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Physicochemical And Bacteriological Variables Of River-Nun Along Three Communities In Bayelsa State Nigeria

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Abstract – Physicochemical and bacteriological parameters were investigated in river nun along its path with stations situate at Amassoma, Tantua and Tombia communities of Bayelsa State. This was undertaken in order to gauge the effect of human intrusions and show site specific patterns in the effect of pollutants on the health of the river. Physicochemical parameters of Temperature, pH, Turbidity, Conductivity and Total Dissolved Solids were investigated. While bacteriological variables of heterotrophic and total coliform bacteria were identified in the water samples. Result was analyzed for means and standard deviation, Analysis of variance was conducted at the 95% confidence to determine the variability and similarities between sampling stations. Turkey post Hoc test was thereafter employed to separate means and show the relatedness between variables. Result from the study indicate that there were significant differences (P<0.05) between all stations in physicochemical variables except conductivity and Total dissolved solids where Amassoma and Tantua were not significantly different (P>0.05) but significantly different from Tombia. There was no significant difference (P>0.05) between all stations in heterotrophic bacteria. Heterotrophic bacteria count show that Tombia>Amassoma>Tantua. There was no significant difference (P>0.05) between all stations in total coliform bacteria count. However, Amassoma>Tantua> Tombia. The Preponderance of total coliform bacteria in Amassoma and Tantua more than in Tombia is attributable to human activities of open defecation into the river. All total coliform bacteria count exceeded international permissible limit. This imply that the safety and health of people living along the river catchments is in grave danger as the consumption of these water without treatment can be fatal.

Keywords – Physicochemical, Bacteriological, River-Nun, Bayelsa State, Nigeria.

I. INTRODUCTION

Anthropogenic inputs into water bodies represents the most potent and valid threat to aquatic ecosystems throughout the world. Apart from the recent emerging threat of the appearance of foreign evasive and hybrid species of animals and plants into ecosystems, man-made intrusive substances account for nearly all aquatic ecosystem dysfunctions. Anthropogenic pollutants include crude oil and its refined derivatives, agrochemicals, fecal matters, dust and smokes, etc. In the Niger Delta, inlands waters are mostly contaminated by human activities originating from its catchment area. [1].

Common activities around river catchments are waste dumping, laundry of cloths, transportation of refined petroleum oils, open defecation and bathing. The study of the effects of land-based pollutants on planktonic populations and water quality, or even directly on fish tissues and blood have been copiously investigated. However, few studies have examined the link between catchment area induced pollution and physiochemical cum microbial quality of receiving waters in Nigeria.

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River nun is one of the most extensive river networks in Nigeria, receiving various waste inputs directly or indirectly into it. Sadly, its importance in transportation, supply of fish and other aquatic products and its use as drinking water is unsurpassed. The need to study its physio-chemistry and bacteriology as regards to specific catchment activity patterns is paramount. Therefore, study locations in Amassoma, Tantua and Tombia communities which play host to the traversing river were identified and used for this study.

This will provide useful information on the spatial pollution status of the river and its implied implication on aquatic biota and human health.

II. Materials and Methods

2.1 Sampling Locations

The coordinates and description of study stations are presented in Table 1 below,

Table 1: Study Area (Sample stations)

S/N	Location	Coordinates	Description of Stations
1	Amassoma	$4^0$58'13.15''N and $6^0$6'32.94''E	Within Community (Human activities)
2	Tantua	$5^0$1'8''N and $6^0$12'7''E	Within Community (Human activities)
3	Tombia	$4^0$47'19''N and $6^0$53'39''E	Upstream (Removed from human interference)

2.2 Measurement of Physicochemical parameters

2.2. 1 Temperature

The temperature of the water was measured on the spot using a thermometer. The end of the thermometer was dipped into about 15-20 cm water, allowed to stay for about a minute and the readings recorded accordingly for the three locations.

2.2.2 Electrical Conductivity

The electrical conductivity of the sample was measured with the conductivity meter. The probe meter was dipped into the water and the control switch was turned on. A steady reading was recorded as the electrical conductivity of the water.

