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Research Article

Metagenomic Analysis of Bacteria Load At Two Locations in Futo and Nekede Otamiri River Imo State.

Ugaya, J. A., Chiegboka, N., Ubah, V.C.S., Nwachukwu, M.O. Adaka, G.S., Nwamba, I.M., and Orji-Kelechi, C.I.

[1]*Department of Biology, Federal University of Technology Owerri Imo State Nigeria.*

Abstract: The study was conducted to determine metagenomic and physiocochemical analysis of bacteria at two locations in FUTO and Nekede in Otamiri River Imo State**.** In present study, water samples were collected from two different sites and coded FUTO and Nekede samples. Various physicochemical parameters were estimated by following the standard methods of APHA and the concentration of heavy metals were measured using Atomic Absorption Spectrophotometer (AAS). In case of physicochemical parameters characterization, the obtained values indicated alteration in the physicochemical properties of the samples. The results revealed that the two study locations water samples contains heavy metal concentrations (Cadmium) below the desirable and admissible levels by the WHO. During analysis of culturable bacteria in the water, a substantial bacterial diversity was observed in the Nekede (976902.43 ± 2987.32) water samples than FUTO samples (873455.78 ± 3245.54). The water samples were subjected to metagenomic analysis which revealed that Proteobacteria (phylum), Betaproteobacteria (class), Burkholderiales (order), Comamonadaceae (family), Hydrogenophaga (genus) and Verrucomicrobiota (species) were found as the most dominant bacterial taxonomic abundance in the Nekede and FUTO Otamiri water samples. The presence of such bacterial communities in water indicates the availability of pollutants and suggests the futuristic use in the field of bioremediation.

Keywords: *Physico-chemical, Heavy metals, Metagenomics, FUTO, Nekede, Imo State.*

1. Introduction

Water is essential for human life and productivity, yet both water quality and security are increasingly under threat globally (Amadi et al., 2022). Unfortunately, while wealthy countries are able to afford effective water treatment, poorer nations are severely hampered by a lack of resources for safe water to protect public health. In both cases, sources of contaminants entering waterways are not sufficiently addressed.

Water pollution is a major problem globally. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily (Matthew, 2016). Water pollution describes the presence of materials in water that interferes unreasonably with one or more beneficial uses of water. Water pollution, especially surface water, can be caused by mining, recreational and agricultural activities. Agents of surface water pollution include bacteria, viruses and other substances present in such concentration or numbers to impair the quality of the water

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rendering it less suitable or unsuitable for its intended use and presenting a hazard to man or to his environment (Nwanebu et al., 2019).

The supply of clean and uncontaminated water is a great challenge facing developing nations. Water bodies in developing countries are predisposed to pollution (Mendes et al., 2019). In Nigeria, pollution is a major threat to both surface and underground water bodies. This emanates mostly from indiscriminate dumping of refuse, untreated sewage, oil spillage, etc. Apart from problem of accessibility of clean water from these contaminated water bodies, it is known that pollution of water could lead to health hazard, sanitary nuisance, and severe economic and social consequences.

Incidence of diseases such as typhoid, paratyphoid, giardiasis, infectious hepatitis, leptospiriosis, schistomiasis, shigellosis, amoebiasis,etc., could be inherent from consumption of contaminated water. The pathogens associated with these diseases have been directly or indirectly detected as having link with contaminated water (Middelbos et al., 2020). Aside microorganisms, water bodies are also known to contain numerous chemical elements at different levels. These chemicals also add to give information on the pollution status of water although; some may not constitute a health hazard to the health of people directly but may give room to the growth or presence of some microorganisms, which may impair health in water.

Bacteria can be described as either pathogenic which causes diseases. Pathogenic bacteria can overcome the body's natural defenses and invade healthy tissues. In addition, opportunistic or secondary pathogens are those that can cause an infection when an unusual opportunity, such as an open wound or suppressed immune system, presents itself (Mikkola et al., 2017). In addition to air, water is essential for human beings, animals and plants. It is believed that man can survive without food but not without water for a long while. It is a group of microorganisms all of which lack a distinct nuclear membrane and hence are considered more primitive than animal and plant cells) and most of which have a cell wall of unique composition (many antibiotics act by destroying the bacterial cell wall). Most bacteria are unicellular. The cells may be spherical (coccus), rod-shaped (bacillus), spiral (spirillum), comma-shaped (vibrio) or corkscrew-shaped (spirochaete). Generally they range in size between 0.5 and 5µm. Motile species bear one or more fine hairs (flagella) arising from their surface. Many possess and outer slimy capsule and some have the ability to produce an encysted or resting form (endospore).

River water can be influenced directly and indirectly by microbial processes which can transform both organic and inorganic constituents. According to Matthew, (2016), single and multi-celled organisms have become adapted to using the dissolved materials and suspended solid matter in the aquifer in their metabolism and then releasing the metabolic products back into the water. There is practically no geological environment at or near the earth's surface where pH condition will not support some form of organic life (Cho et al., 2021).

