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Microbiological And Physicochemical Assessments of Selected Fish Pond Water Sample in South-West, Nigeria

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Abstract

Pathogenic organisms have the ability to reduce or delay fish output. This study aims to assess the microbiological quality and physicochemical quality of fish pond water in Ijebu-North local government of Ogun state, Nigeria. Fish pond water samples were collected from three different locations, which include Ago-Iwoye, Ijebu-igbo and Awa. Physicochemical analysis of fish pond water samples were achieved by standard procedures and all parameters were subjected to World Health Organization standards (WHO). The microbiological examination was determined by different selective agar media and biochemical identification was achieved by conventional analysis. The physicochemical characteristics of the samples such as temperature, pH and conductivity ranged from 33.9-44.2^{0}C, 6.70-7.87 and 163-196 respectively. The presence of trace and toxic heavy metals such as calcium, zinc, copper, iron, lead and so on were detected in this study. The sample from Ijebu-Igbo had the highest bacteria count of 4.28x10^2 cfu/ml. *Esherichia coli, Pseudomonas aeruginosa* and *Bacillus* species have the highest occurrence among the isolates identified. Therefore, there is need for adequate treatment of fish pond water to reduce the risk of fish exposure to pathogens and heavy metals.

Keywords: Physicochemical, Temperature, pH, Heavy metals, Fish ponds

Introduction

Water as proven to be the most expedient and important refreshing resources in the entire globe (Sarker *et al.,* 2020; Onajobi *et al.,* 2021). The most significant resource for aquaculture is water. Water has been known to be one of pathogens-transmitting components. Water of good quality is essential for the viability aquatic lives such as Fish (Onajobi *et al.,* 2015a; Torimiro *et al.,* 2014). Pathogenic organisms are capable of diminishing or retarding fish production (Prosun *et al.,* 2018; Onajobi *et al.,* 2021). When waste water is used for fish rearing, organic matter present in the water serves as a main nutritional constituent for the growth of pathogens (Egbere *et al.,* 2008; Ida *et al.,* 2012).

Pathogenic bacteria such as coliforms, enter the digestive tract of fish through water, food or sediments (Dulfa *et al.,* 2010; Prosun *et al.,* 2018). The presence of faecal coliform in fish indicates the contamination of water by faecal matter from humans or animals because these bacteria are not a

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normal flora of fish (Emikpe *et al.,* 2011; Olalemi and Oluyemi, 2018). The consumption of these infected fish contributes to the causation of foodborne diseases in humans. A study carried out by (Adebayo *et al.,* 2012b), reveal the presence of gastrointestinal disease-causing pathogens in fish. Also, the presences of Mycobacterium species in fish have also been reported by (Hastein *et al.,* 2006).

These bacteria are well known for the cause of Mycobacteriosis which can be transmitted to humans when infected fish are consumed. Most of these pathogens involved are resistance to antibiotics (Hastein *et al.,* 2006; Sarker *et al.,* 2020). As they are consumed, these resistance pathogens are transmitted among humans (Adebayo *et al.,* 2012b). Vast numbers of these bacteria have been found in fish. (Prosun *et al.,* 2019) reported that this is as a result consistence ingestion of contaminated water and food for a long period of time. According to Mandal *et al.* (2018), the presence of *Salmonella* species in sea foods has been reported in Asia and West Africa. Fungi such as *Aspergillus* species have also been reported by Adebayo *et al.,* (2012b) and this could be one of the main contributors of *Aspergillus* transmission causing *Aspergillosis* among consumers.

Environmental parameters such as dissolved oxygen, pH, temperature and others have a great impact on water quality. The growth of pathogens and parasites in water used for aquaculture practices depends largely on these parameters (Emikpe *et al.,* 2008; Prosun *et al.,* 2018). They also affect the growth of fish. Acidic environment does not support the growth of fish. But according to Silapajarn *et al.,* (2004) and Anon (2014), the environment can be neutralized by liming. Dissolved oxygen is also a vital environmental parameter that influences the quality of water and it is required for the viability of fish and other aquatic organisms (Thilza and Muhammed, 2010; Adedeji *et al.,* 2012). As a result Olalemi and Oluyemi (2018) emphasize the need to ensure that water to be used for aquaculture should possess the optimum range of these parameters that will promote fish production.

Waste water from domestic and industrial sources is often times used by famers for their aquaculture activities (Onyango *et al.,* 2009; Onajobi *et al.*, 2020). The Nigerian cattle sector produces non-meat products and wastes that must be recycled into goods that may be used in agriculture and other industrial applications (Onajobi *et al.,* 2020). This waste water contains vast numbers of toxic chemicals such as inorganic chemicals containing heavy metals which pose a great threat to the quality of water and in turn affects the viability of fish. Most of the chemicals originate from waste water containing pesticides, fertilizers and effluents from industries (ECDG, 2002). The purpose of this study is to evaluate the physicochemical and microbiological quality of fish pond water collected in Ijebu-North local government in the state of Ogun.

