Original Research Article

COMPARATIVE STUDY OF METAGENOMIC PROFILE OF BACTERIA STRAINS PRESENT IN AN ABANDONED ARTISANAL REFINERY SITE, IN OBI-AYAGHA COMMUNITY,DELTA STATE

ABSTRACT

Aim: The aim of the study is to identify and compare the bacteria strains present in the control and ~~hydrocarbon~~ Hydrocarbon impacted soil samples in the abandoned artisanal refinery site located in Obi-Ayagha community, Delta State, using high-throughput sequencing of the 16S rRNA gene.

Place and duration of study: The ~~hydrocarbon~~ Hydrocarbon impacted soil was collected from the abandoned artisanal refinery site located in Obi-Ayagha community and analysed in the advanced research laboratory of the Department of Environmental Management and Toxicology, Federal University of Petroleum ~~resources~~Resources, Effurun ~~i~~n 2022.

Methodology: In the present study, soil samples were collected from five pointsto cover the expanse of the site, from depths of 0–15 cm of the abandoned artisanal site, and composited. The bacterial community profile was analyzed using high-throughput sequencing of the 16S rRNA gene, and the bacteria species were identified from Kingdom to species level.

Results: Taxonomical classification of bacteria~~,~~ revealed the most abundant organisms present in each kingdom, phyla, class, in the contaminated and uncontaminated(control) samples.The dominant species at phylum-level microbial diversity identified in the petroleum-contaminated and uncontaminated site~~,~~ is depicted by the dominant groups and were found to be the *Actinobacteriota* (21.94%) for the control ~~while~~. In comparison, the *Firmicutes* dominated the phylum with ~~the~~ apercentage occurrence of (99.86%) for the test samples. At the class level, the dominant group was*Alphaproteobacteria* (16.48%) for the control, while *Bacilli* dominated the class showing (99.76%) for the test samples.

Conclusion: Metagenomic profiling helps to predict the presence and relative abundances of microbes in a sample, whichas this is a critical step in microbiome analysis.

Key words: Metagenomic sequence,16S rRNA gene, Bacteria

1. INTRODUCTION

Soil microorganisms play a decisive role in ecologically significant biogeochemical processes, contributing to plant nutrition and soil health, even in agricultural and extreme environments, and maintaining the matter and energy transfer in terrestrial environments (Lelario *et al*., 2018; Sofo *et al*., 2018). ~~Soil is a very complex and heterogeneous environment for microbiologists, due to its properties and interaction processes, involving mineral and organic particle~~Due to its properties and interaction processes involving mineral and organic particles, soil is a very complex and heterogeneous environment for microbiologists.Soil biota gives rise to the formation and ~~stabilisation~~ stabilization of different sized aggregates, micropores, and clay-organic matter complexes that dominate the soil characteristics and affect the microbial composition (Kachienga, *et al*., 2018).Soil is also considered the most sensitive indicator~~s~~ due to ~~their~~ itsinstant responses to changes in environmental conditions (Jiao *et al*., 2016).

Continuous pollution of the existing freshwater sites across the globe by petroleum and their by-products has catastrophic consequences for the end-users such as animals, humans, plants, and microorganisms. According to(Haritash and Kaushik, 2009), the ongoing pollution by these hydrocarbons and their derivatives results in the accumulation of xenobiotics and other toxic elements in the entire ecosystem.

Widespread oil spill pollution in various water sources and land locations has been reported during the 20th century; oil pollution ~~has been found to be~~is mainly of anthropogenic origin, such as leaks and spills due to oil refining, handling, storage, and transport from the refineries to the point of usage. Millions of tons of petroleum oil are being exploited ~~as a source of energy~~ for the ever-increasing energy demand by the rapidly growing world population.

However, many microorganisms possess the ability to degrade these contaminants (crude oil~~,~~ and low-molecular-weight hydrocarbon metabolites) derived from the degradation of crude oil, and can serve as carbon sources for the growth of microbes (Patowary *et al.*, 2017).

Bacteria are the most abundant and genetically diverse organisms on the planet, and their activity is vital for many aspects of biogeochemical cycling and ecosystem function. ~~As such, u~~Understanding the factors influencing bacterial distributions is paramount to understanding natural systems and predicting ecosystem responses to environmental change. (Pacwa-Polinickak *et al*., 2020).Bacteria exhibit higher versatility than fungi during the biodegradation of hydrocarbons (Kachienga, *et al*., 2018;Pandey *et al*., 2016). Jones *et al*., 2021, found that

the bacterial consortium is responsible for the degradation of saturated and partially aromatic hydrocarbons. Most of the studies have focused on the aerobic degradation of crude oil and the corresponding functional bacteria; however, anaerobic degradation also deserves considerable attention because a large amount of the crude oil contaminants is buried in the sub-surface soil (Kaushik *et al*., 2021). Therefore, a detailed analysis of the bacterial community structure and ~~the identification of~~identifying functional bacteria under anaerobic conditions hold great importance for the bioremediation of crude ~~oil~~ oil-contaminated soil.

