Microbial Analysis of Flood Water at Tombia Community, Niger Delta, Nigeria

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Abstract: **The microbial analysis of flood waters was investigated in Tombia community, Yenagoa, Bayelsa state, Nigeria. This was done to determine the type of microbes associated with flood water in Tombia community and the potential health risk implications of flood waters. Triplicate water samples were collected with sterile bottles from four different locations at Tombia community. Samples were tested using standard procedures and techniques. Result from the investigation reveal that all the samples that were collected from four sampling stations had bacteria and fungi. The total heterotrophic count ranged from 1.80 x 10^6 - 7.10 x 10^6 cfu/ml. Location 4 has the lowest heterotrophic count while Location 2 had the highest heterotrophic count. In this study the coliform and lactose enteric fermenting bacterial count ranged from 1.81 x 10^4 - 3.53 x 10^4 cfu/ml. Location 3 had the lowest count while Location 1 had the highest count. The total fungi count ranged from 1.56 x 10^5 - 8.67 x 10^5 cfu/ml. Location 1 had the lowest count while Location 4 had the highest count. The results show a total of 10 different bacteria isolates. However, they occurred differently in terms of the frequency of isolation. With *E. coli* and *Enterobacter* occurring in every sample tested. The characterization of fungi isolate associated with the four different stations shows a total of 4 different fungi isolate. However, they occurred differently in terms of frequency of isolation, with *Fisarium* and *Mucur* species occurring the most. The general picture of flood water microbial analysis in this study, showed that the flood water samples collected from the four different locations in Tombia community were all contaminated with all sorts of bacteria and fungi. As flooding in low land areas is a perennial event, it is important that more data on pathogen concentration in flood waters should be collected to make more reliable health risk assessment, because of the health risk associated with flood incidents are expected to increase in the future, due to more rainfall induced by climate change.**

***Key words*: Microbial, Flood Water, Tombia, Niger Delta, Nigeria.**

I. INTRODUCTION

Nigeria and most wetland countries of the world have experienced perennial flooding in the recent decade (Bariweni *et al.*, 2012). The reason for this is the rise in sea level globally, caused by global warming as well as the saturated nature of the wetlands in many parts of the world such as Nigeria. The floods that occurred in some parts of Nigeria in 2012 is the country's worst in Nigeria's living memory (Social Action, 2012).

Flooding mostly in areas of massive human development and densely populated areas, is a catastrophe on the people and their livelihood. As an aftermath of flooding, poor hygiene and an increased risk of disease outbreaks is inevitable mostly in poor neighbourhoods. This may result in the intrusion of flood waters into potable water systems resulting in increased risk of waterborne diseases such as typhoid fever and cholera.

Sadly, inhabitants of flood prone areas may not be aware of the health risk posed by these flood waters. Tombia community for instance and indeed most parts of the lower Niger Delta where flooding is a yearly ritual, portray a picture of apathy by the people about the threat posed by flood waters. Water borne diseases that result from direct or indirect interaction with flood waters is rife in the community. There is an acute societal need therefore to investigate microbial characteristics of flood waters in Tombia community in order to identify its make-up and its pathogenicity. This will provide useful information for health professionals, environmentalist and all stakeholders as regards educating the residents, preventing and planning for future floods and its resultant negative fallouts.

II. MATERIALS AND METHOD

2.1 Study Area

This study was conducted in Tombia community, in Yenegoa local Government area, Bayelsa state. Bayelsa state is located in the southern part of Nigeria. The state is geographically located within latitude 04^0 $15^{'}$ North and latitude 05^0 $23^{'}$ South. The state is bounded by Delta state on the North, Rivers on the East and the Atlantic Ocean on the Western and Southern parts. Rainfall in Bayelsa State varies in quantity from one area to another. The mean monthly temperature is in the range of 25^oc to 30^oc.

