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# Abstract

This research study carried out a quantitative and qualitative bacteriological investigations on *sediments sampled from catfish ponds over three seasons: harmattan, hot and wet. Results for quantitative bacteriological analysis recorded high total viable bacterial counts in the sediment samples, with the highest counts observed during the wet season months of August (3.22 x 10<sup>8</sup> CFU/g) and September (2.30 x 10<sup>8</sup> CFU/g). Similarly, results for total and fecal coliform counts also revealed the highest counts in the study during these wet months: August and September, reaching means of 1600 coliforms/100g and 69-138 faecal coliforms/100g, respectively. The lowest mean bacterial counts of 2.92 x 10<sup>7</sup> cfu/g and 3.67 x 10<sup>7</sup> cfu/g were recorded in the months of January and February respectively (harmattan season). Overall, the study recorded a total of 39 bacterial isolates that were identified across the samples through cultural, microscopy and biochemical tests. The predominant species included Escherichia coli, Enterobacter sp., Bacillus sp., Pseudomonas sp., Klebsiella pneumoniae, Aeromonas hydrophila/Aeromonas sp., Salmonella sp. and Micrococcus sp. The high bacterial loads, particularly the high loads of coliforms during wet periods generally indicate potential fecal contamination and seasonal impacts on microbial water quality as a result of anthropogenic activities such as cattle grazing, animal husbandry activities as well as point and non-point sources of pollution around the cluster fish farm. These findings outlined the need for monitoring and mitigating measures to safeguard public health. This is important because some villagers in Monai still use the stretch of Kainji Lake as a drinking water source and this lake receives effluents from the catfish ponds.*

**Keywords:** Bacteria, Coliforms, Dry, Harmattan, Quality, Seasons, Sediments, Water, Wet

## **INTRODUCTION**

The farming of catfish in Nigeria has become an important component of the aquaculture industry because it is the form of aquaculture practice that predominates. This is because Catfish represents about seventy percent (70%) of the total fish species cultured in Nigeria and contributes to approximately one million direct employment opportunities throughout the entire value chain (FAO, 2022).

Furthermore, fish constitutes more than 40 percent of Nigeria's protein consumption, with an average per capita fish intake of 13.3 kilograms per year (Worldfish-Nigeria, 2024). With all the numerous benefits associated with aquaculture, it possess deleterious effect on the environment and one of such negative impacts is the pollution of the environment as a result of excess nutrients and chemicals used in fish farming, which builds up and accumulates in the pond sediments in most cases (Custadio *et al*., 2023).

The impacts of these nutrient-laden wastes from fish ponds to receiving environments have been widely reported in literature (Chopin *et al*., 2012; Omofunmi *et al*., 2016). Sediment accumulation occurs in fish ponds as a result of the gradual accumulation of the sludge that accumulates in pond bottom from uneaten feed and fish excreta during fish culture.

This is because substantial amount of the feed fed to cultured organisms in fish farming is lost and only a little amount of it is consumed and utilized (Hixson, 2014). Similarly, Crab *et al*. (2007) reported that approximately 75% of this feed remains as nitrogen and phosphorus in pond sediment and wastewater effluent.

# **MATERIALS AND METHOD**

## **Study area**

The study area was Monai cluster fish farm in Monai village, New Bussa. Monai village is one of the fishing communities located in the Southern Basin of Kainji Lake with cluster fish farms that are individually owned by villagers and non- villagers. It lies between latitude 9°53.313'N and longitude 4◦32.89ꞌE. Aside from its use for fishing and aquaculture, the Southern basin of the lake provides a source of portable water to the villagers for domestic and irrigation purposes.



**Figure 1:** Map of Kainji Lake showing the study location. **Source:** Omeiza (2018).

# **Collection of Sediment Samples**

About 200g of bottom sediment samples were randomly collected from catfish ponds from the five sub-clusters (CL1 to CL5) in Monai cluster fish farm using the Ekman bottom grab sampler. A total of ninety (90) sediment samples were collected from ponds in each of the subclusters over the span of six months (January to September, 2022) cutting across three seasons: harmattan, wet and dry respectively. All sediment samples were collected in sterile containers, preserved in ice chilled coolers and immediately conveyed to the Central Laboratory Complex of the National Institute for Freshwater Fisheries Research, New Bussa, Niger State, Nigeria.