2.3.3 Total Dissolved Solid

An evaporating dish was washed and oven dried and weighed to obtain a constant weight. 100ml of water sample was transferred into the evaporating dish and placed on a six-hole water bath and evaporated to dryness. The dish and its content were placed in an oven and dried at 105^{o}C to a constant weight. The weight of residue obtained is a ratio of the volume of sample used. The difference in weight is recorded as the total dissolved solid expressed as ppm or mg/L.

2.2.4 pH

The pH-meter (SCHOTT) was used.

2.2.5 Turbidity

Turbidity meter (SCHOTT) was used. Turbidity is expressed in Nephelometric Turbidity unit (NTU).

2.3 Microbial Analysis

2.3.1 Preparation of Nutrient Media

Nutrient Agar was used for the enumeration of the total heterotrophic plate count, while MacConkey agar was used for the cultivation and enumeration of coliform bacteria. Other media were used for the biochemical test and characterization of the bacterial isolates such as Simmon citrate agar (for the detection of citrate utilization), Kliger iron Agar (for the detection of lactose and glucose fermentation, gas and hydrogen sulphide production) Tryptophan water (for the detection of indole production). The powder media were weighed and dissolved in distilled water according to the manufacturer's instruction. The dissolved media were properly shaken to mix well. Thereafter, they were sterilized at 121^{o}C for 15 min.

2.3.2 Bacteriological Analysis

The three samples collected from three points per location were mixed together to form the stock solution. The stock culture was vigorously shaken to mix well. 1ml of the stock culture was collected and poured into the first dilution tube (10^{-1}). From the first dilution tube, 1ml was collected and transferred to the second dilution tube (10^{-2}). The dilution of the samples was done up to the 4th dilution (10^{-4}). Plating of the samples was done in triplicates with the first dilution using pour plate method. 1ml of the inoculum was aseptically collected with a syringe and poured into a sterile plastic dish. Thereafter, 20ml of the molten nutrient media (nutrient agar) was poured into the dishes aseptically. The plates were swirled to distribute the inoculum evenly in the medium, to achieve an even colony distribution after incubation. After solidification of the medium, the plates were inverted and incubated at 37^{0}C for 24 h in aerobic condition.

After incubation, the plates were observed for colony morphology and colony numbers. The number of colonies is expressed as total colony forming unit per ml (cfu/ml). The colonies were randomly selected and were picked off with sterile wire loop. The colonies were sub-cultured on fresh nutrient agar plates by streaking colonies on the agar surface. The sub cultured plates were inverted and incubated at 37^{o}C under aerobic condition to obtain pure isolates.

The pure isolates obtained were subjected to a series of biochemical tests and gram staining. During the biochemical tests, aseptic techniques and good laboratory practice was strictly adhered to in order to obtain the best and accurate results. Also, standardization of the quantity of reagents and media was done in line with set standard operating procedures.

2.3 Characterization and Identification of Bacteria Isolates

2.4.1 Gram Staining Technique

Colonies from different pure culture plates were emulsified into a drop of distilled water on a slide and a thin preparation was made. The smear was allowed to air dry; the smear was covered with crystal violet stain for 60seconds and was rapidly rinsed with running water. Lugol's iodine was added for another 60 seconds and was rinsed again. The smear was decolorized with alcohol and rinsed again rapidly. A counter stain, Safranin was used to stain for 60 s and rinsed. This was allowed to air dry before examined microscopically under the X100 oil emersion objective lens. Gram positive retain the colour of the stain and stay purple whereas gram negative bacteria turn pink or red.

2.4.2 Catalase Test

This test was performed in test tubes. 3 ml of hydrogen peroxide was discarded into sterile test tubes using a sterile glass rod, colony of the pure culture was picked and dipped into the test tube and observed for production of gas bubbles. The production of bubbles indicates a positive result for the presence of catalase.

2.4.3 Citrate Utilization Test

10 ml of Simmon citrate slants were prepared in test tubes as slants. Using a wire loop, the test isolate was picked off and streaked on the slope of the medium. The test tubes were inoculated at 37^{o}C for 24 hours. The alkaline pH turns the pH indicator from green to blue showing a positive reaction.