Freshwater habitats such as lakes, rivers, streams and wetlands offer precious ecosystem services to humans like drinking water, fisheries, recreation as well as affect the global carbon budget via oxidation, storage and release of terrestrial carbon. These lakes present an ecological border between humans and a variety of host organisms. Freshwater lakes consist of 0.26% of total fresh water and 0.007% of total water on earth. The diversity of unculturable lake microbiota provides vast insights for microbiologists to investigate metagenome ecology for taxonomic identification and to study ecological implications.

Metagenomics is defined as the direct genetic analysis of genomes contained with an environmental sample. The field initially started with the cloning of environmental DNA, followed by functional expression screening (Chinedu et al., 2018), and was then quickly complemented by direct random shotgun sequencing of environmental DNA. Recent developments in whole genome sequencing (WGS) technology make it feasible to use DNA sequencing for disease diagnostics and public health surveillance of pathogens. Development of portable sequencing platforms, such as the Oxford Nanopore Technologies MinION device, allow for rapid sequencing of whole genomes, which facilitates metagenomic sequencing to be accomplished in remote locations. Portability can be especially useful during disease outbreaks where laboratory resources are limited, and when coupled with real-time analysis, can facilitate prompt epidemiological study and public health response to epidemic outbreaks.

Metagenomics is a tool for exploring the genetically rich resources of uncultured microbiota without using conventional culturing methods and is based on the principle of direct isolation of DNA from a complex environmental sample containing diverse microbiota to reveal the true microbial composition of that environment (Chinedu et al., 2018). The Next Generation Sequencing (NGS) made these metagenomic studies more reachable via targeted metagenomics, i.e., specifically chosen amplified regions of genomic DNA like 16S amplicon sequencing.

Metagenomic sequencing has been used to study the epidemiology of a variety of infectious disease agents. In one study, a metagenomic approach proved more accurate than conventional genotyping in analyzing an outbreak of tuberculosis, namely by improving identification of single nucleotide polymorphisms and assignment of genome clusters (factors related to the evolution of the outbreak strain) and tracing the spread of the outbreak.

Metagenomics currently is used to describe microbial populations in water and sediment to understand community structure and the role of microorganisms in ecological processes. Metagenomics has also been used to examine water quality to protect public health. Traditional methods for monitoring water quality focus on fecal coliform counts, but methods employing metagenomics provide additional functional and genomic information for species and strains of microbial pathogens. In addition, markers of the potential for antibiotic resistance, and the presence of virulence genes can also be identified in recreational and source waters.

Rather than target identification of a pre-selected group of pathogenic microbes or virulence genes by traditional culture, microscopy, immunoassay, or PCR-based methods, metagenomics employing next generation sequencing allows accurate identification and characterization of all microorganisms within samples for which genomic data are archived. Further, DNA sequence-based identification and characterization can now be done for microorganisms not easily cultured in a diagnostic setting, and can be used to identify multiple pathogens present in a poly-microbial infection or in water bodies.

Otamiri river, one of the major rivers that flow through Owerri the capital city of Imo State in south eastern region of Nigeria and Its environs, is among such water bodies threaten by pollution as a result of waste disposal. Apart from the agrochemicals that are being washed into Otamiri river from farm lands surrounding its banks during rain fall, some industries, institutions, and outlets situated along the river also empty their waste water into the river. Due to its importance as water resource to the populace within Owerri municipality and its delicate environs, the present study will investigate metagenomic analysis of bacteria in the river body with a focus on FUTO and Nekede, Imo state.

2. Materials and Methods

2.1 Study Area

The Otamiri River is a major fresh surface water resource of South-eastern Nigeria. The river takes its name from "Otamiri", a deity who owns all the water that is called by its name, and who is often the dominating god of Mban houses. It is located on latitude 5˚23'N and 5˚30'N, and Longitude 6˚58'E and 7˚04'E (Figure 1). The river runs south from Egbu (its source) pass Owerri and through Nekede, Ihiagwa, Eziobodo, Obowuumuisu, Mgbirichi and Umuagwo (all in Imo state) to Ozuzu in Etche Local Government Area of Rivers state where it has a confluence with Oramirukwa River; both rivers flow from there into the Atlantic Ocean (Fagorite et al., 2019). According to Fagorite et al. (2019), Otamiri River is used for domestic, industrial and agricultural activities. The stream sediments on the river are used for various construction purposes.

2.2 Sampling strategy

Purposive sampling will be employed in this study by focusing on FUTO, Nekede, and Umuagwo axis that will be also being investigated for water chemical composition. The intention will be to use the water chemical composition data to better understand bacteria community structure, abundance and composition. The water samples will be collected from pre-selected sampling areas to generate information on their microbiological water quality in the dry and wet seasons.

2.2.1 Sampling procedure

This will be carried out following the method of Amadi et al., (2022). Two water samples each will be collected in sterile 200 ml falcon bottles from each study location. The bottles will be lowered into the water for water collection using a rope which will be tied to the sterile bottles. Thereafter, the samples will be preserved before analysis.

