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Microbiological Evaluation of Water and Chemical Composition of Fish and Sediment in Surface Water

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Abstract

Anthropogenic activities are the major source of organic and inorganic contaminants carried by surface runoffs and fluvial transport to aquatic bodies. The building-up of these contaminants can make water bodies unfit for inhabitation of living organisms as well as man that relied on these resources. Thus, this study analyzed the microbiological content of water and the chemical composition of fish and sediment collected at four distinct stations in River Benue using standard methods. The results indicated that the highest levels of nitrite (0.01±0.00–5.10±0.14 mg/L), nitrate (1.01±0.01–3.75±0.07 mg/L), and Na (17.15±2.21–186.10±10.14 mg/L) in catfish gills exceeded FAO/WHO standard. The highest values of bacteria load (2.61×10^7 CFU/ml), fungal counts (2.41×10^4 CFU/ml), coliform load (25.02×10^2 CFU/ml) and faecal coliform load (17.06×10^3 CFU/ml) were recorded at Wurukun abattoir station. Five bacterial isolates belonging to the genera were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Salmonella sp* and three fungal isolates: *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp were observed. Sixty percent were Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* sp.) while 40 % were Gram-positive (*Bacillus pumilus* and *Bacillus subtillis*). Prolonged consumption of water and catfish from River Benue may have adverse effects on the people of Benue State as well as commuters (travellers) who buy fish as they traverse Benue State to other parts of the country. It is recommended that the abattoir should be relocated to an area that is not in proximity to River Benue to reduce the level of organic pollutants entering River Benue and people living along the banks should be enlightened on the danger associated with defecating and/or disposing domestic waste into the river to reduce building up of potentially pathogenic bacteria and harmful fungus.

Keywords: Microorganisms, river, fish, sediment, and chemical

Introduction

Water represents ¾ of the earth's surface and only 0.3% can be used by humans as a result of water quality requirements. In September 2015, seventeen Sustainable Development Goals (SDGs) were listed in the 2030 Agenda for Sustainable Development. The agenda includes dedicated goals for water (SDG 6), energy (SDG 7), food security (SDG 2) and others. Water quality is taken into account in SDG 6.3, which is measured by two indicators, namely: 6.3.1: Proportion of wastewater safely treated and 6.3.2: Proportion of bodies of water with good ambient water quality (Bouhezila *et al*., 20200). The discharge of wastes and chemical compounds into rivers is one of the biggest sources of environmental contamination, mainly in developing countries, due to a lack of domestic and industrial wastewater treatment (Olgiun *et al*., 2010; Kora *et al*., 2017). The resultant increase in microorganisms and chemical contaminants enhances the risk of pathogen outbreaks, and harm to aquatic life, bacterial antibiotic resistance, and public health costs (Ramirez Castillo *et al*., 2015). For instance, the United Nations reported that about 1.8 billion people globally use sources of drinking water that are contaminated with faeces (Zziwa *et al*., 2016). Currently, one in five children dies from diarrheal-related diseases, which is more than that of HIV Aids, malaria, and measles combined (UNICEF and WHO, 2009) and chronic diarrhoea hinders child development by impeding the absorption of essential nutrients that are critical to the development of the mind, body, and immune system (Strande *et al*., 2014). In October 2010, about 29,115 cases involving 1,191 deaths of cholera were reported in just 15 out of the 36 states and Federal Capital Territory Abuja, and the figures increased to 1,616 deaths in 2004. It was observed that the outbreak was still in existence in

new areas due to continuous water pollution.

River Benue has great social, economic and ecological importance as it provides water for hundreds of thousands of people in the State, and a habitat for a variety of aquatic animals (Egesi *et al*., 2023). A large number of communities in Benue State are living in proximity and or along the bank of River Benue and the inhabitants of these communities discharge untreated domestic wastewater, fecal matter, and other forms of organic materials directly into the river. Some other activities that generate significant pollution in the River Benue include the Wurukun abattoir and Wadata market which cover hectares of land animals (Egesi *et al*., 2023). Thus, the river receives wastewater that might be having a high load of organic and chemical components from human activities. Furthermore, wastes from agricultural practices that are accumulated in water runoff find their way into River Benue and may lead to large-scale deterioration of the water quality. Consumers to a large extent have no means of judging the safety of water themselves, but their attitude toward drinking water and drinking water supplies will be affected to a considerable extent by the aspects of water quality they can perceive with their senses. It is natural for consumers to regard with suspicion water that appears dirty or coloured or has an unpleasant taste or smell, even though these characteristics may not in themselves be of direct consequence to health (WHO, 2006). Consequently, a good knowledge of the chemical and microbial loads of River Benue is paramount in determining its suitability for public consumption. The main objective of the study, therefore, was to determine the chemical characteristics of fish and sediment and microbiological composition of River Benue, Nigeria.

