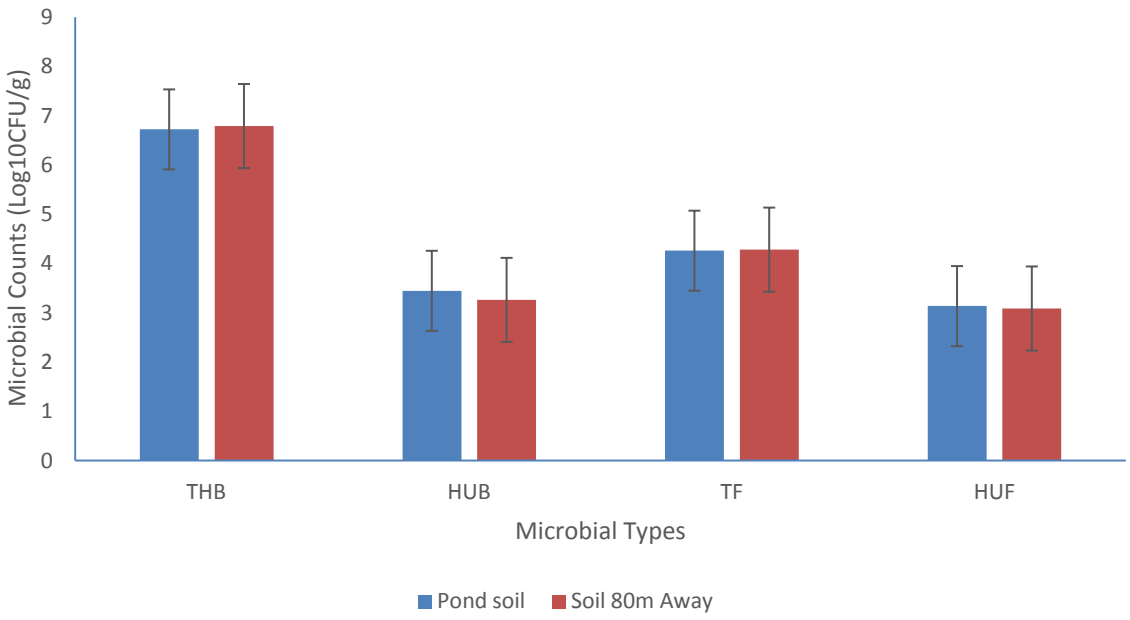

Fig 2. Microbial counts of the Pond soil and Soil 80 m away from the pond during Dry Season

Total heterotrophic bacteria (THB), Hydrocarbon utilizing bacteria (HUB), total fungi (TF), and Hydrocarbon utilizing fungi (HUF)

