

Ikpesu, T.O<sup>1\*</sup>, Uzoekwe, S.A<sup>2</sup>

<sup>1</sup>Department of Biology, Federal University, Otuoke, Nigeria, <sup>2</sup>Department of Chemistry, Federal University, Otuoke, Nigeria,

\**Corresponding Author:* Ikpesu, T.O, Department of Biology, Federal University, Otuoke, Nigeria, Email: tomohwofasa@yahoo.com

### ABSTRACT

Environmental pollution from anthropogenic cause considerable irreparable damage to aquatic ecosystems as well as public health as rapid industrialization and urbanization releases numerous toxic substances into the water bodies without treatment. Despite the sordid state of most of water bodies in the nascent Niger Delta ecological zone, there are some organisms especially microbes and benthos were comfortably thriving, and the species diversity is used as a prime as pectinbio logical monitoring, which is considered to be avaluable parameter in determining the health status of the environment. Three stations within the lower Niger Rivers were chosen for this study. Station A as selected based on its pollution load as a result of sewage disposal and public defecation, B is use for human recreational and agricultural activities, while C has no activities and far away from other stations. Water and sediments samples were collected monthly in triplicates for twelve months and a total of one hundred and forty four were examines for this investigation. Isolates were identified by morphological and biochemical characterization using taxonomy scheme, fungi was based on the microscopic features of hyphal mass, nature of fruiting bodies and morphology of cells and spores and the planktons were microscopically identified using electron microscope, while macroinvertebrates were identified using taxonomic keys. Results show the present of bacteria and fungi with hydrocarbon utilizing potentials, zooplanktons (Cyclops, rotifers, Cladocera) and macro-invertebrates (oysters, leeches and Aporrectodea longa) were abundant varyly. The physiochemical parameters of water samples were above the recommendation limits except the reference station

*Keywords:* Costal pollution, Resistance microbial communities, Public health Monitoring, Physiochemical parameters

### **INTRODUCTION**

The significance of water to terrestrial and aquatic organisms cannot be over emphasized, and there are various experimental and economic truths that water pollution can bring about extreme diminishing in profitability and wellbeing of lives. The fundamental wellsprings of water that is available to people in the developing world are being separated by various anthropogenic variables, of which pollution remain the most fundamental problem (Garba et al. 2008; Galadima et al. 2011).

Water pollution have kept on creating upsetting ramification for wellbeing and economic development in third world by and large, the outcomes of which include millions of death from diarrhea disease and sizeable number of causalities from pathogenic and parasitic organisms (Esrey et al.1991). The nonavailability of good quality water has resulted into a number of health challenges as water is known to be a primary agent of some transmissible diseases (Onweluzo et al.2010; Olaoye et al. 2009).

Most water sources are frequently contaminated with different pollutants like faeces, animal and plant wastes and such harbors different pathogens, manifested in diseases like typhoid fever, amoebic dysentery, cholera amongst others which has caused in deterioration of health and in some cases death (Isikwue et al. 2011).

The release of both organic and inorganic contaminants into the environment by either industrial activities or other form of anthropogenic practices has led to the case of environmental pollution (Mouchet 2004). The discharge of waste into water bodies has rather

become a rapid practice owing to the increasing trend in urbanization and human activities (Tripathi 2014). According to Vidal (2001), environmental quality has a lot to do with the quality of life on earth as this go hand in hand. Recently, the problem of water pollution has been accepted as a general or global issue as the evaluated contaminated areas is significant (Cairney 2000). Seventy percent of the industrial wastes have been estimated to be disposed untreated into the water supplies (UNWAP 2003).

Although the treated sewage allowed to be discharged into the rivers should not exceed the Biochemical Oxygen Demand (BOD) limit of 30mg/L, Total Suspended Solids (TSS) limit of 50mg/L (Tyagi et al. 2013), but only a mere 18% of the 33,000mld sewage spawned daily is treated. Several investigations have been undertaken over time to monitor water quality and more precisely its portability and many studies reporting high levels of contamination in the fresh water bodies (WHO 2006; Mishra et al. 2012; Harnisz et al. 2015; Parvez et al. 2016; Gomes Freitas et al. 2017).

The elevation, decrease or disappearance of certain group of organism within an aquatic habitat is a function of changing biological, chemical, physical and hydro climatologically conditions. Exposure to environmental pollutants and changes in nutrient composition may lead to selection pressures favoring certain organisms or genotypes.