Figure 1: A picture showing the map of Tombia
(Source: www.google.com)

2.2 Designation of Sampling Stations

2.2.1 Sampling Station 1

Station 1 is located at Ikpaikpai street, Tombia community. The GPS coordinates of station 1 are N $4^0$59'47.45" and E $6^0$16'11.388" (Plate 1).

Plate 1: Station 1 (Ikpaikpai street, Tombia community).

2.2.2 Sampling Station 2

Station 2 is located at Christian Avenue, Tombia community. The GPS coordinates of station 2 are N $4^0$59'52.3788" and E $6^0$15'50.6268" (Plate 2).

Plate 2: Station 2 (Christian Avenue, Tombia community).

2.2.3 Sampling Station 3

This station is located at Shepherd's vine church, Tombia community. The GPS coordinates of station 3 are N $4^0$59'49.9668" and E $6^0$15'56.9268" (Plate 3).

Plate 3: Station 3 (Shepherd's vine church, Tombia community)

2.2.4 Sampling Station 4

The fourth sample was collected from the river near Okpo-Uwou water side, Tombia community (Plate 4). The Global Positioning System (GPS) coordinates of station 4 are N $5^0$08. 19" and E $6^0$15'47.862''.

Plate 4: Station 4 (Okpo-Uwou water side, Tombia community)

2.3 Sample Collection

Triplicate water samples were collected from four different locations at Tombia community, Yenegoa, Bayelsa State. Stations 1, 2 and 3 were taken from three different flooded area (station), while station 4(control) was taken from the river. The samples were put in sterile bottles, labelled and transported to the laboratory for analysis.

2.4 Preparation of Nutrient Media

The nutrient media used for the enumeration and isolation of the bacteria isolate associated with the samples include; nutrient agar, MacConkey agar, MacConkey broth, Eosin Methylene Blue. However, Nutrient agar was used as the

preferred media for sub culturing the bacteria isolates. Other media was used for the biochemical test and characterization of the bacteria isolates such as simmon citrate agar (for detection of citrate utilization), kliger iron agar (for detection of glucose and lactose fermentation, gas and hydrogen sulphide production), peptone water (for indole test). The powder media were weighed and dissolve in distilled water according to the manufacturer's instruction. These dissolve media were properly shaken to mix well. Thereafter, they were sterilized by being autoclave at 121^{o}c for 15 minutes.

2.5 Bacteriological Analysis

The bacteriological analysis comprises enumeration of bacteria colony forming unit (cfu). Isolation of bacteria from the samples were performed onto Plate Count (PC). Identification of bacteria was performed by morphological and biochemical tests. The water sample was shaking vigorously, serial dilution was carried out following aseptic techniques. Nutrient agar was used to enumerate the growth of various microbes ranging from fungi like yeast and mould, Bacteria such as *streptococci* and *staphylococci*. MacConkey agar was used to enumerate the growth of gram-negative microorganisms. *Salmonellashigella* agar was used to enumerate the growth of *Salmonella spp* or *shigella spp.*

2.6 Microbiological Analysis

MacConkey agar, Nutrient Agar and Potatoes Dextrose Agar were used to enumerate total coliform, total heterotrophic bacteria count and total fungi. The media were prepared based on the manufacturers' instruction. Enumeration of the microbial density was carried out following pour plate method previously described by Pepper and Gerba (2005) and Benson (2002). Serial dilution was carried out up to 10-6 and approximately 1.0 ml of the diluents was aseptically plated in sterile petri dishes. Later, the prepared media was poured accordingly. The agar plates were swirled (clock wise and anti-clockwise), and allowed to solidify and then incubated inverted at 37°C for 24- 48 (bacteria) 3-5 days (fungi). The resultant colonies were counted and expressed as colony forming units per gram of the sediment sample.