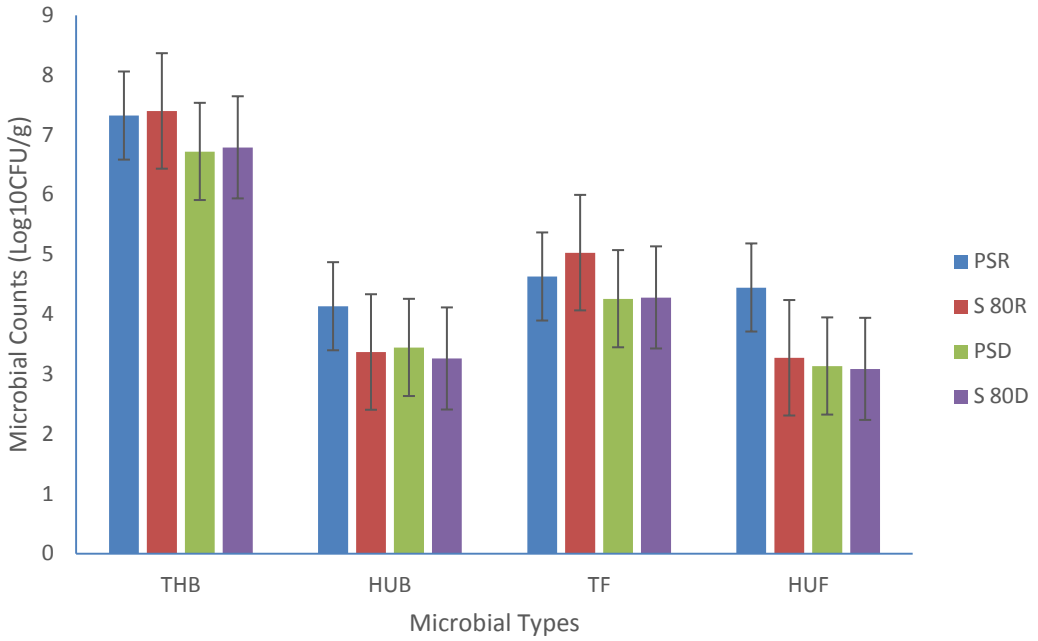

Fig 3. Microbial counts of the Pond soil and Soil 80m away from the pond during Dry and Rainy Season

Total heterotrophic bacteria (THB), Hydrocarbon utilizing bacteria (HUB), total fungi (TF) and Hydrocarbon utilizing fungi (HUF), Pond soil Rainy season (PSR), Soil 80m away from the pond (S80R), Pond soil dry season (PSD), Soil 80 m away from pond in the dry season (S 80D)

Table 1. Bacteria Isolated from Soil Samples

Isolates	Soil around pond	Soil 80 m away from pond
Bacillus spp.	+	+
Aeromonas spp.	+	+
Micrococcus spp.	+	+
Staphylococcus spp.	+	-
Proteus spp.	+	+
Klebsiella spp.	+	+
Pseudomonas spp.	+	+
Rhodococcus spp.	+	+
E. coli	+	+
Actinomyces spp.	+	+
Enterobacter spp.	+	+

KEY: + = isolated, - = not isolated

Table 2. Fungi Isolated from Samples

Isolates	Soil around pond	Soil 80 m away from pond
Aspergillus niger	+	+
A. fumigatus	+	+
A. flavus	+	+
Penicillium spp.	+	+
Fusarium spp.	+	+
Saccharomyces cerevisiae	+	-
Geotricum spp.	+	+
Trichoderma spp.	+	+