Materials and Methods

Study area

Makurdi is the capital city of Benue State located at latitude 7° 41' N and longitude 8° 28' E. The size of the River Benue within Makurdi and the major settlements it runs through is approximately 671 meters (Akaahan *et al*., 2015). Four (4) sampling stations were selected for this study viz: behind Wurukun abattoir, behind Wadata market, major storm drain and upstream at Angbaaye on the outskirts of Makurdi town (i.e. the control).

Sample collection

At each sampling station, five pre-cleaned sampling bottles were rinsed three times with River Benue and filled to the brim at a depth of 20 cm below the surface of the river. The five representative water samples from each sampling station were acidified with 10% HNO_3 analytical grade, covered air-tight, labelled well, placed in an ice-chest container and transferred to the laboratory for pre-treatment and analysis. Samples from each station were mixed separately to form one homogenous representative sample for the station. While in the laboratory; the homogenous water samples were stored in the refrigerator at about 4°C before the analysis (APHA, 1998). Adequate precautions were exercised to avoid contamination of water during sampling, transport, and handling.

Determination of Chemical Content of Fish and Sediments

Sodium and magnesium were determined by flame photometric method Sodium and magnesium were measured by flame photometric method. This was determined using the Technicon auto analyzer flame photometer IV, pre-calibrated using known concentrations of Sodium (Na) and Magnesium (Mg) with Lithium as internal standards. Samples were put in the same cups in the sample tray module and aspirated automatically into the mixing module where the mixing of Lithium and sample occurred, and the Teflon tube was checked regularly for good bubble pattern. The mixed samples were passed to the flame chamber where it was atomized and flared with the aid of propane gas. The concentration of each anion was measured by the colour intensity of the flame and results were obtained from an attached recorder. Fluoride was determined by the SPADNS spectrophotometric method.

Determination of Nitrite and Nitrate in Fish Gill Sample:

The method of Nerdy and De Lux Putra (2018) was adopted in the determination of Nitrite and Nitrate. Twenty-five (25) g of grounded fish sample was transferred into a 50 mL beaker glass, added 25 mL of hot (± 80°C) distilled water, homogenized by stirring, heated and stirred on a hotplate stirrer for 15 minutes, allowed to cool, transferred into 50 mL volumetric flask, added distilled water to the marked line, shaken until homogeneously mixed, and filtered. The 5 mL of the first filtrate was discarded, and the following filtrate was collected. The filtrate obtained was used for Nitrite and Nitrate determination. Each treatment was repeated six (6) times.

- Determination of Nitrite

Ten (10) mL of filtrate was transferred into 100 mL of volumetric flask and 2.5 mL of Sulfanilic acid was added to it, shaken until a homogenous mixture was obtained, and left to stand for 5 minutes. Then 2.5 mL of N – (1 – Naphthyl) Ethylenediamine Dihydrochloride solution was added to the solution, shaken until homogeneous, diluted with distilled water to the marked line, and shaken until homogeneously mixed (dilution factor 10 times). Absorbance was measured at the maximum absorbance wavelength after allowing it to reach the operating time. Each treatment was repeated six (6) times. The concentration (X) of Nitrite was calculated by substituting the absorbance (Y) obtained to the regression equation. Levels of Nitrite in the sample were calculated by multiplication with volume and dilution factor and division by weight.

-Determination of Nitrate in Fish Gills

About 3.5 mL of filtrate was transferred into a separate 100 mL volumetric flask, diluted with distilled water to the marked line, and shaken until homogeneously mixed (dilution factor 28.5 times). The 10 mL of solution was transferred into a 100 mL volumetric flask, added 0.1 g

of Zinc powder, added 1 mL of Hydrochloric acid solution, allowed to stand for 10 minutes (to reduce Nitrate to Nitrite), added 2.5 mL of Sulfanilic acid solution, shaken until homogeneous, left for 5 minutes, added 2.5 mL of N-(1-Naphthyl) Ethylenediamine Dihydrochloride solution, shaken until homogeneous, diluted with distilled water to the marked line, and shaken until homogeneously mixed (dilution factor 10 times). Absorbance was measured at the maximum absorbance wavelength after allowing it to reach the operating time. Each treatment was repeated six (6) times. The concentration of total Nitrate (Nitrite and converted Nitrate) was calculated using the regression equation. The concentration of converted Nitrate (Nitrate that has been converted to Nitrite) is obtained by subtracting the concentration of total Nitrite from a concentration of Nitrite. Levels of Nitrate in the sample were calculated by multiplication with the conversion factor, volume and dilution factor, and division by weight.