2.4.4 Kliger Iron Agar Slant Test

10 ml of Kliger Iron Agar was prepared in test tubes as slants. Using a stab, the butt of the test tubes was first inoculated. Thereafter, the slope was streaked with the test organism with a wire loop. Tubes were closed with cotton wool and incubated at

37°C for 24 h. At the end of the incubation period, the color changes, blackening and cracking of the medium were observed in the tubes and results were interpreted appropriately.

2.4.5 Indole Test

10 ml of the tryptophan broth was prepared in tubes. Using a wire loop, the medium was inoculated with the test organism and incubated for 48 h thereafter, five drops of Kovac's reagent was added to the medium. The development of blue colour within 3 minutes shows a positive reaction while a pink colour shows a negative reaction.

2.4.6 Oxidase test

A piece of filter paper was placed in a sterile petri dish and 3 drops of freshly prepared oxidase reagent was added. Using a plastic wire loop, a colony of the test organism was smeared on the filter paper**.** Microorganisms are oxidase positive when the colour changes to blue within 15 to 30 seconds.

III. RESULT

Table 2: Physicochemical Parameters of water at River-nun

River-nun	Temperature (^{O}C)	pH	Conductivity (s/m)	Turbidity (NTU)	Total Dissolved Solids (mg/l)
Amassoma	27.41^{a}±0.01	7.30^{a}±0.01	53.40^{a}±0.88	32.79^{a}±0.01	26.70^{a}±0.44
Tantua	28.13^{b}±0.01	7.27^{b}±0.01	53.30^{a}±0.62	28.68^{b}±0.01	27.15^{a}±0.31
Tombia	27.30^{c}±0.01	7.39^{c}±0.01	45.16^{b}±0.22	30.72^{c}0±0.02	22.58^{b}±0.11

Data is expressed as Mean ± Standard deviation. Means with the same letter superscripts (a,b,c) on the same column are not significantly different

