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Physicochemical and Molecular Characterization of Bacterial isolates in Earthen Fish Ponds

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ABSTRACT

This study assessed the physicochemical, heavy metals and bacterial content of some earthen fish ponds in Gombe State, to evaluate their suitability for aquaculture and potential health risks. Water samples were collected from six ponds across three locations and analyzed for key physicochemical parameters, including, turbidity, temperature, dissolved oxygen, pH, total dissolved solids and heavy metal concentrations. The results indicated that temperature (27.0–28.0°C), pH (6.23–7.55), and dissolved oxygen (3.85–8.13 mg/L) were within acceptable limits. However, turbidity (7.64–11.52 NTU), nitrate (15.57–51.62 mg/L), phosphate (13.41–18.55 mg/L), and heavy metals such as cadmium (0.01–0.08 mg/L) and lead (0.05–0.08 mg/L) exceeded WHO permissible levels, indicating potential contamination. Bacteriological analysis revealed Escherichia coli (32.8%), Salmonella (15.1%), Klebsiella (16.7%), Proteus (11.1%), Shigella (14.2%), and Pseudomonas (10.1%), suggesting fecal contamination. Molecular analysis confirmed the presence of bacterial strains, emphasizing the need for improved pond management practices. The findings highlight the urgent need for regular water quality monitoring, proper waste management and public awareness campaigns to ensure sustainable aquaculture and food safety in Gombe State.

Keywords: Physicochemical parameters, Bacteria, Earthen pond and water

INTRODUCTION

Fish farming is an important constituent of global food security and aquatic ecosystem management. Fish consumption is an integral part of the Nigerian diet, and stands as the largest consumer of fish in Africa, this substantial demand has historically led to a heavy reliance on both imports and fish cultured in ponds or tanks. Aquaculture is increasingly recognized as a crucial and viable solution to domestic fish production and lessen the dependence on foreign sources (Okochukwu *et al*. 2024). The expansion and development of aquaculture have been prioritized by the Government of Nigeria as a key food value chain to address this issue. With an annual fish consumption reaching approximately 3.2 million metric tons, the aquaculture sub-sector is considered essential for achieving self-sufficiency in fish production and meeting the nutritional needs of the population, (Okochukwu *et al*. 2024). This sector is rapidly expanding within Nigeria, underscoring its growing significance in the national agricultural landscape. The drive towards increased domestic fish production through aquaculture is a strategic move to not only ensure food security but also

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to stimulate economic growth within the nation.

Fish are reared in different controlled environment which could be pond (Concrete or earthen), (wooden or fiber glass) and plastics (Njoku *et al.,* 2015). A fish pond is an artificial lake (reservoir, pond) intended for fish breeding, Pond waters may be polluted due to discharge of effluents from various industries, domestic waste, land, feed and agricultural drainage; this causes a deleterious change in the water quality (Abu *et al.,* 2020). Physical characteristics of a fish pond directly impact pond water quality and indirectly the whole ecosystem and therefore, production management potential for the farmers. Pond water composition may be murky if it has a high number of suspended particles and organisms. Its bottom is usually sediments of sand, decaying matter and microorganisms. Pond ecosystems are often teeming with rich vegetation and a diverse organismal life. Ponds may however be contaminated by direct excretion from fishes (Hoseinzadeh *et al*., 2013). Fish in their natural environment are prone to attack by diseases, predators and other undesirable conditions such as pollutions and aquatic weeds infestation. (Hassan *et al.,* 2022).

Water is the most important resource for aquaculture and can be a significant source for contamination. In some aquaculture practices, the use of chemicals for water treatment or antibiotics for disease control might occur, and these substances can potentially accumulate within the pond environment, impacting water quality and potentially affecting the fish (Okochukwu *et al*. 2024). The natural accumulation of organic matter, such as decaying leaves, dead algae, and other organic debris at the bottom of earthen ponds, can contribute to the biochemical oxygen demand (BOD) and may lead to the development of anaerobic conditions, resulting in the release of harmful gases. (Hassan *et al*., (2022)

These internal sources of pollution within the aquaculture system itself can significantly impact water quality, emphasizing the critical role of implementing proper pond management practices within Gombe State.

Aquaculture in earthen ponds constitutes a significant element of Gombe State's economy and plays an integral role in its food security. The long-term safety and adequate utilization of this practice are inextricably linked to the quality of the water within these ponds. While previous studies have touched upon various aspects of water quality in Gombe State, Earthen ponds in Gombe State are potentially vulnerable to contamination from a multitude of sources, including agricultural runoff carrying fertilizers and pesticides, industrial discharge where relevant, domestic waste, and internal aquaculture activities such as feed waste and fish excretion. Existing research has already indicated the presence of pathogenic microorganisms in some fish ponds within the state, raising concerns about potential health risks. (Abu *et al*., 2020) The absence of a detailed study that integrates both physico-chemical and bacteriological analyses across selected earthen ponds in different locations within Gombe State creates a significant gap in our understanding of the current water quality status and the potential risks associated with it. Without a thorough assessment, the potential for compromised water quality in these earthen ponds could lead to reduced fish health and overall productivity, resulting in economic losses for fish farmers. Also the consumption of fish raised in potentially contaminated water could pose health risks to consumers.