Soil metagenomics is a cultivation-independent molecular approach to explore and exploit the enormous diversity of soil microbial communities. This technology comprises isolation of soil DNA and production and screening of clone libraries.

Metagenomic profiling, predicting the presence and relative abundances of microbes in a sample, is a critical first step in microbiome analysis. Alignment-based approaches are often considered accurate yet computationally infeasible.(Pacwa- Plociniczak, et al., 2018)

Metagenomic predictions from the 16S rRNA gene sequences showed that the introduction of bacteria ~~had a significant influence on~~significantly influenced the predicted pathways (metabolism of xenobiotics, lipids, terpenoids, polyketides, and amino acids).

The metagenomic approach, however help to obtain useful information on the composition and genetic, physiological mechanisms of soil microbiota and their adaptation to specific environments, such as ~~oil~~ oil-contaminated soils, for a better understanding of the alterations of microbial development, biochemical activities, and bioremediation processes (Ahmed *et al.*, 2018; Peng *et al*., 2015.

The introduction of metagenomics can play a vital role in unearthing and monitoring ~~the~~ microbial communities by providing access to ~~the~~ taxonomic and functional gene composition. According to (Gong *et al*.,2013) most of the metagenomic analysis tools have opened new ~~windows of opportunities for researchers to analyse~~opportunities for researchers toanalyze the microbial community as a whole (whole-genome sequencing) and the genetic diversity, which facilitates active metabolic pathways in any given environment.

While studies in the past have focused on species composition of a community, metagenomic studies (a gene-centric approach) enable assessment of the ~~biological function of the gene~~gene's biological function rather than the taxonomic identity(Lelario et al., 2018). The study of microbial biodiversity and whole-genome sequencing (WGS) and analysis of any complicated samples with numerous microorganisms, which are not pure and unculturable in any given laboratory, have made shotgun metagenomics a more efficient

tool than most ~~of the already~~ existing conventional techniques. Furthermore, there is always a well-established direct correlation between microbial diversity and ~~the presence of~~ hydrocarbons in any oil-contaminated sites. Metagenomic studies ~~therefore~~ provide excellent opportunities for finding new microbial strains and genes involved in bioremediation of hydrocarbon contaminants. Recent advances in genomics, transcriptomics and proteomics have led to increased studies on bacterial communities in contaminated soil. Genomic methods include functional bacterial finger printing and ~~next~~ next-generation sequencing (NGS) of hypervariable regions, such as in 16S rRNA genes from bacteria, to determine the genetic diversity of microorganisms within a population without the need for cell culture (Galazka et al.,2018; Malla et al.,2018;Pichler et al.,2018).However, microbes that thrive in the crude oil-contaminated soil have closely related living conditions close to the conditions in the contaminated soil. The ~~aim of the study i~~study aims to identify and compare the organisms(Bacteria) present in an abandoned artisanal refinery site located in Obi-ayagha community, Delta State, Nigeria, with a control sample using high-throughput sequencing of the 16S rRNA gene in Delta State to ascertain the effect of the hydrocarbon contamination of the bacteria genomics of the site.

2.0 MATERIALS AND METHODS

2.1 Sample collection and Preservation

2.1.1 Sample collection of Hydrocarbon impacted and unimpacted(Control)soil

The soils used in this study was collected from the abandoned artisanal refinery site, located in Obi-Ayagha, Ughelli South, Delta State, which has been acquired as a Hydrocarbon Pollution Research/Training site, in affiliation with the Integrated Institute of Environment and Development (IIED), Federal University of Petroleum Resources Effurun Warri.Soil samples from the surface horizons (0-15m), were collected using a soil auger (polluted soil sample).The coordinates of the locations are presented thus: Sample collection site (Hydrocarbon contaminated soil) Latitude 5.3674330 and Longitude 5.8499400.

Composite samples were also collected from ~~an~~ unpolluted soil (0-15m)) which is used as the control sample from FUPRE garden~~, w~~. We have Latitude 5.570334.5 and Longitude 5.840970