Determination of Nitrate in Sediment Samples

The procedure of (Oremo *et al*., 2020) was adopted for the determination of nitrates in sediment samples. Oven-dried sieved sediment samples (2 mm) were accurately weighed (5.0 g) into plastic shaking bottles and to each of the samples, 50 ml of 0.5 M Potassium Sulphate (K_2SO_4) extracting solution was added. Aluminium foil was placed on each bottle and the contents were shaken for one hour. The contents were then filtered through the No.42 Whatman filter paper. 0.5 ml of the sample extract, blanks, and the standard series were transferred into suitably marked test tubes and 1.0 ml of salicylic acid was added to each tube, mixed well and left to stand for 30 minutes. 10 ml of 4 M Sodium hydroxide was then added to each test tube mixed well and left for 1 hour for full yellow color development. The absorbance was measured at wavelength 420 nm. A calibration curve was plotted. The values of the sample and the blank were read. The concentration of nitrates in water and sediment samples was calculated as shown below:

$$\mathrm{NO3}\ (\mu g\ kg - 1) = \frac{(a - b\ /\ g)\ )\ x\ f\ x\ 1000}{w}$$

Where a = absorbance of NO_3 in the solution, b =absorbance of NO_3 in the blank, g = gradient of the calibration curve, v = volume of the extract, w = weight of fresh sediment

-Determination of Nitrite in Sediment Samples

The nitrite in sediment samples was determined by a slightly modified method described by (Sreekumar *et al*., 2003). Approximately 1 g of sediment sample was weighed (0.9876, 0.9899, and 0.9934 g, respectively) placed in a 50 ml beaker and extracted 6 times with 5 ml portions of 1 % sodium carbonate. The extract was filtered and made up to 25 ml with distilled water. For nitrite determination, 5 ml of the final solution (containing not more than 6 µg/ml of nitrite) was directly used by adding 0.5 ml 1 M NaOH and 0.5 ml 0.2 M EDTA. The solution was centrifuged and the centrifugate was transferred to a 10 ml standard flask and directly used for the colour development. The concentration of the nitrite was established by reference to the calibration graph prepared using 0-6 µg/ml of nitrite in 10 ml standard flasks using distilled water.

Microbiological Analyses of Water Samples

- Serial Dilution

The method of (Prescott *et al*., 2005) and (Iyerite *et al*., 2021) was adopted in serial dilution of the samples. One millilitre of each of the water samples was separately added to 9 ml of normal saline (diluents). After thorough shaking, further 10-fold (v/v) serial dilutions were made by transferring 1 ml of the diluted water sample to freshly prepared normal saline diluents to a range of 10^{-3} dilutions.

- Enumeration and Isolation of Total Heterotrophic Bacteria (THB)

The method of Prescott *et al*. (2005) as described by Iyerite *et al*. (2021) was adopted in the enumeration of total heterotrophic bacteria. Bacterial Colonies that appeared on the nutrient agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from 10^{-3} dilutions were counted and the means were calculated and expressed as colony forming unit per millilitre using the formula below:

$$\frac{\mathrm{CFU}}{\mathrm{ml}} = \frac{\text{number of colonies}}{\text{volume plated (0.1)}} \times \text{Dilution}$$

While discrete colonies that developed on the nutrient agar plates were sub-cultured on freshly prepared nutrient agar plates to isolate pure cultures.

-Total coliform counts (TCC)

The method of Prescott *et al*. (2005) as described by Iyerite *et al*. (2021) was adopted in the enumeration of total coliform counts. Bacterial Colonies that appeared on the MacConkey agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from 10^{-2} dilutions and incubated at 37ºC for 24 hours were counted and the mean expressed as CFU/ml (Inana *et al*., 2019).

-Fecal coliform counts

The method of Prescott *et al*. (2005) as described by Iyerite *et al*. (2021) was used for the enumeration of fecal coliform count. Bacterial colonies that appeared on the Eosin Methylene Blue (EMB) agar plates which were inoculated in duplicate with an aliquot of 0.1 ml from 10^{-2} dilutions and incubated at 45.5ºC for 24 hours were counted and the mean expressed as CFU/ml (Inana *et al*., 2019).