Pollutants present in agricultural runoff, sewage and industrial effluent include substances that favor the selection for organic and inorganic resistance microorganisms. Recent studies demonstrated positive correlations between agricultural runoff, sewage and industrial pollution and the spatial distribution of resistance microorganisms (Goiii-Urrizaet et al. 2000; Tuck fieldet al. 2000).

Variations in the populations of organisms may indicate harmful changes due to pollution of the ecosystem. Alteration of the population density may indicate negative impacts to the ecosystem and may be as a result of the relationship between populations and food sources and contamination of the environment (Jain et al. 2010).

Animal indicators also help in detecting the amount of toxinspresentin the tissues of animals (Joanna, 2006, Khatri and Tyagi 2015). The pollution of the water would have effect on the ecology of the aquatic lives and the microbial community may have been affected as well. These activities necessitate resistant mechanism for surviving microorganisms in the water and thus create an avenue for the emergence of mutant strains.

Hence this investigation entails examination of the water quality of one of the major creeks that drains Niger River at Otuoke and to examine the dominant microbial and benthos communities that can be used as sentinel and indices of environmental stress.

### MATERIALS AND METHODS

### **Study Area**

The study area is Otuoke creek that drains River Niger (Figure 1). The area is bounded by latitude 40 51' 05.23" N, Longitude 60 20' 14.2" E and Latitude 40 43' 48.69" N, Longitude 60 20' 19.84"E. It is bounded to the North by Elebele Community, to the East by Emeval and Kolo, to the West by Onuebum and Otuogiri, and to the South by Otuaba and EwoiCommunities; all in Ogbia Local Government Area of Bayelsa State. The creek occupies a central position in the Niger Delta region of Nigeria, cutting through the community from Elebele in the North, and Otuaba in the. By extension, its waters and extended territories are drained by Ekole Creek in the West through Atubu sub-creek and swamp drainage system; in the East by Kolo creek drainage system, and South East by Akoloman creek drainage system (Allison et al., 1999).

The area's climate, being equatorial and the reaches of the creeks draining the study area being freshwater reaches that are not obviously tidal, the vegetation is predominated by peculiar fresh water swamp vegetation observed to have mix of little freshwater vegetation and equatorial high forest, which is called High Equatorial Swamp forest (Horsfall et al. 2015).

### **Sampling Stations**

Three stations within the lower Niger Rivers were chosen for this study. Station A as selected based on its pollution load as a result of sewage disposal and public defecation, B is use for human recreational and agricultural activities, while C has no activities and far away from other stations, thus serves as a reference station (Figure 1).



#### Sampling (Indicators) Organisms

The impact of pollution on the distribution and abundance of microbes (bacteria and fungi), zooplanktons (Cyclops, Rotifera and Cladocera) and macro-invertebrates (Oyster, leeches andA. longa) were investigated at polluted and suspected non-polluted sites along the Creek. The water and bottom sediments samples were collected for physicochemical analysis, microbial and benthic distribution from 4 different sites within the creeks experiencing different anthropogenic activities for 12 months (wet and dry seasons).

#### Samples Collection and Analysis

Samples were collected on monthly basis from January, 2019 to December, 2019, based on 30 days interval and were done between 7:30am and 8:00 am. Ten square meter was map out within the sampling sites and collections was strictly within these spots. The physicochemical parameters and samples were collected in triplicate in an acid washed three liter plastic containers were analyzed using the standard methods for water analysis (APHA 2005). Earthworm samples were collected following the method described by Spiegel (2002). The earthworms were carefully extracted from subsurface terrace of the creek by digging and hand sorting. After which they were washed from soil particles and dried using absorbent filter paper and taken to the laboratory for identification, counting, weighing and analysis, while Oyster and leeches were collected using either sieve nets or metriculousl handpicked For zooplankton distribution and abundance,

subsurface water was collected and sieved through plankton net no. 25 and transferred to 200 ml plastic containers containing 4% formalin solution. The sample was centrifuged at 1500 to 2000 rpm for 10-12 min, were the zooplanktons settled at bottom. The upper water was siphoned out and diluted to concentration that allows the counting of the plankton individually under compound binocular microscope. The zooplanktons from each sampling station were measured and multiplied with dilution factors using Sedgwick rafter cell (Battish, 2002), and identified using the standard list of pollution tolerant zooplankton index by Sladecek pollution indicator index (Khan, 2012). The index showed that;

- $S = \underline{\Sigma(sh)}$ 
  - Σh

S=the saprobicindex; s=indicator value of each species (saprobity value); h=frequency of each species found: 1 = species found only by chance; 3 = species occurring frequently; 5 = species occurring in abundance

### **DATA ANALYSIS**

### **STATISTICAL ANALYSIS**

The student t-test, One-way analysis of variance and Tukey's HSD multiple comparison test were used to compare the seasons and the sampling stations. Minimum significance level was 0.05and the software Statistical 7.0 was used. The correlation analysis between microbial and benthos abundances and contamination level was performed with the software Sigma Plot 11.0.

