Cluster Analysis of Aerobic Heterotrophic Bacteria from Clarias gariepinus and Tilapia zillii in Unwana River, Ebonyi State, Nigeria

Uzoeto Henrietta[1], Ayogu Thomas[2], Iroha Ifeanyichukwu[3], Nwakaeze Emmanuel[4], Ejikeugwu Chika[5]

Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, P.M.B 053, Ebonyi State, Nigeria

Abstract— *Fish is a major source of protein for humans, and it is patronized by many in the tropics – where fishes can either be cultivated in the farms domestically or caught from open water bodies such as rivers, ponds and streams. These various sources of fishes and their attendant diversity of microorganisms particularly the bacteria make fishes as potential sources of pathogens. The maintenance of the microbiological quality of food and water is important to prevent waterborne/foodborne diseases in any community, thus the need for this study. A total of 14 samples of water at different points (upstream, midstream and downstream) and 50 samples of live fishes were used for this study. Each of the fish sample was bacteriologically analyzed using the pour-plate and spread plate techniques on culture media plates. And the isolated bacteria were identified using standard microbiological identification techniques. The water samples were subjected to physicochemical analysis to determine the physical and chemical properties of the water. The relatedness of the isolated bacteria was established using cluster analysis/dendogram. The highest bacterial count was obtained from downstream water sample (5.6x10 cfu/ml), indicating a possible pollution of water at this point. Both aerobic heterotrophic Gram positive and Gram negative bacteria were isolated. The Gram positive bacteria isolated include Staphylococcus lugdunensis, S. hominis, S. cohnii, Streptococcus pyogenes, S. pneumoniae, Kocuria varians while the Gram negative bacteria include Raoutella ornithinolytica, Klebsiella pneumoniae, Aeromonas hydrophila, A. veronii, Proteus vulgaris, Serratia fonticola, and Enterobacter gergoriae. Cluster analysis using dendrogram showed some degree of similarity among the different clusters of isolated bacteria. The result of this study presumptively shows that the water sample is polluted; and this in turn affects fresh water fishes in the river. Therefore the microbiological examination of the water at this study site is necessary for monitoring and controlling the quality and safety of the water for usage by the locals.*

Keywords— Water Microbiology, Water Pollutants, Heterotrophic Bacteria, Cluster Analysis, Nigeria.

I. INTRODUCTION

World Fish Centre showed that about 80 to 90 million people depend solemnly on fish annually as their main source of protein (World Fish Centre, 2002). Fishes have been described as the most important animal protein food available in the tropics (Ali *et al*., 2008). About 40% of the dietary protein intakes of an average Nigerian are sourced from fish (Federal Department of Fisheries, 2007). The most commonly cultivated species of fish in Nigeria include catfish, tilapia and carp, and about 60 % of the people leaving in most developing countries depend on fish as their own source of protein (Olagunju *et al*., 2007; FAO, 2003). Proteins obtained from fish contain essential amino acids and have good cholesterol and also low cholesterol level in the body (Anthonio and Akinwumi, 1991, Fagbenro and Arowosoge, 1998). Proteins derived from fish have been of economic value in Africa especially in less developed or developing areas where other sources of animal protein are scarce and expensive (FAO, 2003). Water quality greatly influences the aquatic environment and is important for the survival of aquatic flora and fauna (Deekae *et al*., 2010). The entire array of aquatic life and water quality are affected by pollution. Human activities and anthropogenic pressures like industrial, community waste disposal, heavy use of insecticides, pesticides and fertilizers in agricultural practices are major causes of pollution in aquatic environment. Studies have shown that the leading heterotrophic and aerobic bacterial flora content of streams and rivers comprises of mostly the Gram-negative rods which include *Aeromonas, Pseudomonas and Enterobacteriaceae* and some Gram-positive spore-bearing rods especially those of the genus *Bacillus* (Rheinheimer, 1985, Olayemi, 1994). Unwana River is a fresh water body in Afikpo North Local Government Area of Ebonyi State, South East of Nigeria. There is paucity of information on the water quality and microflora of fresh water fishes from Unwana River. However, indigenous fishes like *Tilapia zillii and Clarias gariepinus* are widely harvested locally and are easily available in the local markets of Uwana community. This study was therefore aimed at studying the water qualities of Unwana River and its influence on the abundance and diversity of bacteria

being harbored both in the water and the resident fishes especially *Tilapia zillii* and *Clarias gariepinus*. Also cluster analysis of these isolates was studied.