Plate 1: Sample site

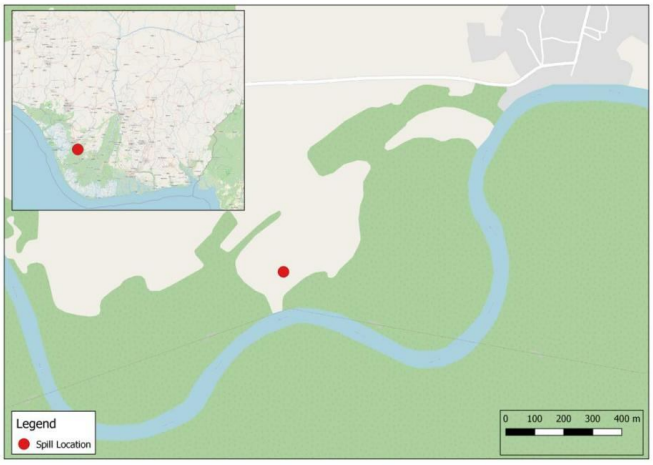

Figure 1: Map of the study area

2.1.2. Bacteria DNA extraction procedure

Bacteria DNA was extracted using a soil genomic DNA isolation kit by adding 50-100mg (wet weight) to bacterial cells that have been resuspended in up to 200ul of water or isotonic buffer (e.g., PBS) or up to 200mg of tissue to a**ZR Bashing™ Lysis Tube, 750ul Lysis Solution**was added to the cell in the tube.

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The tube was secured in a bead fitted with 2 ml tube holder assembly and processed at maximum speed for> 5 minutes. Then the ZR BashingBead™ Lysis Tube was centrifuged in a microcentirifuge at > 10,000 x g for 1 minute. 400 ul supernatant was then transferred to a Zymo-Spin™ IV Spin Filter (orange top) in a collection tube and centrifuged at 7,000 x g for 1 minute.

~~1,200 ul of Bacterial DNA binding buffer was then added to the filterate in the collection tube from the above step~~The above step added 1,200 ul of Bacterial DNA binding buffer to the filterate in the collection tube. 800 ul of the mixture from Step 5 was then transferred to a Zymo-Spin™ IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute.

The flow through from the collection tube was discarded and the previous step was repeated. 200 ulDNA Pre-Wash Buffer was added to the Zymo-Spin™ IIC Column in new collection tube and centrifuged at 10,000 x g for 1 minute. 500ul~~uBacterial~~ bacterial DNA wash buffer was added to the Zymo-Spin™ IIC column and centrifuged at 10,000 x g for 1 minute.T~~ransfer t~~he Zymo-Spin™ IIC Column was ~~transfferd~~ transferred to a clean 1.5 ml microcentrifuge tube and 100ul (35 ul minimum) DNA Elution Buffer was added directly to the column matrix. The extract was centrifuged at 10,000 x g for 30 seconds to elute the DNA, and then the DNA was used for PCR and other downstream applications.

2.1.2.1 PCR cocktail mix

The DNA was subjected to a cocktail mix, and the condition for the PCR ~~are~~were: 10 x buffer (1.0), for $Mgcl_2$ 25m (1.0) ,5pmol forward primer (0.5), 5pmol reverse primer (0.5), DMSO (1.0), 2.5Mm d~~D~~NTPs (0.8), Taq 5u/ul (0.1), H_20 (3.1) all equal to 10µL

2.1.2.2 PCR product purification

Absolute ethanol of 2volume (20ul) was added to polymerase chain reaction (PCR) product, and then it was incubated at room temperature for fifteen (15) minutes~~, i~~. It was centrifuged ~~(spin) down~~ at 10000rpm for 15 minutes as well, then decant spin down again at 10000rpm

for 15 minutes, add 2volume (40ul) of 70% ethanol, decant the supernatant as well, air dry, and add about 10ul of ultrapure water finally you check for amplicon on 1.5% agarose. The PCR product is ready for sequence reaction.

PRIMER: 27F: AGAGTTTGATCCTGGCTCAG
1492R: GGTTACCTTGTTACGACTT

2.1.2.3.PCR Conditions

The PCR undergoes denaturation, annealing, and extension; the initial denaturation was carried out at a temperature of 94˚c for 5mins. ~~later~~ Later denatured again at a temperature of 94˚c for 45sec. The annealing was carried out at the temperature of 56˚c for 30sec~~.~~ followed by an extension which was done at the temperature of 72˚c for 45sec. It went through a number of circles (35), and the final extension was carried out at the temperature of 72˚c for 7mins and ~~.~~finally held at a temperature of 4˚c.

3.0 RESULTS AND DISCUSSIONS

3.1.Taxonomic classification of microbes~~'~~ identified within the abandoned artisanal refinery petroleum oil-polluted site.

3.1.1 Summarized ~~full~~ full-length Metagenomic Analysis of 16S Gene Amplicons

Metagenomic Taxonomic Classification of Bacteria identified within the polluted and unpolluted soil samples was determined based on QIMME2. Report generation command was used.

Molecular database summarized the full length of the metagenomic analysis of 16S gene amplicons; taxonomical classification of Bacteria (4317.0, 27435.0), thus, revealing the most abundant organisms present in each kingdom, phyla, class, order, and species in the contaminated and uncontaminated sample.

