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Metagenomic Analysis of Bacterial Diversity of Different Crude Oil Contaminated Soils in Rivers State

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ABSTRACT

Crude oil pollution is a common occurrence in Rivers State, Nigeria. Several studies have investigated the effect of crude oil pollution to the environment. In this study bacterial diversity of different crude oil contaminated soils (Aged contaminated, recently contaminated and bioremediated soils) and an uncontaminated soil was investigated. The 16SrRNA metagenomic approach was used in identifying bacterial diversity present in the soil. The concentration of total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbon (PAH) and some heavy metals were determined. Alpha diversity and beta diversity index were used to compare diversity of bacterial species within and between samples respectively. The result indicates that the recently polluted soil had higher concentration of TPH and PAH (1836.66 mg/kg and 208.97mg/kg respectively). A total of 13 phyla was identified in all the soil samples, with the uncontaminated soils having eleven identified phyla while the recently polluted, aged polluted and bioremediated soils had seven, five and four phyla observed respectively. The predominant phyla in all soil samples was Proteobacteria and Acidobacteria. Relative percentage abundance of Proteobacteria in the crude oil polluted soil was higher (aged polluted 67.1% > bioremediated soil 38.2% > recently polluted 21.7%,) than the uncontaminated soils (17.3% & 8.2%). Result from Beta diversity showed that all soil samples had shared microbes at different abundance. While the recently polluted soil and one of the unpolluted soil (B5) had more shared microbes at a close abundance with a 0.211 dissimilarity index. Crude oil pollution in soil may play vital role in altering bacterial diversity.

Keywords: *Metagenomics, Bacteria, Diversity, Crude oil, Polluted*

1.0 INTRODUCTION

Exploration of crude oil plays a major role in Nigeria’s economy and has served as a source of energy to run the nation’s economy (Adeyemo & Aliu 2021). Increasing demand for energy and industrialization have led to continuous exploration of fossil fuels. This has led to contamination of many water bodies and terrestrial environments.(Eze, 2021). One major issue that has stirred public and research interest in the Niger Delta region of Nigeria is contamination of the environment with crude oil. Crude oil Pollution usually occurs as spillage into the environment as a result of equipment failure, operational mishaps, transportation or intentional damage to facilities (Adeyemo & Aliu 2021). This greatly damage indigenous fauna and flora, it could also lead to accumulation of the pollutant in the tissues of the fauna and flora, reduction of their population or death.(Ataikiru et al., 2020). Soil microorganisms play active roles in the ecosystem, they are accountable for most biological changes and drive the nutrient cycles to enable the establishment of plant communities. The structure and function soil ecosystem is related to the diversity of the microbial community (Chikere et al., 2019). Soil contains the highest bacterial richness within a single sample (Walters & Martiny, 2020). Physical and chemical characteristics of a particular crude oil determines its toxicity (Okafor, 2023). When a spill occurs the hydrocarbons penetrate soil capillaries that were formerly filled with air or water and bind to soil particles or to minerals. The regions become damaged ecologically as air and water will be expelled from the microenvironment. (Okafor, 2023). When soil gets contaminated with spilled crude oil, microorganisms that are present and can tolerate the concentration and degrade crude oil proliferate and become dominant (Ali et al., 2020) Less than 1% of soil bacteria are culturable. Almost all soil microbial communities represent a source of genetic and metabolic diversity, thus the need to assess and maintain the diversity of soil microorganisms. Investigating the diversity of bacterial species is limited using culture dependent method. Several molecular techniques have been developed to understand microbial communities better. These methods are potent tools and rapid for understanding soil microbial community dynamics and diversity. (Bonomo et al., 2022). Hydrocarbon degrading microorganisms are not easily isolated using the regular nutrient media and laboratory conditions. Culture-independent techniques has become the widely used method to evaluate the taxonomy of microbial diversity in different environments (Chikere et al., 2019). Metagenomics has proven to be a powerful tool for studying soil ecology, evaluating the diversity of multifaceted microbial communities, accessing numerous novel species, genes or new molecules relevant for agricultural and biotechnology applications (Bonomo et al., 2022). This study investigated the bacterial diversity of different crude oil contaminated soils. The concentration of the Total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbon (PAH) and heavy metals was evaluated. In other to advance engineered bioremediation, studying the microbial diversity in crude oil polluted soils, is of great importance. From previous studies, it has been established that bacteria are the predominant microorganisms in soil with a prevalence of 97.5%, eukaryote (1.5%), archaea (1.2%) and virus (0.04%) (Sandhu et al., 2022). Thus bacteria play a major role in activities going on in soil ecosystem. Several 16S rRNA metagenomics studies have been carried out on crude oil contaminated soils in Nigeria but none have compared the microbial diversity between an aged contaminated soil, recently contaminated soil and a bioremediated soil. Hence the choice for this study to investigate the bacterial diversity of an aged, recently and bioremediated crude oil polluted soils.

