

## **Assessment of Physicochemical and Microbial Load of Nworie River Owerri, Imo State, South-Eastern Nigeria.**

Njoku –Tony, R.F<sup>1</sup>, Ebe, T.E<sup>2</sup>, Ihejirika, C.E<sup>3</sup>, Ejiogu, C.C<sup>4</sup> Uyo, C.N<sup>5</sup>  
*Department of Environmental Technology Federal University of Technology, P.M.B 1526, Owerri. Nigeria*

---

**Abstract:** Investigations on the physicochemical properties and microbial load of Nworie River in Owerri Imo state, Nigeria was carried out between January and July, 2014. Water samples from 3 locations namely (Upstream Akwakuma), (Discharge point Federal Medical Center) and (Downstream Holy Ghost College) were collected and taken to the laboratory for analysis. Temperature, Conductivity, pH, TDS, and DO were determined in-situ using Jenway (Model type HANNA 1910) Multipurpose tester. Microbial quality was determined using standard methods. Results showed that temperature, pH, dissolved solids, DO, BOD were all within the WHO standard. Analysis on microbial quality however revealed heavy presence of microbial contamination in the midstream (Discharge point). The total coliform count was highest at the midstream (FMC) and ranged from  $80 \times 10^5$  cfu/cm<sup>3</sup> to  $172 \times 10^5$  cfu/cm<sup>3</sup>, and total faecal coliform count ranged from  $8 \times 10^5$  cfu/cm<sup>3</sup> to  $31 \times 10^5$  cfu/cm<sup>3</sup> while the least was recorded at the upstream (Akwakuma) with a total coliform range of  $12 \times 10^5$  to  $80 \times 10^5$  cfu/cm<sup>3</sup> and total faecal coliform count that ranged from  $4 \times 10^5$  to  $22 \times 10^5$  cfu/cm<sup>3</sup>. The results obtained showed the presence of *Escherichia coli* (8.18%), *Staphylococcus spp* (18.18%), *Bacillus spp* (21.09%), *Klebsiella spp* (10.91%), *Salmonella spp* (1.82%), *Proteus spp* (8.18%), *Pseudomonas spp* (2.72%), *Mucor spp* (4.54%), *Trichophyton spp* (3.63%), *Aspergillus fumigatus* (4.54%), *Candida spp* (2.72%), and *Rhizopus spp* (5.45%). Although most of the investigated physico-chemical parameters were within WHO limits, the presence of high microbial load highlighted the need for routine sterilization and purification of the water before usage.

**Keywords:** Contaminant, physico-chemical parameters, Microbial load, Human health, Nworie river.

---

### **I. Introduction**

A river is a natural watercourse usually freshwater flowing towards an ocean, a lake, a sea or another river. They offer a number of benefits and services to man and the environment. According to [1] pollution of freshwater bodies such as rivers, streams, lakes, and ponds is most experienced as a result of industrial discharges, municipal wastes deposit and surface runoff. Indiscriminate and uncontrolled discharges of wastes into rivers impact negatively on river ecosystems and human health.

Nworie river is subjected to intensive human and industrial activities, and at the same time, is used as a source of drinking water when the public water system fails. The increasing population of the inhabitant of Owerri metropolis has led to the increase in microbial pollution due to the enormous wastes generated from their various activities. The river which runs approximately 5.0km course through Owerri, the capital of Imo state in south-eastern Nigeria is of great importance to the inhabitants of Owerri, serving as a source of water for domestic use such as bathing, washing, drinking etc. The river also support recreational activities and part-time fishing.

The common practice of unregulated wastes disposal into watercourses can affect their normal use by municipalities. Aquatic environment near cities are usually prone to over loading with a variety of pollutants either through direct or indirect discharges. The situation is worsened when the waste is untreated. The public health problems arising from faecal pollution of natural waters have been documented by several workers such as [2].

Reckless dumping of wastes into natural water bodies can overtax the self-purifying capacity of the receiving water. This will not only endanger the resident aquatic life but also impair other amenity purposes and non-consumption uses that the river course might be put into.

Wastes generated as a result of human activities from institution such as Federal medical centre (FMC), Alvan Ikoku federal college of Education (AIFCE), and Holy ghost college in Owerri situated along the river banks as well as wastes from most hotels situated within the municipality do find their ways into the river which increases the microbial load on the river. Washing, bathing and other human activities carried out at different points of the river serve as additional sources of pollution to the river. Runoffs from agricultural farmlands surrounding the river carry agrochemicals like residual pesticides, fertilizers, manures into the river and this leaves the river in a despicable state. [4] in his work also implicated refuse dumping as dwelling places for vectors of diseases.

