



Assessment of Microbial Load in Water and Sediments of Rivers Otamiri and Nworie in Owerri, South Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OJK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AJN and ISK managed the analyses of the study. Authors OJK, AJN, EJE and NPO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Water pollution is a global problem. The study was carried out to assess the microbial constituents in water and sediment of Rivers Otamiri and Nworie during dry and rainy seasons (March and September, 2020) with comparison to WHO benchmark for drinking water. Samples were randomly collected at six (6) sampling points and analyzed using routine microbiological protocols. The results revealed detectable amount of microbial activates in surface water of both rivers during the dry and rainy season. During the rainy season, a THB bioload average of 2.02×10^4 and 5.1×10^4 CFU/mL for Nworie and Otamiri river respectively were measured. For the TCC, the average was 9.8×10^3 and 2.5×10^4 CFU/mL, while in the dry period there was corresponding reduction in the bioload value for both rivers. THB average value for Nworie river was 1.34×10^4 CFU/mL, with corresponding 3.5×10^4 values for Otamiri river, while in same inclination, TCC

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measured 6.1×10^3 and 1.8×10^4 CFU/mL. There were noteworthy variations in the values for the two rivers as well as in the two seasons. TBC value for Otamiri measured about 1.5 folds than the value for Nworie. In same trend, TCC value for Nworie measured about 39%. All values measured were above WHO permissible limit for drinking water. The biochemical and cultural features of the isolated microorganism in water showed the presence of *E. coli* identified in the entire stations (100%), *Salmonella* and faecal coliform occurred 83.3% each, while *Vibrio* and *Shigella* were detected in 4 of the 6 stations. The mean total bacterial count, total coliform count and total *E. coli*, were not in conformity with World Health Organization (WHO) Standard for drinking water and thus constitute a threat to the River; these were attributed to indiscriminate waste dumps around the rivers. The study underscores the need for adequate waste management system to forestall outbreak of pathogenic diseases in the area.

Keywords: Microbial load; Otamiri; Nworie; water; sediment.

1. INTRODUCTION

Water is an essential natural resource for sustainability of all life forms on earth [1]. Water pollution is a major challenge facing developing countries such as Nigeria [2-5]. Water pollution occurs when unwanted materials with potentials to threaten human and other natural systems find their ways into rivers, lakes, wells, streams, boreholes or even reserved fresh water in homes and industries [6]. There are numerous scientific and economic evidence such as industrial production, recreational activities that water pollution can cause severe decrease in productivity and deaths of living species and subsequent alteration of ecosystem services [7-11]. According to Food and Agricultural Organization (FAO) African countries, particularly Nigeria, water related diseases had been interfering with basic human development [12]. Improper management of huge amount of wastes generated by various anthropogenic activities [13-15] indiscriminate dumping of refuse [16]; faecal, agricultural and industrial contamination or pollution which is continually threatening aquatic ecosystem due to increasing exposure of untreated wastes [17] and urbanization in developing countries has gradually led to the deterioration of water bodies in recent years [18].

Major agents of surface water pollution include bacteria, viruses and other substances present in such concentration or numbers to impair the quality of the water rendering it less suitable or unsuitable for its intended use and presenting a hazard to man and other components of the ecosystem [19-21]. The increase in microorganisms and anthropogenic contaminants enhances the risk of pathogen outbreaks, bacterial antibiotic resistance, and public health costs [17]. Incidence of diseases such as *typhoid*, *paratyphoid*, *giardiasis*, *infectious hepatitis*, *leptospirosis*, *schistosomiasis*,