II. MATERIAL AND METHOD

2.1 Study Area

The study was Unwana River in Afikpo North Local Government Area, Ebonyi State, South East Nigeria. Unwana town is situated at about 80 Km South East of Abakaliki, with geographical co-ordination of latitude 5^o 48` 51'' and longitude of $7^o$ 55` 20''.

2.2 Collection of Samples

Live fishes were collected using glass aquarium for two years (2013- 2014). Particularly, fifty samples of the two fishes comprising catfish (n=28) and tilapia (n=22) were examined for physical injuries and disease signs like ulcerations and necrotic lesions on the skin surfaces, gills or fin rot, abdominal dropsy and pop eye. Water samples (n=14) from each point were also collected at different points of the river using sterile bottle; and the water samples were collected from upstream, midstream and downstream part of the water.

2.3 Determination of Physical Parameters of the water sample

The physical parameters of the water sample that were determined include temperature, pH, specific gravity, ascorbic acid, sodium, potassium, chloride, phosphate, calcium, copper, magnesium, nitrates, ammonium, dissolved oxygen concentration (DOC) were determined following the method described by Al-Harbi and Uddin (2004).

2.4 Analysis of fish samples

Each of the parts of the fish sample including the gills, intestines, and skin was examined. Briefly, live fish was killed through cervical puncture and dissected; and the skin, gill and intestine samples of each fish were removed aseptically and collected separately in a sterile container. Exactly 50 g of each of the part was weighed and crushed and 450 ml of distilled water added to already crushed sample and allowed to stay for few minutes. These formed the stock solution. Then, 10-fold serial dilutions were prepared from this stock solution up to 10^3. Samples were plated onto nutrient agar and different selective media including Cystein lactose electrolyte deficient (CLED) medium, MacConkey agar, Sorbitol agar, Vogel and Johnson agar, *Salmonella-Shigella* agar, Cetrimide selective agar, *Staphylococcus aureus* chromogenic agar, blood agar, Mannitol salt agar, Tryptone soy agar (TSA), Thiosulphate citrate bile sucrose (TCBS) agar. Bacterial enumeration was done using the pour plate and streak plate technique (Cheesbrough, 2004). All the culture plate was incubated at 27^oC for 24 - 48 hrs.

2.5 Water analysis and bacteria isolation

Water samples collected in sterile bottles were plated in various culture media including CLED, MacConkey agar and incubated as in the fish samples. The bacteria growths (colonies) were separated into diverse types according to the colonial appearance (colour, elevation, shape, size). Bacterial colonies from these plates were then streaked on TSA plate continually until distinct colonies were obtained. Cultures on TSA slant were further stored in the refrigerator at 4^oC as stock for further use. These bacteria colonies were always transferred to fresh slant to avoid contamination every 6 weeks.

2.6 Heterotrophic bacterial count and identification

The heterotrophic bacteria count of the water samples was done using pour plate method. Ten (10^{-1}) fold serial dilution of the water samples were carried out and samples were collected from the fourth tube (10^4) and the samples were inoculated on nutrient agar and incubated for 18 – 24 hrs at 27^oC. All culture plates were prepared in triplicates. Quebec colony counter with built-in grid to simplify counting were used for the enumeration of the heterotrophic bacteria. Bacterial identification was done using standard microbiological techniques; and the commercially available identification kit, Analytical Profile Index (API) kit (BioMerieux, France) was also included in the identification protocol for bacterial identification.

2.7 Cluster Analysis of Bacterial Isolates

Simple matching coefficient (S_{SM}) method was used statistically to produce a dendrogram which showed the groupings and relatedness of the various bacterial isolates.

III. RESULTS

The physicochemical property of the water samples is shown in Table 1. The physicochemical properties investigated include the appearance, pH, temperature, specific gravity, ascorbic acid, sodium, potassium, chloride, phosphate, calcium, copper, magnesium, nitrate and ammonium. And the result showed high contents of phosphate, chloride, and nitrates – which indicate possible pollution of the river. Table 2 shows the heterotrophic bacteria count of water sample collected from upstream, midstream and downstream. The result obtained showed colony forming unit between 2.4 X 10^4 cfu/ml, 3.4 X 10^4 cfu/ml and 5.6 X 10^4 cfu/ml for water samples collected in the upstream, midstream and downstream respectively (Table 2). The colony count of bacteria was highest at downstream compared to the midstream and upstream which had lower bacterial counts.

