



## **Diversity and Levels of Bacteriological Contamination in Orashi River, Mbiama Community, River State, Nigeria**

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### **Authors' contributions**

*This paper was based on part of undergraduate project work of the lead author EPK, supervised by the second author EIS. Author EIS wrote the initial draft based on author EPK project work. Author EIS edited the manuscript. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

This study evaluated the level and diversity bacteria contamination in River Orashi in Mbiama community, Rivers state of Nigeria. Triplicate samples were obtained from three stations viz: upstream, downstream and midstream. Standard bacteriological methods were employed for the analysis. Total heterotrophic bacteria, total coliform and fecal coliform ranged from 3.00 to 7.00 x 10<sup>6</sup> cfu/ml, 12.00 to 15.67 MPN/100 ml and 7.00 to 14.00 MPN/100 ml respectively. Analysis of variance showed that there were no significance difference (P>0.05) among the various location apart from total heterotrophic bacteria that was significantly different (P<0.05) among the different location. The bacteria density exceeded the Standard Organization of Nigeria and World Health Organization/Food and Agricultural Organization allowable limit of 1.0 x 10<sup>2</sup> cfu/ml for potable water. The bacteria isolates identified include *Pseudomonas*, *Proteus*, *Micrococcus*, *Shigella*, *Salmonella*, *Enterobacter* species, *Staphylococcus aureus* and *E. coli*. The findings of this study showed that the water quality of Orashi River at Mbiama community is not suitable for consumption based on bacteriological analysis. As such the water should be treated through boiling and chlorination prior to consumption.

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## 1. INTRODUCTION

Water resources include rain, ground and surface water. In developing country like Nigeria, these three water resources is used as potable water [1]. Typically, the choice of potable water depends mostly on its availability. For instance, in areas that ground water is grossly inadequate and or unavailable surface water is mostly used during the dry season and rainwater is trapped through roofing sheet during the wet season.

Surface water is frequently contaminated through several routes including direct deposition of wastes and indirectly through runoff [2-3]. The compositing of the wastes and runoff affects the water quality parameters including physicochemical, heavy metals and microbial diversity and density.

Several activities are carried out in water bodies in the Niger Delta including sewage deposition through pier toilet system [4-7], effluents from food processing such as oil palm processing [8], cassava processing and deposition of refuse/wastes in unsustainable manner. Several categories of refuse are deposited in surface water in the central Niger Delta including domestic and market wastes especially in communities aligning surface water. Depending on the composition of the wastes it could lead to acidification and eutrophication.

Surface water being an habitats of several biodiversity including aquatic plants such as water hyacinths [6,7,9], aquatic mammals, reptiles, fisheries (fin and shelled fish), planktons [10], their composition/diversity and abundance could be altered due to change in water quality. Beside the water, the sediment of the surface water could also be impacted when they receive pollutants from the environment. Typically, the sediment occurs as deposited or suspended sediment depending on the composition of substances deposition of the water [11-13].

Several studies have been carried out in the water quality in the Niger Delta especially in Bayelsa state [6,7,9,14-19]. Furthermore, previous studies have been carried out in Orashi River with regard to the sediment [20] and water quality [21]. Therefore, these present study focus

on the level of bacterial contamination in Orashi River, Rivers state, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

River Orashi passes through several communities. Mbiama which is situated in the Ahaoda West Local Government Area of Rivers State is one of the communities the River Orashi passes. River Orashi lies between latitudes 50° 45" and 60° 35" N and longitudes 40° 50" and 50° 15" E [20,21]. The River Orashi is one of the major rivers in the Easter Niger Delta [20]. The geology of the area is characterized by a vast flood plain built up sedimentary deposits [21], the area has two predominant seasons including wet (April to October) and dry (November to March of the following years). Several economic activities are carried out in Mbiama including trading, dredging, boating. Most pier toilet system is also built in the river.

### 2.2 Sampling Techniques

Triplicate samples was collected from three different locations (Upstream, midstream and downstream). The sampling stations were approximately 2 km apart [22]. At each sampling locations, three samples were collected using sterile container; at the town side banks, the middle of the river and in the opposite bank of the river [22]. Sampling was done at the early hours of the day (between 8.30 am-10 am). All the samples were appropriately labeled at the sampling stations. Water samples collected were taken to the laboratory for analysis.

### 2.3 Bacteriological Examination of the Water Samples

#### 2.3.1 Examination of total and faecal coliform

The most probable number guide by Pepper and Gerba [23], Benson [24] Akubunenyi et al. [25] was employed for determining the population density of total and fecal coliform in the water samples. The test was carried through presumptive, confirmatory and completed test. The results were compared with the most probable number table of Pepper and Gerba [23].

### **2.3.2 Enumeration of total heterotrophic bacteria population**

The total heterotrophic bacteria counts were enumerated using Nutrient Agar, which was prepared based on manufacturers' instruction. Pour plate techniques described by Pepper and Gerba [23] and Benson [24] was adopted for the enumeration of the total heterotrophic bacteria population. About 1.0 ml of serially diluted sample was aseptically plated, then after the agar plates were incubated inverted at 37°C for 24- 48 hours. The resultant colonies were counted and expressed as colony forming units per the water sample.