shigellosis, *amoebiasis* etc., have been implicated in the consumption of contaminated water [20]. Some of these pollutants are decomposed by the action of micro-organisms through oxidation and other biochemical processes. The major problem is the re-concentrations of these harmful substances in natural food chain [22]. During the decomposition process, natural bacteria and protozoan in the water source utilize the oxygen dissolved in the water. This could significantly reduce the oxygen level to less than two parts per million (<2ppm), therefore the respiratory conditions of aquatic species would be seriously affected. Consequently, fishes, bottom-dwelling animals and even marine plants can be contaminated and/or killed, creating significant disruption in the food chain. On the other hand, when this contaminated water is directly consumed without proper treatment (a common practice to local communities), spread of diseases such as typhoid, dysentery, cholera, hepatitis e.t.c will occur [23]. Recently, [24] have documented varying levels of microbial contaminations in drinking water from western parts of the country. Total bacteria and coliform counts were found to be between 2.86 -4.45 and ≤ 1.62 log cfu/ml respectively. The major issues of national and international interest are how these water pollution problems could be fully assessed and mitigated, proper knowledge and planning are thus essential. A study by Lye [25] showed that 48% of the people in Katsina-Ala Local Government area of Benue state are affected by urinary *schistosomiasis*, due to increased in water pollution index. Some previous investigations indicate that 19% of the whole Nigerian population is affected, with some communities having up to 50% incidence. This has raised serious concerns to World Health Organization, in an attempt to improve cultural and socio-economic standards of people in the tropical region [12].

Constant monitoring of the quality of surface water cannot be overstressed especially now that increase in population has resulted in generation of more waste thus exposing the water to more pollutants [19]. According to Eja. [20] key waste sources at these two Rivers are offices, markets, households, hotels, hospitals but exclusive of toxic wastes. This problem has its roots to the indiscriminate disposal of industrial waste, runoff of oil and grease from the increasing filling stations, mechanic workshop and also the dumping of huge amount of refuse in water bodies [26]. Due to the increasing population in Owerri metropolis, there has been an increase in waste generation and also more modern industries are on the increase, but the fact still remains that the waste disposal system has not grown as fast as the waste being generated [20]. The discharge of municipal, industrial and agricultural wastes into fresh water and the resultant deleterious changes in water ecology have been reported by several researchers [13].

As opined by Eja [20], previous investigation on Otamiri and Nworie River have been basically limited to just the water quality and bioload [27]; effect of untreated sewage effluents on the water quality [28] without taking cognizance to the sediment content of the water bodies. The present study was undertaken to bridge this gap in knowledge. The aim of this research was to analyze and identify microbial constituents/load in the water and sediment samples of Otamiri and Nworie Rivers in Owerri, Imo state Nigeria with a view to ascertaining the potential ecological risks organisms and humans are exposed to as a result of consuming/using these waters.

2. MATERIALS AND METHODS

2.1 Description of Study Area

Study area is Owerri metropolis. Its metropolitan class informed its selection [29]. A typical representation of an urban area [30,29] lying on coordinates 5°28'3.59"N, 7°02'06.0E on a land spanning over 550 km² and comprising of three Local Government areas of the twenty seven in Imo State, namely Owerri West, Owerri municipal and Owerri North [29]. The study was carried out on the two Rivers in Owerri: Otamiri and Nworie Rivers. Otamiri River is one of the main rivers in Imo State that runs through Owerri Municipal City (see Fig. 1). Otamiri and Nworie rivers bound the metropolis. Otamiri river spans south from Egbu

through Owerri, Nekede, Ihiagwa, Eziobodo, Obowuumuisu, Mgbirichi and Umuagwo to Ozuzu in Etche Local Government Area of Rivers state into the Atlantic Ocean, covering a watershed of approximately 10,000 km² [31]. Depleted rain forest vegetation make up key portion of the watershed [32,31]. Otamiri river (see Fig. 2) confluences with Uramiriukwu at Emeabiam, 30km from its origin [32]. Nworie river spanning over 9km in length stream into Otamiri at Nekede in Owerri [32,29]. Contamination could be generated due to high anthropogenic activities that include some waste discharge [29].