2.0 MATERIALS AND METHOD

2.1 Study area and samples

Soil samples were gotten from two Local government areas in Rivers State Nigeria (Eleme and Gokana). Three different levels of pollution were considered within the area and selected for the study. First a soil that has not been contaminated with crude oil, second an aged crude oil contaminated site, recently contaminated site and a bioremediated site. Soil samples were collected using soil auger at different depths (top soil, 0.5 m, 1 m,) from five different points for each site and bulked together. The collected samples were put in a cool bag and transported to the laboratory.

2.2 Physicochemical analysis

Total petroleum hydrocarbon, Polycyclic aromatic hydrocarbon were quantified using a gas chromatograph–mass spectrophotometer (GC-MS)) as described by (Ehis-Eriakha et al., 2020) Heavy Metal (Cd, Cr, As, Cu) concentration was determined using Atomic Absorption Spectrophometer method (AAS) as described by Tanee & Albert, 2017). pH probe was used to determine the pH of the soil samples.

2.3 DNA extraction and sequencing

Extraction of DNA was carried out using ZR Soil Microbe DNA MicroPrep Kit (zymo research) following the manufacturer's instruction. Nanodrop Spectrophotometer was used in quantification of the DNA. The 16SrRNA gene amplification was done according to Illumina protocol for 16S Metagenomic Sequencing Library Preparation protocol. The variable region aimed at was the V3 and V4 variable regions with primers 16S-341F 5′-CCTACGGGNGGCWGCAG-3′ and 16S-805R 5′-GACTACHVGGGTATCTAATCC-3′. MiSeq Illumina platform was used for sequencing. Trimmomatic software program was used to remove the sequencing primers and reads of low quality (Bonomo et al., 2022)

2.4 Bioinformatics analysis of sequenced data

QIIME (v.1.9.0) pipeline was used in processing the data obtained, reads containing less than 50 nucleotides and reads with more than 2% of ambiguities were excluded during the course of analysis. Sequences were clustered into operational taxonomic units (OTUs) at a 97% identity threshold using the UCLUST algorithm (Edgar et al. 2011). For open reference operational taxonomic unit picking and taxonomic classification, version 132 SILVA data base was used (Chikere et al., 2019). Alpha diversity (Shannon, Simpson, Simpsons reciprocal index and Berger parker index) and beta diversity analysis was done using web based tool provided by Bacterial and Viral Bioinformatics Resource Center (BV-BRC) (https://www.bv-brc.org). Beta diversity was done using the Bray–Curtis index for estimation of beta diversity. This was used to compare the similarities between and within the samples.

3. RESULTS

3.1 Physicochemical analysis

The average Total petroleum hydrocarbon and polycyclic aromatic hydrocarbon for all samples ranged from 5.13 mg/kg -1836.66 mg/kg with the recently polluted having the highest value. The crude oil contaminated soils had slightly higher concentration of the four heavy metals evaluated (Table 1).