#### **RESULTS**

Water samples from the three different sites within the investigated area were colorless, odorless and with the following properties; pH range of 7.0-7.5, temperature range between 23-29oc %, total dissolve solid (mg/l (809 - 1120),

dissolve oxygen (mg/l) (1.50 - 3.90), chemical oxygen demand (30 -35) and nitrate (15 -18).The frequency of occurrence of microbial isolates (Fig. 2 & 3), planktons and macro invertebrates (Table 1-3) from urban creek at Otuoke, Niger Delta Nigeria is shown below, with further illustration in figure 2-6.

**Table1.** Distribution and abundance of planktons and macro-invertebrates in downstream of Otuoke Creek at Otuba, Niger Delta Nigeria

Months	Cyclops Mean ± SE	Cladocera Mean ± SE	Rotifers Mean ± SE	Oyster Mean ± SE	Leeches Mean ± SE	Aporrectodea longa Mean ± SE
Jan.	7±0.13	$22 \pm 1.10$	$31 \pm 1.20$	ND	ND	$3 \pm 0.40$
Feb.	13±1.10	$26\pm0.40$	$40 \pm 1.30$	ND	ND	$7 \pm 0.30$
March.	$15 \pm 0.60$	$30 \pm 0.02$	$34 \pm 2.10$	ND	ND	$7 \pm 0.10$
April	$16 \pm 0.20$	$33 \pm 0.01$	$41 \pm 1.12$	ND	ND	$11 \pm 0.30$
May	$18 \pm 1.02$	$38 \pm 0.30$	$43 \pm 3.20$	$7 \pm 0.20$	$12 \pm 2.90$	$30 \pm 1.10$
June	$21 \pm 2.13$	$40 \pm 1.60$	$56 \pm 1.60$	$13 \pm 1.30$	$16 \pm 1.80$	$37 \pm 2.30$
July	$32 \pm 1.28$	$47 \pm 2.10$	$66 \pm 2.10$	$15 \pm 2.10$	$26 \pm 0.20$	$38 \pm 1.20$
August	$36 \pm 1.20$	$49 \pm 1.90$	$68 \pm 0.10$	$18 \pm 2.80$	$31 \pm 0.23$	$41 \pm 2.10$
Sept.	$40 \pm 3.10$	$56 \pm 1.10$	$72 \pm 2.20$	$23 \pm 1.50$	$28 \pm 1.20$	$47 \pm 1.10$
Oct.	$43 \pm 2.10$	$63 \pm 2.70$	$78 \pm 2.70$	$26 \pm 2.20$	$37 \pm 3.40$	$53 \pm 2.00$
Nov.	$49 \pm 1.10$	$71 \pm 2.10$	$82 \pm 1.40$	$12 \pm 1.70$	ND	$56 \pm 2.10$
Dec.	3 ± 2.10	$12 \pm 1.10$	$21 \pm 1.50$	ND	ND	$18\pm2.40$