2.6.1 Tentative Identification of the Microbial Isolates

i. **Bacteria**: The resultant colonies were streaked onto differential media and then subjected to biochemical test using the guide of Chesbrough (2006). Furthermore, the samples were also streaked onto Blood Agar, Mannitol salt Agar, *Salmonella-Shigella* Agar, Levine's eosin Methylene Blue (EMB). The distinct isolates from the various media were also streaked onto nutrient agar and further subjected to another biochemical test. The presence of yellowish pigments in Mannitol Salt Agar indicates *Staphylococcus* aureus and this was confirmed by streaking the colonies in nutrient agar and subjected to coagulase and gram reaction test. From the EMB agar, the presence of small nucleated colonies with greenish metallic sheen indicates *E. coli*, while large nucleated colonies without the metallic sheen is an indication of *Enterobacter* species. From the blood agar, the presence of swarming and haemolytic characteristics after incubation depicts *Proteus* species and *Streptococcus* species respectively. Furthermore, the presence of black colonies in *Salmonella-Shigella* Agar after incubation indicates *Salmonella* species.

ii. Slants in a Triple Sugar Iron Agar. The presence of cracks and blackening in the tube depicts *Salmonella s*pecies. The resultant colonies from the biochemical tests were compared with that of known taxa using Bergey's Manual of Determinative Bacteriology scheme by Holt *et al*. 1989

iii. Fungi: Macroscopic/colonial and microscopic characteristics were used for fungi identification. The scheme of Benson (2002) was used for the macroscopic identification. While the wet mount preparation using Lactophenol cotton blue stain as indicator previously described by Pepper and Gerba; Benson was used for the microscopic identification.

2.7 Biochemical Characterization

2.7.1 Gram Staining Technique

Colonies from different pure culture plate 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 60 seconds and was rapidly washed off with clean water. The lugol's iodine was added for 60 seconds and was washed off. The smear was decolorized with acetone alcohol and washed off rapidly. The smear was counter stained with safranine for 60 seconds and washed off. The smear was examined microscopically under the x100 objective lens.

2.7.2 Catalase Test

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

2.7.3 Citrate Utilization Test

10ml of simmon citrate slants were prepared in test tubes using a stab, the bath of the test tubes was inoculated and a wire loop was used to streak the slope with the test organism on slope. The test tubes were inoculated at 37^{o}c for 24 hours.

2.7.4 Kliger Iron Agar Slant Test

Kliger Iron Agar was prepared in slant test tubes containing 10ml of another medium using a stab. The butt of the test tube was first inoculated. Therefore, the slope was streaked with

the test organism with a wire loop. Tubes were closed with a cotton wool and incubated at 37°c for 24 hours. At the end the incubation period, the colour changes, blackening and cracking of the medium were observed in the tubes and results were interpreted appropriately.

2.7.5 Indole Test

10ml of tryptophan broth was prepared in tubes using a wire loop; the medium was inoculated with the test organism and incubated for 48 hours. Five drops of Kovac's reagent were added.

2.7.6 Coagulate Test

1 -in- 6 dilution of the plasma in saline (0.85%) was prepared; 1ml volume of the diluted plasma was placed in a test tube. An isolated colony of the test organism was emulsified severally in the diluted plasma. The tubes were incubated at 37°c for 24 hours.

III. RESULTS AND DISCUSSION

All samples analysed had different bacteria counts. The total heterotrophic count ranged from 1.80 x 10^6 - 7.10 x 10^6 cfu/ml. Location 4 has the lowest heterotrophic count while station 2 had the highest heterotrophic count as shown in (Table 1) below. The coliform and lactose enteric fermenting bacterial count ranged from 1.81 x 10^4 - 3.53 x 10^4 cfu/ml. Location 3 had the lowest count while Location 1 had the highest count as shown in (Table 1) below. The total fungi count ranged from 1.56 x 10^5 - 8.67 x 10^5 cfu/ml. Location 1 had the lowest count while Location 4 had the highest count.