According to Imo State Government [33] and [29], the average yearly rainfall ranges 1500-2200 mm with the heaviest period around June-August. So moisture content of waste is high and similar to other countries in the tropics with comparable rain pattern such as in Malaysia, where 53-66% moisture content has been reported [29]. Characteristic average annual temperature and humidity is 31°C [33,29] and 75%, with humidity soaring upto 90% during the rainy regime [33]. There is elevated hydraulic, transmissive, conductive and storage coefficient, due to the configuration of the area's stratigraphy, which conforms with the thick, friable earth of low intercalations of sandy clay lens and beds of Benin formation [34,29,33].

3. METHODOLOGY

3.1 Sample Collection

Water and sediments of Rivers Otamiri and Nworie were sampled 2 km apart during the dry and rainy season (March and September, 2020). All protocols were aseptically observed including ensuring all screens or aerators are removed and taking at least 100 ml of water sample after moderate flow of about 2-3 minutes, making sure about 2.5cm of head space is available in sample container. The samples were collected randomly from six (6) sampling points designated as [NRS1], [NRWS2], [NRWSS3], [ORWS1], [ORWS2] and [ORWS3]. The samples were corked under water immediately after collection so as to avoid the oxidation of the constituents and later sent to the laboratory within 24 hours for analysis. The sediment were collected by manual dredging method using stainless hand trowel pre-cleaned with detergents, dilute HNO₃, and de-ionized water and transferred into the polyethylene bags and stored in the refrigerator prior to analysis [35].