**Table2.** Distribution and abundance of planktons and macro-invertebrates at Otuoke Creek Niger Delta Nigeria

Months	Cyclops Mean ± SE	Cladocera Mean ± SE	Rotifers Mean ± SE	Oyster Mean ± SE	Leeches Mean ± SE	Aporrectodea longa Mean ± SE
Jan.	58±1.10	$86 \pm 2.10$	$11 \pm 1.20$	ND	ND	$10 \pm 0.40$
Feb.	53±3.20	$50 \pm 1.10$	$70 \pm 1.30$	ND	ND	$18 \pm 0.30$
March.	$32 \pm 0.30$	$42 \pm 0.20$	$41 \pm 2.10$	ND	ND	$24 \pm 0.10$
April	$25 \pm 0.70$	$36 \pm 1.10$	$35 \pm 1.12$	ND	ND	$15 \pm 0.50$
May	$21 \pm 0.12$	$20 \pm 0.10$	$48 \pm 3.20$	$13 \pm 0.50$	$19 \pm 0.30$	$31 \pm 0.10$
June	$29 \pm 1.13$	$49 \pm 0.30$	$78 \pm 1.60$	$17 \pm 0.70$	$21 \pm 1.80$	$42 \pm 1.30$
July	$72 \pm 0.40$	$76 \pm 1.20$	$96 \pm 2.10$	$21 \pm 0.10$	$29 \pm 0.10$	$48 \pm 0.20$
August	$77 \pm 2.10$	$92 \pm 1.20$	$103\pm4.10$	$26 \pm 2.80$	$33 \pm 0.30$	$52 \pm 1.10$
Sept.	$87 \pm 1.50$	$99 \pm 2.20$	$107\pm2.20$	$31 \pm 1.50$	$38 \pm 0.50$	$58 \pm 1.10$
Oct.	$91 \pm 1.20$	$102\pm0.30$	$118\pm2.70$	$34 \pm 2.20$	$41 \pm 1.40$	$65 \pm 2.00$
Nov.	$71 \pm 2.10$	$94 \pm 1.50$	$101 \pm 1.40$	$25 \pm 1.70$	$47 \pm 2.10$	$71 \pm 2.10$
Dec.	$16\pm0.30$	$23\pm2.00$	$34 \hspace{0.1in} \pm 1.50$	$5 \pm 0.30$	ND	33± 1.10

**Table3.** Distribution and abundance of planktons and macro-invertebrates in upstream of Otuoke Creek, Niger Delta Nigeria.

Months	Cyclops Mean ± SE	Cladocera Mean ± SE	Rotifers Mean ± SE	Oyster Mean ± SE	Leeches Mean ± SE	Aporrectodea longa Mean ± SE
Jan.	58±1.10	86 ± 2.10	$11 \pm 1.20$	ND	ND	$10 \pm 0.40$
Feb.	53±3.20	$50 \pm 1.10$	$70 \pm 1.30$	ND	ND	$18 \pm 0.30$
March.	$32 \pm 0.30$	$42 \pm 0.20$	$41 \pm 2.10$	ND	ND	$24\ \pm 0.10$
April	$25 \pm 0.70$	$36 \pm 1.10$	$35 \pm 1.12$	ND	ND	$15 \pm 0.50$
May	$21 \pm 0.12$	$20 \pm 0.10$	$48 \pm 3.20$	$13 \pm 0.50$	$19 \pm 0.30$	$31 \pm 0.10$
June	$29 \pm 1.13$	$49\pm0.30$	$78 \pm 1.60$	$17 \pm 0.70$	$21 \pm 1.80$	$42 \pm 1.30$
July	$72 \pm 0.40$	$76 \pm 1.20$	$96 \pm 2.10$	$21 \pm 0.10$	$29 \pm 0.10$	$48 \pm 0.20$
August	$77 \pm 2.10$	$92 \pm 1.20$	$103\pm4.10$	$26 \pm 2.80$	$33 \pm 0.30$	$52 \pm 1.10$
Sept.	$87 \pm 1.50$	$99 \pm 2.20$	$107\pm2.20$	$31 \pm 1.50$	$38 \pm 0.50$	$58 \pm 1.10$
Oct.	$91 \pm 1.20$	$102\pm0.30$	$118\pm2.70$	$34 \pm 2.20$	$41 \pm 1.40$	$65 \pm 2.00$
Nov.	$71 \pm 2.10$	94 ± 1.50	$101 \pm 1.40$	$25 \pm 1.70$	$47 \pm 2.10$	$71 \pm 2.10$
Dec.	$16 \pm 0.30$	$23\pm~2.00$	$34\ \pm 1.50$	$5\pm0.30$	ND	33± 1.10

The following bacteria (*Esherichi coli*, *Bacillus* spp, *Proteus* spp, *Pseudomonas* spp, *Micrococcus* spp and *Salmonella* spp) and fungi (*A. niger*, *A. fumigatus*, *Mucor* spp and

*Geotrichum* spp) with hydrocarbon utilizing potentials were observed in the investigated creeks (Figure 2 and 3).