Water quality stands as the most critical determinant influencing the success and long-

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term viability of aquaculture ventures. Fish, entirely is an aquatic organisms, depend on their surrounding water for all essential life processes, including feeding, respiration, growth, and reproduction. Maintaining a high standard of water quality within earthen pond systems is therefore crucial for ensuring optimal growth rates, health and overall productivity of the fish being cultured (Yusoff *et al*., 2024) any deterioration in water quality can induce stress in fish populations, leading to outbreaks of diseases, reduce growth, and substantial economic losses for fish farmers, monitoring and thorough assessment of various water quality parameters are indispensable for the effective management of aquaculture operations. The release of untreated or poorly treated water from these ponds could have negative consequences for the surrounding environment. Therefore, a comprehensive physico-chemical and bacteriological assessment of selected earthen ponds in Gombe State is needed to provide baseline data.

MATERIALS AND METHODS

Area of Study

The study was performed in Akko and Yamaltu Deba local govement areas of Gombe State, Nigeria. Akko and Yamaltu Deba local government areas have a total population of 337,853 and 254,726 respectively (NPC 2006). Akko local government area is approximately 2,627km2 while Yamaltu Deba local government area has a total land area of 1,981km2. The communities (stations) selected for this study were Kalshingi & Tukulma in Akko local government area and Kwadon & Deba in Yamaltu Deba local Government area.

Research design

Criteria used for choosing sampling station was a good representative of the area, earthen ponds which were the main type of ponds used for aquaculture in the area. Out of the 3 stations 6 ponds were selected from in the stations. Random sampling procedure was employed to select 6 ponds from which water samples were collected from. They were labelled as Pond sample 1-6 all the ponds were stocked with catfish, 5-6 months old. Water samples were collected manually at each sampling ponds to represent the composition of the ponds. Sampling was carried out within six (6) months (April – September). During the sampling seasonality was not considered.

Sample Collection

Eight earthen fish ponds from four sampling stations were collected in a plastic bottles. Each sample bottle was rinsed with the pond water before it was finally collected according to the standard methods by (APHA, 2015). When collecting the water sample, the base of each sterilized sample bottle was held with one hand, and plunged about 30cm below the water surface. After the samples were collected, they were labeled and immediately carried in a cooler packed with ice blocks for analysis.

Determination of Physicochemical and heavy metals

Physico-chemical parameters of the ponds water was analyzed using standard methods (APHA 2005). The parameters include electrical conductivity, chloride, temperature, pH, total dissolved solids, turbidity, phosphate, sulphate, dissolved oxygen, nitrate, biological oxygen demand, total hardness, acidity, cadmium, lead, copper, and iron.

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Determination of Bacteria

The inoculating loop was sterilized by flaming in the Bunsen burner until it turns red hot. Similarly, microbial load on the working surfaces were reduced by application of disinfectant solution (90 % ethanol).

Media Preparation

The media used were prepared according to manufacturer's instruction and sterilized in an autoclave at 121°C for 15 minutes.

Bacterial isolation

Ten-fold Serial dilutions of all the samples were made. 0.1 ml of the diluted water sample was inoculated in duplicate onto the sterile nutrient agar and swirled. After solidification, the plates were incubated at an optimal temperature. The colonies on the plates were counted using colony counter and recorded. After the colonies were counted, the mean for each sample was expressed as described by Umeh *et al*. (2020).

Isolation and Identification of Bacteria

To have distinguishable colonies from the initial culture, the colonies were cultured in selective and differential media which include MacConkey agar and Salmonella Shigella (SS) agar, and then incubate at 25^0C.vGrowth of distinct colonies was achieved (Nura *et al*, 2021). The colonies were morphologically identified. Subsequently sub-culture was carried out to increase the chance of obtaining a pure culture. The isolates were transferred onto agar slant in bijou bottles and kept in the refrigerator at 4 °C to serve as stock culture for subsequent test during identification. This process was carried out aseptically to prevent contamination.

Incubation

All the newly inoculated plates were incubated according to the optimal growth temperature for the suspected organism (usually 35-37 °C for most bacteria). Typical incubation times 24 hours.

Gram-staining and microscopic examination

This was done according to the procedure described by Cheesbrough (2010).

Biochemical tests

The confirmatory tests such as catalase, coagulase, citrate utilization, oxidase and urease tests were carried out to identify the isolated microorganisms. The results obtained were then compared with Bergy's manual for determinative bacteriology for confirmation

Identifying bacteria isolate using molecular approach

Discrete isolate from stock culture were molecularly identified to ascertain the genetic makeup of the organisms. This was done involving these major steps which include DNA extraction, 16S rRNA Amplification, Sequencing, Phylogenetic Analysis.

DNA Extraction

QIAamp extraction kit was used to extract the genomic DNA in accordance with the manufacturer's instructions. a 10-milliliter broth culture of the test organism was made over night. A 560 µl of prepared lysis buffer (AVL + RNA) was pipetted into 1.5 ml microcentrifuge tube containing 140 µl of the sample and vortexed for 15 seconds. The mixture was then incubated for ten minutes at room temperature. 560 µl of absolute ethanol was added to the sample and mixed by vortexing for 15 seconds and centrifuged briefly. After that, 630 µl of the solution was placed in a spin column and spun for one minute at 8000 rpm. A new collection tube was installed in lieu of the old one, together with the flow-through. This previous step was repeated using the remaining solution and the

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same spin column. The column was put in a fresh collection tube and buffer AW1 (500 ml) was added. The mixture was then centrifuged at 8000 rpm for one minute. After adding 500 ml of buffer AW2, the mixture was centrifuged for three minutes at 14000 rpm. Centrifugation was used to dry the spin column for one minute at 14000 rpm. After that, the spin column were put in a fresh 1.5 ml microcentrifuge tube, 60 µl of Buffer AVE was added, and it was left to incubate for a minute at room temperature. After centrifuging it for a minute at 8000 rpm, the column was disposed of. Until it was needed for PCR, the DNA sample was kept in a Freezer at -20C, (Saleh *et al*., 2024).