-Total fungal counts

The method of Okerentugba and Ezeronye, (2003) as described by Prescott *et al*. (2005) was adopted in the determination and counting of total fungal counts. It was determined using Sabouraud Dextrose Agar (SDA) amended with Tetracycline to suppress bacterial growth. The spread plate technique as described by Prescott *et al.* (2005) was adopted. An aliquot zero point one (0.1 ml) millilitre from 10^{-2} dilution of the serially diluted samples was inoculated onto pre-dried SDA agar plates

in duplicates. The inocula were then spread evenly on the surface of the media using a flamed bent spreader. The plates were then incubated at room temperature (25°C) for 5 days after which the colonies that developed were counted and the mean of total fungal counts were recorded accordingly.

Purification and maintenance of Isolates

After incubation, pure isolates were obtained by picking (with a sterile inoculating loop) distinct culturally and morphologically different colonies from the various plates. These were subjected to streaking on sterile nutrient agar in plates and incubated at room temperature for 24 hours until pure distinct colonies developed (Iyerite *et al*., 2021).

Identification of bacterial isolates
-Biochemical characterization

The method described by Collins *et al.* (1998) and Cheesebrough (2006) was adopted for the identification of pure bacterial isolates. The pure bacterial isolates were subjected to biochemical tests including the Oxidase test, Catalase test, Indole test, methyl red test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate test, Sugars fermentation test Triple sugar iron agar test. Bacterial isolates were identified according the Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994). The Gram staining procedure modified by Rueckert and Morgan (2007) was used in the microscopic identification of bacterial contaminants.

Quality assurance and quality control

Quality assurance and quality control were carried out with parallel experiments, blank tests and recovery tests. The recovery rates were between 90% and 110%, and the relative deviations of parallel tests were within 10%. All used acids and reagents were of analytical grade. The reagents used were ultrapure, and the water was de-ionized to a resistivity of 18.2 MΩ·cm in a Direct-Q UV3 Ultrapure Water System apparatus (Millipore, France). Suitable safeguards were applied to evade contamination of river samples during sampling, conveyance and conduct of the experiment.

Statistical analysis and Data presentation

The data from Laboratory analysis was subjected to one-way analysis of variance (ANOVA) with statistical package for social sciences (SPSS) v. 18 and means were separated by the Duncan New Multiple Range Test (DNMRT) according to (Steel and Torrie, 1980). Results are presented as mean ± SD.

Results and Discussion

Chemical characteristics (mg/L) of fish harvested in River Benue

The result of the chemical properties of gills extracted from fishes harvested from the various sampling stations of River Benue is summarized in Table 1. The result indicated that significant differences ($P<0.05$) were evident in the chemical content of fish gills from the four sampled stations. The highest values of nitrite (5.10±0.14 mg/L) and sodium (186.10±10.14 mg/L) were recorded in fish gills harvested at the major storm drain station. The high content of nitrite in fish gills harvested at the major storm drain station may be attributed to the nitrite level in sediment (Table 2) as well as dermal contact, and ingestion of nitrite-contaminated water. Nitrite is a common pollutant in surface water and it accumulates in fish tissues such as gills, liver, brain and muscle (Kroupova *et al*., 2005). The highest values of Mg (20.01±0.01 mg/L) and F^- (1.01±0.01 mg/L) were observed in fish gills harvested at the Wurukun abattoir station, which may be attributed to their level in sediment at the abattoir station of River Benue (Table 2). The values of Mg in fish gills increased from 2.05±0.07–20.01±0.01 mg/L, which is higher than 0.21–0.32 mg/g for fish sampled from Lake Kainji, Nigeria (Effiong and Fakunle, 2011). Sodium increased from 17.15±2.21–186.10±10.14 mg/L, which is higher than 2.8–3.2 mg/g for fish sampled from Lake Kainji, Nigeria (Effiong and Fakunle, 2011). The recommended dietary allowances (RDA) of Na and Mg for males and females (9–50 years) are 1.3–1.5 and 240–420 mg per day (FAO/WHO, 2001), respectively. In this study, the level of Na (17.15±2.21 to 186.10±10.14 mg/L) in fish gills exceeded the regulatory standard. The values of fluoride in fish gills in this study increased from 0.01±0.00–1.01±0.01 mg/L. When people ingested fluoride contaminated aquatic organisms like fish, some part of the fluoride is excreted but the rest is deposited in the bones and teeth and is capable of causing crippling skeletal fluorosis, non-skeletal fluorosis and dental fluorosis (Kaur *et al*., 2017). The values of nitrate in this study increased from 1.01±0.01–3.75±0.07 mg/L. The acceptable daily intake for nitrite is 0.07 mg of nitrite per kg body weight per day while nitrate is 3.7 mg of nitrate per kg body weight per day (FAO/WHO, 2011). The level of nitrite (0.01±0.00–5.10±0.14 mg/L) and nitrate (1.01±0.01–3.75±0.07 mg/L) in fish gills exceeded the regulatory standard, and this might be detrimental to the health of consumers. Generally, the order of abundance of the chemical properties of fish tested in this study is as follows: $Na>Mg>NO^-_2>NO^-_3>F^-$.

