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PHYSICOCHEMICAL ANALYSIS OF BACTERIA AT TWO LOCATIONS IN FUTO AND NEKEDE OTAMIRI RIVER IMO STATE

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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.*

Introduction

The problem under investigation is that the microbial water quality of otamiri water and its metagenomic analysis is unknown [1]. However, previous research had focused on microbial load analysis and hydro-geochemical processes respectively, and did not pay particular attention to the microbiological metagenomics of water quality [2]. Based on this, there is a knowledge gap in relation to the microbial water quality in the otamiri river of Imo State. This is undesirable since water is a habitat for some pathogenic microorganisms. Moreover, disease outbreaks transmitted through contaminated drinking water are of grave concern worldwide especially in underdeveloped countries which experience 99.8% deaths of the cases annually [3]. In addition, lack of knowledge on the microbial water quality and safety of this river is a barrier towards relating the cause of water borne disease outbreaks to the water quality and the development of appropriate remediation strategies [4]. Furthermore, there is a poor understanding of the ecology of microbial communities in river body. Hence, this study employed culture-dependent (enrichment and culturing) and culture-independent (Metagenomics) bacterial analyses of otamiri river to reveal the entire microbial communities [5], and the metagenomic identity of the species present.

Materials and methods

Brief Description of Study Area

The study was carried out in Imo State Nigeria from August to November, 2024. The Otamiri River is a major fresh surface water resource of Southeastern 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. 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 [6]. According [7], Otamiri River is used for domestic, industrial and agricultural activities. The stream sediments on the river are used for various construction purposes.

Sampling strategy

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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 were collected from pre-selected sampling areas to generate information on their microbiological water quality in the dry and wet seasons.

Sampling procedure

This was be carried out following the method of [8]. Two water samples each was collected in sterile 200 ml falcon bottles from each study location. The bottles were lowered into the water for water collection using a rope which was tied to the sterile bottles. Thereafter, the samples were preserved before analysis.

Culture of bacteria from water

The collected water samples was 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 was 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 was 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 was sterilized using an autoclave at 121° C for 15 minutes. The agar was 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 was 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 was inverted and incubated at 35° C for 48 hours for total coliform counts. With regards to thermotolerant coliform counts, the plates was 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.

Biochemical Tests

Citrate Utilization Test

This was carried out using the method as described by [8]

Sugar Fermentation Test

This was determined according to [9]

Motility Tests

This will be done according to Fawole and Oso, (2004) method[10]

Catalase Test

This was carried out according to the method by [10]

Indole Test

This was done according to the method adopted [10]

Oxidaze Test

The method of [10 was adopted

Determination of the relative abundance of bacteria species

According to the method of [11] the relative abundance of each microbial phylum in each location was 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 was 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.

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.

Results

Physicochemical Composition of water samples contaminated with crude oil (Mean ± SD)

The physicochemical properties from crude oil contaminated waters samples from from FUTO and Nekede is presented in Table 1. The physicochemical parameters determined in the samples were pH, Temperature, Electrical Conductivity (µS/cm), Total Dissolved Solid(S/m), Alkalinity (mg/l), and Turbidity (NTU). Their levels were compared with World Health Organization (WHO, 2016) permissible limits for these parameters in effluent discharged into fresh water bodies. Results obtained showed that the mean values of pH across the sampled areas ranged between 7.9 ± 1.34 in FUTO samples to 8.4 ± 1.42 from Nekede with corresponding highest values obtained from Nekede. This pH level did not differ significantly ($p>0.05$) across the two sampling locations. The mean pH range across the sampled areas were observed to be within WHO permissible limit of 6.5 – 8.5 in effluent. Average values of Temperature ranged from 28.5 ± 0.41 in FUTO to 28.3 ± 0.31 in Nekede; no significant difference exist in EC between the two locations as the values ranged between 0.110 ± 0.07 in FUTO to 0.110 ± 0.07 in Nekede; TDS ranged from 0.030 ± 0.015 in FUTO to 0.050 ± 0.029 in Nekede; mean values for Alkalinity and Turbidity differed as follows: 6.12 ± 1.02 to 5.34 ± 0.93 and 0.012 ± 0.001 to 0.007 ± 0.001 respectively.