16Sr RNA Amplification

The 16s rRNA region of the DNA isolates were amplified using the 20F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 20R:5' ACGGCTACCTTGTTACGACTT-3' primers, Master mix include a 20ml reaction mixture made up of 2µl genomic DNA, 1 µl of each primer, 4 µl of Thermoscientific DreamTaq Green PCR master mix (5X), and 12 µl sterile distilled water, sample were then mix by vortexing. The PCR conditions for amplification were as follows: three minutes of initial denaturation at 95°C, thirty seconds of denaturation at 95°C, thirty seconds of annealing at 58°C, one minutes of extension at 72°C, and five minutes of final extension at 72°C for 35 cycle, (Saleh *et al*., 2024).

Agarose Gel Electrophoresis

Agarose gel (1%, w/v) was prepared by dissolving 1g of agarose powder in 99 ml of 1X TAE buffer. The powder was fully dissolved after five minutes of microwave heating of the mixture. The solution was allowed to cool down to about 60o C and 5 µl (0.5 µg/ml) ethidium bromide was added. Mixture was poured into a gel casting tray to solidify with a gel comb inserted into the agarose gel to make wells into which the PCR products were loaded. The gel-containing casting tray was put into a gel electrophoresis tank with the same TAE buffer concentration. An aliquot of 5 µl of the PCR products containing each of the detected isolated bacterial genes was loaded into the wells alongside 1 kb DNA ladder or molecular marker (BioLabs Quick-Load Purple DNA ladder). The electrophoresis was ran by using a voltage of 80 V and a current of 200 mA for 40minutes and viewed using Flou-link UV transilluminator (Saleh *et al*., 2024).

Sequencing

The isolated bacteria DNA was sent to Inqaba Biotec West Africa Ltd, Oreoluwa Oladele Oyo Road, Ibadan 200001 Oyo State for the sequencing.

Phylogenetic Analysis

Obtained sequences were edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using multiple sequence alighnment. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei 1993). The tree with the highest log likelihood (-5585.38) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 7 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1788 positions in the final dataset.

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Evolutionary analyses were conducted in MEGA X (Kumar *et al* 2018).

Data analysis

The collected data was initially entered into a data sheet before being organized and inputting it into a database using the Statistical Package for Social Sciences (SPSS). Data analysis was conducted using two-Way ANOVA to identify differences between pond means of water parameters in aquaculture ponds, with significant differences. The results were presented using tables, and chart.

RESULTS

Physical parameters result from the study area

Temperature

The temperature values are slightly similar across all stations. The lowest temperature in within the station was observed in station one 1 (27.00±0.00^{0}C), whereas the highest temperature was recorded in station two (2) (28.00±0.00^{0}C), analysis of variant revealed no significant different among stations (p-value=0.214). The values are reasonable and are within the WHO limit range of 25-35^{o}c.

pH

The variation between sampling stations revealed that lowest pH was recorded in station 1 (6.23±0.01) while highest was in station 2 (7.55±0.00) as presented at table 1. Analysis of variance indicate no significant variation between stations with p-value= 0.325. Which is within the WHO recommended range of 6.5-8.5.

Electrical Conductivity

The electrical conductivity showed variation between stations, where low conductivity was recorded in station 2 (263.00±0.94) and station 1 having the highest value (825.00±1.43). Also the obtained result has a significant variation between stations (p-value= 0.001). Whereas are within the WHO maximum value set 2700uS/cm.

Total Dissolve Solid (TDS)

The results of TDS revealed stations two (2) having the lowest value of (118.79±0.13) while the highest was in station one (1) (212.50±2.12), analysis of variance revealed a significant difference exist between stations with p-value=0.001. Recorded values are within the WHO specified values (500 mg/L)

Turbidity

The result reveals that lowest turbidity of the pond water was recorded in station 2 (7.64±0.00) while highest in station 3 (11.52±0.00) respectively. Turbidity varies significantly among stations. The Turbidity levels are above the minimum value set WHO (2018).

Total suspended solid (TSS)

The TSS values results revealed that station (1) has the lowest value (114.00±1.52) and the highest as in station 2 (215.00±0.00), with significance different among stations (p-value= 0.001). Values are within the WHO maximum level set (150mg/L).

Chemical Parameters Result from the Study Area

Dissolve oxygen

Variation in dissolved oxygen concentration based on sampling station shows that lowest value was recorded at station 3 (3.85±0.00) while highest was at station 2 (8.13±0.42), and the record obtained are within maximum value set by 8mg/L WHO (2018). With significant difference between stations at (p-value=0.051)

Biological oxygen demand

Biological Oxygen Demand (BOD) values across sampling stations and months were

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presented in table 1 where lowest value was observed in station 1 (2.16±0.03) while highest value was obtained in station 2 (5.3±40.21) Also, results reveal that there was significant variation in stations at p-value=0.014). And Values are within the WHO standard 30mg/L

Chemical oxygen demand (C.O.D)

The COD concentration of the ponds revealed the lowest in station 3 (4.27±0.00) while highest was at station 2 (7.22±0.02). Analysis of variance showed no significant different across stations with p-value= 0.244.