Chemical characteristics (mg/kg) of sediment in river Benue

The results of the chemical properties of sediments in River Benue are shown in Table 2. The highest values of nitrate (14.11±2.01 mg/kg), nitrite (20.01±4.01 mg/kg), and sodium (109.01±11.01 mg/kg) were observed in sediments collected at the major storm drain station and the values are significantly ($p<0.05$) higher than their corresponding values at Wurukun abattoir station, Wadata market station, and control area. The high nitrate, nitrite, and sodium at the major storm drain station may be attributed to water runoff from nearby farms subjected to agricultural anthropopressure such as chemical fertilizer, animal husbandry as well as soaps and detergents used in washing clothes, vehicles, and dishes in the homes of people living along the bank of the river. Human activities release chemical substances to surface waters that are accumulated in sediments via sedimentation (Szydowski *et al*., 2017). The values of nitrite in sediments increased from

4.01±1.01–20.01±4.01 mg/kg while nitrate increased from 0.85±0.07–14.11±2.01 mg/kg. The values of nitrate in sediments of River Benue are higher than 0.75±0.02–1.93±0.05 mg/kg in sediments of River Isiukhu, Kenya (Oremo *et al*., 2020). The values of sodium in sediments of River Benue increased from 22.01±3.01–109.01±11.01 mg/kg, which is higher than 0.081–0.415 g/kg in sediments of Brody Hzeckie reservoir and 0.142–0.206 g/kg in sediments of Zalew Zemborzycki (Wojcikowska-Kapusta *et al*., 2018). The values of fluoride in sediments increased from 0.01±0.00–3.51±0.01 while magnesium increased from 15.01±1.01–51.01±10.01 mg/kg. The value of magnesium in the sediments of River Benue is higher than 1.02 to 2.22 g/kg in the sediments of the Zalew Zemborzycki reservoir (Wojcikowska-Kapusta *et al*., 2018). Generally, the order of abundance of chemical parameters of sediments tested in this study followed a decreasing order: $Na>Mg>NO^-_2>NO^-_3>F^-$.

Microbiological composition of water

The results of microbial counts indicated that a significant difference ($P<0.05$) was evident in mean total heterotrophic bacterial and fungal counts, total coliform counts and faecal coliform counts among the four stations studied. Figure 2 indicated that the total heterotrophic bacteria count from water samples increased from 0.11×10^7 CFU/ml to 2.61×10^7 CFU/ml with the Wurukun abattoir station having the highest bacteria load of 2.61×10^7 CFU/ml followed by major storm drain station 2.41×10^7 CFU/ml, Wadata market station 1.52×10^7 CFU/ml while control station with no visible anthropogenic pressure had the lowest with 0.11×10^7 CFU/ml. The high bacteria load in water samples in the Wurukun abattoir station may be attributed to the deposition of abattoir wastewater at the abattoir station of the River Benue. The introduction of wastewater high in organic matter and essential nutrients brings about the proliferation of microbial growth (Adieze *et al*., 2016) in aquatic bodies. Figure 3 showed that total fungal counts (2.41×10^4 CFU/ml) for the Wurukun abattoir station were higher than 2.25×10^4 CFU/ml observed at the major storm drain station, and 1.31×10^4 CFU/ml obtained at Wadata market station as well as 0.26×10^4 CFU/ml recorded at the control station. The result indicates that anthropogenic activities were the major factor responsible for the high fungal load observed in River Benue. The result is in relationship with Iyerite *et al*. (2021) who studied the effect of anthropogenic activities on the microbiological quality of Lobia Creek in southern Ijaw of Bayelsa State, Nigeria. Figure 4 unveiled that total coliform counts increased from 2.01×10^2 CFU/ml to 25.02×10^2 CFU/ml with the Wurukun abattoir station having the highest coliform load (25.02×10^2 CFU/ml) followed by the major storm drain station (18.02×10^2 CFU/ml), Wadata market station (13.5×10^2 CFU/ml) while the lowest coliform counts were observed in control area (2.01×10^2 CFU/ml). The high coliform counts recorded from the river samples indicated the occurrence of faecal contamination (Sanders *et al*., 2013). Figure 5 showed that faecal coliform counts increased from 0.98×10^2 CFU/ml to 17.06×10^2 CFU/ml with Wurukun abattoir station having the highest faecal coliform load (17.06×10^3 CFU/ml) followed by major storm drain station (11.3×10^2 CFU/ml), Wadata market station (7.14×10^2 CFU/ml) while the least coliform count was recorded in the control station (0.98×10^2 CFU/ml). The high faecal load in the Wurukun abattoir station may be attributed to the washing away of faeces deposited by animals awaiting to be slaughtered in the abattoir and water used in cleaning the intestines of slaughtered animals by runoff into the abattoir section of River Benue. For instance, rivers are contaminated by anthropogenic activity via the release of faecal waste and organic pollutants (Garcia-Armisen and Servais, 2004). The presence of fecal coliform in the four sampled stations showed that River Benue is greatly polluted with fecal matter and potential pathogens which suggested that the water is not potable and safe for domestic use. Fecal coliforms are normally not pathogenic and are indicator organisms but pathogenic diseases associated with fecal contamination include typhoid fever, viral and bacterial gastroenteritis and even Hepatitis A (Ejiogu *et al*., 2014). The biochemical tests are shown in Table 3 and identified as shown in Table 4. Five bacterial isolates belonging to the genera were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Salmonella sp* and three fungal isolates: *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp were found in the samples tested in this study.