TABLE 1
AVERAGE PHYSICOCHEMICAL PARAMETERS OF WATER SAMPLES

Water Sample	Appearance	P^H	Temp	Specific gravity	Ascorbic acid (mgl-l)	Sodium (mgl-l)	Potassium (mgl-l)	Chloride (mgl-l)	Phosphate (mgl-l)	Calcium (mgl-l)	Copper (mgl-l)	Magnesium (mgl-l)	Nitrates (mgl-l)	DOC(mgl-l)	Ammonium
A	Brownish & clear	6.5	26.1 ± 1.16 ^{0}C	1	6.03 ± 0.12	184.07 ± 0.34	3.75 ± 0.03	81.33 ± 1.25	4.23 ± 0.23	25.23 ± 0.29	1173.33 ± 20.55	0.38 ± 0.37	1.93 ± 0.05	4.20 ± 0.08	0.06 ± 0.00
B	Brownish & clear	6.5	24.4 ± 0.94 ^{0}C	1	5.87 ± 0.12	175.73 ± 0.87	5.40 ± 0.16	75.27 ± 0.34	10.47 ± 0.21	28.07 ± 0.77	1213.33 ± 14.48	0.60 ± 0.08	2.67 ± 0.12	4.50 ± 0.12	0.11 ± 0.00
C	Brownish & clear	6.5	25.3 ± 0.69 ^{0}C	1	5.87 ± 0.12	17.53 ± 0.26	5.20 ± 0.16	75.43 ± 0.24	10.43 ± 0.25	28.57 ± 0.17	1208.33 ± 6.24	0.58 ± 0.02	2.70 ± 0.16	4.60 ± 0.16	0.16 ± 0.01

Key: A= Upstream, B= Midstream and C= Downstream. The values were expressed in Standard mean deviation (±).

TABLE 2
AVERAGE COLONY FORMING UNIT COUNT (HETEROTROPHIC BACTERIA COUNT) ANALYSIS ON THE WATER SAMPLES

Sample	Average CFU Count
Upstream	2.4 X10^4 CFU/ml
Midstream	3.4 X10^4 CFU/ml
Downstream	5.6 x10^4 CFU/ml

Key: CFU/ml = Colony Forming Unit per millilitre

Figure 1 shows the results of the dendrogram on bacteria isolate from *Clarias gariepinus*. Bacterial species were clustered based on the similarity in their phenotypic and morphological characteristics. It was observed that some bacteria showed similarity and relatedness while some had dissimilarity to certain degrees. The results of the dendrogram on bacteria isolate from *Tilapia zillii* is shown in Figure 2. Bacterial species were clustered based on the similarity in their phenotyic and morphological characteristics (Figure 2). It was also observed that some bacteria showed similarity and relatedness while some had dissimilarity to certain degrees. The pictorial representations of the frequency of the bacteria isolated from *Clarias gariepinus* is shown in Figure 3. The results showed that more bacteria were isolated from the intestines of *Clarias gariepinus* than from the gills and skins of the same fish sample. Figure 4 shows the pictorial representations of the frequency of the bacteria isolated from *Tilapia zillii*. It was also observed that more bacteria were isolated from the skin of *Tilapia zillii* than the gills and intestine of the same fish sample. The frequency of bacteria isolated from water samples is elucidated in Figure 5. The bacterial species isolated from the water sample analyzed in this study include *Raoultella ornithinolytica, Staphylococcus aureus, Staphylococcus hominis, Staphylococcus lugdunensis, Aeromonas hydrophila, Proteus vulgaris, Klebsiella pneumoniae,* and *Kocuria varians*.