Table 1: Result for Total Microbial Count from Water Samples

Station	Total heterophic bacteria x ^{106}cfu/ml	Total coliform x 10^4cfu/ml	Total Fungi x10^5cfu/ml
1	1.86 ± 0.11a	3.53 ± 1.24c	1.56 ± 0.08a
2	7.10 ± 1.03b	2.51 ± 0.19a	1.78 ± 0.17ab
3	5.93 ± 0.61b	1.81 ± 0.14a	8.07 ± 0.78bc
4	1.80 ± 0.07a	2.45 ± 0.26a	8.67 ± 2.93c

Data is expressed as mean ± standard error. different alphabets (a, b, c & d) along the same column indicate significant variation ($P < 0.05$) according to turkey honesty significance distance.

Table 2: Characterisation of Bacteria Isolates from Water Sample (Station 1)

Bacteria Isolate	1	2	3	4	5	6
Gram	-veRod	+veRod	+veCocci	-veCocci	-veRod	-veRod
Indole	-ve	-ve	-ve	-ve	+ve	-ve
Citrate	+ve	+ve	+ve	-ve	-ve	-ve
Oxidase		-ve	-ve	-ve	+ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve
Butt	Y	Y	Y	Y	Y	Y
Slant	R	Y	Y	Y	Y	R
H_2S	-ve	+ve	-ve	-ve	-ve	+ve
Gas	+ve	-ve	-ve	-ve	+ve	+ve
Lactose	-ve	+ve	+ve	+ve	+ve	-ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve	+ve
Tentative Identification	*Enterobacter Spp*	*Bacillus Spp*	*Staphylococcus Spp*	*Micrococcus Spp*	*E. coli*	*Proteus Spp*

Table 3: Characterisation of Bacteria Isolates from Water Sample (Station 2)

Bacteria Isolate	1	2	3	4	5	6	7	8
Gram	-veRod	-veRod	+veRod	+veCocci	+veRod	_veRod	-veRod	-veRod
Indole	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
Citrate	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Oxidase	-ve		-ve	-ve	-ve	+ve	-ve	-ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve
Butt	R	Y	Y	Y	Y	Y	Y	Y
Slant	R	R	Y	Y	R	Y	R	R
H_2S	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve
Gas	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve
Lactose	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Tentative Identification	*Pseudomonas spp*	*Enterobacter spp*	*Bacillus spp*	*Staphylococcus spp*	*Corne Bacterium spp*	*E.coli*	*Proteus spp*	*Salmonella spp*

Table 4: Characterisation of Bacteria Isolates from Water Sample (Station 3)

Bacteria Isolate	1	2	3	4
Gram	-ve	-ve	+ve	-ve
Indole	-ve	-ve	-ve	+ve
Citrate	+ve	+ve	+ve	-ve
Oxidase	-ve		-ve	+ve
Catalase	+ve	+ve	-ve	+ve
Butt	R	Y	Y	Y
Slant	R	R	Y	Y
H_2S	-ve	-ve	-ve	-ve
Gas	+ve	+ve	-ve	+ve
Lactose	+ve	-ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve
Motility	+ve	+ve	-ve	+ve
Tentative Identification	*Pseudomonas spp*	*Enterobacter spp*	*Streptococcus spp*	***E.coli***

Table 5: Characterisation of Bacteria Isolates from Water Sample (STATION 4)

Bacteria Isolate	1	2	3	4	5	6	7	8
Gram	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Citrate	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve
Oxidase	-ve		-ve	-ve	-ve	-ve	+ve	-ve
Catalase	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
Butt	R	Y	Y	Y	Y	Y	Y	Y
Slant	R	R	Y	R	Y	Y	Y	R
H_2S	-ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve
Gas	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve
Lactose	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Motility	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Tentative Identification	*Psuedomonas spp*	*Enterobacter spp*	*bacillus spp*	*Salmonella spp*	*Staphylococcus spp*	*Micrococcus spp*	*E.coli*	*Proteus spp*