Gram staining and microscopic characteristics of bacteria isolates

Five bacterial microbes were isolated, 60 % were Gram-negative and 40 % Gram-positive. Gram-positive bacteria were *Bacillus pumilus* and *Bacillus subtillis* (Table 5). Gram negative bacteria were *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* sp. The percentage of occurrence was indicated as follows: *Staphylococcus aureus* (48.8%), *Escherichia coli* (30.4), *Pseudomonas aeruginosa* (9.4%), *Bacillus subtillis* (5.87%), *Salmonella sp* (5.53%) and fungal isolates: *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp.

Conclusion

The results of the study imply that human activities have resulted in the contamination of River Benue with chemical contaminants and potentially pathogenic bacteria and harmful fungi at the four sampled stations of the River Benue. The level of nitrite and nitrate in fish gills exceeded the regulatory standard. The highest values of bacteria load, fungal counts, coliform load, and faecal coliform load were recorded at Wurukun abattoir station. Five bacterial isolates belonging to the genera were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtillis*, *Salmonella sp* and three fungal isolates: *Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp were observed. Sixty percent were Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella* sp.) while 40 % were Gram-positive (*Bacillus pumilus* and *Bacillus subtillis*). Prolonged consumption of water and

catfish from River Benue may likely have adverse effects on the people of Benue State as well as commuters (travellers) who buy fish as they traverse Benue State to other parts of the country. It is recommended that people living along the banks of River Benue should be enlightened on the danger associated with defecation and disposal of organic and inorganic wastes into the River to avert possible pathogen outbreaks, eutrophication, harm to aquatic life, and public health costs.

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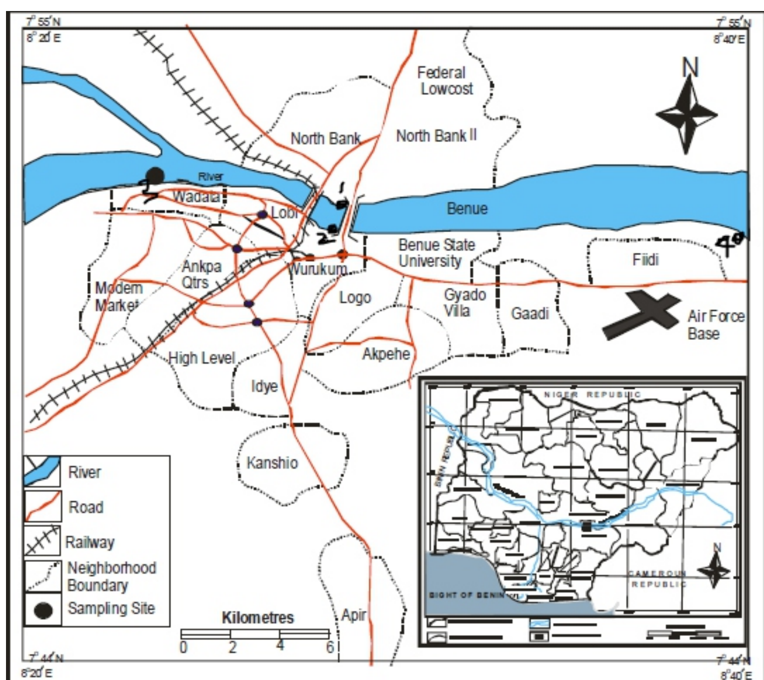