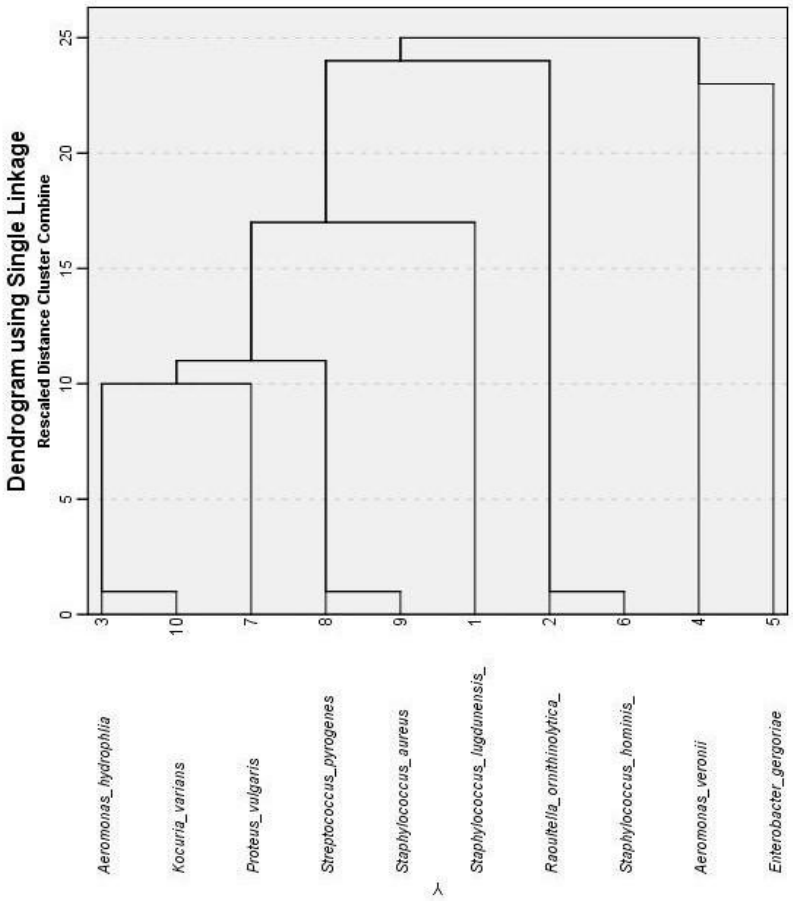

FIGURE 1: DENDROGRAM OF BACTERIA ISOLATES FROM CLARIAS GARIEPINUS USING SIMPLE MATCHING COEFFICIENT (SMM).

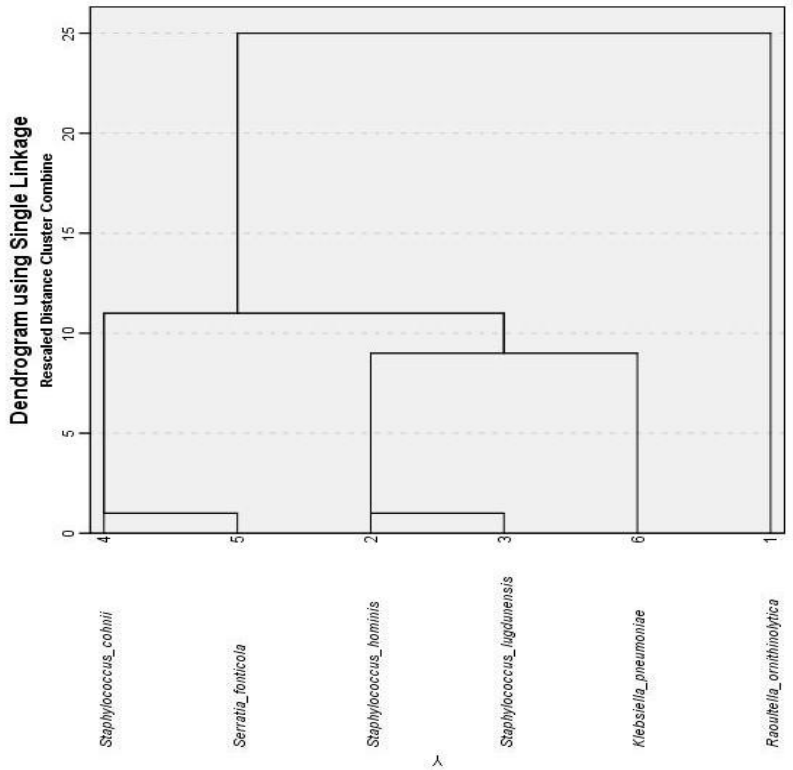

FIGURE 2: DENDROGRAM OF BACTERIA ISOLATES FROM TILAPIA ZILLII USING SIMPLE MATCHING COEFFICIENT (SMM).