3.1.1.1 Kingdom classification of Bacterial (Contaminated and uncontaminated sample

Top kingdom classification

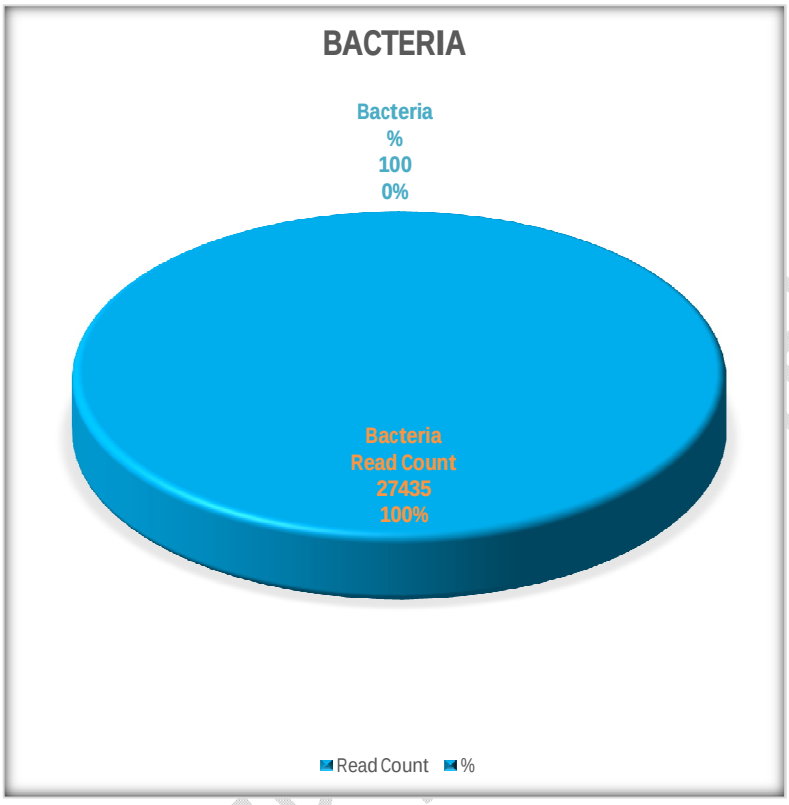

Fig.2: Top species of Bacteria present in contaminated soil sample

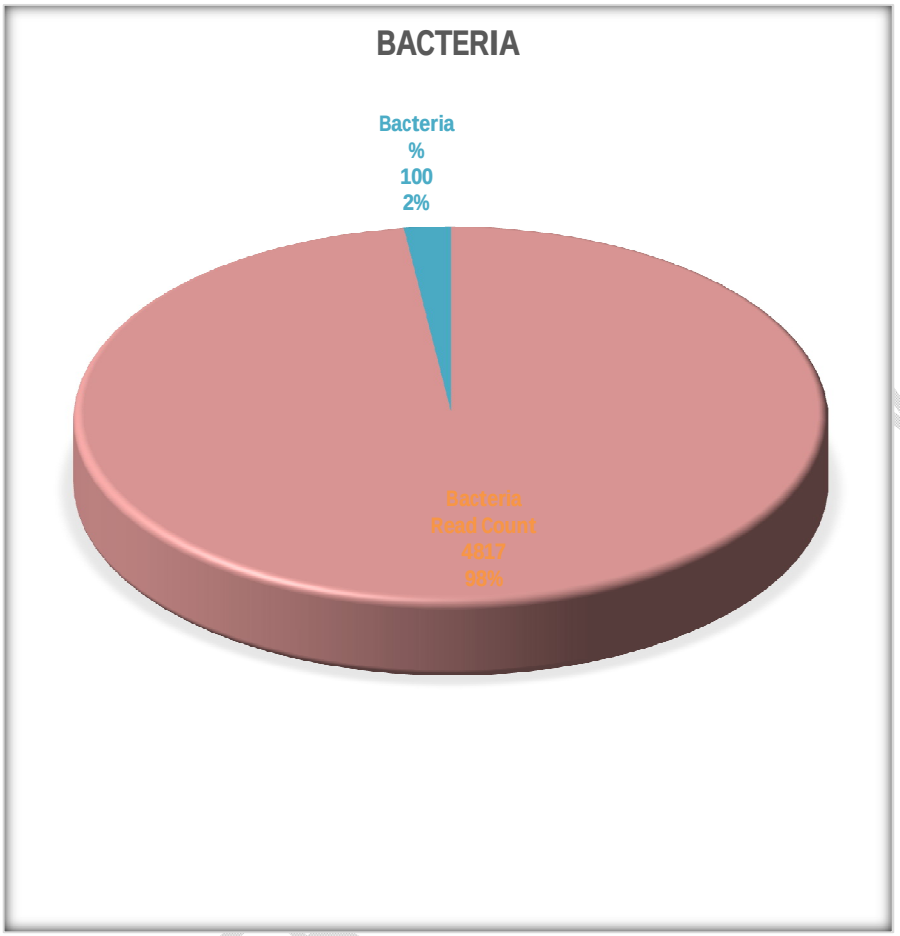

Fig.3:Top species Bacteria present in uncontaminated (control)soil sample

A representation of top kingdom classification of microbial diversity identified in the contaminated and uncontaminated soil (control) is depicted in Fig. 2 and 3, respectively, Molecular database summarized the full length of the metagenomic analysis of ITS1F and 16S gene amplicons; of the taxonomical classification of Bacteria in the various samples (27435.0, and 4317.0,)