*Corresponding Author: **UGAYA, J. A**

Table 1: Physicochemical Composition of water samples contaminated with crude oil (Mean ± SD)

Parameters	FUTO Sample	Nekede Sample
pH	7.9 ± 1.34	8.4 ± 1.42
Temp (^{0}C)	28.5 ± 0.41	28.3 ± 0.31
EC (µS/cm)	0.110 ± 0.07	0.110 ± 0.07
TDS (mg/L)	0.030 ± 0.015	0.050 ± 0.029
Alkalinity	6.12 ± 1.02	5.34 ± 0.93
Turbidity (TU)	0.012 ± 0.001	0.007 ± 0.001

EC, Electrical Conductivity; TDS, Total Dissolved Solids; TSS, Total Suspended Solids.

Heavy Metals in Water Contaminated with Crude Oil

Heavy metal concentrations in crude oil contaminated water from FUTO and Nekede study locations are presented in Tables 4.2. The concentration of Mercury, and Arsenic, varied significantly ($p<0.05$) among the sampling locations. The highest concentration of detected heavy metals was Cadmium from FUTO (0.001 ± 0.0001) and Nekede (0.003 ± 0.001). The result from this study shows that the detected heavy metals were within maximum limit standard set by WHO.

Table 2: Heavy Metals in Water Contaminated with Crude Oil

Sample Locations/Stations	WHO standard	FUTO Sample	Nekede Sample
Mercury (ppm)	0.001	ND	ND
Cadmium	0.01	0.001 ± 0.0001	0.003 ± 0.001
Arsenic (ppm)	0.02	ND	ND

ND, Not Detected; values is insignificant compared to WHO standard.

Information on phyla groups into respective sources with number of samples per group

Information on phyla groups into respective sources with number of samples per group is presented in Table 3. The results shows that a total of 12 bacteria phyla belonging to different species were identified from the study locations. Out of the 12 phyla identified, V*errucomicrobiota* from FUTO location recorded the highest mean value of 976902.43 ± 2987.32 followed by Nekede (873455.78 ± 3245.54) while the least phyla identified from the study locations was Planctomycetota with a mean value of 154153.19 ± 1231.07 from FUTO and 354218.05 ± 1841.82 from Nekede.

Table 3: Information on phyla groups into respective sources with number of samples per group

Phyla	Source	N	Mean ± SD	Mean difference	P - value
Verrucomicrobiota	FUTO	3	873455.78 ± 3245.54	745.32	0.002*
	Nekede	3	976902.43 ± 2987.32	233.71	
Proteobacteria	FUTO	3	632753.87 ± 2126.38	425.76	0.002*
	Nekede	3	643457.42 ± 3245.21	345.32	
Actinobacteriota	FUTO	3	736551.35 ± 2374.60	333.31	0.002*
	Nekede	3	772853.72 ± 2245.33	325.21	
Bacteroidota	FUTO	3	474955.55 ± 3349.21	282.43	0.002*
	Nekede	3	576902.43 ± 2443.11	271.72	
Cyanobacteria	FUTO	3	522789.03 ± 2276.54	217.32	0.015
	Nekede	3	522845.74 ± 3533.14	209.24	
Desulfobacterota	FUTO	3	64302.43 ± 2237.29	233.48	0.015*
	Nekede	3	702323.72 ± 2240.32	217.63	
Armatimonadota	FUTO	3	323454.80 ± 2178.19	249.25	0.034
	Nekede	3	323900.83 ± 2422.40	233.71	
Acidobacteriota	FUTO	3	325753.15 ± 1122.01	198.87	0.034*
	Nekede	3	732333.73 ± 1144.82	134.53	

*Corresponding Author: **UGAYA, J. A**

Chloroflexota	FUTO	3	130532.16 ± 1022.88	198.87	0.012*
	Nekede	3	183223.32 ± 1034.04	134.53	
Bdellovibrionata	FUTO	3	247552.33 ± 1432.01	198.87	0.002*
	Nekede	3	291123.70 ± 1724.03	174.43	
Firmicutes	FUTO	3	323453.32 ± 1312.01	158.89	0.014*
	Nekede	3	325338.41 ± 1832.05	134.53	
Platescibacteria	FUTO	3	323753.13 ± 1242.33	177.86	0.002*
	Nekede	3	522333.21 ± 1144.82	134.53	
Planctomycetota	FUTO	3	154153.19 ± 1231.07	151.80	0.013*
	Nekede	3	354218.05 ± 1841.82	134.53	

* = Sig. at P < 0.05

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 [12]. According to [12], 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 [13].

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 [14].In related to this study, [15] 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 [16]. 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, [3] most aquatic ecosystems involving mixed fish fauna can tolerate TDS levels of 1000 mg/l. This corroborates the report [18]

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 [19].

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 [11] but within the limit of 0.03mk/kg reported by [20] However, the

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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.

Conclusion

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

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