## II. Materials and Methods

### 2.1 Study Area

Owerri Municipal Council is located on latitude  $5^{\circ}25'50.23''N$  and longitude  $7^{\circ}2'149''E$ . Nworie River is about 9.2km in length and it originates at Egbeada in Mbaitoli local government area in Imo state. The river flows through Owerri the capital city of Imo state in south-eastern Nigeria and its environs lies between latitude  $5^{\circ}28'N$  and  $5^{\circ}31'N$  Atlas map of Imo state. The river flows in the city through Federal Medical Centre (FMC), Alvan Ikoku Federal College of Education (AIFCE) and Holy Ghost College all in Owerri and empties into another river, the Otamiri river at Nekede in Owerri West local government area. Nworie River is within the rainforest zone of Owerri.

According to the 2006 National census, Owerri municipal has a population of 127,213 inhabitants. It is bounded on the North by Amakohia, on the North-East by Uratta, on the East by Egbu, on the South-East by Naze, on the south by Nekede and on the North-West by Irete. Owerri municipal inhabitants are mainly traders, few artisans, civil servants and farmers who are predominantly natives.

Owerri Municipal has two geological regions namely a coastal plain and a plateau portion. The vegetation is typical rainforest. It has a mean annual rainfall of about 2,250-2,500mm, the mean temperature is 25-27 °C and the relative humidity is 80%

### 2.2 Sample Collection

Using sterile bottle containers, water samples were collected from three (3) different points of the river namely Upstream (Akwakuma), Point of discharge (FMC) and Downstream (Holy Ghost College). The water samples were labelled thus:  $U_M, P_M, D_M$  indicating samples from the three (3) different sampling points for morning section and  $U_E, P_E, D_E$  for evening section respectively.

### 2.3. Materials and Equipment used

Materials and equipment used in the analysis includes the following: Weighing balance, Autoclave, Inoculation needles, Forceps, Wire loops, Bunsen burner, Glass-wares (Beakers, Conical flask, Petri-dishes, Test-tubes, Measuring cylinders etc.), Cotton wool, Whatman filter paper, Incubator.

The reagents used are distilled water, iodine, crystal violet, safranin, KOVAC's reagent, ethanol etc. Media used include: Nutrient agar, MacConkey agar, Eosine Methylene Blue agar, Sabouraud dextrose agar for microbiological studies. Diluents include distilled water and some biochemical reagents.

### 2.4. Sterilization of Materials

Glass-wares were sterilized as described by [4] and [5] using hot air oven at 170<sup>0</sup> C for two (2) hours. Moist heat, dry heat, direct flaming and chemical methods of sterilization as described by [6] Cruickshank *et al.*, (1982) and [7] Ogbulie *et al.*, (1998) were also adopted for the sterilization of materials.

### 2.5 Physicochemical Analysis

#### 2.5.1 Insitu Measurement

Temperature, Electrical conductivity, pH, Total dissolved solids, (TDS), and Dissolved Oxygen (DO) were determined in-situ using Jenway (Model type HANNA 1910) multipurpose tester in each sampling points.

#### 2.5.2. Total Suspended Solids (TSS):

TSS was determined by photometric method using HACH DR/2010 spectrophotometer at a wavelength of 810nm and program number 630.

#### 2.5.3 Turbidity:

Turbidity was determined by photometric method using HACH DR/2010 spectrophotometer at a wavelength of 860nm and programme number 750.

#### 2.5.4. Dissolved Oxygen (DO):

The DO (Dissolved Oxygen) was determined using Dissolved Oxygen meter. The DO meter was calibrated using 5% Sodium sulphate solution. The probe of the meter was then inserted into the sample after the meter was put on for about 10 minutes. The reading was recorded in mg/dm<sup>3</sup>.

#### 2.5.5 Biochemical Oxygen Demand (BOD<sub>5</sub>)

The BOD<sub>5</sub> was determined using DO meter. The DO meter was calibrated using 5% Sodium sulphate solution. The probe of the meter was then inserted into the sample after the meter was put on for about 10 minutes. The reading was recorded in mg/dm<sup>3</sup>.

### 2.5.6 Microbiological Analysis

Media prepared included Nutrient agar, MacConkey agar, Eosine Methylene Blue agar and Sabourand dextrose agar for the study and the preparations were done in accordance with the manufacturer's directives.

### 2.5.7 Identification of Isolates

Bacterial isolates were identified based on their physical morphologies and reactions to series of biochemical tests as described by [4] and [6] to determine their probable genus by observing under the microscope using X 100 objectives.

### 2.5.8 Motility test

Stab culture technique as described by [7] was adopted. It involved the stab-inoculation of the test isolates into semi-solid media with the aid of a sterile straight wire on the Centre of the tubes about half the depth of the media. The stab cultures were incubated at 37<sup>0</sup> C for 24 hours. Motility was observed by the migration of the bacteria from the stab lines into the media causing turbidity and thereby rendering the media opaque, while non-motile organisms gave growths confined to the path of inoculation.