3.1.1.2. Top phylum classification

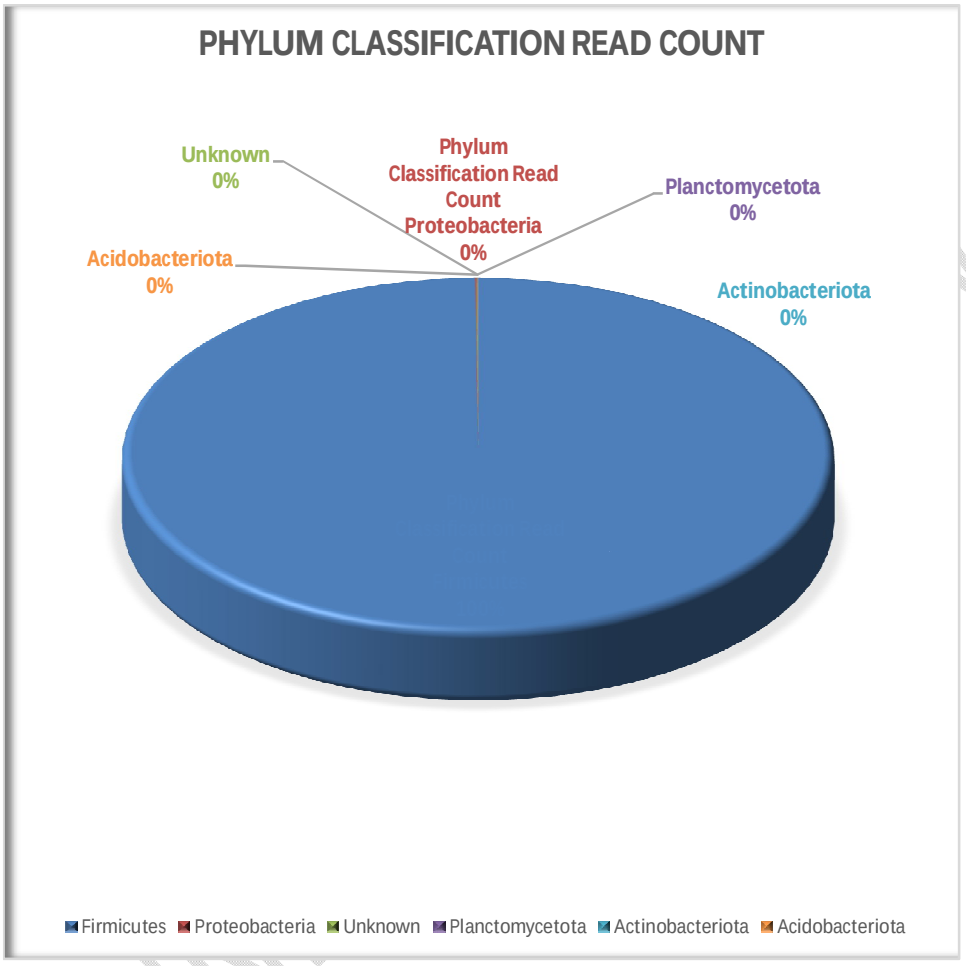

Fig.4: Top phylum Bacteria present in uncontaminated(control) sample

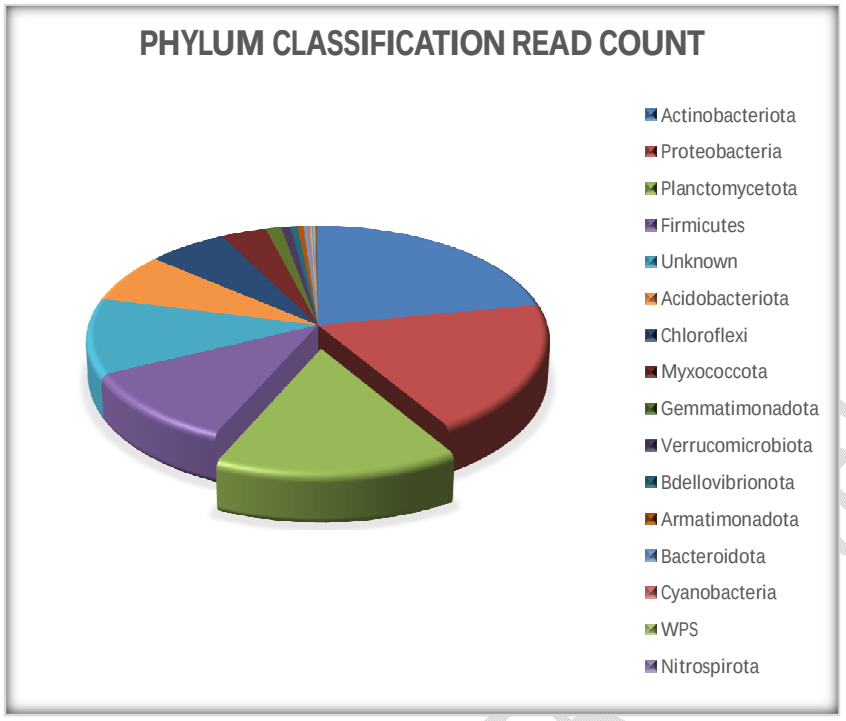

Fig.5:Top phylum Bacteria present in contaminated sample

The dominant species of phylum-level microbial diversity identified in the uncontaminated site and petroleum-contaminated site is depicted in Fig. 4 and 5 respectively. The dominant groups were found to be the Actinobacteriota (21.94%) followed closely by the Proteobacteria (19.31%), then the Planctomycetota (15.49%), the Firmicutes (11.11%) and the unknown were (11.11%) respectively. Others were Acidobacteriota (7.25%), Chloroflexi (6.21%), Myxococcota (3.55%), Gemmatimonadota (1.20%), while the remaining phyla were considered insignificant as showed in (Fig. 5). But for the control, the Firmicutes dominated the phylum with the percentage occurrence of (99.86%),while other phyla recorded were all insignificant, a marked reduction in the quantitative number of the phyla were observed.