Fig. 1: Map showing the study area

Table 1: Chemical characteristics (mg/L) of fish gills

	Sampling stations			
Parameters	**Major storm drain**	**Wadata market**	**Abattoir station**	**Control**
Nitrate	4.75^{a}±0.07	2.01^{c}±0.01	3.05^{ab}±0.07	1.01^{d}±0.01
Nitrite	5.10^{a}±0.14	1.01^{b}±0.01	2.01^{b}±0.01	0.01^{c}±0.01
Fluoride	0.15^{b}±0.07	0.01^{b}±0.00	1.01^{a}±0.01	0.01^{b}±0.01
Sodium, Na^{2+}	186.10^{a}±0.14	51.00^{c}±9.40	69.10^{b}±0.14	17.15^{d}±0.21
Magnesium, Mg^{2+}	16.15^{b}±0.21	13.10^{b}±0.14	20.01^{a}±0.01	2.05^{c}±0.07

Values were expressed as mean ± standard deviation of 3 replicates*; *abcd Means in a row with different superscripts are significantly different (P<0.05)

Table 2: Chemical properties (mg/kg) of sediments

	Sampling stations			
Parameters	**Major storm drain**	**Wadata market**	**Abattoir station**	**Control**
Nitrate	14.11^{a}±2.01	4.01^{c}±0.01	8.02^{b}±0.02	0.85^{d}±0.07
Nitrite	20.01^{a}±4.01	7.03^{c}±0.04	13.01^{b}±2.01	4.01^{c}±1.01
Fluoride	0.68^{b}±0.01	0.11^{bc}±0.01	3.51^{a}±0.01	0.01^{c}±0.00
Sodium, Na^{2+}	109.01^{a}±11.01	77.01^{b}±6.01	80.01^{b}±7.01	22.01^{c}±3.01
Magnesium, Mg^{2+}	31.02^{b}±5.02	21.01^{c}±3.01	51.01^{a}±10.01	15.01^{d}±1.01

Values were expressed as mean ± standard deviation of 3 replicates*; *abcd Means in a row with different superscripts are significantly different (P<0.05)