# **Bacteriological Analyses**

# **i. Total viable bacteria count**

Total viable bacterial and coliform counts, as well as identification were carried out on sediment samples using bacteriological culture technique as described by Chessbrough (2012). Total viable bacterial count was done using plate count agar while coliforms were enumerated on lactose and BGLB broths respectively. Total viable bacteria enumeration was done on plate count agar which involved making serial dilutions from the original sample, after which plating of aliquots of the prepared serially diluted tubes was made using the pour plate technique (Chessbrough, 2012). Six serial dilutions were prepared (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>,  $10^{-5}$  and  $10^{-6}$ ) but only the last three dilutions ( $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ) were plated onto the agar plates as described in standard procedure (FAO, 1987). Each dilution had 1 ml inoculated from it onto sterile petri dishes and each dilution had three replicate plates, after which 15ml of molten agar that had been cooled to about  $40-45°C$  was poured and the mixture was gently swirled on the laboratory bench in circular motion for about five seconds. All inoculated plates were then allowed to fully solidify after which they were incubated in an inverted position for 3-7 days at 30◦C.

## **i. Coliform enumeration**

Coliform enumeration was conducted using the multiple tube fermentation technique. This was a three in one test (presumptive, confirmatory and completed tests) and was conducted using standard procedure by (APHA, 1998).

## **Presumptive test**

The untreated water MPN procedure was employed to conduct the presumptive test for coliform bacteria in sediment samples (APHA, 1998).

Firstly, Lactose broth was prepared in single (SS) and double strength (DS) concentrations and the 10ml of the double strength medium was dispensed into 5 tubes accordingly. Conversely, the single strength medium was dispensed into 10 tubes of 10 ml in each case. Durham tubes were gently placed into each tube and covered with cotton wool plugs, then subsequently sterilized at 121◦C for 15 minutes in an autoclave. After autoclaving, all tubes were allowed to cool down to room temperature prior to inoculation. With the aid of sterile new 5 ml syringe in each case, 10 ml of sample was measured and added to the 5 tubes containing 10 ml of double strength (DS) medium in each case. Similarly, the remaining 10 tubes containing 10 ml of single strength (SS) medium were inoculated with 1ml and 0.1 ml respectively in each case where 5 tubes were inoculated with 1ml and the remaining 5 tubes with 0.1 ml each. All inoculated tubes were incubated at  $35 \text{°c} \pm 0.5$  for 24 hours. In the event that no tube appear to be positive (no growth), the set-up was re-incubated for another 24 hours, making 48 hours  $\pm$ 3 hours according to the procedure described by APHA (1998) in the manual of standard methods for examination of water and wastewater.

All tubes that gave positive result (with growth) per dilution were compared to standard MPN (most probable number) table and recorded against the number of bacteria present in the sample to obtain the MPN index of coliform per 100ml of the sample.

## **Confirmatory Test**

All acid and gas positive tubes from the presumptive test were passed on to this stage of testing where brilliant green lactose bile broth (BGLBB) was used. After gently shaking or rotating the test tube to re-suspend the organisms, a loop full of inoculum from each gas positive tube from presumptive test was inoculated into a tube containing 10ml of BGLBB with the inverted Durham tube that had been previously sterilized by autoclaving at 121℃ for 15 minutes. This was done using a preheated and sterilized wire loop and the setup was incubated at  $35 \text{°c} \pm 0.5$  for 24 hours. If no evidence of fermentation was observed at 6 hours, 18 hours or 24 hours after incubation, the setup was left for another 27 hours, making  $48 \pm 3$ hours.

## **Completed Test**

All tubes that appeared positive on BGLBB and with gas formation from the confirmatory test were passed on to this test. This test involved incubating BGLBB tubes at two temperatures (37◦c and 44.5◦c) for total coliforms and fecal coliforms respectively. Additionally, gas positive tubes from confirmatory test were streaked onto Eosin Methyl Blue Agar (E.M.B).

## **i. Differential staining (Gram staining)**

All bacterial isolates isolated from sediment samples were subjected to gram staining procedures to determine the gram property of the bacterial isolates.

#### **ii. Biochemical test**

A total of eleven (11) Biochemical tests were conducted on bacterial isolates from samples of sediments. The biochemical tests were; catalase test, oxidase test, indole test, urease test, motility test, starch hydrolysis test, Vogues Proskauer test (VP). Methyl red test, triple sugar iron test (TSI) and Simon's Citrate test

#### **Statistical Analysis**

Statistical analysis was done using SPSS (IBM, USA, version 10) statistical software package where descriptive and inferential statistical tests were conducted. Means and standard deviation of bacteriological counts in sediments were calculated. Similarly, the means of monthly bacteriological counts were subjected to one way analysis of variance (ANOVA). Significant means were separated using least significant difference (LSD) as the post-Hoc test.