3.1.1.3. Top Class Classification

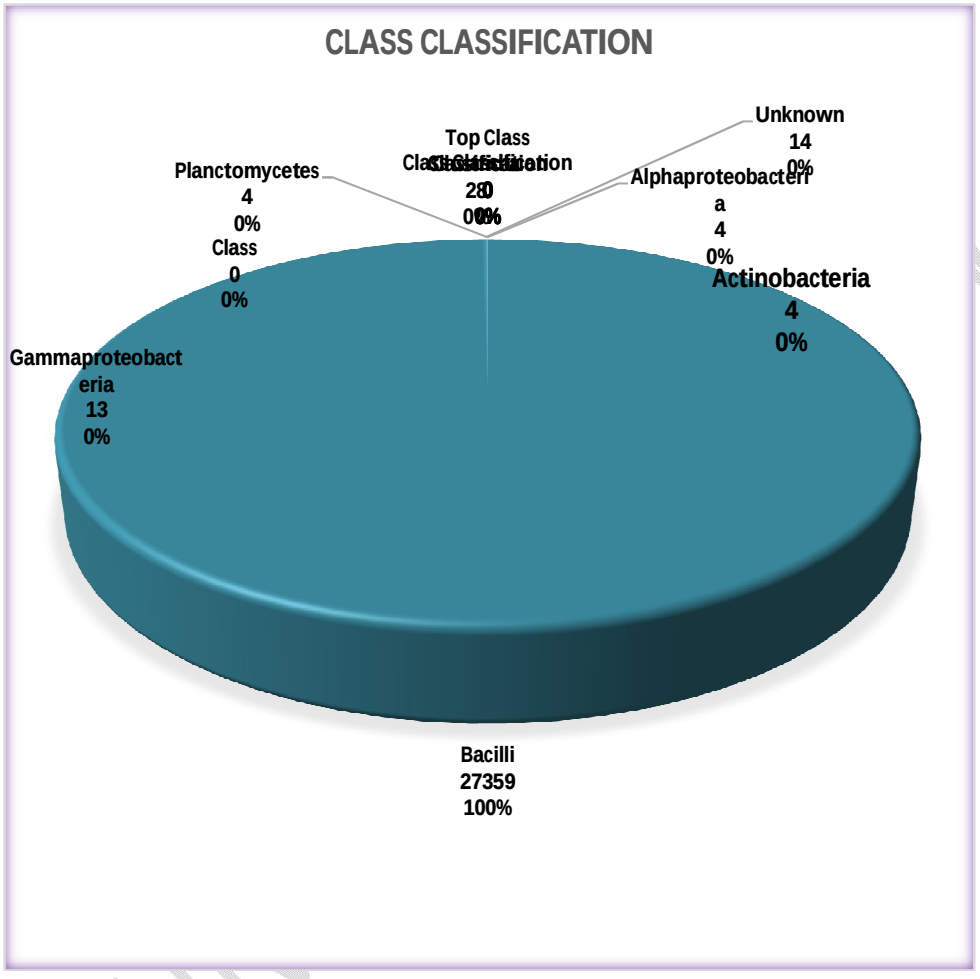

Fig.6: Top species Bacteria present in contaminated sample

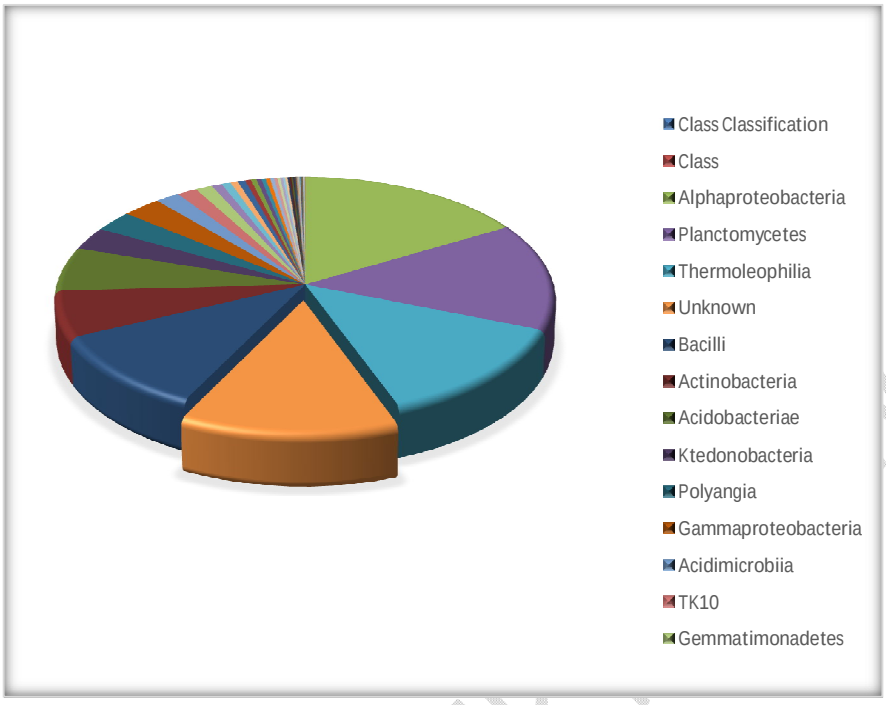

Fig.7: Top species Bacteria present in uncontaminated sample

In accordance to the classification at the class level, the dominant group was*Alphaproteobacteria* (16.48%) followed closely by *Planctomycetes* (14..59%)next is the *Thermoleophilia* (13.31%),unknown (12.95), *Bacilli* (10.95%) , *Actinobacteria* (6.27%) *Acidobacteriae* (5.9%) *Ktedonobacteria* (3.09%) *Polyangia* (2.82%), *Gammaproteobacteria* (2.80%) *Acidimicrobiia* (1.74%) TK (1.45%), *Gemmatimonadetes* (1.20%) other class were all insignificant(Fig 6). ~~, and whereas.~~ In contrast, for the control *Bacilli* dominated the class showing (99.76%) as other class such as *Clostridia* (0.10%), unknown (0.05%), *Gammaproteobacteria* (0.05%), *Alphaproteobacteria* (0.01%), *Planctomycetes* (0.01), *Actinobacteria* (0.01%) were all insignificant were significantly low and drastically lower in the control site (Fig. 7).

3.2. Discussion

Fossil fuel contamination ~~at large~~ is on the increase and of public health concern as huge amounts of these compounds are being deposited during processing, storage, and transportation either accidentally, through sabotage, or intentionally. Its exploration has led

to an enormous of these product directly in the soil and water bodies~~, majority~~; most of these hydrocarbons are normally toxic to the flora and fauna in the ecosystem in line with the report of (Jin *et al.*,2012). Despite the fact that the product is toxic, it still ~~have~~ hasits positive side, where the affected sites contain ~~a~~ varied types of microbes with genes and enzymes that catalyze the degradation process~~, .~~According to the report of (Abbasian *et al.*,2015), the relevance of metagenomic analysis tools in the affected oil-spill sites provides a promising approach that allows analysis of these microbial communities and their mode of adaptation to petroleum oil contamination in the environment.

Results of the present study have demonstrated from the results obtained from the metagenomic sequence that the microbial community of the petroleum oil-polluted soil (site)in the top kingdom classification~~,~~ showed that various microbial domains in the impacted soil ~~has~~ havethe dominating number of various bacteria specie(27435.0) compared to the control (4817.0). Petroleum hydrocarbon pollution requires the collaboration of numerous ~~useful~~ beneficial microbes to accomplish the most excellent environmental cleaning practices. (Dombrowski *et al.*, 2016).