**Table 1 Physicochemical Parameters**

S/N	PARAMETER S	UPSTREAM (MORNING)	UPSTREAM (EVENING)	MIDSTREAM (MORNING)	MIDSTREAM (EVENING)	DOWNSTREAM (MORNING)	DOWNSTREAM (EVENING)	WHO STD (2003)
1	TEMPERATURE	30.7	30.3	28.2	28.4	28.5	29.3	20-30
2	Ph	8.6	7.6	8.8	6.0	8.2	8.6	6.5-8.5
3	CONDUCTIVITY (uS/cm)	93	103	196	110	180	188	100
4	TDS (mg/dm <sup>3</sup> )	60.5	67.0	127.4	71.5	117.0	122.2	250
5	TURBIDITY (NTU)	172.50	246.45	418.75	730.00	1089.0	113.00	5.0
6	TSS (mg/dm <sup>3</sup> )	95	136	232	404	602	615	50
7	COLOUR (PCU)	330	410	770	250	2850	2150	15
8	NITRATE (mg/dm <sup>3</sup> )	68.0	49.0	86.7	85.5	77.3	74.0	10
9	NITRATE – NITROGEN (mg/dm <sup>3</sup> )	15.0	11.0	24.8	25.1	21.4	20.8	15
10	PHOSPHATE (PO <sub>4</sub> <sup>3-</sup> ) (mg/dm <sup>3</sup> )	12.2	11.6	18.9	16.0	16.8	15.6	5.0
11	PHOSPHORUS (P)	4.0	3.8	6.1	5.7	5.8	5.1	5.0
12	PHOSPHATE (P <sub>2</sub> O <sub>5</sub> ) (mg/dm <sup>3</sup> )	9.1	8.7	11.2	10.8	10.9	10.3	-
13	SULPHATE (mg/dm <sup>3</sup> )	15	10	45	20	30	20	400
14	IRON (mg/dm <sup>3</sup> )	1.00	0.30	2.61	1.64	2.28	2.16	0.3
15	LEAD (mg/dm <sup>3</sup> )	0.026	0.018	0.284	0.277	0.153	0.172	0.05
16	Hg (mg/dm <sup>3</sup> )	0.000	0.001	0.031	0.028	0.150	0.132	0.001
17	Cu (mg/dm <sup>3</sup> )	0.10	0.15	0.16	0.12	0.08	0.11	1.0
18	DO (mg/dm <sup>3</sup> )	10.7	10.1	11.1	11.5	10.5	10.8	>4
19	bod (mg/dm <sup>3</sup> )	2.0	2.2	3.5	5.0	3.4	4.0	10

**Table 2: Population of Total viable Bacteria, Total Coliform, and Faecal Coliform and Isolated From Water Samples (Morning Section)**

SAMPLES	TOTAL VARIABLE COUNT CFU/cm <sup>3</sup>		TOTAL COLIFORM COUNT (CFU/cm <sup>3</sup> )		FAECAL COLIFORM COUNT (CFU/cm <sup>3</sup> )			
	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>5</sup>		
U <sub>M</sub>	14	22	10	12	3	4		
P <sub>M</sub>	13	51	15	80	4	8		
D <sub>M</sub>	13	16	20	40	3	6		

**KEY:**

U<sub>M</sub> = Upstream (Akwakuma)

P<sub>M</sub> = Discharge Point (FMC)

D<sub>M</sub> = Downstream (Holy Ghost College)

**Table 3: Population of Total viable Bacteria, Total Coliforma, FaecalColiform Isolated From Water Samples (evening section)**

SAMPLES	TOTAL VARIABLE COUNT CFU/cm <sup>3</sup>		TOTAL COLIFORM COUNT (CFU/cm <sup>3</sup> )		FAECAL COLIFORM COUNT (CFU/cm <sup>3</sup> )	
	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>5</sup>	10 <sup>6</sup>	10 <sup>5</sup>
U <sub>E</sub>	144	155	24	80	31	22
P <sub>E</sub>	220	280	40	172	14	31
D <sub>E</sub>	190	256	20	100	10	43

**KEY:**

U<sub>E</sub> = Upstream (Akwakuma)  
 P<sub>E</sub> = Discharge Point (FMC)  
 D<sub>E</sub> = Downstream (Holy Ghost College)

**Table 4: Percentage Occurrence of Bacteria Isolated From Waste Water Samples**

ORGANISMS	D	U	P	TOTAL	FREQUENCY %
<i>Bacillus spp</i>	10	8	14	32	21.09
<i>Klebsiellaspp</i>	2	4	6	12	10.91
<i>E. coli</i>	2	3	4	9	8.18
<i>Pseudomonas spp</i>	2	-	1	3	2.72
<i>Proteus spp</i>	3	2	4	9	8.18
<i>Staphylococcus spp</i>	8	7	5	20	18.18
<i>Salmonella spp</i>	-	1	1	2	1.82