#### **RESULTS**

Results for quantitative bacteriological investigation of sediment samples revealed high total viable bacteriological counts in sediment samples, with a mean range of  $2.92 \times 10^{7}$  cfu/g to  $3.22 \times 10^8$  cfu/g of sediment. Similarly, total coliform and fecal coliform bacteria counts were also high, particularly in sediment samples that were sampled in the months of August and September (during the wet season), with mean counts of 844 to 1600 coliforms/100g of sediments for total coliforms and 14 to 138 coliforms/100g of sediments for faecal coliforms respectively. Result for total viable bacteria count from sediment samples is presented in Table 1. The highest bacterial count was recorded in the month of August, with a mean count of 3.22  $x$  10<sup>8</sup> cfu/g of sediment. Similarly, the lowest bacterial count was recorded in the month of January, with a mean bacterial count of  $2.92 \times 10^7$  CFU/g of sediment.





\***Values are Means ± SD**

Means within same column with dissimilar superscripts are significantly different

Similarly, result for mean seasonal total viable bacteria count is presented on figure 1 where, the highest bacterial count in sediment was recorded in the wet season, with mean count of  $2.75 \times 10^8$  cfu/g of sediment while the lowest mean total viable count was recorded in the harmattan season, with mean count of  $3.29 \times 10^7$  cfu/g of sediment (figure 2).



**Figure 1:** Mean seasonal total viable bacteriological count in sediment samples

## **Total Coliform Count**

Results for total coliform count showed that the months of August and September, coinciding with the wet season recorded the highest total coliform counts with mean coliform counts of (1600) coliforms/100g of sediment respectively. Conversely, the months of January and February, coinciding with the harmattan season recorded the lowest coliform counts, with mean coliform counts of 844 coliforms/100g of sediment for the month of January and 803 coliforms/100g for the month of February respectively (Table 2).





\*Values are Means ± Standard deviation.

\*Means with different superscripts on same column are significantly different (p<0.05)

## **Fecal Coliform Count**

Result for fecal coliform count also showed that the highest fecal coliform bacteria count was recorded in the months of August and September, with mean faecal count of 69 coliforms/100g of sediment for the month of August and 138 faecal coliforms per 100g of sediment for the month of September respectively, during the wet season. This was closely followed by samples that were sampled during the hot season in the months of March and April. Throughout the entire study, the highest fecal coliform count was recorded in the month of August with a mean fecal coliform count of  $69 \pm 29.3$  fecal coliforms per 100g of sediments. Similarly, the lowest fecal coliform count was recorded in the month of January, with a mean fecal coliform count of  $14 \pm 3.8$  coliforms per 100g of sediments respectively (Table 3).

**Table 3**: Mean Monthly Fecal Coliform Bacterial Count in Sediment Samples (Coliforms/100g)

$\cdots$ $n = 90$	Minimum count $\text{(coliform/100g)}$	Maximum count $\text{(coliform/100g)}$	Mean ± Std. (coliform/100g)
January	8	17	$14.0 \pm 3.8$
February	14	22	$17.0 \pm 2.6$
March	17	33	$22.0 \pm 7.0$
April	8	63	$27.0 \pm 19.0$
August	33	110	$69.0 \pm 29.3$
September	79	180	$138.0 \pm 147.8$

Furthermore, result for seasonal fecal coliform count in sediments samples revealed high counts in fecal coliforms in the wet season (Months of august and September) with a mean fecal coliform count of  $138 \pm 147.8$  per  $100g$  of sediments. Similarly, the lowest fecal counts were obtained in the harmattan season, with a mean fecal coliform count of 19 ± 15.4 per 100g of sediments (Table 4).





## **Table 5:** Results for Biochemical Tests





**Table 6:** Frequency of occurance of bacterial isolates over the seasons.



## **Discussion**

Overall, the total viable bacterial counts, total coliform counts and fecal coliform counts were high in the sediment samples, particularly during the wet season months of August and September. Similar observation was recorded for total and fecal coliform counts respectively. This elevated counts in bacteria during the wet season of the study is in close agreement with findings from previous studies that have reported increased bacterial growth and abundance in freshwater aquatic systems during the wet season (Dirisu *et al.,* 2020; Abija *et al*., 2021; Kelechi *et al.,* 2021; Ibinabo *et al*., 2022).