2.2.2 Membrane Filtration

The membrane filtration technique is used to examine water samples from different sources. An appropriate volume of the sample is filtered through a membrane with a pore size of 0.45 mm. The membrane is incubated on an agar plate. Bacterial (and other) cells trapped on the membrane will grow into colonies that can be counted, and a bacterial density can be calculated. When using the membrane filtration technique to test for the presence of indicator microorganisms, different filtration volumes are suggested depending on the source of the water sample. The Membrane Filter (MF) technique is an effective and accepted technique for testing fluid samples for microbiological contamination. The technique was introduced in the late 1950s as an alternative to the Most Probable Number (MPN) procedure for the microbiological analysis of water samples. The MF technique offers the advantage of isolating discrete colonies of bacteria, whereas the MPN procedure only indicates the presence or absence of an approximate number or organisms (indicated by turbidity in test tubes).

This membrane filtration method was accepted by the U.S. EPA for microbiological testing of potable water in the 11th edition of Standard Methods for the Examination of Water and Wastewater. In the 1978 publication, Microbiological Methods for Monitoring the Environment, the U.S. EPA stated that the MF technique is preferred for water testing because it permits analysis of larger samples in less time. The method follows some very simple steps such as. The sample is poured onto a filter membrane, usually inside a funnel, and then filtered through. The funnel is then rinsed, and the membrane filter placed on a culture medium for culture and analysis.

2.2.3 Culture of bacteria from water

The collected water samples will be processed for bacterial culture. In order to widen the scope of bacterial isolation, the water in each 200 ml falcon tube will be centrifuged at a speed of 7 000 xg for one hour to concentrate the bacteria. After centrifugation, each volume will be reduced to 10 ml by discarding the supernatant thereby leaving the pellet suspended in 10 ml and then 0.1 ml will be streaked on the selective and differential MacConkey agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA). This media will be used to isolate and detect gram-negative bacteria according to manufacturer's guidelines.

Briefly, MacConkey agar was prepared by suspending 52 g of the medium in 1000 ml of distilled water and boiled to dissolve completely. The media will be sterilized using an autoclave at 121° C for 15 minutes. The agar will be left to cool at room temperature (26° C) in a fume hood, upon which about 30 ml of agar was poured per petri dish left to solidify. After solidifying, the sterility test will be performed at key points where contamination can potentially be introduced such as the incubator where microbial growth is enhanced, and the fume hood where inoculation will be done. The sterility test will be performed by placing uncapped petri dishes containing Tryptone soya agar (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for seven days at the work stations to detect contamination by bacteria before inoculation. After confirmation of sterility, inoculation will be performed under the fume hood and the plates will be inverted and incubated at 35° C for 48 hours for total coliform counts. With regards to thermotolerant coliform counts, the plates will be incubated at 45° C for 48 hours. This media provides a basis for total coliform counts and thermotolerant coliform counts, and also distinguished between lactose-fermenting and none lactose fermenting gram-negative enteric bacilli.

2.2.4 Biochemical Tests

- **Citrate Utilization Test**

This was carried out using the method as described by Cheesborugh (2005).

- **Sugar Fermentation Test**

This was determined according to Ochei and Kolhatkar, (2000).

- **Motility Tests**

This will be done according to Fawole and Oso, (2004) method

- **Catalase Test**

This was carried out according to the method by Cheesbrough, 2005.

- **Indole Test**

This was done according to the method adopted by Cheesbrough 2005

- **Oxidaze Test**

The method of Cheesbrough 2005 was adopted

2.2.5 Determination of the relative abundance of bacteria species

According to the method of Boamah, (2018), the relative abundance of each microbial phylum in each location will be determined by dividing the sum of counts of each phylum from all samples by the total counts of all the phyla from all the samples and multiplying the product by 100 to get a percentage. The percentages will be rounded to two decimals places and subsequently used to generate a Sunburst chart. This chart was display the relative abundance of each bacterial phylum detected. Hence, the representation of each phylum was explored and only the phyla with a significant (> 1%) representation was shown on a Sunburst chart while the rest were combined to form the “Others” group on the Sunburst chart.

2.2.6 bacterial species diversity, evenness and richness

Species diversity is the number of species and abundance of each species present in a particular area, while species richness is the number of species present in a particular area (Pielou, 1975). Species evenness is the measure of the relative abundance of different species in a particular area (Pielou, 1975). Species diversity, evenness and richness are fundamental in determining ecosystem health, and in the present study it gave an indication of contamination levels. In each location, bacterial species richness was counted, and Shannon-Wiener diversity indices, Simpson's diversity indices and species evenness was calculated to determine the species diversity, richness and evenness using the formulas described by Uthappa et al., (2016).