Table 6: Characterization of Fungi Isolates

Station	Macroscopic Appearance	Microscopic Appearance	Colour	Rapidity of Growth	Hyphae/ Morphology	Tentative Fungi
1.	White surface velvety texture	Rough conidia	Grey	Rapid	*Filamentous* and septate	***Aspergillums spp***
	Wholly growth	Broad hyphae	Grey	Rapid	Simple & non-septate	***Mucur spp***
	Wooly cotton flat spreading	Short conidia	White	Rapid	*Filamentous* & non-septate	***Fisarium spp***
2.	Wholly growth	Broad hyphae	Grey	Rapid	Simple & non-septate	***Mucur spp***
	Wooly cotton flat spreading colonies	Short conidia	White	Rapid	*Filamentous* & non-septate	***Fisarium spp***
3.	Wholly growth	Broad hyphae	Grey	Rapid	Simple & non-septate	***Mucur spp***
	Cotton candy like	Broad hyphae	White	Non-Rapid	Branching mycelium and septate	***Rhizopous spp***

4.	White surface velvety texture	Rough conidia	Grey	Rapid	*Filamentous* and septate	***Aspergillums spp***
	Cotton candy like	Broad hyphae	White	Non-Rapid	Branching mycelium and septate	***Rhizopous spp***
	Wooly cotton flat spreading colonies	Short conidia	White	Rapid	*Filamentous* & non-septate	***Fisarium spp***

Table 7: Percentage of Occurrence of Bacterial Isolate

Bacterial Isolates	Frequency of Occurrence	Percentage
Pseudomonas sp	3	12%
Enterobacter spp	4	15%
Bacillus spp	3	12%
Salmonellaspp	2	8%
Staphylococcus spp	3	12%
Micrococcus spp	2	8%
E.coli	4	15%
Proteus spp	3	12%
Streptococcus spp	1	3%
Corne bacterium spp	1	3%
Total	26	100%

Percentage of occurrence of bacterial isolate is presented in table 7. E. coli and Enterobacter had the highest number of occurrence (15%). Streptococcus and Corne bacterium had lowest number of occurrences 3%.