Figure2. Occurrence and distribution of hydrocarbon utilizing bacteria in different Stations of Otuoke Creeks, Niger Delta Nigeria



Figure3. Occurrence and distribution of hydrocarbon utilizing fungi in different Stations of Otuoke Creeks, Niger Delta Nigeria

Spatial distribution revealed that station 1 had the highest occurrences of the bacteria and fungi, followed by station 2 and the reference station (station 3) having the least in bacteria and fungi distribution. *E.coli, Bacillus* spp, *Micrococcus* spp and *Salmonella* spp. were present in all the three stations and well distributed. While, *Proteus* spp, *Pseudomonas* spp were only found in polluted station, absent in the reference station. The most dominant bacterium species in the polluted stations is *Bacillus* spp with 52% of occurrence, closely followed by *Pseudomonas* spp (52% of occurrence), the least was *Micrococcus* spp with 32% of occurrence. Fungi distribution revealed the dominancy of Mucor spp with 67% of occurrence, in the investigated river was rotifers, while A.longa was the most abundant macro invertebrates. The order of occurrence of the zooplankton was rotifers more than Mucor spp (62% of occurrence), with A. Niger having 12% of occurrence. A. fumigates and Geotrichum spp were not detected in the reference station (Figure 3). The distribution of the bacteria varies significantly between the reference station and the polluted stations (p < p0.05, F= 7.80), but not within the polluted sites (p > 0.05, F= 6.20). Similar trend was observed in fungi distribution between the reference station and the polluted sites (F < 0.05, F=

9.20), within the polluted sites (p > 0.05,F= 8.60). Spatial distribution of zooplankton and macro invertebrates differs from microbial distribution with the reference station having more of them than the polluted stations (Table 1-3), with further illustrations in figure 4 and 5.The most abundance zooplankton in the investigated river was rotifers, while A.longa was the most abundant macro invertebrates. The order of occurrence of the zooplankton was rotifers (43.94%) >cladoceras (29.40%) >cyclops (26.30%) and the occurrence of the macro invertebrates A.longa (47.37%) >leeches (30%) > oyster (22.63%).



Figure4. Spatial distribution of planktons and macro invertebrates in different stations of Otuoke Creeks, Niger Delta Nigeria during the dry season



Figure5. Spatial distribution of planktons and macro invertebrates in different stations of Otuoke Creeks, Niger Delta Nigeria during the wet season



Figure6. Seasonal distribution of planktons and macro invertebrates in different stations of Otuoke Creeks, Niger Delta Nigeria

Seasonal distribution during the seasons showed that the investigated organisms were more abundance in the wet season than dry season, with oyster and leech not detected in some of the months in the dry season in all the stations (Figure 6). The distribution of the planktons varies significantly between the control station and the polluted stations (p < 0.05, F= 6.40) and between the two polluted stations (p < 0.05, p = 8.70)

### DISCUSSION

Various human activities, such as rapid urbanization, industrialization and indiscriminate use of agri cultural products are causing significant pollution of aquatic environment leading to deterioration of water quality and hence decline of aquatic biota (Khan2012).

Microbial evaluation of the urban creeks at Otuoke and its environment revealed the presence of the following bacteria (E. coli, Bacillus spp, Proteus spp, Pseudomonas spp, Micrococcus spp and Salmonella spp) and fungi (A. niger, A. fumi gatus, Mucor spp and Geotrichum spp). These microbes are ubiquitous, diverse in nature, hydrocarbon utilizing potentials, able to adapt to extreme environment and overcome environmental stress (Watanabe et al. 2002).

They have different mechanisms depending upon the environmental factors and the nature of chemicals either to degrade or to remove the toxic contaminants from the environment (Boopathy, 2000). The differences in the distribution of the microbes could be attributed to the extreme environmental conditions which may change microbial count and the diversity as well (Ashboltet al. 2010).

Certain resistance indigenous microbes have shown to have the capability to degrade waste materials in polluted water and provide an effective, economical, versatile and environmental friendly means of reclaiming polluted environment by a complete mineralization of organic and inorganic contaminants into other simpler organic compounds e.g. carbon dioxide and water and a measure for public and aquatic health monitoring.

Faecal polluted and agricultural polluted sites do harbor a vast array of microbial flora that show resistant traits to the environmental contamination. The existence of these microorganisms in sewage and other agricultural and industrial polluted substances that find their way or are disposed in ground water of urban creek at Otuoke and its environs suggests that the isolated microbial communities could utilize the pollutant constituent as energy and carbon source (Das and Mukherjee 2007).