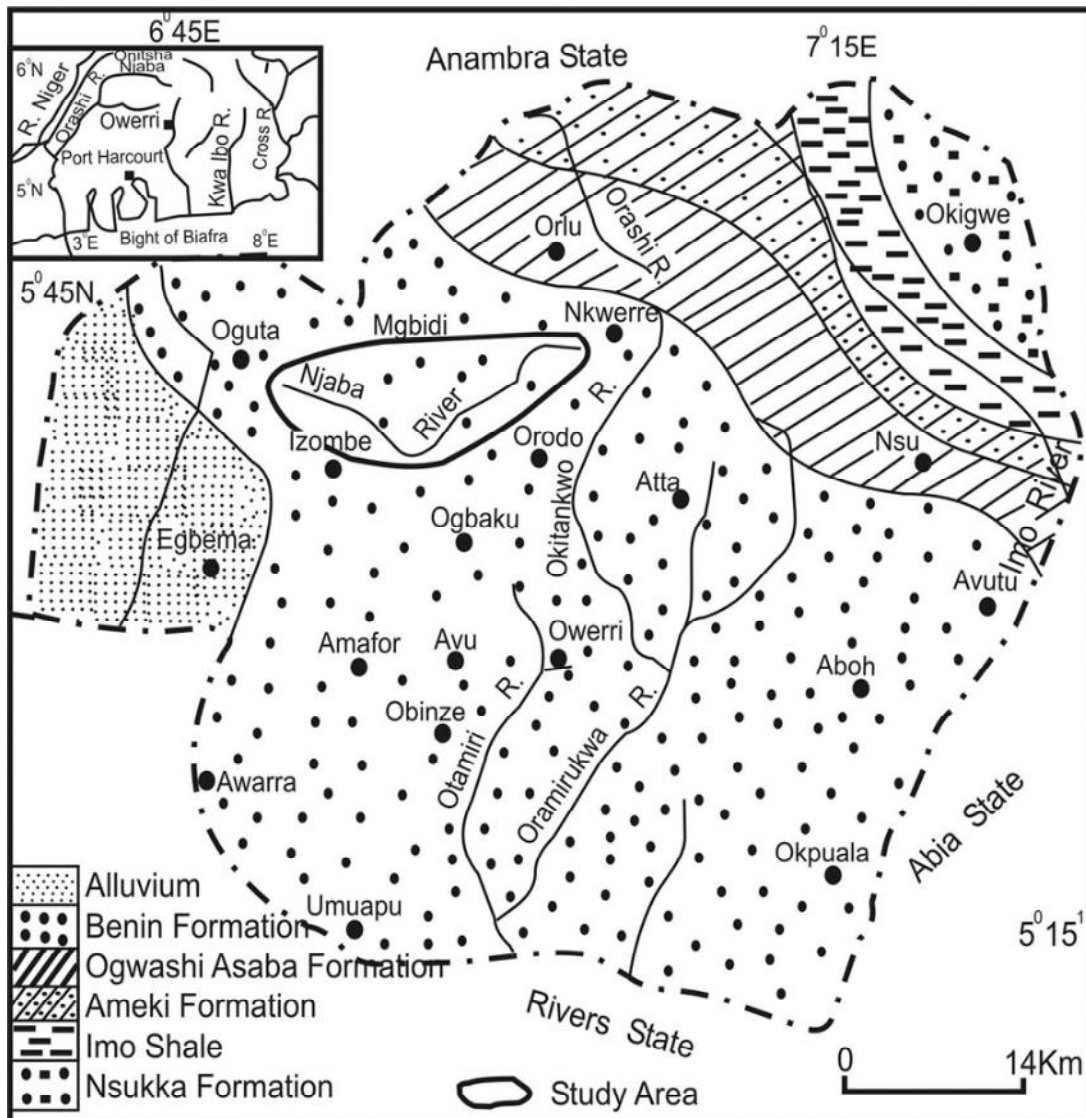


Fig. 1. Showing Otamiri river as one of the rivers in Imo State, Nigeria

3.2 Laboratory Analysis

Evaluations included presumptive, confirmatory as well as completed experiments [36]. Total and faecal coliform groups were ascertained using multi-tube fermentation [37]. Confirmatory test was used to ascertain faecal coliforms applying brilliant green broth, while to enumerate total coliform, presumptive test experiment was carried out using MacConkey broth [37]. To determine heterotrophic bacteria- *E. coli*, *Salmonella* and *Vibrio cholerae*, study used Dihydroxycholate hydrogen sulphide lactose agar, *Salmonella-shigella* agar and Thiosulphate citrate bile salt sucrose agar respectively. Plates

used were 24 hours incubated at 35°C with morphological characterization as basis for colonies identification. As used in [37], gram staining and biochemical reactions were employed for confirmation of presumptive colonies. With each plate assigned a positive or negative score, isolates were confirmed incorporating some conventional biochemical investigation [38]. Nikon SMZ1500 stereomicroscope aided the examination of colonies formed as a result of growth during incubation. The microscope has a magnification capacity of 3.75x-540x reliant on the oculars and objectives nominated.