Table 1: Concentration of physico-chemical parameters

S/N	Parameters	Bioremediated (B1)	Recently contaminated (B2)	Aged contaminated (B3)	Uncontaminated (B4)	Uncontaminated (B5)
1	**pH**	6.7	7.0	6.2	5.5	5.7
2	**TOC (%)**	0.4	1.70	0.43	0.5	0.53
3.	**PAH (mg/kg)**	42.06	208.97	57.11	5.00	5.13
4	**TPH (mg/kg)**	238.9	1836.66	243.98	56.33	69.24
5	**Cd (mg/kg)**	0.061	0.094	0.065	0.045	0.017
6.	**Cr (mg/kg)**	0.112	0.173	0.106	0.039	0.060

7	**As (mg/kg)**	1.081	2.041	1.902	0.327	0.183
8	**Cu (mg/kg)**	1.476	1.654	1.497	1.385	1.333

3.2 Taxonomical classification of bacteria and diversity

Proteobacteria and Acidobacteria were the two predominant phylum observed in all soil samples. Actinobacteria, chloroflexi and Planctomycetes were observed in all soil samples except the bioremediated soil sample (Figure 1). A total of 72 genera was identified in all sample. The genera observed for all samples were; Rhodoplanes, Candidatus solibacter, Streptomyces and Bacillus. Identified genera and species was more in the bioremediated sample(Figure 2 & 3). The alpha diversity compared how similar or different the bacteria are at species level within each sample (Figure 4). while the beta diversity compared the dissimilarity between all samples using the Bray- Curtis index of dissimilarity (Figure 5). If there are no shared bacteria between the sample the dissimilarity is one.