The high occurrences of the bacteria and fungi in the polluted sites (station A and B) indicates that the indigenous microbes in the polluted soil and water have catabolized part of the organic and inorganic substances present in the pollutants and used them for metabolic process to grow. According to Ojo (2006), the activities of the indigenous microbes could be responsible for the bioremediation and degradation of pollutants in polluted sites.

The unpolluted samples (control station) had low microbial population suggests that natural unpolluted water environment do not support the abundance of microorganisms because there are no sufficient carbon and energy source in the environment that could encourage them to multiply. Corroborating this finding was Okerentugba and Ezeronye (2003), Onifade and Abubakar (2007) and Boboyeet al. (2010) and according to them, high numbers of certain faecal and agricultural wastes degrading microorganisms from an environment implies that those organisms are the active degraders and indicators of such pollutants.

In particular, the proportion of microbial population with plasmids containing genes for the utilization of these pollutants increased (Atlas 2000). Similarly, Singh (2006) reported a group of terrestrial fungi namely Aspergillus spp., and Mucor spp. that are potential degrader of sewage, industrial and other agricultural waste. The yeast species, namely Geotrichum spp. Isolated from contaminated water were noted to degrade agricultural and petroleum compound.

Species number and community composition of organism have been found to be more reliable monitoring parameters than abundance and biomass (Joschkoet al. 2006). Environment, climate, and food are the three main factors that dictate the distribution of organism in aquatic habitat.

The environmental quality is determining the physical and chemical habitat quality in relation to the species-specific preference and tolerance. The provision of food is determining the carrying capacity/maximum population size. Weather is decimating populations and initiates

phases of recovery. Some species are known to have particular requirements with regard to nutrients or levels of dissolved oxygen. Once defined, the presence of species indicates that the given parameter is within the tolerance limits of that species.

In this investigation, we observed the trends of species diversity declined, the reference station had the highest number of organisms. We observed that the composition and distribution of microbial load (bacteria and fungi) was inversely proportional to the zooplanktons and macro invertebrate's population in the investigated creeks.

As stated earlier; the bacteria and fungi detected in this study can resist environmental contamination and utilize the pollutant constituent as energy and carbon source, but could not adequately settled in clean environment because of insufficient energy source to survive, whereas pollution inhibit multiplication and even survival of planktons and aquatic animal as a result of eutrophication.

Rotifer was the most abundance plankton observed in all the stations, which can be attributed to its high degree of pollution resistance. Similar observation was reported by Sunil (2017) in assessing the quality of water in the nagpokhari of Kathmandu valley, Nepal, using zooplankton.

The progressive decline in the plankton and macro-invertebrates may be attributed to pollutants that altered the hyro-chemical composition of aquatic life (Alimov 2010).Similar finding, but with different habitat was reported by Timmermann et al. (2006) that earthworm numbers in wet grassland in the Netherlands fluctuated by a factor of 5.8 during a 10 years observation period, and that the fluctuation correlated with the sum of daily average temperature values below 0oC in the preceding winter. Other authors found soil moisture to be the key limiting factor for earthworm populations (Eggleton et al. 2009).

### CONCLUSION

This study had shown the present of zooplanktons (cladocera, copepod, rotifers) and macro-invertebrates (oysters, leeches and A. longa) in the creek. These organisms can be used as indicator's species considering their abundance and distribution pattern. The bacteria and fungi identified in this study along with the hydro-chemical properties of the creek observed revealed its pollution status showed that the creek is polluted because for the fact that they are hydrocarbon utilizing microbes. The sampled organisms had shown to be useful indicators of water shed health as they are not difficult to identify in the laboratory and can live for more than one year, have restricted mobility, integrators of ecological condition.

### SIGNIFICANCE STATEMENT

This investigation established that the Otuoke creek is highly polluted mainly by anthropogenic activities as shown by the control and contaminated stations. The microbes, planktons and benthic organisms distribution and abundance had depict their significance as bio-indicators of the health status of aquatic ecosystem

### **AUTHOR'S CONTRIBUTION STATEMENT**

DR. IKPESU, THOMAS OHWOFASA: Design the research; participate actively in visibilities studies and samples analysis

DR. UZOEKWE, S.A: Carryout the physicochemical analysis of the investigated creek and actively involved in the writing of the manuscript.

### **CONFLICT OF INTERESTS**

Dr.Ikpesu,T,O declares that there is no conflict of interest. Dr.Uzoekwe, S.A.declares that there is no conflict of interest. The work was a PhD thesis and was self-sponsored.

### ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

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