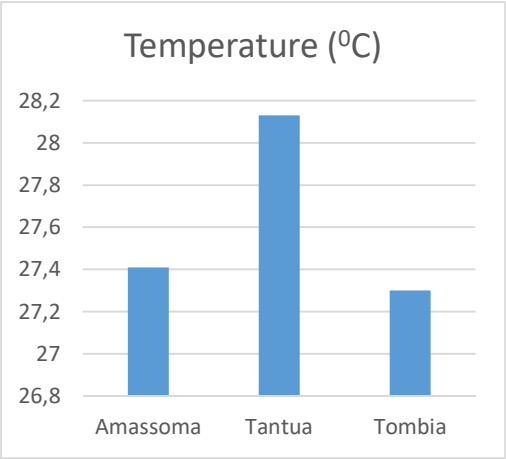

Figure 1: Temp of study stations

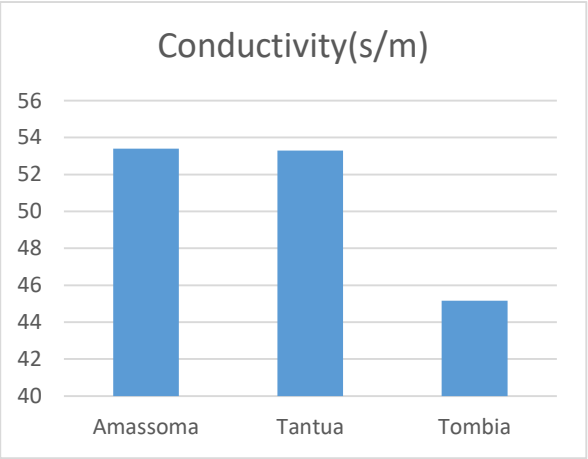

Figure 2: Conductivity of study stations

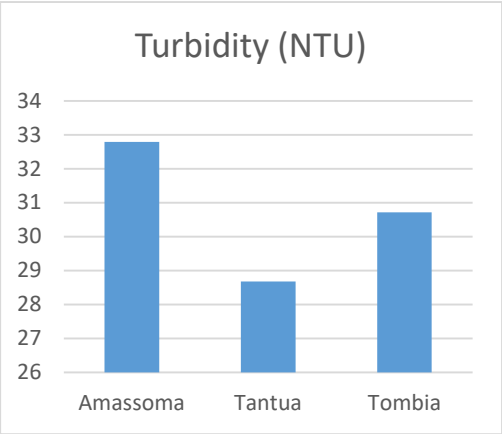

Figure 3: Turbidity of study stations

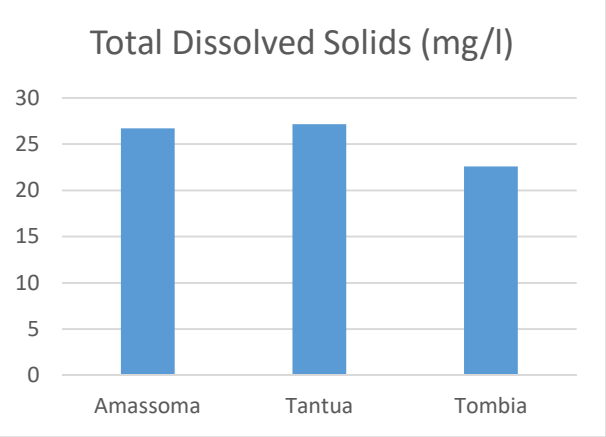

Figure 4: TDS of study stations

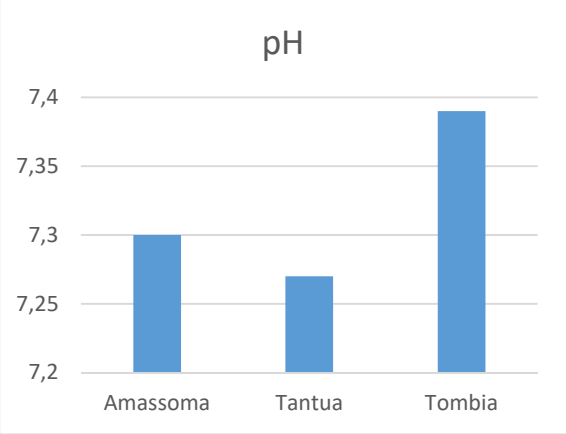

Figure 5: pH of study stations.

Table 3: Total heterotrophic bacteria and Total coliform bacteria

S/N	Location	Total heterotrophic bacteria (CFU/ml)	Total coliform bacteria (CFU/ml)	*WHO Standard [2].
1	Amassoma	130.66[a]±48.05	165.33[b]±20.13	3coliform/100ml for TCB and <500 cfu/ml for THB
2	Tantua	82.66 [a] ± 8.32	165.33 [b] ±36.29	3coliform/100ml for TCB and <500 cfu/ml for THB
3	Tombia	296.00 [a] ±183.69	153.33 [b] ±2.30	3coliform/100ml for TCB and <500 cfu/ml for THB

Data is expressed as Mean ± Standard Deviation. Means with the same letter (a, b) superscript on the same column are not significantly different. *WHO, [2].