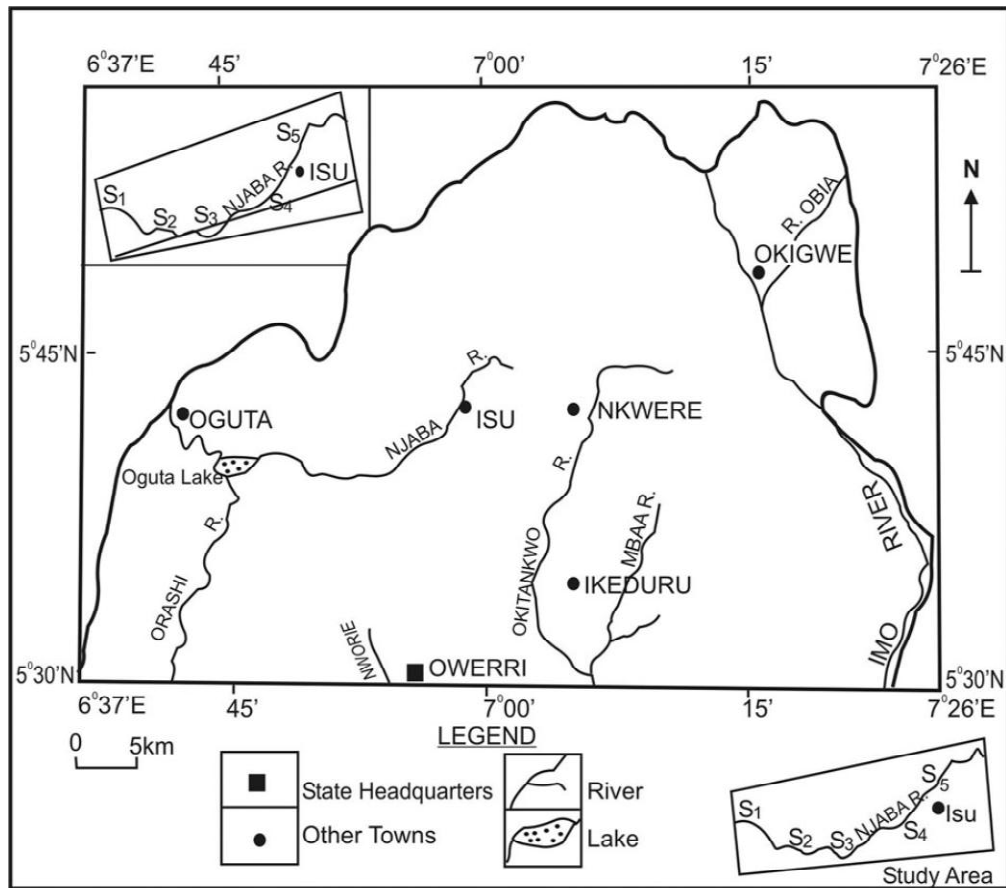


Fig. 2. Showing Nworie River as one of the rivers in Imo State, Nigeria

3.3 Method of Result Analysis

The mean of three samples per station (NRS1 - ORWS3) was taken for three sampling points. The results obtained were compared with World Health Organization (WHO, 2011) standard for drinking water while Federal Ministry of Environment (FME, 2006) was used for the sediment.

4. RESULTS

The mean surface water microbial features for Nworie and Otamiri Rivers for dry and rainy seasons are depicted in Tables 1 and 3 while the occurrence of bacteria isolates from Otamiri and Nworie rivers is displayed in Tables 2 and 4. There are detectable amount of microbial activities in surface water of both rivers during the dry and rainy season. As shown in Table 1, during the rainy season, a THB bioload average of 2.02×10^4 and 5.1×10^4 CFU/mL for Nworie and Otamiri river respectively were measured. For

the TCC, the average was 9.8×10^3 and 2.5×10^4 CFU/mL, while in the dry period there was corresponding reduction in the bioload value for both rivers. THB average value for Nworie river was 1.34×10^4 CFU/mL, with corresponding 3.5×10^4 value for Otamiri river, while in same inclination, TCC measured 6.1×10^3 and 1.8×10^4 CFU/mL. There were noteworthy variations in the values for the two rivers as well as in the two seasons. TBC value for Otamiri measured about 1.5 folds than the value for Nworie. In same trend, TCC value for Nworie measured about 39% the value for Otamiri river. All values measured were above WHO permissible limit. The biochemical and cultural features of the isolated microorganism are as in Table 5, while the various sample stations and their corresponding domiciled organisms are displayed in Tables 6 and 7. In all samples of water, *E.coli* was identified in entire stations (100%), *Salmonella* and faecal coliform occurred 83.3% while *Vibrio* and *Shigella* were detected in 4 of the 6 stations.

Table 1. Surface water microbial features for Nworie and Otamiri rivers (Rainy season)

	NRWS1	NRWS2	NRWSS3	ORWS1	ORWS2	ORWS3
GPS Location	N05. 46974 E07.03165	05.46485 07.03084	05.46037 07.03018	N05. 47045 E07.03924	N05. 47178 E07.04170	N05.47297 E07.04249
THB	15360	28020	17300	29050	110095	12850
TCC	9520	10205	9804	10200	60750	5610

Note: NRWS = Nworie River Water Samples 1 - 3; ORWS = Otamiri Water Samples 1-3

Table 2. Occurrence of the bacteria isolates from Otamiri and Nworie rivers (Rainy season)