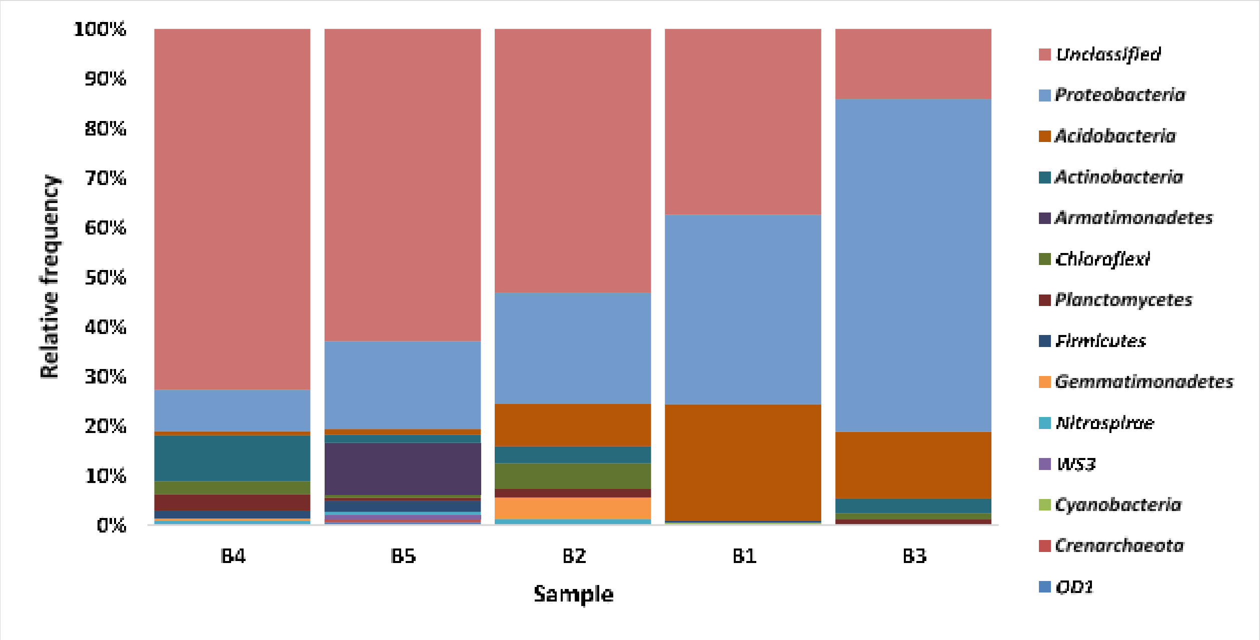

Figure 1: Percentage relative frequency of phylum level in all samples

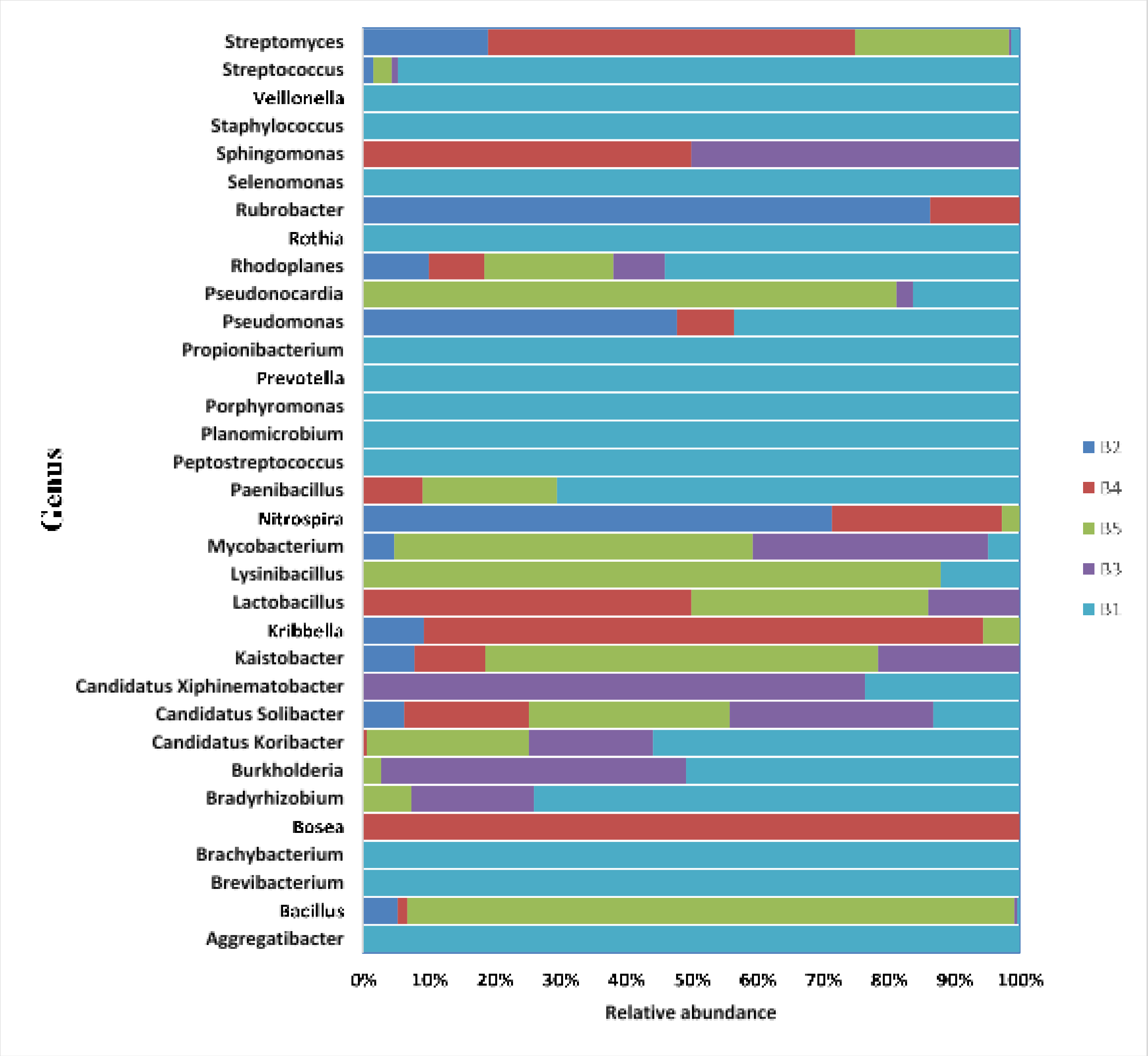