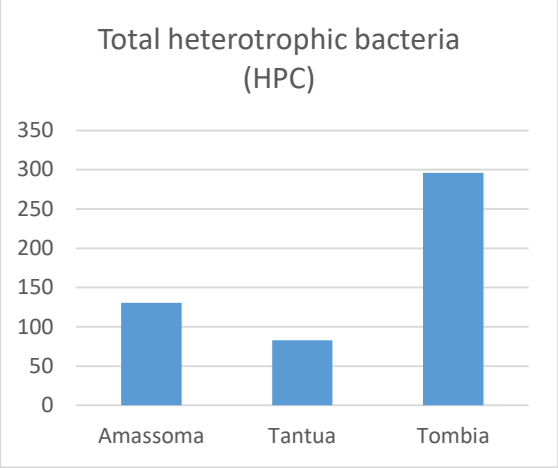

Figure 6: THB in study stations

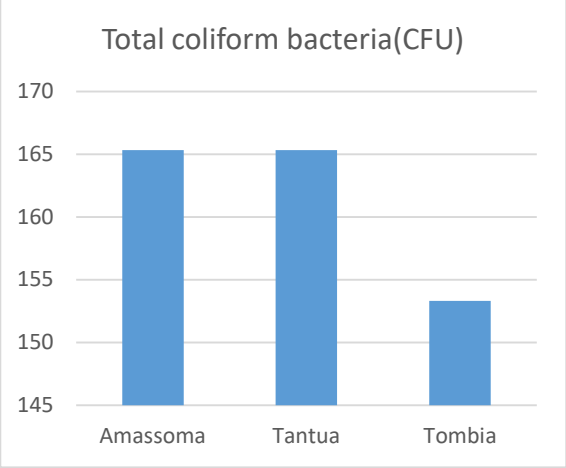

Figure 7: TCB in study stations

Table 4: Biochemical Test and Characteristics of Bacteria Isolate from Amassoma.

Bacteria Isolate	Colonies Morphology	Gram reaction	Motility test	Oxidase test	Citrate test	Indole test	Catalase	KIA Glucose	KIA Lactose	KIA Gas	KIA H_2S	Tentative Identification
I	Rod	-	+	-	-	-		+	-	+	+	*Salmonella* sp.
II	Rod	-	-	-	-	+	+	+	-	-	-	*Shigella* sp.
III	Rod	-	+	+	+	-	+	-	-	-	-	*Pseudomonas aeruginosa*
IV	Rod	-	+	-	-	+	+	+	+	+	-	*Esherichia Coli*
V	Cocci	+	-	+	+	-	+	-	-	-	-	*Micrococcus Luteas*
VI	Rod	-	+	-	+	+	+	+	-	+	+	*Proteus Vulgaris*
VII	Rod	-	+	+	+	-	+	+	-	-	-	*Pseudomonas Flourescens*
VIII	Cocci	+	-	-	+	-	+	+	+	-	-	*Staphylococcus* sp.
IX	Rod	+	+	-	+	-	+	+	-	-	-	*Bacillus* sp.

Table 5: Biochemical Test and Characteristics of Bacteria Isolate from Tantua

Bacteria Isolate	Colony Morphology	Gram reaction	Motility test	Oxidase test	Citrate test	Indole test	Catalase	KIA Glucose	KIA Lactose	KIA Gas	KIA H_2S	Tentative Identification
I	Cocci	+	-	+	+	-	+	-	-	-	-	*Micrococcus luteas*
II	Rod	-	+	-	+	+	+	+	-	+	+	*Proteus vulgaris*
III	Rod	-	+	+	+	-	+	+	-	-	-	*Pseudomonas flourescens*
IV	Rod	-	+	-	+	-	+	+	+	+	-	*Enterobacter cloacae*
V	Rod	-	+	-	-	+	+	+	+	+	-	*Escherichia coli*
VI	Rod	+	+	-	+	-	+	+	-	-	-	*Bacillus* sp.
VII	Rod	-	-	-	-	+	+	+	-	-	-	*Shigella* sp.

Table 6: Biochemical Test and Characteristics of Bacteria Isolate from Tombia

Bacteria Isolate	Colony Morphology	Gram reaction	Motility test	Oxidase test	Citrate test	Indole test	Catalase	KIA Glucose	KIA Lactose	KIA Gas	KIA H_2S	Tentative Identification
I	Cocci	+	-	+	+	-	+	-	-	-	-	*Micrococcus luteas*
II	Rod	-	+	-	+	+	+	+	-	+	+	*Proteus vulgaris*
III	Rod	-	+	+	+	-	+	+	-	-	-	*Pseudomonas flourescens*
IV	Rod	-	+	-	+	-	+	+	+	+	-	*Enterobacter cloacae*
V	Rod	-	+	-	-	+	+	+	+	+	-	*Escherichia coli*
VI	Rod	+	+	-	+	-	+	+	-	-	-	*Bacillus* sp.
VII	Rod	-	-	-	-	+	+	+	-	-	-	*Shigella* sp.