In accordance ~~to~~ withthe classification at the class level, the dominant group was*Alphaproteobacteria* (16.48%), followed closely by *Planctomycetes* (14..59%)next is the *Thermoleophilia* (13.31%),unknown (12.95),Bacilli (10.95%), *Actinobacteria* (6.27%) Acidobacteriae (5.9%) *Ktedonobacteria* (3.09%) *Polyangia* (2.82%), *Gammaproteobacteria* (2.80%) *Acidimicrobiia* (1.74%) TK (1.45%), *Gemmatimonadetes* (1.20%) other classes were all insignificant(Fig 6), and whereas for the control *Bacilli* dominated the class showing (99.76%) as other class such as Clostridia (0.10%), unknown (0.05%), *Gammaproteobacteria* (0.05%), *Alphaproteobacteria* (0.01%), *Planctomycetes* (0.01), *Actinobacteria* (0.01%) were all insignificant were significantly low and drastically lower in the control site (Fig. 7).

From the result obtained looking at the top phylum classification of the control samples, the firmicutes has the highest occurrence(27390.0) followed by Proteobacteria(17.0) and the unknown (11.0), Planctomycetota (4.0) and Actinobacteriota(4.0). For the contaminated sample, the Actinbcteriota topped the list (1057.0) in the metagenomic sequence, followed closely by Proteobacteria with (930.0) read, Planctomycetota has the record of (746.0), followed by the Firmicutes (535.0).Others like Acidobacteriota, Chloroflexi, Myxococcota, Gemmatimonadota, Verrucomicrobiota, Bdellovibrionota, Armatimonadota, Cyanobacteria and were present at reduced population compared to the aforelisted above in the top phylum group.

The representation of different Kingdom, phyla and class of microbes obtained in the petroleum-contaminated soil sites showed the dominant phyla to be the prokaryotes (*Firmictes* and the *Actinobacteriota)* in both samples (where a high number (>20%) of unknown sequences were observed in the top kingdom, phyla and class classification (Fig. 2, 3 and 4). According to (Atlas and Widdel *etal.*, 1981), the majority of prokaryotic family are reported to be more closely monitored in order to evaluate the potential for both aerobic and anaerobic oil biodegradation, since they are known for their utilisation of petroleum hydrocarbons. They reproduce rapidly and show a wide tolerance against environmental extremes in temperature, oxygen supply, pH and the presence of toxins. The phylum *Actinobacteriota* and *Bacilli* play an important role in removing hazardous hydrocarbon compounds from the soil. This is the desired ecological niche for Bacteria, which inhabit substrates, and in biodegradation processes, they are known as consumers of the carbon sources from hydrocarbons found in petroleum oil spill site sources. Population of indigenous oil degrading micro biota increased rapidly, ~~which corresponds to high availability of hydrocarbons during these perphylogenetic novelty observed during this study, which makes an important contribution towards a better understanding of the microbial diversity that exists~~corresponding to high availability of hydrocarbons during these perphylogenetic novelty observed during this study, which makes an important contribution towards a better understanding of the microbial diversity in an oil-spill affected site.

Conclusion

Petroleum hydrocarbon degrading bacteria are ever-present in the environment and ~~they utilize these elements as sole carbon and energy source~~utilize these elements as sole carbon and energy sources. Bacteria that have these ~~capability~~ capabilities(Pseudomonas species, Bacillus species etc) are frequently brow beaten for the bioremediation of fossil fuel environment. Recently researchers were able to identify various bacteria from additional seventy-nine genus that has the competence to degrade petroleum hydrocarbon (Trembley, *et al.*,2017).The novelty of this study is that the majority of these microbes were found to be Bacteria (Prokaryotes).It is envisaged that prokaryotes will form part of the bioremediation process, hence the soil bacteria are known to be social organisms that live in complex communities with extensive interactions within and between species. Microorganisms conceal surfactants that make easy emulsification of the petroleum oil, therefore attracting the uptake of hydrocarbons into the microbial cells.The uniqueness of this study is that the bulk of these microbes were Bacteria (Prokaryotes) found in the site. It is envisaged that Bacteria form~~s~~ part of the bioremediation process. However, the study confirms the collection of different Bacteria phyla, Class and Kingdom which were never before known to ~~be playing~~play any role in hydrocarbon degradation or inhabiting these petroleum oil-

contaminated areas. The phylogenetic novelty observed during this study makes an important contribution towards a better understanding of the microbial diversity that exists in such sites. The findings however provide a scientific background and technology display for further research and utilisationHowever, The findings provide a scientific background and technology display for further research andutilization of microbial species, especially Bacteria strains, since they are ubiquitous in nature.

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