Figure 2: Percentage relative frequency of some Genus level in all samples

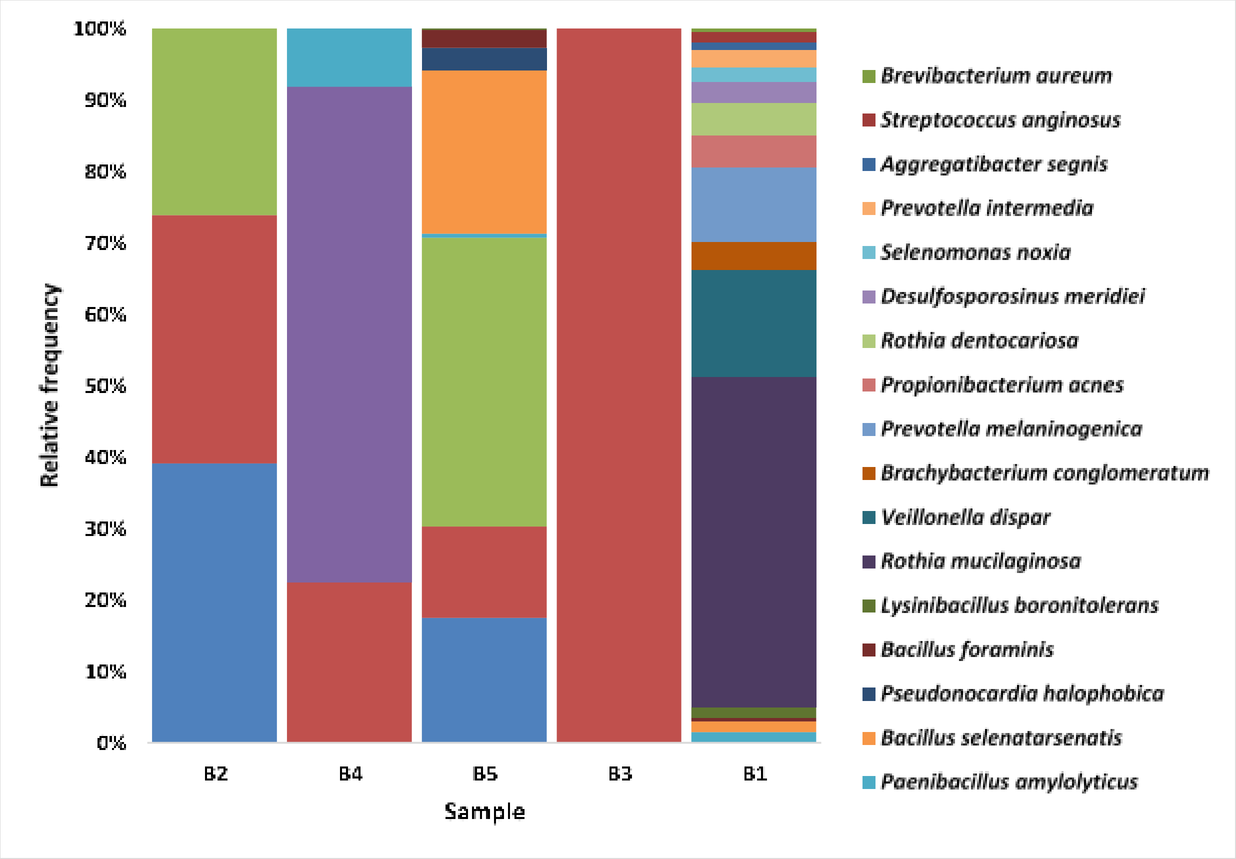

Figure 3: Percentage relative frequency of species identified in all samples