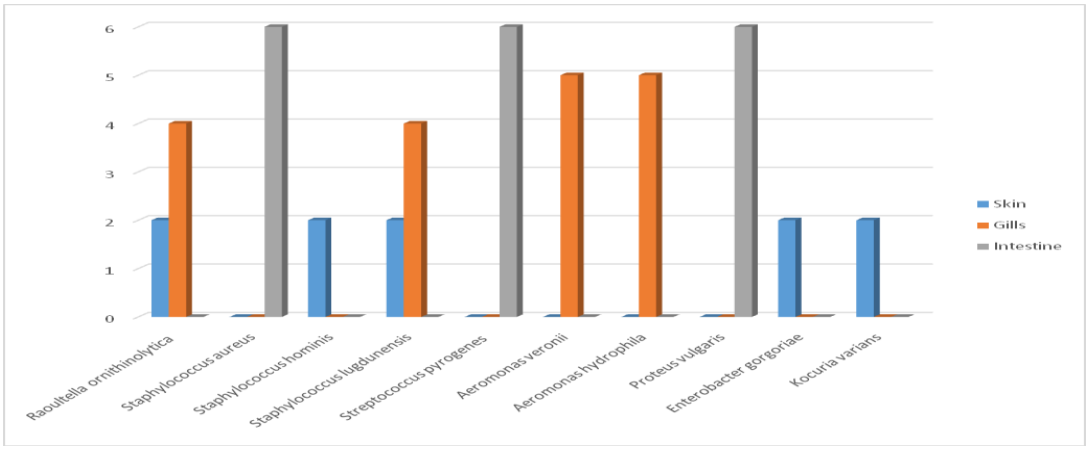

FIGURE 3: FREQUENCY OF BACTERIA ISOLATED FROM CLARIAS GARIEPINUS

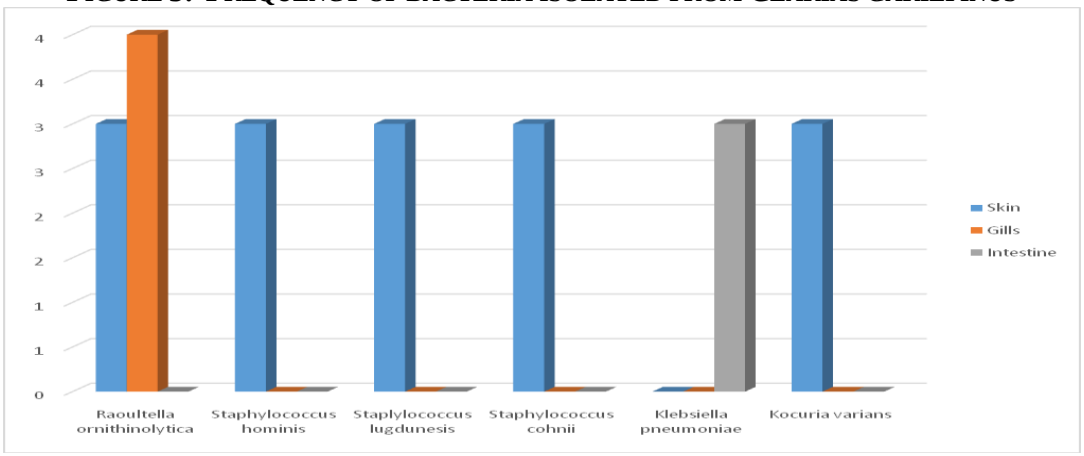

FIGURE 4: FREQUENCY OF BACTERIA ISOLATED FROM TILAPIA ZILLII

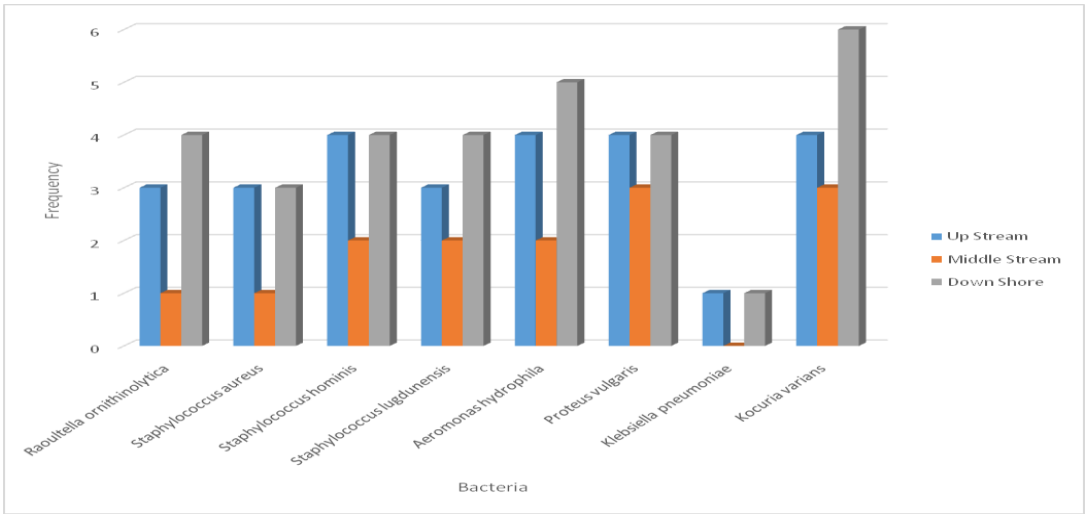

FIGURE 5: FREQUENCY OF BACTERIA ISOLATED FROM WATER SAMPLES

IV. DISCUSSION

The aquatic environment is usually affected by pollution which may arise from either natural cause or human activities. Some human activities and other anthropogenic pressures including but not limited to industrial wastes disposal into water bodies, community waste disposal, and heavy use of insecticides, pesticides and fertilizers in agricultural practices are major causes of contamination in aquatic environment. The pollution of aquatic environment affects aquatic life such as fish – which is a major source of protein to mankind. Several studies abound which reported that the contamination of the aquatic

environment affects aquatic life – which may in turn affect the health of man that depends on it for his source of food especially proteins from fish (Olafsen, 2001, El-Shafai, 2004, Inglis *et al.*, 1994, Al-Harbi and Udadin, 2012). It is in view of this that this investigated the water qualities of Unwana River and its influence on the abundance and diversity of bacteria being harbored both in the water and the resident fishes especially *Tilapia zillii* and *Clarias gariepinus*. The result of the physicochemical parameters of water samples from upstream, midstream and downstream show that the average temperature of water at the three different points of collection ranged between 25.3 ± 0.69 to 26.1 ± 3^{o}C. This temperature range of water showed appropriate condition for the growth of mesophilic bacteria and optimal temperature for aquatic animals; and this result agrees with the study of Rheinheimer, (1985) and Al-Harbi and Udadin, (2012). The average pH of water sample in this study ranged between 6.0 to 6.5, and this shows a slightly acidic condition. In India, similar pH conditions of fresh water bodies have also been reported (Chakraborty, 1998). The dissolved oxygen (DO) concentration of the three water samples ranged between 4.5 ± 0.08 to 4.6 ± 0.16 mg/1. This result is similar to the