**KEY:**

D = Downstream (Holy Ghost College)  
 U = Upstream (Akwakuma)  
 P = Discharge Point (FMC)

**Table 5. Characterization and Identification of Bacteria Isolates**

Isolate	M	G.S	U.R	M.R	IN	V. P	C I	C A	O X	CO A	M OT	GL U	LA C	SUC	P.I
1	Rod	-	-	+	+	-	-	+	-	-	+	A/G	AG	+	<i>E. coli</i>
2	Rod	-	+	+	+	-	-	-	+	-	+	A	-	A	<i>Proteus spp</i>
3	Rod	-	-	-	-	-	-	+	+	-	+	A	-	AG	<i>Pseudomonas spp</i>
4	Cocci	+	-	+	-	+	-	+	-	+	-	AG	AG	A	<i>Staphilococcuspp</i>
5	Rod	-	+	-	-	+	+	+	-	-	-	AG	AG	AG	<i>Klebsiellaspp</i>
6	Rod	+	-	+	-	-	+	+	-	-	+	AG	A	A	<i>Bacillus spp</i>
7	Rod	-	-	+	-	-	+	+	-	-	+	AG	-	-	<i>Salmonella spp</i>

**Table 6: Frequency and Incidence of Bacterial Pathogen Isolated From Water Samples of Nworie River**

ORGANISMS	DOWNSTREAM (%)	MIDSTREAM (%)	UPSTREAM (%)	TOTAL (%)
<i>Bacillus spp</i>	10(37.04)	14(40.00)	8(32.00)	32(36.78)
<i>Klebseillaspp</i>	2(7.41)	6(17.14)	4(16.00)	12(13.79)
<i>E. coli</i>	2(7.41)	4(11.43)	3(12.00)	9(10.34)
<i>Pseudomonas spp</i>	2(7.41)	1(2.86)	0(0)	3(3.45)
<i>Proteus spp</i>	3(11.11)	4(11.43)	2(8.00)	9(10.34)
<i>Staphylococcus spp</i>	8(29.63)	5(14.29)	7(28.00)	20(23.00)
<i>Salmonella spp</i>	0(0)	1(2.86)	1(4.00)	2(2.30)
<b>TOTAL</b>	<b>27(100)</b>	<b>35(100)</b>	<b>25(100)</b>	<b>87(100)</b>

**III. Result.**

TABLE 1 shows the result of the physicochemical parameters at the various sampling points for both the morning and evening section.

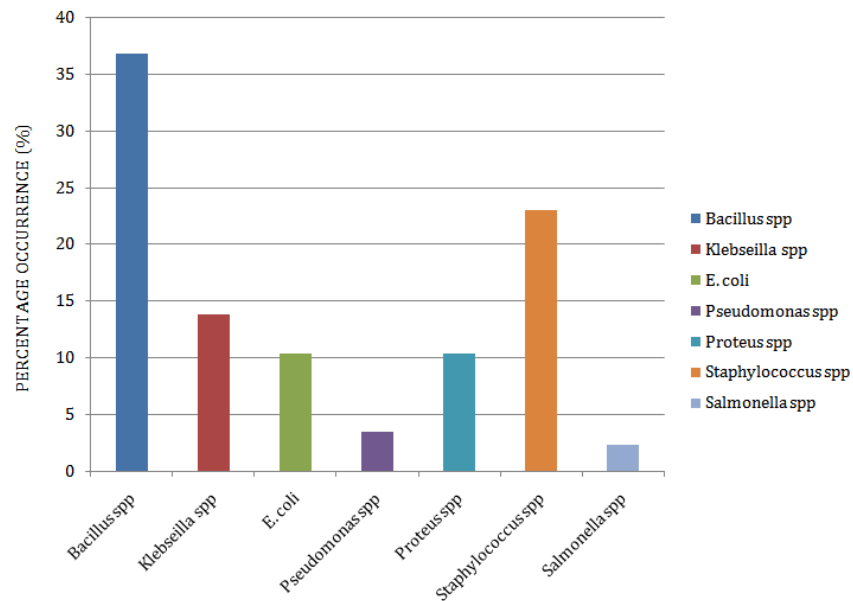
TABLE 2 shows the population of total viable bacteria, total coliform, faecal coliform isolated from the water samples for morning section.

TABLE 3 shows the population of total viable bacteria, total coliform ,faecal coliform isolated from the water samples for the evening section.

TABLE 4 shows the occurrence of microorganisms isolated from the water samples. From the table, it was observed that *Bacillus spp*(21.09%)was highest followed by *Staphylococcus spp*with (18.18%) while the lowest were *Pseudomonas spp*(2.72%) and *Salmonella spp*(1.82%) .