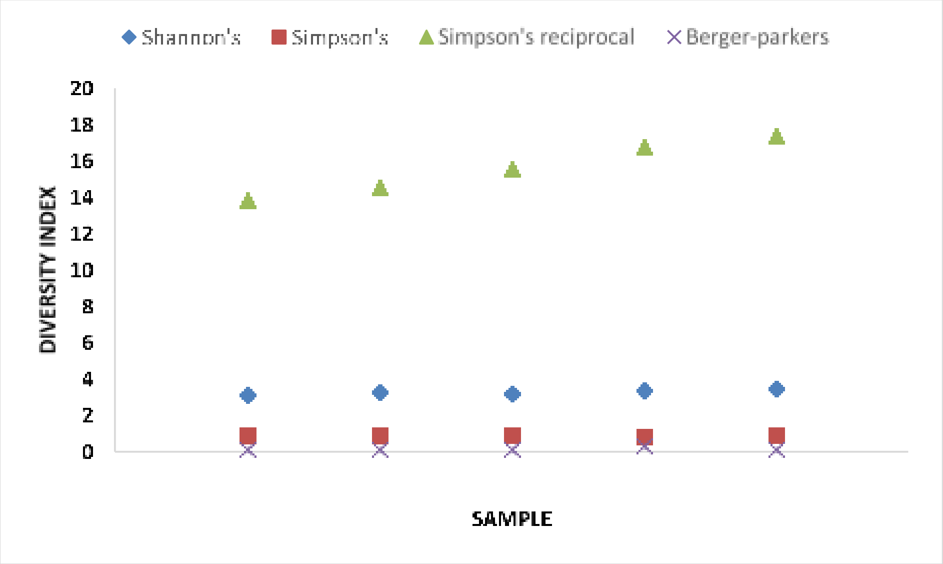

Figure 4: Alpha diversity index

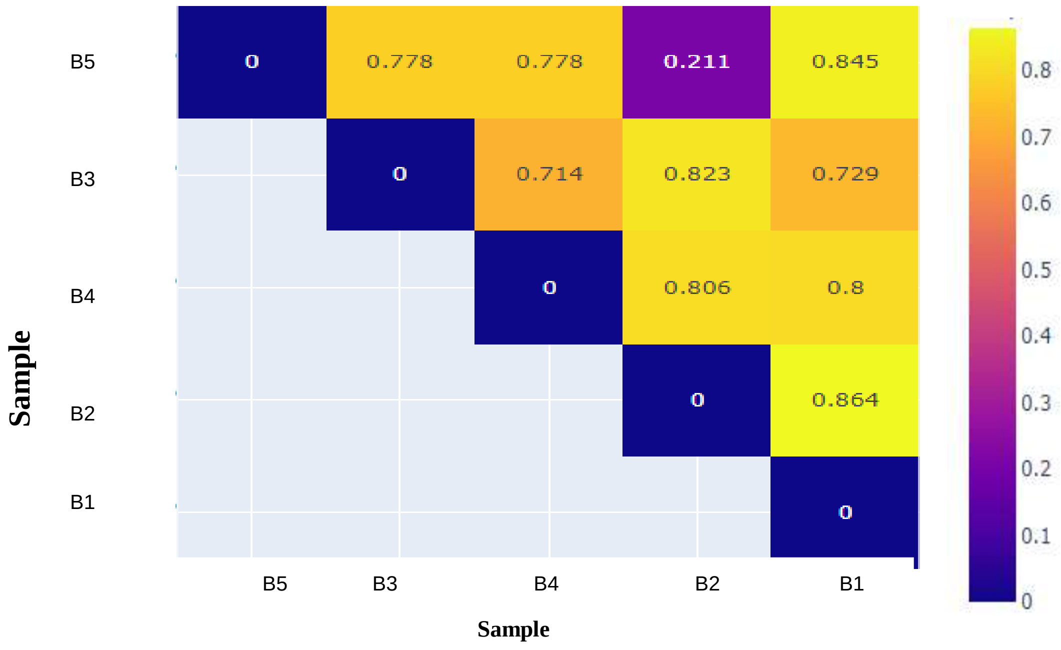

Figure 5: Beta diversity index (Bray Curtis)