KEY: + = isolated, - =not isolated

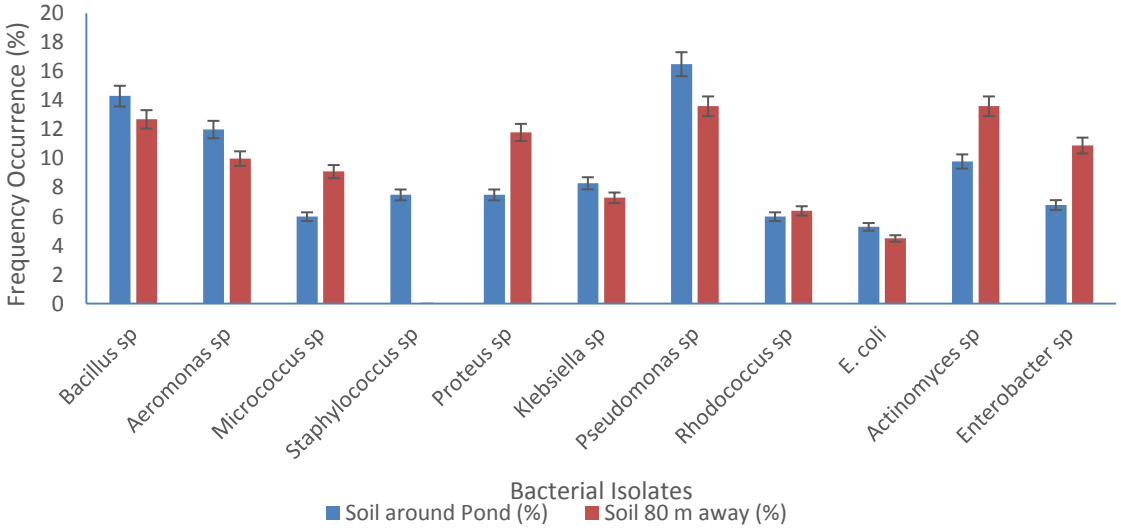

Fig 4. Percentage Occurrence of Bacterial Isolates from Samples

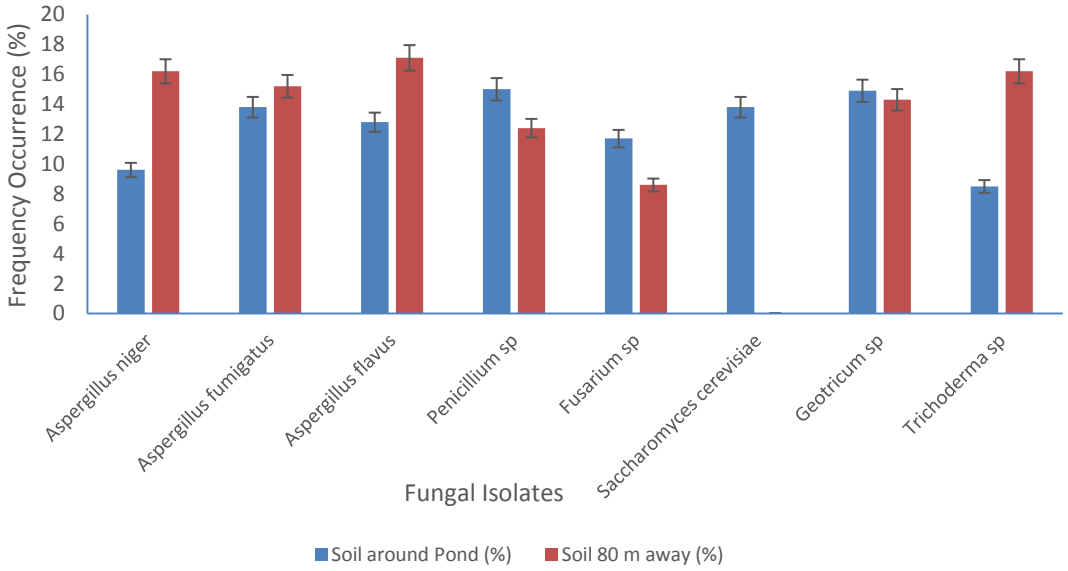

Fig. 5. Percentage Occurrence of Fungal Isolates from Samples

The percentage occurrences of bacterial and fungal isolates from the samples are shown in figures 4 and 5, respectively. Except for *Staphylococcus* spp., which was not isolated in soil samples 80 m away from the pond, all the bacterial isolates were identified in both locations (Table 1). For the fungi (Table 2) *Saccharomyces cerevisiae* was not isolated in soil samples 80 m away from the pond, all the fungal isolates were identified in both locations. *Pseudomonas* spp., had the highest percentage occurrence of 16.5% while *E. coli* recorded the lowest percentage occurrence of 5.3% in soil around the pond. For soil 80 m away from the pond, *Pseudomonas* spp., and *Actinomyces* spp., recorded highest percentage occurrence of 13.6%, while E. coli recorded lowest percentage occurrence of 4.5% (Fig. 4).

For the percentage occurrences of fungal isolates as shown in Fig 5, *Penicillium* spp., had highest percentage occurrence of 15 %, in the

soil around the pond, the lowest percentage occurrence was Trichoderma (8.5%). In soil 80 m away Aspergillus flavus recorded highest percentage occurrence of 17.1%, while *Fusarium* spp., had the lowest percentage occurrence of 8.6%.