2.3 Metagenomics analysis of bacteria

2.3.1 DNA extraction and 16S rRNA gene amplification.

Each water sample containing a volume of 200 ml will be centrifuged at a speed of 7 000 xg for one hour in order to concentrate the bacteria. Each volume will be reduced to 10 ml of water after centrifugation by discarding the supernatant. DNA will be extracted from a two microliter volume using SEEPREP 12 TM kit (Seegene, Rockville, USA), and its concentration and quality determined by the NanoDrop-2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

2.3.2 Metagenomics bacterial species groupings and analysis

The taxonomically classified sequences with bacterial identity was separated into four categories namely; zoonotic, humans, livestock, and grey with various emphases as described below;

1. Zoonotic categories was focused on bacteria which cause diseases in both humans and livestock.
2. Human’s category was focused on bacterial species that cause diseases in humans only.
3. Livestock category was focused on bacterial species that cause diseases in livestock only, and
4. Grey category was focused on bacterial species not known to cause diseases in both humans and livestock.

2.3.3 Metagenomics Data Analysis

Microbial BLAST (Basic Local Alignment Search Tool) analysis was carried out following the method of Khatiebi et al., (2023).

2.4 Statistical analysis

Data collected from this study was analyzed using routine statistical tools, percentages, standard deviation, graphs, t-test and analysis of variance (ANOVA) and the differences were determined at 95% level of confidence.

3. Results and Discussion

Alpha and beta diversity

The Alpha diversity indexes for the water samples of FUTO and Nekede were analyzed and the results are summarized in Figure 1. Indices were consistent between the two sampling sites of the FUTO samples and showed a higher level of prokaryotic diversity compared to the water of the Nekede. The number of observed species from Nekede was 402% higher when compared to the FUTO samples indicating not only a greater number of species but also a wider spread of taxonomic units.

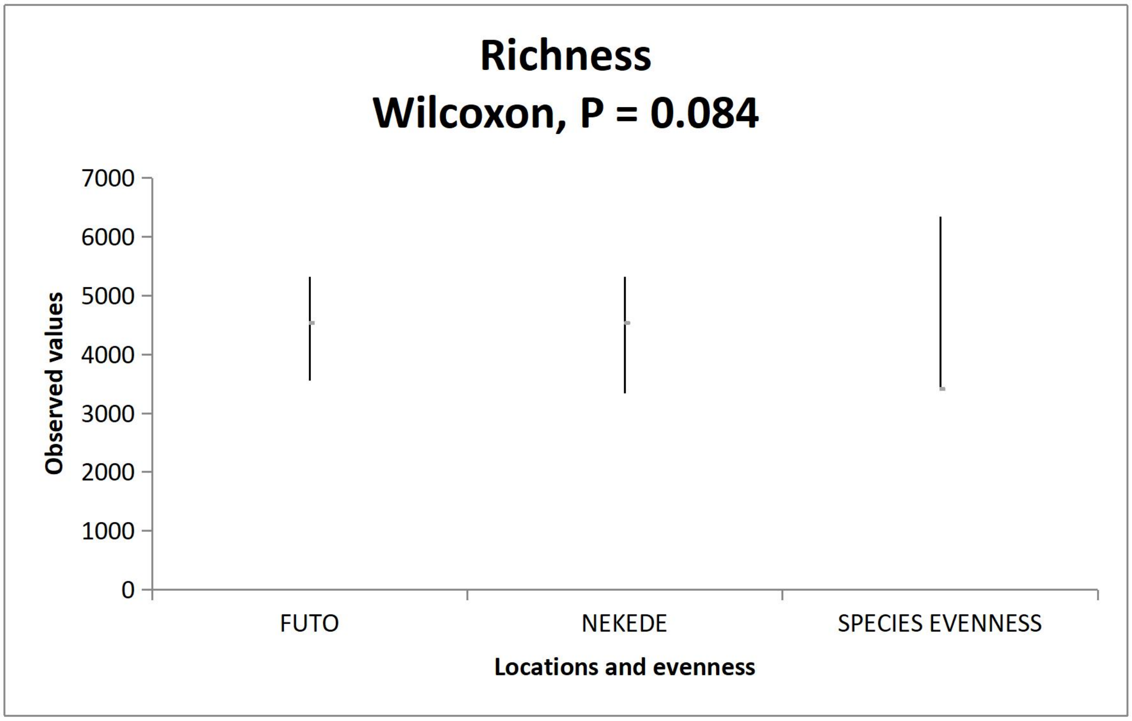

Figure 1: Alpha-diversity metrics on species richness and evenness in FUTO and Nekede Otamiri River