Organisms	Stations					
	NRWS1	NRWS2	NRWS3	ORWS1	ORWS2	ORWS3
<i>Shigella</i> sp.	+	+	-	+	+	-
<i>Salmonella</i> sp.	+	+	-	+	+	+
<i>Vibrio cholerae</i>	-	+	-	+	+	-
Faecal coliform	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+

Table 3. Surface water microbial features for Nworie and Otamiri rivers water (Dry season)

	NRWS1	NRWS2	NRWS3	ORWS1	ORWS2	ORWS3
GPS Location	N05. 46974 E07.03165	05.46485 07.03084	05.46037 07.03018	N05. 47045 E07.03924	N05. 47178 E07.04170	N05.47297 E07.04249
THB	10415	19508	11087	20047	68290	17056
TCC	6005	6550	5846	8400	43072	3852

Note: NRWS = Nworie River Water Samples 1 - 3; ORWS = Otamiri Water Samples 1-3

Table 4. Occurrence of the bacteria isolates from Otamiri and Nworie rivers water (Dry season)

Organisms	Stations					
	NRWS1	NRWS2	NRWS3	ORWS1	ORWS2	ORWS3
<i>Shigella</i> sp.	+	+	-	+	+	-
<i>Salmonella</i> sp.	+	+	-	+	+	+
<i>Vibrio cholerae</i>	-	+	-	+	+	-
Faecal coliform	+	+	+	+	+	-
<i>E. coli</i>	+	+	+	+	+	+

Note: + and - 'identified' and 'not identified' respectively

5. DISCUSSION

The values of THB detected in Nworie and Otamiri river are close to those observed by [39] who recorded Total Aerobic Bacteria value range of 4.5×10^3 - 5.0×10^5 CFU/mL but a lower TCC concentration of 1.1×10^3 - 1.7×10^3 CFU/g against the prevailing research value of 9.8×10^3 - 2.5×10^4 CFU/mL obtained in Nworie and Otamiri river respectively. A recent result by [40] documented TBC; Total *E.coli* and total faecal CFU/mL of 1600-3400; 520-920 and 60-210 respectively.

The level of bacteria in present study could have been responsible for the low DO measured in the rivers especially the Otamiri river, where the concentration of anions such as SO_4^{2-} , NO_3^- , PO_4^{3-} including Ca and Mg were higher and consequently could be related to the eutrophication status observed [39]. According to [41], total coliform bacteria are a collection of relatively harmless microorganisms that are found mainly in the intestine of man; warm and cold blooded animals [41]. A known sub group of total coliform is the faecal coliform with

Escherichia coli as the most common member. Their ability to grow at increased temperature is a factor that enhances their isolation from total coliform. They are non spore forming, Gram-Negative, lactose-fermenting at 44.5°C within 24 hours with ability to grow even in anaerobic conditions [42]. Faecal coliforms are normally not pathogenic and are indicator organisms [42], and by implication when present in aquatic environment, it indicates contamination due to the presence of faecal materials of man and other animals which may have been domiciled by pathogen contaminants or diseases producing bacteria or viruses [42-44]. Some pathogenic diseases associated with faecal contamination include typhoid fever, viral and bacterial gastroenteritis and even Hepatitis A [40]. Consequently individuals exposed to faecal contamination, potentially face health risk. Chlorine could be used to treat faecal contamination. Though disinfection could also cause some issues as some necessary fauna or flora that helps to balance the aquatic ecosystem could be killed or destroyed thereby altering the ecological balance of the water [41]. But where faecal material containing faecal coliform is not treated, it could add to organic load of the water and consequently deplete the available oxygen due to increased BOD in the water [39]. The implication of oxygen depletion could be fish kill and possible adverse impact on other sensitive aquatic animals. USEPA has put guidelines for body contact recreation, fishing and boating as well as domestic water supply and treatment at 200; 1000; and 2000 colonies/100 mL while the drinking water standard is less than 1 colony total coliform bacteria/100mL with no presence of *E. coli* [39]. Hence both rivers are unfit for both recreational and drinking need.