TABLE 5 shows the characterization and identification of bacteria isolates. The identified bacteria were *Escherichia coli*, *Proteus spp*, *Pseudomonas spp*, *Staphylococcus spp*, *Klebseillaspp*, *Bacillus spp*, and *salmonella spp*.

TABLE 6 shows the frequency of bacterial pathogens isolated from the water samples of the river. The table shows that the most dominant of the bacteria is the *Bacillus spp*(36.78%) and the least was *salmonella spp*(2.30%). A total of 87 bacteria were isolated, the highest occurred at the midstream (FMC) with 35 isolates, followed by the downstream (Holy ghost college) with 27 isolates while the least occurred at the upstream (Akwakuma) with an isolates of 25.



**Bacterial pathogens**

figure 1: bar chart showing occurrence of bacterial pathogens isolated from water samples of nworie river.

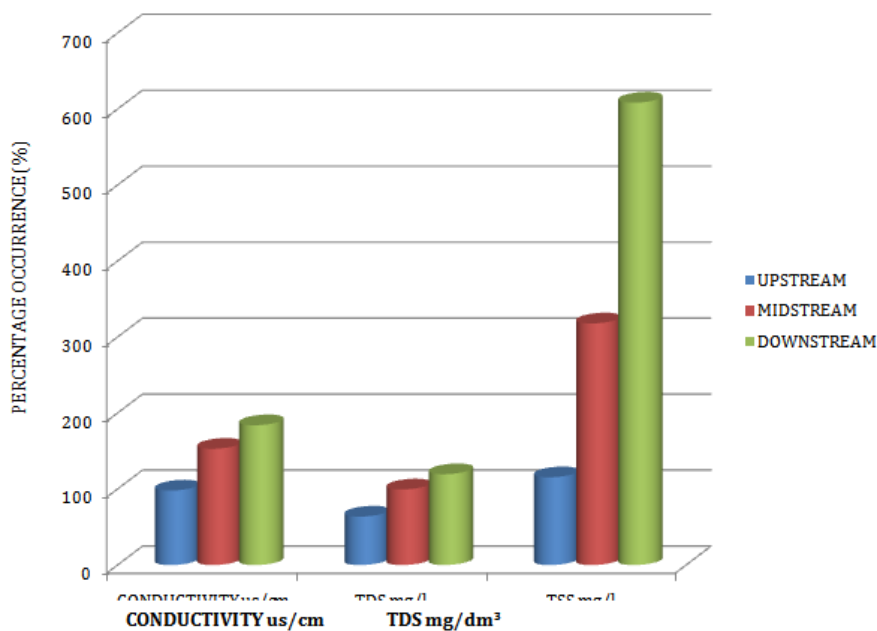


figure 2: mean variation in conductivity, TDS, and TSS

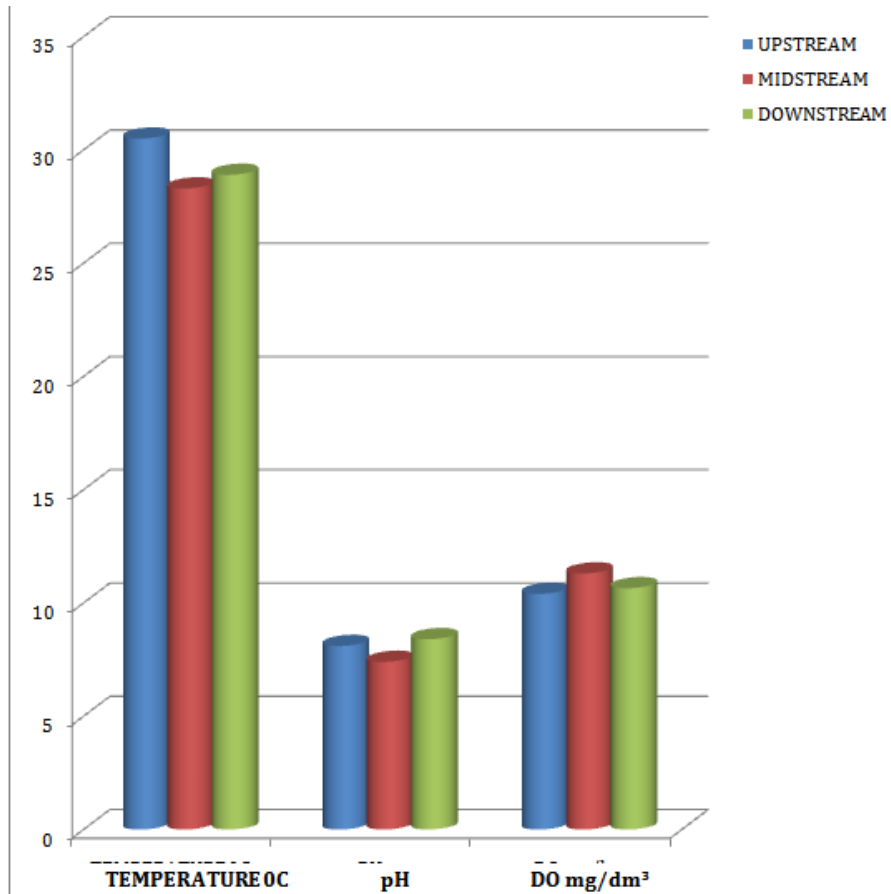


figure 3: mean variation of temperature, pH and DO

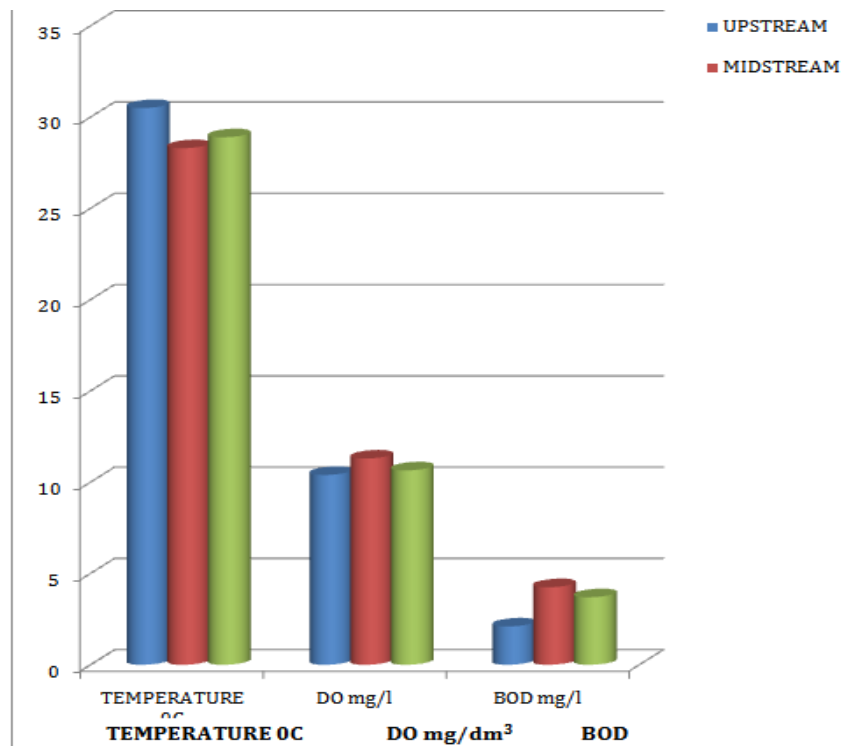


figure 4: mean variation in temperature, DO and BOD

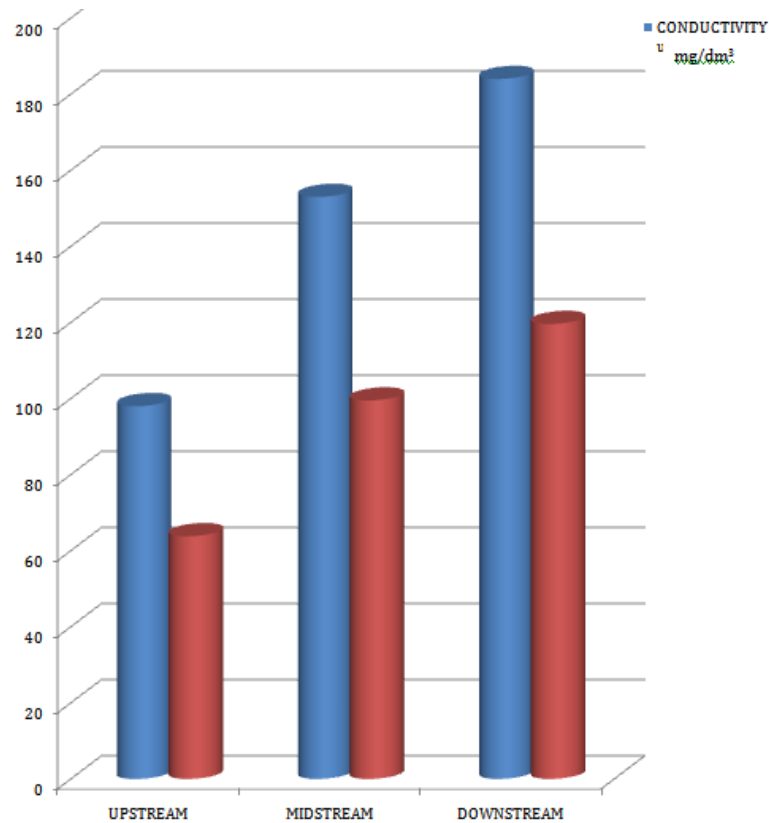


figure 5: mean variation in conductivity and total dissolved solids

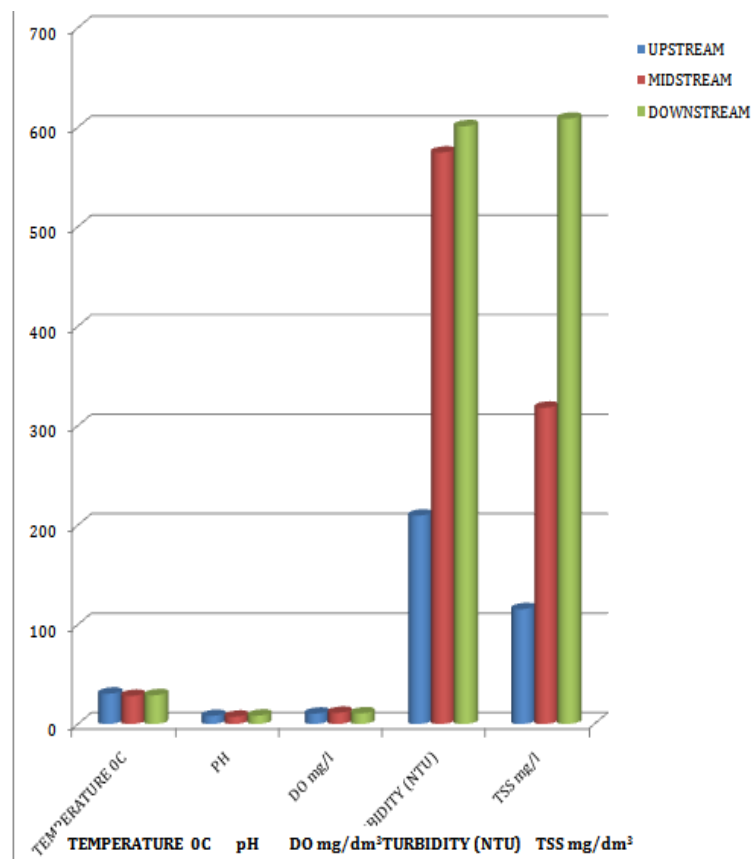


figure 6 : mean variation in temperature , pH, DO, turbidity and TSS .