Total Hardness

Total Hardness values based on station shows that lowest value was obtained in station 3 (201.00±0.00) while the highest was in station 2 (453.50±5.32), and the result recorded is above the minimum value set by WHO standard 100mg $CaCO_3$/L Total hardness indicates a significant differences among stations at p-value= 0.001.

Acidity

The variation among sampling stations revealed that lowest acidity was recorded in station 3 (0.58±0.00) while highest was in station 1 (0.85±0.03) as presented in Table 1. The acidity values are relatively consistent among the stations. Analysis of variance result revealed there is no significant different between stations

Phosphate-phosphorus

Concentration of Phosphate throughout stations was shown in table 1 where the lowest and highest values were recorded at station 1 and 3 (13.41±0.18 and 18.55±0.04) respectively. With significant difference among the stations having p-value of 0.001, also all value obtained from the stations are above the WHO standard limit of 10mg/L.

Nitrate ($NO3^-$)

Nitrate concentration value varied among stations where the lowest and highest values were recorded at station 3 and 1 (15.57±0.05 and 51.62±1.14) respectively. Level of nitrate obtained are above the minimum value 45mg/L set WHO (2018), with significant different between stations (p-value=0.001).

Sulphate (SO34)

The variation between sampling stations shows in table 1 where the lowest recorded was in station (2) (15.64±0.05) while highest was in station 1 (64.40±2.12) as presented in Table 2, the values obtained are within the WHO standard limit of 100mg/L with significant variation between stations at (p-value= 0.001).

Chloride (Cl^-)

The concentration of chloride values varied among the sampling stations. The lowest value of chloride was recorded in station to 2 (29.00±0.00), whereas the highest was recorded in station three (1) (72.00C±0.1.23). The result shows significant variation between stations at p-value= 0.002) also values are within the WHO limit of 250mg/l.

Heavy metals parameters results based on stations

Cadmium

The cadmium values are relatively low as the lowest cadmium value was recorded in station 1 (0.01±0.02) while highest was in station 2 (0.08±0.07), the result obtain have exceed the WHO guideline value of 0.003mg/L Analysis of variance revealed there is no significant variation between the stations with p-value= 0.232.

Copper

The variation of copper concentration are relatively low, the lower values obtained were

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recorded in station 3 (0.11±0.42) and higher in station 2 (0.36±0.20), in table 1 Also the result shows there is no significant different (at p-value=321) between the stations. The values are below the WHO guided value of 2 mg/l.

Iron

The content of iron in fish pond water sample was presented in table 1. The results reveal that station three (3) recorded lowest value of (5.22±0.00) while the highest was in station two (2) (12.08±0.50), there is a significant different between the stations with p-value= 0.000.

Lead

Table 1 showed the mean variation of lead between stations, the result reveals that lowest lead concentration of the water was recorded in station 1 (0.05±0.02) while highest was in station 3 (0.08±0.89). Analysis of variance revealed values obtained have no significant different among the stations, Also all the values recorded are higher than the WHO guided value 0.01mg/L.

Table 1: Characteristics of Physical Parameters of Ponds water within stations.

Samples	pH	Temp	E. Conductivity (µS/cm)	Total Dissolve Solid (mg/L)	Turbidity (NTU)	Total Suspended Solid (mg/L)
Kalshingi	6.23 ± 0.01 [a]	27.00 ± 0.00 [a]	825.00 ± 1.43 [a]	212.50 ± 2.12 [a]	8.77 ± 0.13 [a]	114.00 ± 1.52 [a]
Kwadom	7.55 ± 0.00 [a]	28.00 ± 0.00 [a]	263.00 ± 0.94 [b]	118.79 ± 0.13 [b]	7.64 ± 0.00 [a]	215.00 ± 0.00 [b]
Tukulma	6.52 ± 0.00 [a]	27.10 ± 0.00 [a]	486.00 ± 0.47 [c]	143.00 ± 0.47 [c]	11.52 ± 0.00 [b]	186.00 ± 0.00 [c]
P value	0.325ns	0.214ns	0.001*	0.001*	0.000*	0.001*
WHO limits	6.5 – 8.5	25 – 35	1000	500	5	150

Means with different alphabet(s) along Colum are significantly different

** Significant difference, ns – not significant at P>0.05.

Mean Value ± Standard Deviation.

Table 2: Presentation of Chemical Parameters of Pond waterfrom the study area.

Samples	D.O (mg/L)	BOD_5 (mg/L)	COD (mg/L)	Hardness (Mg/L $CaCO_3$ eqv.)	Acidity (mg/L)
Kalshingi	5.64 ± 0.12 [a]	2.16 ± 0.03 [a]	4.60 ± 0.02 [a]	246.50 ± 1.75 [a]	0.85 ± 0.03 [a]
Kwadom	8.13 ± 0.42 [b]	5.34 ± 0.21 [b]	7.22 ± 0.02 [b]	453.50 ± 5.32 [b]	0.83 ± 0.02 [a]
Tukulma	3.85 ± 0.00 [c]	2.65 ± 0.00 [a]	4.27 ± 0.00 [a]	201.00 ± 0.00 [a]	0.58 ± 0.00 [a]
P value	0.051ns	0.014ns	0.244ns	0.001*	0.0214ns
WHO limits	8	30	250	100	

Means with different alphabet(s) along Colum are significantly different

** Significant difference, ns – not significant at P>0.05.

Mean Value ± Standard Deviation

Table 3: Chemical Parameters of Water Samples from the study Area.