Table 7: Pearson's Correlation of Physicochemical Parameters

	River nun	Temperature	pH	Conductivity	Turbidity	Total Dissolved Solids
River nun	1	-0.122	0,687*	-0.811**	-0.505	-0.811**
Temperature	-0.122	1	-0.779*	0.667*	-0.795*	0.667*
pH	0.687*	-0.779*	1	-0.952**	0.259	-0.952**
Conductivity	-0.811*	0.667*	-0.952**	1	-0.083	1.000**
Turbidity	-0.505	-0.795	0.259	-0.083	1	-0.083
Total dissolved solids	-0.811**	0.667*	-0.952**	1.000**	-0.083	1

***Correlation is significant at the 0.05 level (2-Tailed)**

****Correlation is significant at the 0.01 level (2 Tailed)**

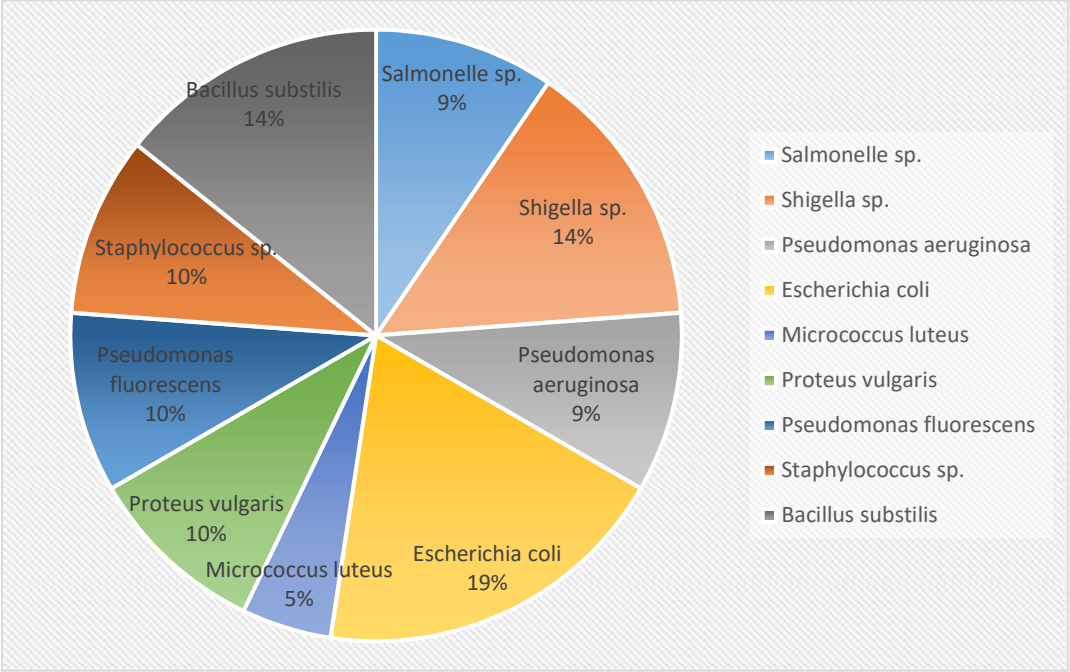

Figure 8: Percentage of occurrence of bacterial isolates in study stations

IV. Discussion

The result shows the physico-chemistry and bacteriological properties of river nun at stations located at Amassoma, Tantua and Tombia. The result of this study is comparable to the findings of previous workers in a similar environment [3]. Agbabiaka and Oyeyiola [4] also reported similar isolates; shigella sp, salmonella sp, klebsiella, pseudomonas, aspergillus sp, Enterobacter sp, Escherichia coli, bacillus, micrococcus, Rhizopus, curvularia, peicillium, Corynebacterium in the surface water of river nun Amassoma axis.

The findings reveal that physicochemical parameters in all sample sites were within normal and permissible international limits for surface waters. There were significant differences ($P<0.05$) between all stations in physicochemical variables except

conductivity and Total dissolved solids where Amassoma and Tantua were not significantly different (P>0.05) but significantly different from Tombia.