#### IV. Discussion

There is a range of physical, chemical, and biological components that affect water quality. These variables provide general indication of water pollution, whereas others enable a direct tracking of pollution sources.

The pH of water samples collected from Nworie River vary along sampling points with the highest recorded at the midstream/point of discharge (8.8) during the morning section. The pH for the three sampling points except midstream were within the WHO standard (6.5-8.5). The temperature of the water samples except the upstream were within the range of WHO (20-30), and falls within the range supportive of good surface water quality which is 0°C to 30°C. Hence the temperature of the water from Nworie River could be implicated as influencing the observed variation in the bacterial population as well as other physicochemical parameters.

The significantly high total suspended solids (TSS) are implicative of high level of pollution of the River when compared to the WHO standard limit for good water quality which is 50mg/dm<sup>3</sup>. TSS and TDS are indicative of materials carried in suspension and solid respectively. Suspended solids in streams are often as a result of sediments carried by the water whose source includes natural and anthropogenic (human) activities in the watershed, such as natural or excessive soil erosion from agriculture, forestry and industrial discharges into the river. The significant decrease in the TSS and TDS content of water samples observed at the upstream section could be linked to the observed correspondingly low count of heterotrophic bacteria enumerated at these sampling points.

The dissolved oxygen (DO) observed at the three locations were within the WHO standard (>4) which indicates that the water can support the growth of aquatic organisms. The BOD values recorded were all below the WHO standard.

As *Escherichia coli* was isolated from the water samples, it indicated recent faecal contamination of the water sources. This result is supported by the works of [8] and [9]. While most strains of *E. coli* are non-pathogenic, some can cause serious diarrheal infections in human [10]. The presence of *Salmonella typhi* in the water samples could be traced to contamination from domestic sewage, agricultural waste and storm water runoffs. This is in line with the report of [11] and [12]. According to [13], *Salmonella typhi* is responsible for salmonellosis. This implies that controlled sewage water systems and personal hygiene will help reduce the incidence of gastroenteritis and typhoid fever [14]. *Klebsiella spp.* are ubiquitous in the environment [8], *Klebsiella spp.* can cause human diseases ranging from asymptomatic colonization of the intestine, urinary or respiratory tract to fatal septicemia [15]. Total coliform bacteria include the faecal coliforms associated with faecal materials, species of *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter* are important members of the total Coliform group [16].