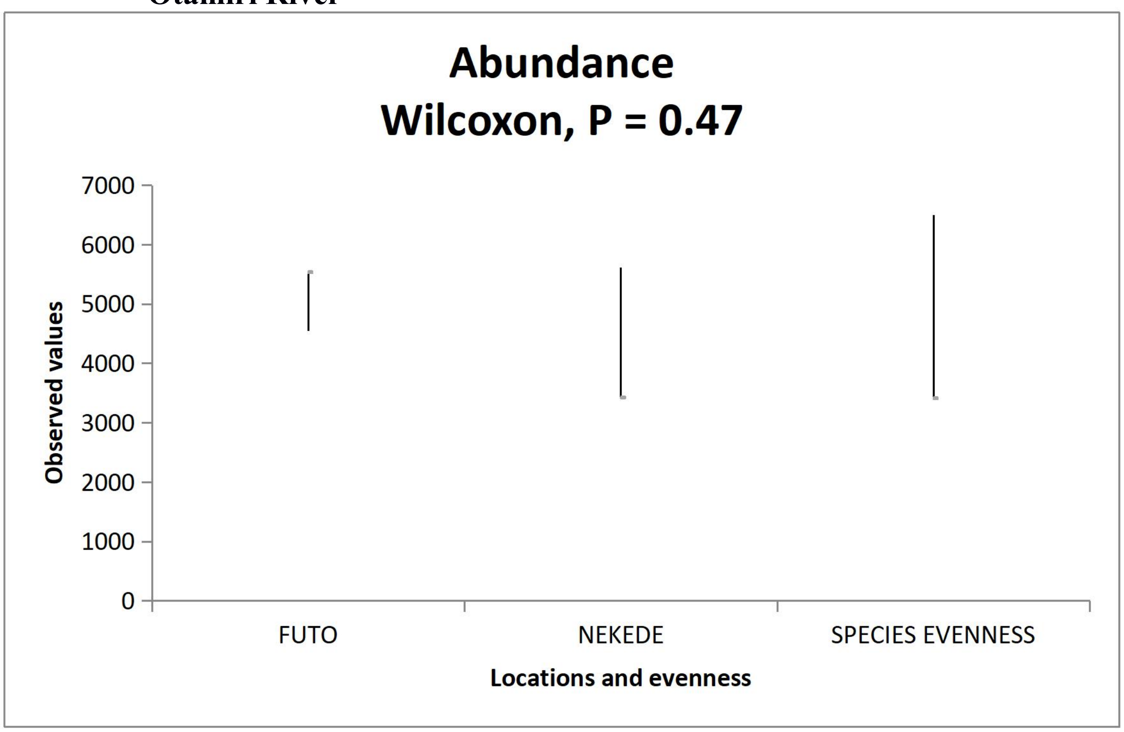

Figure 2: Alpha-diversity metrics on species abundance and evenness in FUTO and Nekede Otamiri River