work of Mondal and Barat, (2004) who obtained a DO concentration of 5.0 mg/l in their study. The dissolved oxygen content at the three water sampling points from the river showed similar trend, irrespective of the different dates of sample collection. Ion and anion concentration is also an important factor in determining water quality; and the chloride concentration in the water samples is between 75.43 ± 0.24 to 81.33 ± 1.25 mg/l - which falls within the permissible limit of 250 mg/l according to WHO standard (WHO, 2003). Studies have shown that chloride concentration in river acts as good indicator ion for pollution sources as well as organic waste of animal origin (Kegley and Andreus, 1997, Olayemi, 1994). The high concentration of chemicals and other physical parameters investigated in this study may be as a result of sewage and waste from the surface run-off and effluent discharging from surrounding inhabitants and agricultural fields. The result of the heterotrophic bacteria count show higher bacterial load above the acceptable limits; and this result is similar to that obtained by Olayemi, (1994) and Atobatele and Owoseni, (2012). The bacteria isolated from both the water and fish samples in this work were similar to those found by Obiajuru and Ogbulie, (2006) who reported the presence of similar bacteria in their study. The dendogram result show that the bacterial species were clustered based on the similarity in their phenotypic and morphological characteristics. However, the type and number of bacteria species in both the water and fish samples show the relationship between water quality and microbial load of the fishes. Fishes interact and come in contact with the water body through breathing, feeding, and swimming; and once the water body is polluted it affects aquatic life. This study presumptively establishes the presence of bacteria in *Clarias gariepinus and Tilapia zillii* that are of public health importance. Regular monitoring of water bodies for the presence of biological and physicochemical factors that affect their suitability for either domestic or industrial usage is required to make them free from disease-causing organisms and other substances that may affect human health directly or indirectly.