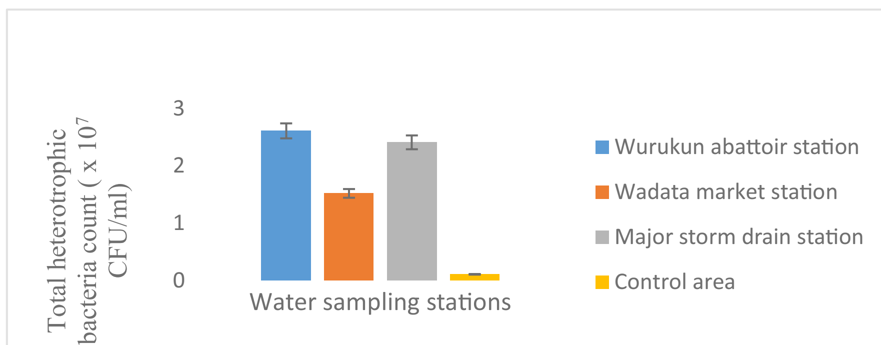

Figure 2: Total heterotrophic bacteria count of sample stations

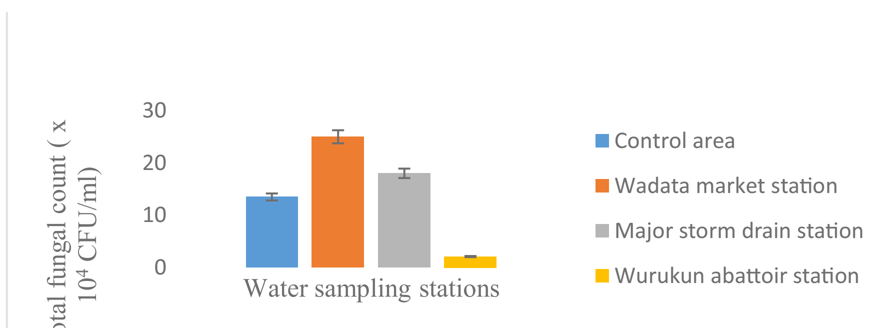

Figure 3: Total fungal count of sample stations

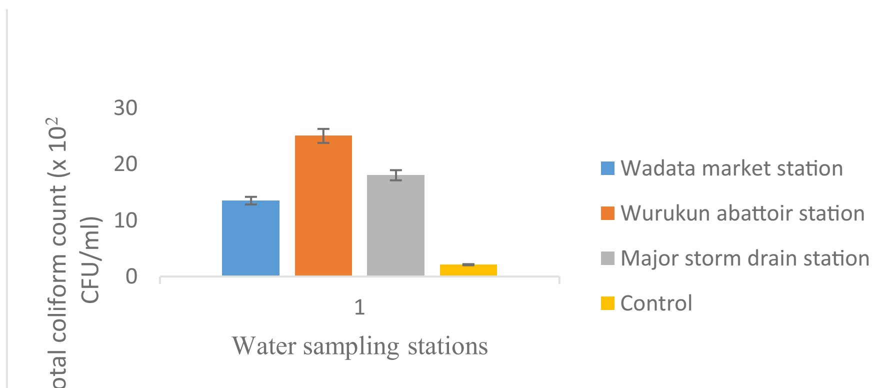

Figure 4: Total coliform count of sample stations

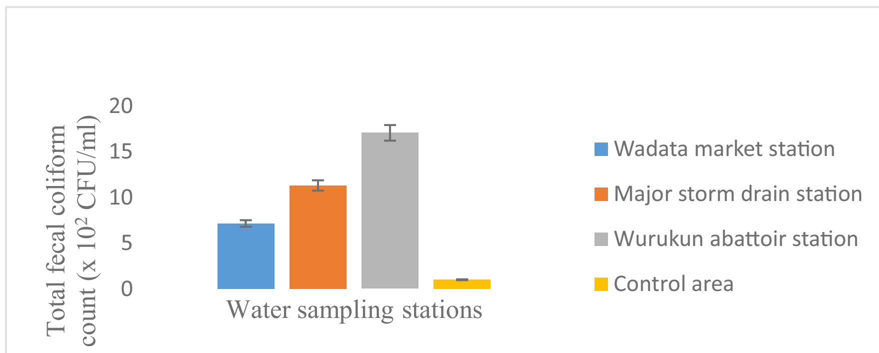

Figure 5: Total coliform count of sample stations

Table 3: Biochemical identification of bacteria

Colony on NA	H_2S gas.	Motility	Gram stain	Catalase	Starch hydrolysis	Citrate utilization	Indole	Lactose	Oxidase	Isolate identity
Green, glossy pigmented and thin	-	-	- Bacillus	+	-	+	-	-	+	*Pseudomonas aeruginosa*
Clear, small, round and irregular	-	-	- Bacillus	+	-	+	-	+	-	*Enterobacter sp.*
White, smooth, creamy and round	-	-	+ Coccus in clusters	+	-	-	-	-	-	*Staphylococcus aureus*
White, moist with glistening growth	-	+	+ Cocci	+	-	-	+	AG	-	*Escherichia coli*
White glossy membranous	-	-	+ Bacillus	+	+	-	-	-	-	*Bacillus subtilis*

+ Positive; - Negative; AG Acid Gas

Table 4: Identified bacteria and fungi species in Abattoir, Wadata, major storm drain, and control sites stations

Sampling stations	Bacteria identified	Fungi identified
Wurukun abattoir	*Pseudomonas aeruginosa*, *Staphylococcus aereus*, *Escherichia coli*, *Bacillus subtilis* *Salmonella sp*	*Aspergillus niger*, *Penicillium* sp., and *Fusarium* sp.
Wadata market	*Pseudomonas aeruginosa*, *Staphylococcus aereus*, and *Escherichia coli*	*Penicillium* sp., *Fusarium* sp.
Major storm drain	*Pseudomonas aeruginosa*, *Staphylococcus aereus*, *Salmonella sp.*, *Escherichia coli*	*Penicillium* sp., Aspergillus *niger*,
Control	*Escherichia coli*, *Pseudomonas aeruginosa* *Staphylococcus aereus*	*Fusarium* sp.

Table 5: Microscopic and Gram staining characteristics of identified bacteria

Bacterial species	Shape	Arrangements	Gram reaction	Motility
Pseudomonas aeruginosa	Straight and slightly curved rods	Singles	G-ve	Motile
Escherichia coli	Straight rods, cocobacilliary	Singles/ pairs	G-ve	Non-motile
Staphylococcus aureus	Cocci	Singles, pairs and irregular clusters	G+ve	Non-motile
Bacillus subtillis	Rods	Singles, pairs	G+ve	Motile
Salmonella sp.	Straight rods	Paired	G-ve	Motile

G – ve Gram-negative; G + ve Gram-positive