Samples	Concentrations (mg/L)			
	Phosphate (PO_4^{3-})	Nitrate (NO_3)	Sulphate (SO_4^{3-})	Chloride (Cl^-)
Kalshingi (s1)	13.41 ± 0.18 [a]	51.62 ± 1.14 [a]	64.40 ± 2.12 [a]	72.00 ± 1.23 [a]
Kwadom (s2)	16.34 ± 0.03 [b]	27.52 ± 0.07 [b]	15.64 ± 0.05 [c]	29.00 ± 0.00 [c]
Tukulma (s3)	18.55 ± 0.04 [a]	15.57 ± 0.05 [b]	34.57 ± 0.06 [b]	36.00 ± 0.00 [a]
P value	0.01*	0.01*	0.003*	0.002*
WHO limits	10	45	100	250

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Means with different alphabet(s) along colum are significantly different

** Significant difference, ns – not significant at P>0.05.

S1, 2 and 3= Station 1, 2 and 3 respectively

Mean Value ± Standard Deviation

Table 4: Heavy metal concentration of Water Samples from the study Area.

Samples	Concentrations (mg/L)			
	Cd	Cu	Fe	Pb
Kalshingi	0.01 ± 0.2 [a]	0.30 ± 0.17 [a]	11.38 ± 0.80 [a]	0.05 ± 0.02 [a]
Kwadom	0.08 ± 0.07 [a]	0.36 ± 0.20 [a]	12.08 ± 0.50 [b]	0.08 ± 0.09 [a]
Tukulma	0.02 ± 0.30 [a]	0.11 ± 0.42 [a]	5.22 ± 0.00 [c]	0.08 ± 0.89 [a]
P vaue	0.232ns	0.321ns	0.000*	0.214ns
WHO limits	0.003	2	0.3	0.01

Means with different alphabet(s) along Colum are significantly different

** Significant difference, ns – not significant at P>0.05.

Mean Value ± Standard Deviation

Bacteriological Result

The pond water samples showed different genera result represented in Table 5. These include *Escherichia coli, Salmonella sp., proteus, shigella, Klebsiella sp.,* and *pseudomonas.* *E.* coli had the highest occurrence of 32.8% (n=65) followed by *salmonella* with 15.1% (n=30) occurrence, *proteus* with 11.1 % (n=22) occurrence, *shigella* with 14.2% (n=28) occurrence, *klebsiella* with 16.7% (n=33) occurrence, and pseudomonas with 10.1% (n=20) the least occurrence. Also Klebsiella showed positive reaction to catalase test, Citrate test, and urease test. Whereas *proteus* displayed positive to Citrate test, catalase test, and urease test. *Pseudomonas* responded to some of the biochemical tests like catalase, Citrate as well as the oxidase test. *E. coli* showed positive response to catalase test, Indole test. *Salmonella* showed positive response to Catalase tests. Based on the microscopic studied it was conformed that the above identified bacteria were found to b e gram negative (Table 6).

Table 5: Frequecy parcentage of bacterial species identified from sampling station

Organisms	Number of Colonies Isolated	Percentage (%)
E. coli	65	32.8
Salmonella	30	15.1
Proteus	22	11.1
Shigella	28	14.2
Klebsiella	33	16.7
Pseudomonas	20	10.1
Total	**198**	**100**

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Table 6: Biochemical Test of bacteria of the pond water.

Bacterial species	Morphology	Shape	Gram Stain	Catalase test	Coagulase test	Citrate test	Oxidase test	Indole test	Urease test
E. coli	Pink to red colonies	Rod	-	+	-	-	-	+	-
Salmonella	Colourless colonies with black centers	Rod	-	+	-	-	-	-	-
Proteus	Colourless colonies with black centers	Rod	-	+	-	+	-	-	+
Pseudomonas	smooth green colonies	Rod	-	+	-	+	+	-	-
Shigella	Round Colouless colonies	Rod	-	+	-	-	-	-	-
Klebsiella	Mucoid pink colonies	Rod	-	+	-	+	-	-	+

Key: - negative, + positive

Molecular and Bioinformatics Analysis of the Bacteria Isolates

The stained PCR products were readily detected from the five (5) extracted bacteria DNA as illustrated (figure 3) with indication of shade protien contamination lane 1-5, also lane (M) representing molecular marker size of 1kb.

Genetic background were investigated from the 3 selected DNA, their genomes were sequenced. The strains were observed via complete 16S rRNA gene sequence and Multiple Sequence alignment, copy core gene products as *stenotrophomonas* species, *lysinibacillus* and uncultured bacteria.

Members of the Strains identified as *stenotrophonas* species, clustered into two groups (*KM009131.1, LN23587.1, and MG674336.1, FJ765513.1*). The gene shared 90% nucleotide sequence identity with askb, the tree showed the strains has a distant relationship (Fig.1). While E02askb genes isolates from different ponds water strain identify as *lysinibacillus macroides* cluster into two groups (KJ882413.1, OP860984.1, and MW173351.1, MZ413597.1).