REFERENCES

[1] Ali, E. A., Gaya H. I. M. and Jampada, T. N. (2008). Economic Analysis of Fresh Fish Marketing in Maidugri Gamboru Market and Kachallari Alau Dam Landing Site of Northeastern Nigeria. *Journal of Agriculture and Social Science*. 4: 23-26.

[2] Atobatele, O. E and Owoseni, A. A. (2012). Distribution and Diversity of Bacteria in a Small Tropical Fresh Water Body (Aiba Reservoir) in Iwo,Osun State Nigeria. *Nature and Science;* 10 (12).

[3] Anthonio, O. R. and Akinwumi, J. A. (1991). Supply and Distribution of Fish in Ibadan. *Nigeria*. *Geogology Journal* 14(2): 16.

[4] Al-Harbi, A. H. and Uddin, M. N. (2004). Quantitative and Qualitative Study of the Bacterial flora of Farmed Freshwater Prawn (*Macrobrachium rosenbergii*) Larvae. *Journal of Applied Ichthyol*. 20: 461–465.

[5] Al-Harbi, A. H. and Udadin, M. N. (2012). Bacterial Content of Intestine of Frozen Common Carp *Cryrinus carpio*. *African Journal of Bacteriology;* 11 (30): 7751 – 7755.

[6] Chakraborty, D. (1998). A study on the WATER and Sediment Quality as Well as Macro Population of Natural Hill in the Darjeeling Hill. Indian. *Journal of Environment and Ecoplanology*. 1: 69-72.

[7] Cheesbrough, M. (2004). District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press, UK. p. 434.

[8] Deekae, S. N., Abowei, J. F. N. and Alfred-Ockiya, J. F. (2010). Seasonal Variation of Some Physical and Chemical Parameters of Luubara Creek, Ogoni Land, Niger Delta, Nigeria. *Journal of Environmental Earth Science* 2 (4):208-215.

[9] El-Shafai, S. A., Gijzen, H. J., Nasr, F. A., and El-Gohary, F. A. (2004). Microbial Quality of tilapia reared in fecal- Contaminated Ponds. *Environmental. Research*. 95, 231–238. 10.

[10] FAO (2003). The Role of Aquaculture in Improving Food Security and Nutrition. Committee on World Food Security, Food and Agriculture Organization of the United Nations, Rome.

[11] Federal Department of Fisheries, FDF (2007). Federal Department of Fisheries. *Fisheries Statistics of Nigeria*, 4th edition: Pp 49.

[12] Fagbenro, O. A. and Arowosoge A. I. (1998). Utilization of Agricultural Waste and by Products in the Fish Feed Production Nigeria. Proceedings of the 6th Annual Conference of the Fisheries Society of Nigeria (FISON) Lagos. *Fisheries Society of Nigeria*. Pp 121-130.

[13] Inglis, V., Roberts, R. J. and Bromage, N. R. (1994). Bacteriological Diseases of Fish. Blackwell Science Ltd., University Press, Cambridge, UK.

[14] Kegley, S. E and Andreus, J. (1997). The Chemistry of Water University Science Books. California.

[15] Mondal, D. and Barat, S. (2004). Effect of Varying Stocking Density of Sword Tail (*Xiphophorous helleri* Heckel) on the Water Quality and Growth Rate. *Journal of Inland Fish Society.* 36 (2):25-30.

[16] Olayemi, A. B. (1994). Bacteriological Water Assessment of an Urban River in Nigeria. *International Journal of Environmental Health Research* 4:156-164).

[17] Olagunju, F. I., Adesiyan I. O. and Ezekiel, A. A, (2007). Economic Viability of Catfish Production In Oyo State, Nigeria. *Ecology;* 21 (2):121-124.

[18] Obiajuru, I. O. C and Ogbulie, J. N. (2006). Bacteriological Quality of Some Fishes and Crab from Rivers within Imo River Basin. *Journal of Aquatic Sciences* 21 (1):9-14.

[19] Olafsen, J. A. (2001). Interactions Between Fish Larvae and Bacteria in Marine Aquaculture. *Aquaculture* ; 200, 223–247.

[20] Rheinheimer, E. (1985). Aquatic Microbiology. 3rd edition. John Wiley and Son Toronto. Pp 257.

[21] WHO, (2003). The World Health Report 2003: Shaping the Future, World Health Organization, 1211 Geneva 27 and Switzerland.

[22] World Fish Center (2002). An Issue for Everyone. A Concept Paper for "Fish for All". Penang.