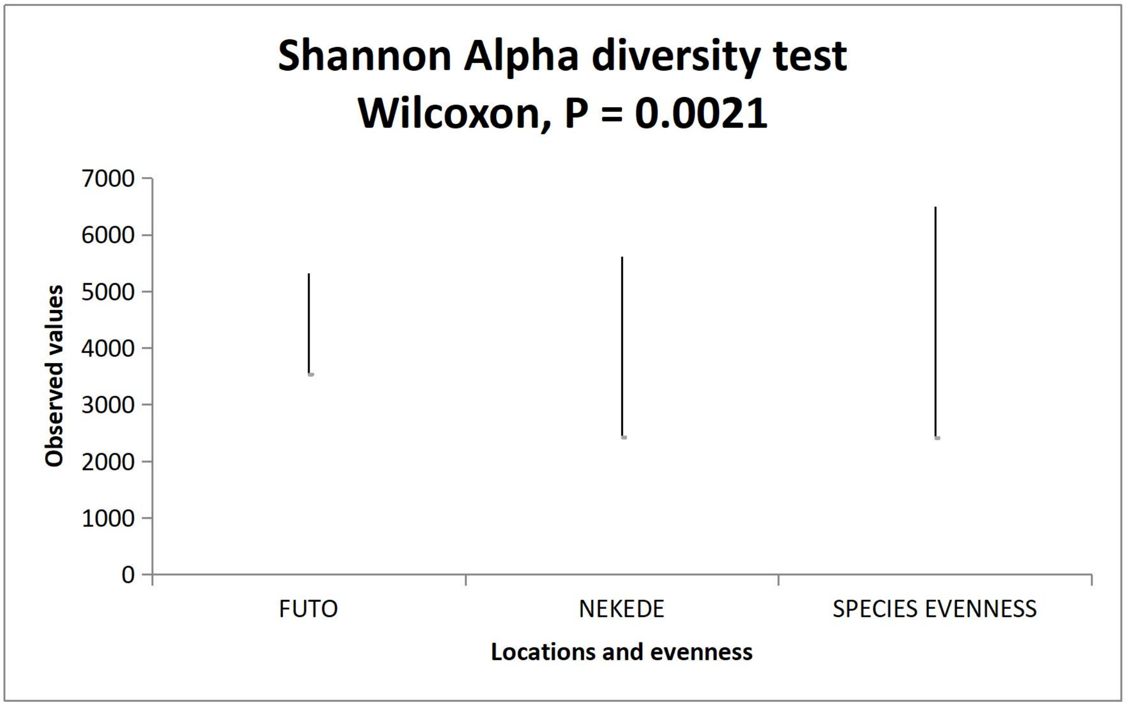

Figure 3: Shannon Alpha-diversity and evenness in FUTO and Nekede Otamiri River

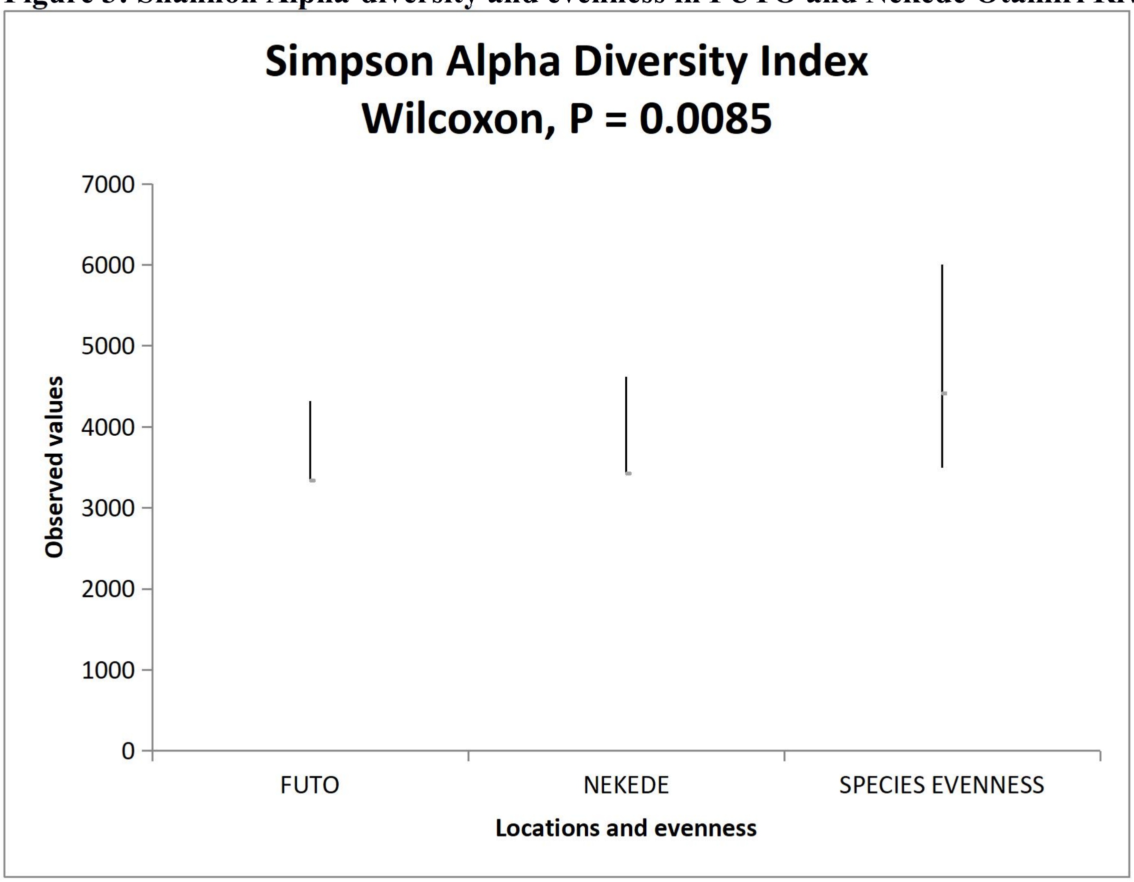

Figure 4: Simpson Alpha-diversity index and evenness in FUTO and Nekede Otamiri River