On this regard, several factors might have been responsible for the significant bacterial proliferation in the months of August and September (Wet season) of the study. One of the most important resource for growth and survival of all living things is food, in the form of nutrients. Therefore, the counts observed in August and September, which both occurred in the wet season, could be linked to the high levels of nutrients such as nitrites, nitrates, phosphates and ammonia. However, Boyd *et al*. (2012) reported that the high abundance of nutrients in aquaculture systems may likely be connected to surface runoff from agricultural

fields. In relation to this, it is likely that high nutrients concentrations in the sediments which are essential for bacterial growth and metabolism may have likely originated from external sources like runoff from numerous crop farms such as rice and maize fields around the vicinity of Monai cluster fish farm. Therefore, the increased nutrient availability, coupled with favorable temperature conditions and inputs from soil particles during runoff in the wet season could have likely created an environment conducive for bacterial proliferation (Zhang *et al.,* 2019). Similarly the increased concentrations of organic matter and organic carbon during the wet seasons than other season due to leaching and point sources of pollution may be connected to increased rates of bacterial decomposition during the wet season, which could have provided a rich source of nutrients for bacterial growth (Abija *et al*., 2021; Wolińska *et al*., 2022). Furthermore, it is important to note that the presence of high bacterial counts in aquaculture systems, particularly in habitat components like the sediments is not necessarily detrimental because many bacterial species play important roles in nutrient cycling, organic matter decomposition and maintaining a balanced ecosystem (Bentzon‐Tilia *et al.,* 2016; Infante‐Villamil *et al*., 2021). However, excessive bacterial growth can lead to oxygen depletion, increased turbidity and the potential accumulation of harmful metabolites, which can negatively impact water quality and fish health (Loh *et al.,* 2017; Wang *et al*., 2017).

The high counts in total viable bacteria, total coliform and fecal coliform counts that were observed in the wet season (August and September) can be associated with anthropogenic activities around the vicinity of the cluster fish farm. This is because surface runoff during the wet season can introduce additional sources of fecal contamination from surrounding areas into the catfish ponds (Bera , 2022; Haldar *et al*., 2022). Livestock operations such as grazing by cattle herds around the ponds, indiscriminate defecation by humans and runoff from animal farms can all lead to situations where fecal matter and associated coliform bacteria can be transported into the ponds and become trapped in the pond sediments (Dirisu *et al.,* 2020; Olalemi *et al*., 2023). Furthermore, heavy rainfall events can lead to the re-suspension of sediments, releasing bacteria from the sediments into the water column, contributing to higher coliform counts (DesRosiers *et al*., 2022; Acharya, 2023).

Additionally, higher water temperatures and increased nutrient levels which emanate from crop farms such as rice and maize fields around Monai cluster fish farm during the wet season can also promote the growth and proliferation of coliform bacteria. Reitter *et al.* (2021) reported that higher water temperatures in wet season correlated with increased coliform bacteria growth from their study. This is because coliforms, including fecal coliforms like *Escherichia coli* prefer warm, nutrient-rich environments (Qiao *et al*., 2022). The combination of high temperatures and increased organic matter input from runoff and fertilizer residues from crop farms can create favorable conditions for coliform growth, leading to higher counts in the sediments (Rashmi *et al.,* 2020; Tiwari and Pal, 2022).

## **CONCLUSION AND RECOMMENDATIONS**

Sediments from catfish ponds are reservoirs of nutrients which support the growth of bacteria including fecal coliforms. High loads of fecal coliforms in the sediment samples could be attributed to anthropogenic activities such as cattle fattening, cattle grazing by herders as well as farming activities such as fertilizer application, which all take place around the vicinity of the catfish ponds in Monai cluster fish farm. These activities do not only cause environmental pollution but also constitute a potential threat to public health safety in Monai village because sediment re-suspension can permit the exchange of these high bacterial loads from the sediment interphase to the water column. These high bacterial loads  $(3.22 \times 10^8 \text{ CFU/g})$  for August and September (2.30 x 10<sup>8</sup> CFU/g) could be directly discharged into the receiving stretch of the Lake that passes through the village where some villages use the water as a drinking water source. It is important that these sediments from catfish ponds be collected and utilized as viable resources where the nutrients contained in them can be safely recycled back into the aquaculture value chain. This can also ensure that the high bacteria loads contained in the sediments can be reduced by subjecting the sediments to various technologies that could help reduce the bacteria loads in them so they don't cause environmental pollution and subsequent public health hazard.

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