#### V. Conclusion

The evaluation of the bacteriological quality of the water samples has confirmed the major contaminant as faecal. Contaminated water is an established source of most enteric disorders which in most cases manifest as diarrhoea. Diarrhoea

The implication of these findings may be that people dependent on this river water for domestic use including cooking, bathing, washing and even drinking or for agricultural uses like fishing and farming may be exposed to public health risks. We therefore suggest that to maintain healthy living, health education, proper waste disposal system and environmental sanitation remain key factors to eradicating this menace of water pollution.

#### Acknowledgment

The authors are grateful to our field assistants and laboratory Scientists for their doggedness in helping to organize the practical materials for this paper.

#### References

- [1]. Akaninwor, J.O., and Egwim, O. (2006). Effect of industrial effluent discharge on physicochemical properties of new Calabar river in Choba River State. *JNES*, 3 (3): 174-182.
- [2]. Ihejirika, C.E, Ogbulie, J.N, Nwabueze, R.N, Orji, J.C, Ihejirika, O.C, Adieze, I.E, Azuwuikwe, O.C, Ibe, I.J (2011) Seasonal influences on bacterial pathogens and waterborne disease transmission Potentials of Imo River, Nigeria. *Journal of res. In Biol.* 3, 163-17
- [3]. Duncan, J.W.K (1996). Sewage and Refuse Disposal in Africa. In. Sofoluwe, G.O, Schram R. and Ogunmekan, D.A.(eds). *Principles and Practice of Public Health in Africa*. Vol. 1 Pp 162-16
- [4]. Cheesbrough M., (2000). *District Laboratory Practice in tropical countries Part II*, Co-published by the press syndicate of the University of Cambridge, pp: 36-37, 187-189 and 398-400.
- [5]. Cruickshank R., Duguid J.P., and Swain R.H., (1982). *Medical Microbiology: The Practice of Medical Microbiology*, 12<sup>th</sup> Edn., London: Longman group, Vol. 11
- [6]. Pelczar, M.J, Cean, E.C.S, and Krieg, N.R (1993). *Microbiology: Concepts and applications*. McGraw-Hill, USA.
- [7]. Cruickshank, R, Duguid, J.P, and Swain, R.H, 1982. *Medical Microbiology: The Practice of Medical Microbiology*, 12<sup>th</sup> Edn., London, Longman group, Vol. 11.



- [8]. Ogbulie J.N., Uwaezuoke J.C., and Ogiehor S.I., (1998).Introductory Microbiology Practical.Nigeria: Springfield Publishers, pp: 24-157.
- [9]. Cabral JP. (2010).Water Microbiology.Bacteria Pathogens and Water.Int. J. Environ. Res. Public Health **7**, 3657-3703
- [10]. Ihejirika, C.E, Ogbulie J.N, Ihejirika, O.C, Azuwuike O.C, Ibe, J.N(2011b) Contributory pattern of papermill effluents on the population and distribution of enteric pathogens in Owerrinta River, Eastern Nigeria. Int. J. Biosci (4):74-79
- [11]. Health Canada(2006). Bacterial Waterborne Pathogens- Current and Emerging organism of concern, Guildlines for Canadian Drinking water Quality: Guildline Technical Document, Ottawa, Ontario.
- [12]. World Health Organization(1998) The state of the World Health. In the world Health Reports, Life in the 21<sup>st</sup> century.A vision for all. WHO Geneva. 57 58
- [13]. Arvanitidov M.(2005.) Diversity of Salmonella spp and Fungi in Northern Greek Rivers and their correlation to fecal indicators. Environ. Res.99:278-284
- [14]. Le Minor, L.E (2003).In the rokaryotes. An Evolving Elctronic Resource for the Microbiological Community, electronic release 3.14, 3<sup>rd</sup> ed., Dworkin,M.,Falkow, S., Rosenberg, E., Eds., SringerVerlag. New York, USA.
- [15]. Popoff MY, LE.(2005).Genus Salmonella. In: Bergey's Manual of Systematic Bacteriology, 2<sup>nd</sup>ed; Brenner, D.J, krieg, N.R., Staley, J.T.; Eds Springer: New York, USA Vol 2, Part B. P764-799
- [16]. Grimont F, Grimont PAD, Richard C (2003). The genus Klesiella. In the Prokaryotes: An evolving Electronic Resource for the Microbiological community, electronic release 3.14.3<sup>rd</sup> ed.; Dworkin, M.; Falkow, S.; Rosenberg, E Eds.; Springe Verlag: New York, USA.
- [17]. Curds, C.R. and Grothier, J.I (1990).Wastewater Biology.TheMicrolife. A socialublication reared by the Force on Wastewater Biology, under the supervision of the operations and Maintenance Subcommittees, technical Practice Committee, Michigan.