According to [45], though positive species such as *Staphylococcus aureus* have high pathogenic effect but in recent times *Staphylococcus epidermidis* has emerged as a nosocomial pathogen in individuals with compromised immune system. *Staphylococcus epidermidis* is associated with septicemia and other polymer related diseases [45]. Moreover *Salmonella* sp., *E. coli*, *Staphylococcus* were also implicated in fish borne [46] and shrimp borne [47] diseases of human. *Staphylococci* are gram positive facultative anaerobic bacteria. They are widespread among mammalian where they belong to the healthy microbial flora of skin and mucosa.

The results in present study also conform with work by [43,44] and [45] who isolated *Staphylococcus*, *E. Coli* and *Salmonella* sp. and hinted of the public health significance.

Present study aspect of Nworie river is in contrast to bacteriological study of [46] that established presence of *Salmonella*, faecal count and *Vibrio* with just staggered and small amount of *E. coli* in 50% of sampled stations. However with consistency of present study with studies such as [7], it may imply that the river pollution trend is on the increase. There were significant differences in the values for the two rivers as well as in the two seasons. THB value for Otamiri measured about 1.5 folds than the value for Nworie. In same trend TCC value for Nworie was about 39% the value for Otamiri River. All values measured were above permissible limit. The coliform value obtained is in alignment with the fact that microbial contamination varies with time as documented by [4] and further enhances the general conception that faecal contamination is generally higher during the rainy season [39]. Reduction of bioload concentration is observed during the dry season as could be correlated in Tables 1 and 3. This direction of variation had been reported by [47-49]. It may have been a bit challenging to make a very quality comparison. This is due to non uniformity in quantitative data reported or even enough descriptions for a meta-analysis of effect size [50].

Aquatic ecosystems are influenced by the putrefaction of biological matter to inorganic form and the cyclic distribution of nutrients via ocean living things sustaining microorganisms [51]. Conversion of biological waste by the action of microorganisms has gained research focus due to its importance in the [52]. Moreover majority of benthic biomass are due to the processes of aquatic microbes, as they play significant ecological and biogeochemical part through regulating the conversion of key organic active elements such as nitrogen, oxygen, carbon, sulfur, as well as impacting on biodegradation [53,54] Station ORWS2 had the highest microbial load in both seasons with THB and TCC reaching 6.8×10^4 - 1.1×10^5 CFU/mL and 4.2×10^4 - 6.0×10^4 CFU/mL respectively. This may be due to a conglomeration of siltation and chemical accumulations due to a barricade built around the sample station on Otamiri river, which was supposed to enhance water flow [40].

Table 5. Identification of bacteria isolates from Otamiri and Nworie rivers surface water

Features of the colony	Gram Reaction	motility	Spore stain	Catalase	Coagulase	urease	VogesProsk	citrate	H ₂ S	Methyl orange	Oxidase	Glucose	Maltase	Lactose	Sucrose	Manitol	
Moist, all edge smooth surface cream coloured raised convex colony	Cocci inclusive Gram +ve rods	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	<i>Staphylococcus epidermidis</i>
Pinkish flat lactose fermenting colonies on Macconkey agar cream coloured on agar nutrient	Single patterned Gram -ve rods	+	-	+	-	-	-	-	-	+	+	+	+	+	+	+	<i>Escherichia coli</i>
Pinkish mucoid non lactose fermenting colonies in Macconkey agar exhibiting dark decoloration on BCA	Gram -ve rods	+	-	+	-	-	-	-	-	-	+	-	-	-	-	-	<i>Salmonella</i> species
Average sized pinkish colonies raised on DCA	Gram -ve rods	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	<i>Shigella</i> species
Creamy raised colonies on nutrient agar	Single short Chained Gram +ve rods	+	+	-	-	-	+	+	+	-	-	+	+	-	+	+	<i>Basilus</i> species