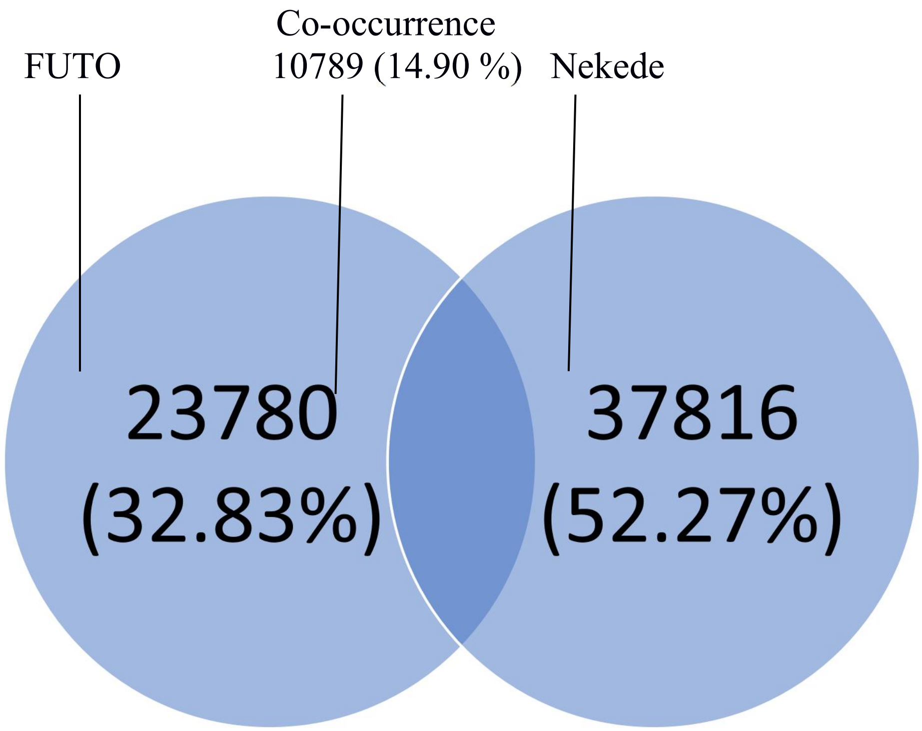

Figure 5: Overall microbial diversity in FUTO and Nekede Otamiri River

4. Discussion

Rivers have played a very important role in development of civilization, culture, settlement of urban area thus it plays a critical and crucial role in the prosperity of a nation affecting the different aspects of its economic status. Otamiri River is one of the most sacred still most polluted rivers of Imo State.

pH indicates the intensity of the acidic or basic character of a solution and is controlled by the dissolved chemical compounds and biochemical processes in the solution. It is an important indicator of water quality and pollution level in the aquatic environment. It is closely linked with biological productivity. In the present study, the pH values from both locations shows slightly alkaline nature (pH 7.9 ± 1.34 to 8.4 ± 1.42). The alkaline nature of these crude oil polluted waters may be relatable with biological activity in both waters. pH range obtained in this study is in line with previous work of Arya, Bohra, Ashish & Tewari (2020). According to Abbas & Al-Seria (2020), pond water released into the environment at this pH is not likely to pose any harm to the environment. These values are within the permissible limit of 6.5 – 8.5 by WHO for drinking and irrigation purposes, respectively. The statistical analysis at 95% confidence level also showed no significance differences among the studied sites with respect to pH levels from both FUTO and Nekede environments. This is in line with the works of Mgbemena, Ilechukwu, Amalu, & Anierobi (2021).

Electrical conductivity reflects the ability of water to conduct electricity. This electrical conductivity is due to the substances dissolved in the water which breaks down into positively and negatively charged ions. The conductivity of the two study locations environments varied significantly ($p<0.05$). It was generally lower in FUTO water than in Nekede crude oil contaminated water. Contaminated waters have access to many agricultural run-offs, i.e dissolved chemicals summarizing the very reason for higher electrical conductivity observed in this study. Similar trend had been observed by Saborni, Kamal, Mustafa , Afroza, Badhan, Pritika & Mohammad (2020).In related to this study, Ramulu & Benarjee (2019) observed high and low values of conductivity in crude oil conytaminated waters respectively.

Total Dissolved Solids of water is the amount of dissolved inorganic salts and organic matter present in water. Water containing more than 500 mg/L of TDS is not considered as a potential source of pollution. Water with high TDS is undesirable or harmful for human and aquatic life. It may taste bitter, salty, or metallic and may have unpleasant odors. High TDS water is also less thirst quenching and interferes with the taste of foods (Famoofo, &Adeniyi, 2021). The present study showed that the mean TDS values varied in the two sampling environments (FUTO and Nekede) with FUTO aquatic environment having slightly lower values than the Nekede environment. These values were with the permissible limits of WHO. The statistical analysis at 95% confidence level showed no significant differences between the two sampling sites. The elevated levels of TDS recorded in the water might be due to agricultural runoff, discharge of wastes from the town, and other human activities like washing of different vehicle at and around the study areas. According to Ilechukwu, Olusina, Echeta (2021), most aquatic ecosystems involving mixed fish fauna can tolerate TDS levels of 1000 mg/l. This corroborates the report of Mgbemena, *et al.,* (2021).

The turbidity of the samples in all the study locations was higher in Nekede crude oil contaminated water than the FUTO crude oil contaminated water. Highly turbid waters require some treatment before release into environment. The high turbidity of some of the samples may be due to poor housekeeping. Ponds that are always washed and kept clean will be less turbid. Turbidity may also be as a result of over population. Introduction of feeds and metabolic activities of fishes in the pond such as excretion contribute to the turbidity of fish pond water. Release of high turbid fishpond water into the environment destroys the aesthetic nature of the environment. This is in line with the report of Abideen et al., (2022).

Heavy metals such as Cadmium are a known cause of long-term health effects in humans. Such effects include not only nefarious effects of acute and/or chronic toxicity, but also special cases of toxicity such as carcinogenicity and genotoxicity. From analysis of the waters of the FUTO and Nekede there were relatively low concentrations of certain elements, the highest being cadmium. Heavy metals have been associated with many deformities in natural populations and laboratory specimens. Mean values of cadmium obtained from this study is higher than 1.7m/kg reported by Abideen et al., (2022) but within the limit of 0.03mk/kg reported by Njoku et al. (2024). However, the study also established higher microbial diversity and heavy metal load in Nekede sample than FUTO sample. This may be attributed to difference in anthropogenic activities in the two sampling locations under study.