4.0 DISSCUSION

Results from physicochemical analysis reveals that the Total petroleum hydrocarbon (TPH) and Polycyclic aromatic hydrocarbon for the recently polluted soil (1836.66mg/kg and 208.97 mg/kg respectively) was higher than the aged polluted (243.98 mg/kg, 57.11 mg/kg) and bioremediated soil (238.9mg/kg, 42.06 mg/kg) (Table 1). The TPH value of the recently polluted was above the intervention limit of National Oil Spill Detection and Response Agency (NOSDRA) and Hydrocarbon pollution remediation project (HYPREP) in Nigeria. The bioremediated soil was gotten from one of the communities of the Local government area of ogoni land where HYPREP had carried out bioremediation. The concentration of the TPH was within the target threshold for bioremediation (Independent Monitoring of the Ogoniland Clean-up: Biannual Progress Report). The low concentration of TPH for the aged contaminated soil is as a result of natural attenuation that must have taken place over time since hydrocarbon degraders are naturally present in soil and water bodies (Tiku et al., 2016). Results for the 16SrRNA metagenomics analysis indicated Proteobacteria and Acidobacteria as the predominant phyla for all samples with Proteobacteria having the highest prevalence (Figure 1). Relative percentage abundance of proteobacteria in the crude oil polluted soil was higher (aged polluted 67.1% > bioremediated soil 38.2% > recently polluted 21.7%,) than the uncontaminated soils (17.3% & 8.2%). Studies carried out by (Yang et al., 2016) indicated that proteobacteria were dominant in soil samples and their prevalence increased after pollution with crude oil. Phylum richness was observed in the uncontaminated soils with eleven phyla, and eight phyla identified. while the recently polluted, aged polluted and bioremediated soils had seven, five and four phyla observed respectively. This was similar to the work of Guneshwari et al., 2023 were it was reported that freshly contaminated oil sludge had higher microbial diversity than an aged contaminated oil sludge. The effect of the crude oil contamination may have encouraged the proliferation of bacteria phyla that can degrade hydrocarbon thus leading to the eradication of some phylum that may not be able to degrade hydrocarbon. The bioremediated soil had the least number of phyla this could be as a result of bioaugumentation and biostimulation during the bioremediation process. Thus augumenting with bacteria species known to degrade hydrocarbon. The phylum Firmicutes was identified in the uncontaminated and the bioremediated soil but was absent in the recently and aged polluted soils. This was also observed by Camacho-montealegre et al., 2021 where it was noted that increase in crude oil concentration caused a decrease of Firmicutes. The phylum Nitrospirae which plays an important function in nitrification was not identified in bioremediated and aged polluted soils. Indicating that long term pollution may have effect on nitrogen cycle. The multifunctionality of soil ecosystems is highly dependent on soil microbial diversity. The shift or loss of biodiversity can lead to diminishing of natural ecosystem functions and quality of services they provide (Cheng & Tan, 2023).

At genus level Rhodoplanes, Streptomyces, Bacillus and Candidatus solibacter were prevalent in all samples with higher prevalence in bioremediated soil. Other genera that had higher prevalence in bioremediated and aged polluted soils were Candidatus koribacter, Bradyrhizobium and Burkholderia. These genera are known to be crude oil degraders (Xu et al., 2018)

At the species level (Figure 3) twenty-one bacterial species were identified with the bioremediated soil having more identified species (sixteen). Alpha-diversity indices which is an essential tool for describing and comparing biodiversity (Finn, 2024) was implored to test bacterial diversity within each sample. It was done using the Shannon, Simpsons, Simpson's reciprocal and the Berger-parker's diversity index. The Simpson's reciprocal index which is more intuitive and indicates that a higher value represents higher diversity showed that the uncontaminated soils had higher bacterial diversity than the polluted soils followed by the aged polluted, recently polluted and the bioremediated (B5> B4 >B3> B2 >B1) (Figure 4). This aligns with the work of Huang et al., 2021 , where it was observed that most bacteria presented abundance advantages in unpolluted soils than in crude oil polluted soils.

The Bray-Curtis index of dissimilarity was used to analyzed the beta diversity. In application of this index, it is said that if two samples have the same microbe at the same abundance the dissimilarity will be zero. If there are no shared microbes between the dissimilarity is one. Result from Beta diversity showed that all soil samples had shared microbes at different abundance as seen with the different index value. While the recently polluted soil (B2) and one of the unpolluted soil (B5) had more shared microbes at a close abundance with a 0.211 dissimilarity index than other samples compared. The comparism between polluted soils (aged and recently polluted) with the bioremediated indicated a dissimilarity index of 0.729 and 0.864 respectively indicating that the abundance of shared microbes is with the aged contaminated soil where biodegradation has been going on for a while (Figure 5). Studies have shown that original soil microbial diversity is the key to harnessing the potential of remediation(Correa-García et al.,2021)

5.0 CONCLUSION

From the data obtained in this study, it was observed that even without a clean-up process the concentration of total petroleum hydrocarbon of the aged polluted crude oil soil was lesser than the recently polluted. Heavy metal concentration differs from each sample with the polluted soils having slightly higher concentration. The age of crude oil pollution in soil may play vital role in bacteria diversity as indicated in the percentage relative abundance of the phyla, especially proteobacteria. Long term crude oil pollution could also affect different bacteria that play significant roles in biogeochemical cycle.

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