### **2.3.3 Tentative identification of the microbial isolates**

The isolates from the Nutrient agar was subculture into pure cultures. The isolates were subjected to biochemical tests following guide of Cheesbrough [26] and Benson [24]. The water samples were also streaked in Blood Agar, Salmonella shigella agar and Mannitol Salt Agar plate. On Blood agar the presence of swarming characteristics suggests the presence of Proteus species. The presence of black and pink growth in Salmonella- Shigella Agar indicates the presence of Salmonella and Shigella species respectively [22,27]. While yellowish pigments in Mannitol Salt Agar indicates *Staphylococcus aureus* [28]. Furthermore, the positive tubes based on colour change and gas production were shaken and streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37°C for 24 hours. The presence of small nucleated colonies with greenish metallic sheen indicates *E. coli* [23,24]. All growth in the different media used for the identification was streaked in nutrient agar from where the biochemical test was carried out. The scheme of Cheesbrough [26] and Bergey's Manual of Determinative Bacteriology by Holt et al. [29] was used to compare the results of the biochemical test carried out. Also, the Salmonella species were further identified using Triple Sugar Iron Agar. A positive tube was confirmed by presence of cracks and blackening of the medium indicate [22,30].

### **2.4 Statistical Analysis**

SPSS software was used to carry out the statistical analysis. Univariate analysis was

carried out. Data were expressed as mean  $\pm$  standard error (n=3). On way analysis of variance was carried out at P = 0.05. Mean separation was carried out using Tukey Honestly Significance Difference (HSD).

## **3. RESULTS AND DISCUSSION**

Table 1 presents the bacteria density in water samples from Orashi River at Mbiama Axes, River state Nigeria. Total heterotrophic bacteria, total coliform and fecal coliform ranged from 3.00 to 7.00 x 10<sup>6</sup> cfu/ml, 12.00 to 15.67 MPN/100 ml and 7.00 to 14.00 MPN/100 ml respectively. There was no significance difference (P>0.05) among the various location apart from total heterotrophic bacteria that was significantly different (P<0.05) among the different location. The variation with regard to total heterotrophic bacteria counts could be due to difference in anthropogenic activities of the river during the sampling period. The water is used for several purposes including transportation (using canoe and engine boats), pier toilet system, bathing and cooking. In some instances indigenous people in the region also drinks it. The density exceeded the limit specified by World Health Organization/Food and Agricultural Organization allowable limit of 1.0 x 10<sup>2</sup>cfu/ml for drinking water (for total heterotrophic bacteria) [22,31–35] and 10 cfu/ml and 0 cfu/100 ml for total coliforms and Thermo tolerant Coliform/*E. Coli*/ faecal streptococcus for total and fecal coliforms respectively [22,31,32,36]. The occurrence of coliforms suggests that the water contain fecal materials. This is evident of pier toilet system in the water. The bacteria density is comparable to the values in the order of 10<sup>6</sup> previously reported in some surface water around Wilberforce Island, Bayelsa state [5], River nun at Amassoma axes in Bayelsa state [22]. Similarity in the microbial density in this study compared to previous study could be due to the fact that the residence of the area practice similar wastes management processes. Authors have variously reported that surface water is major recipient of domestic wastes and sewage [2,3,5,6].

Table 2 present the bacteria isolates from Orashi River at Mbiama station, River state, Nigeria. *Pseudomonas*, *Enterobacter*, *Staphylococcus aureus* and *E. coli* occurs in all the locations (downstream, mid and up stream).

**Table 1. Bacteriological mean density of Orashi River at Mbiama section**

Location	Total heterotrophic bacteria (10 <sup>6</sup> ), cfu/ml	Total coliform, MPN/100 ml	Fecal coliform, MPN/100 ml
Upstream	3.00±1.11 <sup>a</sup>	15.67±2.49 <sup>a</sup>	14.00±3.33 <sup>a</sup>
Midstream	5.00±1.9 <sup>b</sup>	12.33±2.15 <sup>a</sup>	14.70±3.15 <sup>a</sup>
Downstream	7.00±1.97 <sup>c</sup>	12.00±2.62 <sup>a</sup>	7.00±1.8 <sup>a</sup>

Data is expressed as mean ± Standard Error (n=3); The same superscripts letters along the column indicate no significance variation (P>0.05) according to Tukey Honestly Significance Difference statistics

**Table 2. Bacteria isolates from Orashi River at Mbiama station**

Microbes	Up-stream	Mid-stream	Down-stream
<i>Pseudomonas</i> sp	+	+	+
<i>Enterobacter</i> sp	+	+	+
<i>Staphylococcus aureus</i>	+	+	+
<i>Micrococcus</i> sp	+	-	+
<i>E. coli</i>	+	+	+
<i>Proteus</i> species	+	-	+
<i>Salmonella</i> sp	+	-	+
<i>Shigella</i> sp	-	-	+

+ = Present; - = Absent

While *Proteus*, *Micrococcus* and *Salmonella* species occurred in only upstream and downstream. Furthermore, *Shigella* occurred in only downstream. The diversity of bacteria in the various water sample location is evident of anthropogenic activities. Typically, upstream of a location may be downstream of another community (location). The lower of bacteria diversity in the midstream could be to the fact its receives lower effect of anthropogenic activities compared to the upstream and downstream. The various bacteria isolates found in the water have been reported in river Nun at Amassoma axes in Bayelsa state [22] and In a review study in different potable water sources (surface, ground and rain water) in Nigeria [31]. Some of the isolates are pathogenic to human health. Majority of the isolates have been implicated in diarrhea related diseases (*E. coli*, *Salmonella* sp).

#### 4. CONCLUSION

Water is a valuable resources needed for the sustenance of life. This study assessed the level and impacts of bacterial contamination in Orashi River at Mbiama axes. The study found that total heterotrophic bacteria, total coliform and fecal coliform exceeded the World Health Organization/ Standard Organization of Nigeria limits for drinking water. Some of the bacteria isolates are pathogenic to human health. As such the water should not be consumed with appropriate treatment such as boiling and chlorination. Furthermore, wastes deposition in

the surface water should be avoided to minimize its potential effect on bacteriological quality of the river.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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