4. DISCUSSION

The microbial quality of soil around the oilfield wastewater discharge pond and soil 80m away from the pond as evaluated in this study showed a disparity in microbial counts both in the rainy and dry seasons. The low total heterotrophic bacterial counts recorded in the soil around the pond compared to the high counts of total heterotrophic bacterial counts in soil 80 m away from the pond could be attributed to the chemicals and other substances present in the effluent which had impacted the diversity of microbial populations around the pond. The soil is one of nature's most dynamic sites of biological interactions and microorganisms whose activity is controlled by the soil environment mediate many of the biochemical reactions involved in the mineralization of soil organic matter and the nutrition of plants, particularly crops. Various species of bacteria and fungi play a role in improving soil fertility. These microbes increase organic matter that boosts the availability of N.P.K. and Fe in soil [16]. Any element that harms the activities of soil microorganisms might thus harm plant development [12]. Thus, the substances contained in the effluent could have exerted a negative influence on the microbial population thereby limiting the growth of microbes that are unable to tolerate or utilize them [17]. This agreed with the counts recorded for both the hydrocarbon utilizing bacterial and fungal counts which were higher in the soil around the pond where the effluent is discharged than those 80 m away from the pond. More so, the reduced population of the total heterotrophic bacteria could be attributed to the high toxicity of the wastewater which has forced the selection of microorganisms that could withstand or utilize the chemical substances inherent in it. This agreed with Aleruchi and Obire [17] who had reported that the toxicity of oilfield wastewater on the soil around the pond may have resulted in the reaction of microorganisms that can survive the toxic effects, thereby leading to an overall heterotrophic bacterial population. Obire and Anyanwu [18] reported a decrease in species variety as the concentration of added crude oil increased, which they used as an indicator of oil hydrocarbon environmental stress. According to Prescott et al*.,* [7], environmental factors and nutrients such as temperature, pH, nitrate, phosphate, carbon source could influence microbial growth and activities. Thus, the higher counts of hydrocarbon utilizers could mean that the nutrients and environmental conditions favored their growth while making the environment unfavorable for none utilizers. The mean counts of total heterotrophic bacterial and fungal counts in this study are lower than the values of $8.25x10^6$ in the peak of dry season reported by Obire and Wemedo [19]. More so, the mean fungal count of 4.63×10^4 CFU/g for the rainy season is lower than the $5x10^4$ CFU/g reported by Obire and Wemedo [12].

The bacterial diversity in this current investigation was more in the heterotrophic bacteria than the hydrocarbon utilizing bacterial diversity. This could be attributed to the abundant nutrient and favorable environmental conditions of the unpolluted soil (soil 80m away from effluent receiving pond). This agreed with Obire and Wemedo [12] who had earlier reported that microbial densities or numbers are influenced to a large extent by the organic matter content of the soil as well as by the available nutrients. *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Proteus* spp., and *Pseudomonas* sp which were isolated as hydrocarbon utilizing bacteria have been reported in previous studies (19,17]. *Pseudomonas* spp, *Proteus* spp., *Micrococcu*s spp., and *Bacillus* spp., were reported by Nrior and Wosa [20]. Because of their wide catabolic abilities, resilience in harsh environmental conditions, and ability to produce bio-surfactant, these organisms are known to be of considerable environmental and biotechnological importance [21]. More so, the fungal isolates such as *Aspergillus niger, A. flavus, Fusarium* spp*., Penicillium* spp., and *Saccharomyces cerevisiae* which were characterized as hydrocarbon utilizers in the current investigation have been reported in previous studies to be able to degrade hydrocarbon [22,12]. *Staphylococcus* spp., and *Saccharomyces cerevisiae* were not isolated from soil 80m away, probably because the soil did not favour their growth.

5. CONCLUSION

In conclusion, most of the types of bacterial and fungi isolated from the soil 80 m away from the pond effluent were also isolated in the soil around the oilfield wastewater discharge pond. While this investigation revealed high microbial

population in the soil 80 m away from the pond than those within the soil around the wastewater pond. The study revealed that the microbial population was affected by the season with the rainy season having a higher microbial population than the dry season.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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