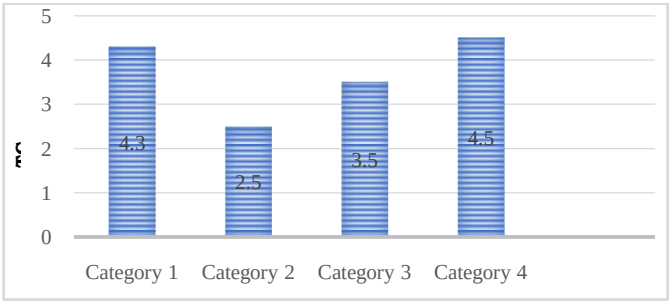

Figure 1: Graphical Representation of Total Coliform count

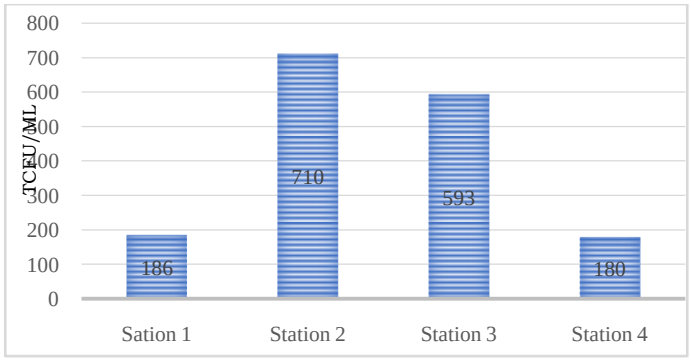

Figure 2: Graphical Representation of Heterotrophic Counts in the sample

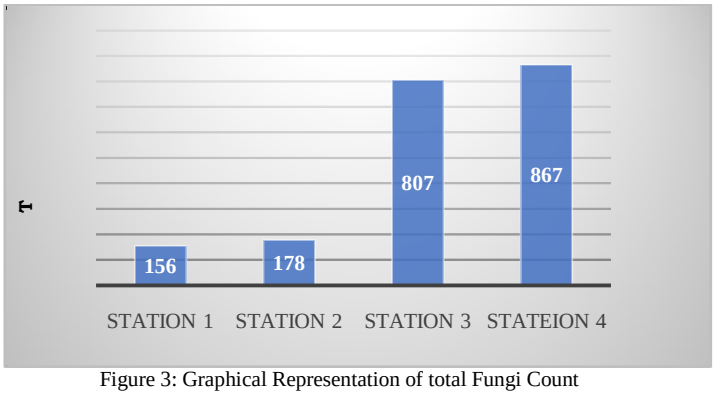

Figure 3: Graphical Representation of total Fungi Count

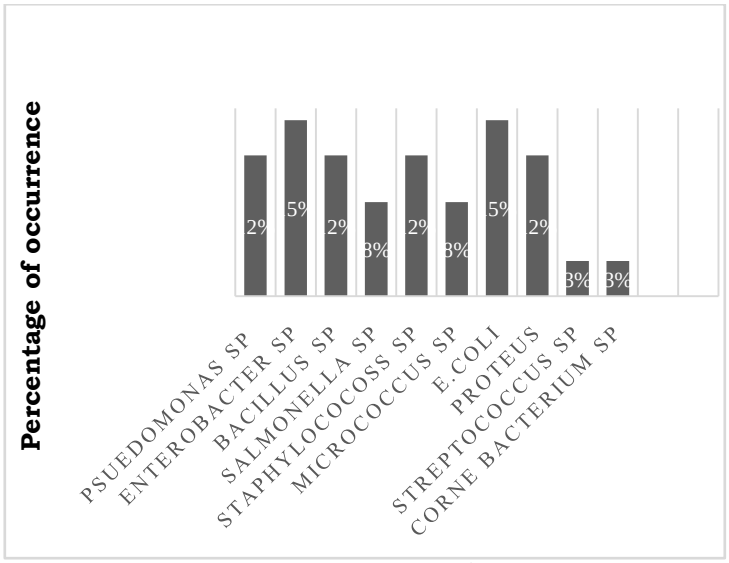

Figure 4: Bacterial Isolates and Frequency of Occurrences.

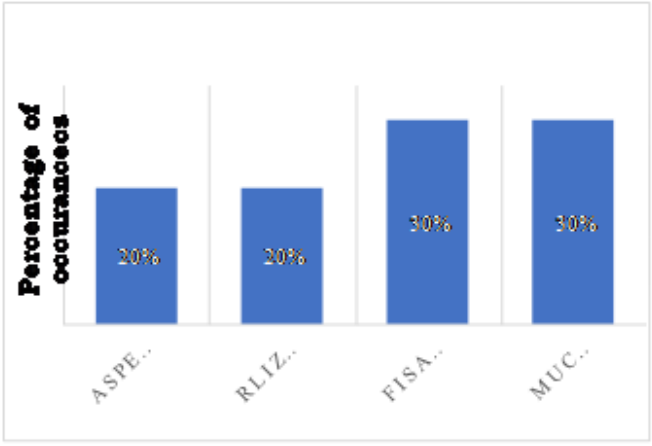

Figure 5: Fungi Isolate and Frequency of Occurrence

The total fungi count ranged from 1.56 x 10^5 - 8.67 x 10^5 cfu/ml. station 1 had the lowest count while station 4 had the highest count. The general picture of flood water microbial analysis in this study, showed that the flood water sample collected from four different locations in Tombia community were all contaminated with all sorts of bacteria and fungi.