There was no significant difference (P>0.05) between all stations in heterotrophic bacteria. Heterotrophic bacteria count show that Tombia>Amassoma>Tantua. Heterotrophic bacteria count in all the stations were within the permissible limit of <500 cfu/ml. Although heterotrophic bacteria are not indicators of pathogenic conditions, some of them like pseudomonas can cause some infections such as skin and lungs ([5], [6], [7]). However, heterotrophic bacteria are considered as an accessory indicator of measuring of coliform in water. If there is a high concentration of heterotrophic bacteria (>1,000CFU/ml) in the water, it will be hard to determine the fecal coliform and pathogenic conditions since organisms like *pseudomonas* and *flavo* bacterium can prevent the growth of total coliform and prevent the observed production of lactose fermentation. This is not the case in this study.

There was no significant difference (P>0.05) between all stations in total coliform bacteria count. However, Amassoma>Tantua> Tombia. All total coliform bacteria count exceeded international permissible limit. The World Health Organization (WHO [2]) stipulates that there should be no fecal coliforms in 100 ml drinking water or more realistically, coliform in water should not exceed 3coliform/100ml.

Higher Total coliform counts were recorded in stations situated at Amassoma and Tantua communities than in Tombia. The higher presence of Total coliforms at stations in Amassoma and Tantua than in Tombia in the river could be traced to the use of pier toilet systems that are built on the water surfaces of the two locations as these stations are situated within the communities. Izah and Angaye [3] also reported high total coliform of river nun at Amassoma axis, in which they implicated human activities like the use of pier toilet systems built on water surfaces in coastal communities in Bayelsa state. This is further corroborated by the lower values of electrical conductivity and total dissolved solids obtained from Tantua. (Figures 2 and 4). Also, pH values were higher in the sampling station at Tonbia than in Amassoma and Tantua. The importance of hydrogen ion concentration (pH) of water is evident in the manner by which it affects the chemical reactions and biological activities that occur only within a narrow range [8]. Typically, the presence of microbial isolates, its distribution, abundance and growth of microbes in water is greatly influenced by environmental conditions such as the ones been carried out in this study; pH, total dissolved solids (TDS) and electrical conductivity [9]. Hence the variation of the microorganisms from the three locations; Amassoma, Tantua and Tombia in this study could be associated to the environmental conditions of the river water.

The high abundance of bacteria such as *Enterobacter, Escherichia, Salmonella* and *Shigella* that were recorded in the river water in this study could be related to one or to a combination of sewage effluents, such as agricultural run-off and direct fecal contamination from humans [10].

In this study, the presence of Escherichia coli is the major indicator of fecal pollution. This constituted 19% of the identified bacterial isolates in the examined water samples, an indication that, the river water has received fecal pollution (Figure 8).

The result indicates an environment under mild threat from land-based activities in its catchment. However, as heterotrophic bacteria are not only decomposers but also competitors with phytoplankton for nutrients, the continued enrichment of the river by anthropogenic activities may portend a greater threat for the ecosystem in the near future.

V. Conclusion

The physiochemical bacteriological properties in this study were evaluated in Amassoma, Tantua and Tombia axis of river nun in Bayelsa state. The results showed no significant variation of the physicochemical parameters of Temperature, pH, and Turbidity between all stations except for conductivity and total dissolved solids were significant differences occurred between Tombia and the other two stations. There were no significant variations as well in total heterophilic bacteria and total coliform bacteria at all stations. However, bacteriological parameters reveal that there were no significant differences (P>0.05) in total heterotrophic and total coliform counts in the three studied sample stations. Amassoma>Tantua> Tombia in total coliform count suggesting that areas far removed from direct human interference even on the same river are not as impacted as areas with direct human activities. In this study the presence of total coliform above recommended international standards suggests a river impacted by fecal inputs among other pollution sources.

It can be concluded that the water quality of river nun is polluted and therefore portends grave consequences for ecosystem and human health.

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