Features of the colony	Gram Reaction	motility	Spore stain	Catalase	Coagulase	urease	VogesProskal	citrate	H ₂ S	Methyl orange	Oxidase	Glucose	Maltase	Lactose	Sucrose	Manitol	
All colonies on TCBS agar-yellow creamy	Gram –ve curves rods	+	-	+	-	-	-	+	-	-	+	+	-	-	+	+	<i>Vibrio cholerae</i>

Table 6. Nworie and Otamiri rivers sediment microbial features (Dry season)

	NRSS1	NRSS2	NRSS3	ORSS1	ORSS2	ORSS3
GPS Location	N05. 46974 E07.03165	05.46485 07.03084	05.46037 07.03018	N05. 47045 E07.03924	N05. 47178 E07.04170	N05.47297 E07.04249
THB (cfu/g)	2.45	3.79	2.16	1.120	1.274	1.158
THF (cfu/g)	1.10	3.21	1.03	1.252	0.917	0.983

Note: NRSS = NWORIE RIVER SEDIMENT SAMPLES 1 - 3; ORSS = OTAMIRI SEDIMENT SAMPLES 1-3

Table 7. Nworie and Otamiri rivers sediment microbial features (Rainy season)

	NRSS1	NRSS2	NRSS-C	ORSS1	ORSS2	ORSS3
GPS Location	N05. 46974 E07.03165	05.46485 07.03084	05.46037 07.03018	N05. 47045 E07.03924	N05. 47178 E07.04170	N05.47297 E07.04249
THB (cfu/g)	1.59	3.07	1.76	1.008	1.144	1.015
THF (cfu/g)	0.91	2.59	0.87	1.301	1.008	0.860

Note: NRSS = NWORIE RIVER SEDIMENT SAMPLES 1 - 3; ORSS = OTAMIRI SEDIMENT SAMPLES 1-3

Meanwhile the high presence of human activities very close to this sample station especially activities such as swimming, washing of cars as well as washing of domestic wares as some homes are less than 50 metres from the river bank could also have contributed to the high THB and TCC recorded. *E. coli* was most dominant isolated bacteria as it was found in all stations. This is consistent with study by Nwanebu [39] and [40].

The health implication of bacteria isolates presented in Tables 4, from both rivers have been documented by some studies. *E.coli*, according to [29] is an enterobacterium, gram positive rods. It causes primary and opportunistic or hospital acquired infections in humans. The pathogenicity of *E.coli* include urinary tract infections, infections of wounds, peritonitis, sepsis, endotoxin induced shock, meningitis and bacteraemia in neonates, diarrhoeal diseases like infantile gastroenteritis, travelers' diarrhea, dysentery and hemorrhagic diarrhea which may progress to haemolytic uraemic syndrome. *Vibrio Cholerae* causes cholera. Chesbrough [52] and [55] documented that *Vibro cholerae* is a gram positive motile curved rod. There is serious dehydration due to the consistent and high loss of fluid and electrolytes in throw-ups and stool, which if not medically checked could be critical as a result of renal failure and shock [52,7,56]. Some medical conditions such as nephrotyphoid, abscess of spleen and liver, enteric fever, gastroenteritis, food poisoning and septicemia could be activated by *Salmonella* species and as well, severe bacillary dysentery could be caused by *Shigella* which could lead to dehydration, inflammation and ulceration of the large intestine [29].

6. CONCLUSION

Evaluation of microbial load of water and sediment at rivers Otamiri and Nworie were carried out in this study. There were noteworthy variations in the values for the two rivers as well as in the two seasons in both water and sediment samples. All values measured were above WHO permissible limit for drinking water and Federal Ministry of Environment (FME, 2006) for the sediment. From the study, it is evident that the two rivers contain various forms of pollutants mostly from sewage and industrial discharge, indiscriminate disposal of domestic waste into and/or along the river courses. This calls for proper waste management practices and pre-use treatment of the river water with chlorine

as well as boiling before usage in order to reduce the microbial constituents of the water.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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