Phylogenetic tree of G02askb3 genes isolates from different ponds tress selected group members of uncultured bacterium (DQ915580.1 and JF18742.1) with *paenibacillus* polymyxa cluster into two (PQ454950.1 and PQ454948.1) figure (6).uncultured bacterium shared <90 per identification as shown in the blast result.

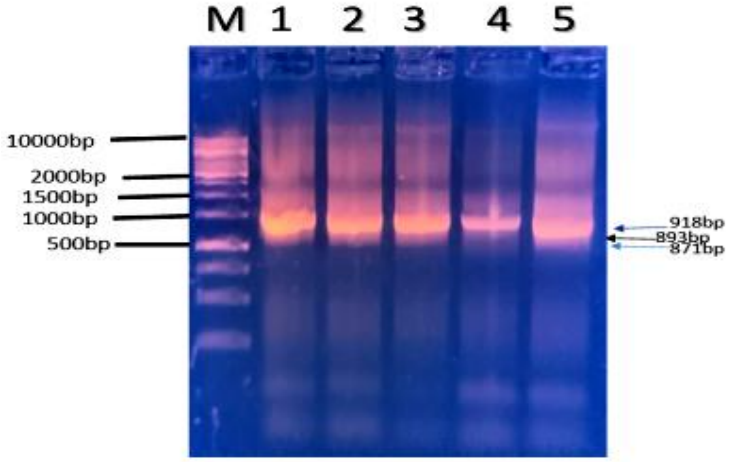

Figure 2: 16srRNA genes amplified from bacterial M: 1kb DNA Marker, lane 1- lane 5. The sample 1 gene (918bp), sample 3 gene (893pb) and sample 5 (871pb) are indicated on the right.

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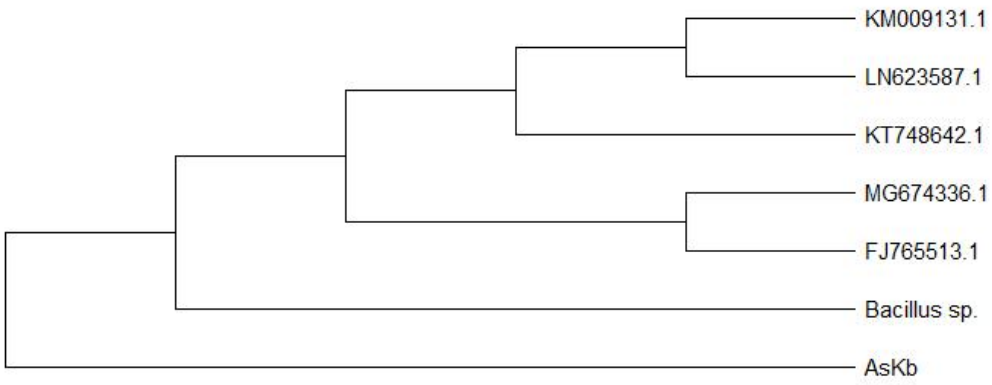

Figure 1: Phylogenetic tree of Askb DNA isolates from ponds water, similar strained identify in Streptrophomonas species cluster into two groups (KM009131.1, LN23587.1, and MG674336.1, FJ765513.1).

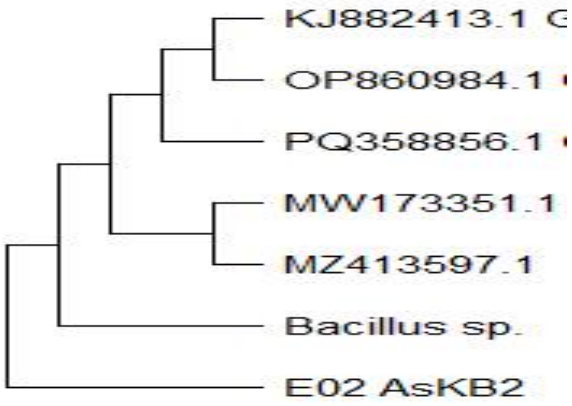

Figure 2: Phylogenetic tree of E02askb genes isolates from different ponds water strain identify as lysinibacillus macroides clustered into two groups (KJ882413.1, OP860984.1, and MW173351.1, MZ413597.*1).*

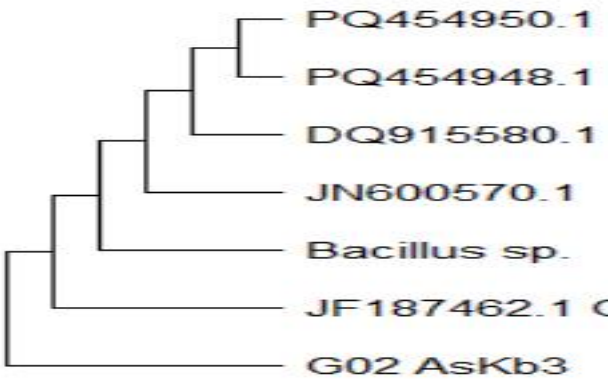

Figure 3: Phylogenetic tree of G02askb3 genes isolates from different ponds selected members of uncultured bacterium (DQ915580.1 and JF18742.1) with paenibacillus polymyxa clustered into two(PQ454950.1 and PQ454948.1).