Fisarium and *Mucur* species have the highest percentage of occurrence (30%) among the other fungi species. While *Aspergillums* and *Rhizopous* both had the lowest percentage of occurrence (20%). While on the other hands for bacteria species. *E. coli* and *Enterobacter* were identified in every sample we tested. They both had the highest percentage of occurrence (15%) compare to the rest organisms present. *Bacillus* species were detected in similar proportion to *Proteus*, pseudomonas and *Staphylococcus* species, all having (12%) number of occurrences. *Salmonella* species was also identified having similar proportion to *Micrococcus* species, both having (8%) number of occurrences. While *Streptococcus* and *Corne* bacterium species was also detected in small quantity having (3%) number of occurrences. These organisms mentioned poses grave public health concern because exposure to them can cause acute illness. For example, USEPA requires that these organisms must not be present in drinking water (Kistemann *et al*. 2002). Although it is common to find normal gastrointestinal microorganisms such as *E. coli* in normal surface water, their preponderance during the floods, imply that people are expose to these pathogenic diseases when they come in contact with this contaminated flood waters. This report agrees with that of Donovan *et al*. (2008) who provided a report on health risks associated with flood water. Their investigation found a probability of contracting gastrointestinal illness from incidental ingestion of water near flooded environment.

The result of this study agrees with the findings of Abraham & Wenderoth. (2005) who found high concentration of bacteria and fungi in flooded building and on a play grounds days after river Elbe floods in 2002. The result of this study indicates that flooding leads to the presence of pathogens in our environment over prolong periods of time.

The quantitative analysis (number of microorganism) present in the flood water show the presence of potential pathogenic microorganisms. The characterization of bacteria isolate associated with the four different station is shown in table 2, 3, 4 and 5 respectively. This shows a total of 10 different bacteria isolates. However, they occurred differently in terms of the frequency of isolation with *E. coli* and *Enterobacter* occurring in every sample tested. This work also correlates with the findings of Chandra Yard *et al*. (2014) who investigated microbial contamination during and after floods. They reported that *E. coli, Salmonella, Enterococci* species were present in every sample they tested. Veldhuis *et al*. (2010) also investigated the microbes associated in flood water and found out the presence of *E. coli*, *Enterococci and Campylobacter* in all investigated samples.

The characterization of fungi isolate associated with the four different station is shown in table 6, which shows a total of 4 different fungi isolate. However, they occurred differently in terms of frequency isolation, with *Fisarium* and *Mucur* species occurring the most. This result also agrees with the findings of Mhuantong *et al*. (2011) who survey the microbial diversity of flood areas. They reported the presence of fungi and bacteria in the flood waters. The report shows a total of four different fungi occurring at different station, with *Ascormycota* occurring the most among other fungi.

In lowland areas like Tombia community where flooding is a frequent phenomenon, exposure of people to contaminated flood waters during daily activities, swimming or washing of clothes is a serious reason for concern because of the microbial content present in it (Abraham & Wenderoth, 2005).

IV. CONCLUSION

The study evaluated the microbial content present in flood waters in Tombia community. Concentration of different microbes were found in the different respective locations. They include bacteria and fungi. A total of ten different bacteria isolates were present in all different locations. They include *psuedomnonas*, *Enterobacter*, *Bacillus*, *Salmonella*, *Staphylococcus*, *Micrococcus*, *E. coli*, *Proteus*, *Streptococcus* and *Corne* bacterium. However, they occurred differently in terms of frequency of isolation. *E. coli* and *Enterobacter* were present in all the sample tested. While for that of fungi a total of four different fungi were present in all different locations. They include *Aspergillus*, *Rhizopous*, *Fisarium* and *Mucur*. However, they occurred differently in terms of frequency of isolation, with *Fisarium* and *Mucur* species occurring the most.

This study has established that flood waters are likely to be contaminated and may pose potential health risks to residents expose to pathogens in this water.

Given the regular occurrence of flooding in low land areas, it is especially important that more data on pathogen concentration in flood water should be collected to make more reliable health risk assessment, because of the health risk associated with flood incident are expected to increase in the future, due to more intense rainfall induced by climate change (Ashley *et al*. 2005).

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