Many recent studies have investigated the bacterial communities of particularly with an ecological interest (Massello et al., 2020, Escuder-Rodríguez et al., 2022, Rupasinghe et al., 2022, Chen et al., 2023). In this study, results showed sizable variation in prokaryotic community structure between the FUTO and Nekede, as demonstrated by the alpha and beta diversities between the two sites. These differences are likely driven by the variations in environment factors, such as temperature, pH and concentration of certain elements. The warmer temperature of the two study locations clearly could have had an impact on the prokaryotic diversity.

There are many metagenomic researches done on polluted water sample to identify the bacterial contamination (Ding et al. 2017; Saleem et al. 2018; Samson et al. 2019; Behera et al. 2020). This study of metagenomic sequencing of water sample from FUTO Otamiri River and Nekede revealed different organisms for the evaluation of microbial diversity and abundance. The results showed that Proteobacteria (phylum), Betaproteobacteria (class), Burkholderiales (order), Comamonadaceae (family), Hydrogenophaga (genus) and Verrucomicrobiota being the most dominant bacterial taxonomic abundance in the FUTO and Nekede samples. The major phylum (Proteobacteria) with predominant class of Alpha-, Beta-, Gamma-, and Delta-Proteobacteria were present in the sample in high abundance. This is similar to other studies performed on freshwater systems (Tsagaraki et al. 2018).

A similar study has been done on Yamuna River at Kalindi Kunj, Delhi in which Proteobacteria (33.68%) and Bacteriodetes (24.07%) showed maximum abundance at phylum level. Similar results were also observed by Wang et al. (2014), Miyashita (2015) and Liu et al. (2012) who reported Proteobacteria as the most prevalent phylum found in domestic sewage water, drinking water as well as in soil. Many researchers (Kent et al. 2001; Li et al. 2006 and Schnetzer et al. 2011) reported the dominance of Proteobacteria in wastewaters and soils. α-Proteobacteria is the dominant Proteobacterial class in the marine microbial community found at the surface of the Yellow Sea (Bai et al. 2009; Sogin et al. 2006).

Metagenomic study for identification of microbial communities in water helps in the exploration of novel genes with different metabolic functions related to respective organism. The alarming levels of pollution in rivers necessitate the functional analysis of microbial communities. These results could conclude that sample may be rich in hazardous pollutants. Hence, we can state that a substantial bacterial diversity was observed in the FUTO otamiri river and Nekede water samples collected from different sites. From previous studies, it is clear that the two study locations harbor species that are not just of interest ecologically and environmentally, but likewise in an applied sense.

From the results and observation it can be concluded that the quality of FUTO Otamiri and Nekede water samples is highly contaminated at all sampling sites that may be due to small and large scale industries, disposal of untreated sewage and agricultural sectors. This study is helpful in addressing the issues of polluting rivers contaminated with pathogenic bacteria and the respective pollutants. The information will help in making of effective plans in order to improve the water quality for its consumption. Also, the study helps in understanding of different microbial communities or microbial population dynamics in the contaminated water which cannot be cultured and help in the development of future remediation strategies related to them.

The Otamiri river water of is heavily contaminated with toxic pollutants including heavy metal that causes severe damage to ecological and social aspects. At present, the direct use of the river water for domestic use causes severe hazards due to anthropogenic activities causing environmental pollution in the river. The physicochemical properties of the crude oil contaminated waters from FUTO and Nekede locations show the waters to be a complex environment and with the use of

16 S rRNA metagenomic sequencing this study has highlighted a highly diverse bacterial and archaeal community in the study locations, which differed significantly between the FUTO and the Nekede. Current study results help in recognizing the structure of bacterial communities at the FUTO and Nekede area in relation to their surroundings for planning for environmental protection and future restoration of affected ecosystems. The findings highlight the dominance of various bacterial phyla, classes, families, and genera, with remarkable species richness in some areas. These results underscore the influence of human activities on microbial diversity, as well as the significance of monitoring and conserving the reserve's natural ecosystems.

Metagenomics is a high useful tool for understanding the uncultured microbial fraction and can help guide further studies and more detailed analysis. This not only has application and benefit for understanding global microbial diversity, but also can be beneficial for applied studies, for example in biotechnology or anti-microbial resistance. Whilst metagenomics is a highly beneficial tool for understanding microbial diversity, it is best applied in a holistic approach, incorporating culture based studies, modelling and microscopy, to gain a full understanding of community structure and function.

Broadly, this study aimed to contribute to the use of metagenomics in the field of environmental microbial ecology, in terms of providing both methodological advancements and to broaden understanding of microbial diversity using FUTO and Nekede areas of Imo State as a case study.

Recommendations

1. Analysis of Metagenomics of bacteria species from other areas of Imo State not covered in this study.
2. Further studies should be carried out to establish potential risks of heavy metals detected in the study areas.
3. The use of transcriptomics is recommended as useful addition to metagenomics analysis, which involves sequencing the microbial community mRNA

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