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DISCUSSION

Physicochemical and Heavy Metals Parameter

The result of physicochemical constituent of earthen pond water samples indicate that Temperature detected in the fish ponds ranged from 27.00±0.00^{0}C to 28.00C±0.00 (table 1a) and are between the range of W.H.O (2018) limit 25°C-35°C. These temperatures was similar to the result of Umeh *et al*. (2020), also Kabiru *et al*. (2019) reported average temperature range of 27.5±0.60°C, to 27.40±0.56°C. lower pH value symbolized acidity of water while higher value give rise to alkalinity of a solution or water sample. The pH values obtained for fish ponds ranged from 6.23±0.01 to 7.55±0.00 in (table 1) based on stations. The water samples were within the W.H.O limits with no significant different between the stations. It falls within the normal range compared to the findings of Kabiru *et al*. (2019). Additionally, Umeh *et al*. (2020) observed pH values ranging from 6.21 to 8.15. The concentration of salts in an aquatic environment is directly proportional to its conductivity. Conductivity detected reported in the three stations ranged from 263.00±0.94 to 825.00±1.43 (table 1). Analysis of variance revealed a significant different exist both between stations and months. These values fall within the WHO limits of 1000 µS/cm, and agreed with the report of Kabiru M *et al*. (2019), umeh *et al*. (2020) also reported low conductivity values ranged from 166.36 µS/cm to 394.00 µS/cm for physicochemical analysis from Selected Fish Pond Water Samples in Awka and Its Environment, Anambra State**.** The TDS mean values from three stations ranged from 118.79±0.13 to 212.50±2.12. These values were within W.H.O maximum containment level goal of 500mg/L TDS result shows a significant variation among months and stations. The values exceed the findings of Kabiru M *et al*. (66.90+12.28 to 113.90±75.86), for pond. Fish feeds have been known to increase the total dissolved solids in a water body (Ogbeibu and Edutie, 2006).

The total suspended solids has influence in the turbidity of aquatic habitat and the results indicate from the three stations falls within

114.00±1.52 to 215.00±0.00. Analysis of variance indicates significant variation between months and stations. The values obtained were within the W.H.O permissible limit of 150 mg/L with the exception of station 3 which is above the stated limit. Turbidity values reported in the fish pond waters ranged from 7.64±0.00 to 11.52±0.00 as given in table 1a. The turbidity values were above the W.H.O. 2018 permissible limit of 5 NTU. Values indicating significant different between the stations and the months. The values are slightly above the findings of umeh *et al*. (2020) suggest the high turbidity may be as a result of stocking density, time frame for removal/exchange of pond water, and organic matter. Nitrate concentrations in the fish ponds ranged from 15.57±0.05 to 51.62±1.15 in table 1. Analysis of variance shows significant different between stations and the nitrate values were within W.H.O. permissible limit of 45mg/L while station1has exceeded the stated limit and higher than the values (0.25± 0.10 to 0.42±0.31) recorded by Ya Qin *et al*., (2016) also, Umeh *et al*. (2020) observed value from 3.10 mg/L to 28.00 mg/L.

Phosphate: The values observed from the sample station ranged from 13.41±0.18 to 18.55±0.04 (table 1c). Result indicates significant variation among stations and months. And of the phosphate values were above W.H.O. permissible limit of 10mg/L and therefore the water is unsuitable for fish farming. The sulphate values observed in the fish pond water samples in all the three

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stations ranged from 15.64±0.05 to 64.40±2.12 (table 1. Analysis of variance revealed there is significant variation between stations and the months and values recorded are within W.H.O permissible limit of 250 mg/L and values are above the findings of Umeh *et al*. 2020 who observed values range from 0.39 mg/l to 4.37 mg/L. Chloride content obtained from the three stations ranged from 29.00±0.00 to 72.00C±0.1.23. Analysis of variance indicate significant different between stations and months. These values are within permissible limits of 250 mg/L. Elevated concentrations may affect aquatic life (Umeh *et al*. 2020).

Dissolved Oxygen (DO): The DO values obtained from the three stations ranged between 3.85±0.00 to 8.13±0.42 (table1.the result recorded showed no significant difference between stations, it also observed that the DO levels is within the standard limit (8.5mg/L) except station 2 which is slightly above the stated limit. Obtained result has lower values compared to the findings of Ehiagbonare and Ogunrinde (2010) who reported DO value of 9.3 mg/L - 16.2 mg/L. and slightly higher than the work reported by (Ya Qin *et al*., 2016). The Biochemical Oxygen Demand (BOD) values obtained ranged from 2.16±0.03 to 5.34±0.21 table 1. The results revealed no significant variation in stations and the months, although close to being significant. These values obtained are within the W.H.O limit of 30mg/L These BOD values are therefore within the values for optimum fish activities. Recorded result are similar to the values (2.98 to 4.27) reported by Umeh *et al*. (2020). Total hardness of water is used to describe the effect of dissolved minerals (mainly Ca. and Mg.) suitable for domestic and industrial purposes which is attributed to the presence of bicarbonates, sulphates, chlorides and nitrates (Umeh *et al*. 2020). Total hardness of the water samples in the three stations ranged from 201.00±0.00 to 453.50±5.32. Analysis of variance indicate significant different between stations and months. These values are within the W.H.O (2018) permissible limit of 150 mg/L Acidity observed in the in the three stations ranged from 0.58±0.00 to 0.85±0.03 (table 1b). There are no significant variations between stations the obtained acidity result the values were

Heavy metals: lead, copper, iron, and cadmium concentrations in ponds water in all the sampling stations were compared with W.H.O 2018 standard. The obtained results indicate that, with the exception of cupper, the heavy metal concentrations in the pond water has exceed WHO 2018 standard. Cadmium content observed in the stations ranged from 0.01±0.02 to 0.08±0.071 (Table 1. Analysis of variance shows no significant different between stations and months. The fish pond water samples exceeded the WHO guideline value of 0.003 mg/L and the cadmium value obtained has a slightly higher value compared to the work reported by Umeh *et al*. (2020). Lead content observed in the fish pond water samples from the stations range from 0.05±0.02 to 0.08±0.89 (table 1). Lead concentrations indicate no variation between stations and months, and values were above the WHO recommended standard of 0.01 mg/l and therefore not fit for fish farming. Copper was found in all the 3 stations values ranged from 0.11±0.42 to 0.36±0.20 (table 1d). Result indicates significant different between stations and the months. These values were within the WHO recommended standard of 2 mg/L and therefore fit for fish farming, Umeh *et al*. (2020). Iron content observed in the fish pond water samples ranged from 5.22±0.00 to 12.08±0.50. Analysis of variance revealed a significant variation among stations and between months. The results exceeded the WHO 2018 recommended value of 0.3 mg/l

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the iron content has exceeded the observed report by Umeh *et al*. (2020) with value ranged from 0.01 mg/L to 1.19 mg/l.

Bacteriological analysis: Escherichia coli was the most dominant organism occurring in the studied fish pond waters (Table 2). The fish pond waters revealed the presence of E. coli 32.8 % (n=65), *salmonell* 15.1 % (n=30), *proteus* with 11.1 % (n=22), *shigella* with 14.2 % (n=28), *klebsiella* 16.7 % (n=33), and pseudomonas 10.1 % (n=20) (table 2. The presence of *Salmonella* and *Escherichia coli* in water or food indicates the possible presence of causative agents of many gastrointestinal diseases. The biochemical result showed that Gram negative bacteria were dominant in the studied waters. The bacterial identification revealed the presence of six isolates; *Escherichia coli, Salmonella, Shigella, Pseudomonas, Proteus*, and *Klebsiella,* (Tables 2. Nura *et al*, (2021) reported a total of eight bacteria species isolated from the fish ponds with their percentage occurrence; *Escherichia coli, Flavobacterium spp., Psuedomonas spp., Samonella spp., Steptococcus spp. Escherichia coli* in pond are an indicator of fecal pollution which could be attributed to human activities or excrete from the fishes (David *et al*. 2020). Torimiro *et al*.2014 also Identified *Escherichia coli, Aeromonas sp., Klebsiella sp., Staphylococcus aureus*, and *Shigella spp*. in fish pond water samples stocked with *Clarias gariepinus* in Ile-Ife. They noted that this could pose a health risk to both the fish and consumers.

Molecular Analysis: PCR products from the five (5) extracted bacteria DNA as illustrated (figure 2) with indication of protein contamination in lane 1-5, lane (M) representing molecular marker size of 1kb. The genetic background of the 3 selected sequenced DNA, The copy core gene products as *stenotrophomonas* species, *lysinibacillus* and uncultured bacteria. Members of the Strains identified as *stenotrophonas species,* clustered into two groups (*KM009131.1, LN23587.1, and MG674336.1, FJ765513.1*). The gene shared 90% nucleotide sequence identity with askb Showing that the strains has a distant relationship (Fig.3). While E02askb genes isolates from different ponds water strain identify as *lysinibacillus macroides* cluster into two groups (KJ882413.1, OP860984.1, and MW173351.1, MZ413597.1) in figure (4). And uncultured bacterium shared <90 per identification as shown in the blast result. Lysinibacillus macroides, Myoides odoratimimus and Chryseobacterium cucumeris have little or no information as regards to fish pond water (David *et al*. 2020). Ya Qin *et al*., 2016 also reported the abundance of bacterial 16S rRNA genes from ponds with grass carp fed sudan grass and pond with grass carp fed commercial feed. The microbial communities were dominated by Proteobacteria, Cyanobacteria, Bacteroidetes, and Actinobacteria in both ponds. Saleh *et al.* (2024 state that Isolates of *Enterobacteriaceae* in our locality creates significant therapeutic problems prompting an immediate need for the establishment of local guidelines. (Adelowo, *et al*. 2018) result underscores the role of the Nigerian environment as reservoir of bacteria isolates from Nigerian wetlands and selected members of Pseudomonas putida group isolated from fish in Asia (DSM 15088, NZBD9, XDHY-P, NB2011 andDJ-1), soil/ sediment in USA (KCJK7865), China (NyZ12) and India (TND35).

CONCLUSION

The physicochemical and bacteriological assessment of earthen fish ponds highlights the critical need for proper sanitary practices and water quality management to ensure healthy aquaculture. The study reveals

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varying levels of bacterial loads and the presence of heavy metals, which may pose risks to fish health and consumer safety. To enhance the reliability of microbiological assessments, incorporating sensitivity or resistance testing before conducting molecular analyses is crucial. This step ensures that any resistant or sensitive microbial strains are adequately identified, contributing to more accurate diagnostics and informed management decisions. Ultimately, fostering better environmental and aquaculture practices will not only improve fish health and productivity but also safeguard public health and promote sustainable fish farming. Evaluating water quality is a fundamental prerequisite for ensuring the sustainability of earthen pond aquaculture in Gombe State.

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