Research Methods

Study Area

Three study sites in Ogun State, South West, Nigeria were used for this study, which were all commercial farm fish ponds

Sample Location

The water samples were collected from three different sources; one sample from each locations in Ago-Iwoye, Ijebu-Igbo and Awa, Ogun State, Nigeria as shown in Figure 1 below. Three commercial farm fish ponds culturing *Clarias glarienpinus* (African Cat fish) all situated in Ogun State Nigeria.

Figure 1
Map showing the location of the Ijebu North, Ogun State, Nigeria fish ponds for water sampling.

Source: Onajobi *et al.* (2023)

Collection of fish pond water samples

Water samples were collected from three different fish ponds to assess the physiochemical parameters and microbial quality present. The water samples were collected in labeled universal bottles and taken to the Microbiology Laboratory of Olabisi Onabanjo University for microbial analysis.

Physicochemical Analysis of Water Sample from Fish Pond

Standard procedures established by APHA (1998) and Anon (2014) were utilized to screen the physiochemical characteristics of the water samples. The pH of the samples was measured using a pH meter. A mercuric thermometer was used to measure the temperature of the samples. The dissolved oxygen was measured using the Winklers iodometric titration method. The 5 days biochemical oxygen demand (BOD) measurement was utilized to measure the sample BOD. Dichromate reflux condenser was utilized to measure the chemical oxygen demand (COD). The alkalinity was measured by subjection 100ml of the sample to 0.02ml of hydrochloric acid solution through titration with the use of an indicator (methyl

orange). The sulphate, ammonia and phosphate constituents of the sample were measured by using Turbidimetric, Nessler reaction and Ascorbic acid method respectively (Njoku *et al.,* 2015).

Isolation of Microbial Species

Isolation of bacteria was performed by making serial dilution of the collected fish pond water samples and the dilution used for studies were 10^{-2} to 10^{-8}. One mililitre (1.0 ml) of the dilution samples was inoculated separately in duplicate plates of nutrient agar for total viable bacteria count, MacConkey agar for *Enterobacteria* count and *Salmonella*/*Shigella* agar for *Salmonella* determination, using the spread plate method. All media were prepared according to manufacturer's instruction. The nutrient agar plates were incubated at 37^{o}C for 24 hours. Distinct colonies growing on each plate were selected, subcultured and stored on a slant (Khasabuli and Kibera, 2014).

Gram Staining of Isolated Bacteria

Gram staining was used to determine the nature of the bacterium, all different colonies in nutrient agar obtained after incubation at 37^{o}C overnight were used to make smears on clean labeled slides Colonies grown on nutrient agar were gram stained using method describe by Todar (2004). The growth characteristics on nutrients agar for the pure bacterial isolate which included the forms, colors, margins elevations of the bacterial colonies, and also the cell shapes and arrangements under light compound microscope were observed and recorded (Cheesebrough, 2010).

Biochemical Characterization of Isolated Bacteria

Biochemical test and morphological characterization of the bacteria were carried out to determine their probable identities. Colonies exhibiting clear zones of inhibitions were isolated and re-streaked over a fresh sterile medium to generate pure cultures (Onajobi *et al.,* 2019; Onajobi *et al.,* 2020). The pure isolates were collected from the sample, the plates were examined and the suspec examined and the suspected colonies were gram stained. Gram-positive and negative, rod-shaped organism were observed and subsequently selected for further identification. Biochemical tests which include starch hydrolysis, sugar fermentation, gelatin, motility, Indole production, catalase, hydrogen sulphide production were carried out, results obtained were recorded (Cheesebrough, 2010; Onajobi *et al.,* 2013a).

Research Results and Discussion

Table 1 and Figure 2 shows the total bacteria count (TBC) was determined in the three locations under study, with Ijebu-Igbo having the highest 4.28 x 10^{2} CFU/ml followed by Ago-Iwoye 4.0 x 10^{2} CFU/ml and Awa with the least total bacteria count of 1.61 x 10^{2} CFU/ml. Coliforms of Ago-Iwoye, Awa and Ijebu-Igbo were also analyzed, with Ijebu-Igbo having a countable coliforms of 1.31 x 10^{2} CFU/ml while coliform in fish pond water sample of Ago-Iwoye and Awa were too numerous to count (TNTC). The numbers of Non Lactose Fermenters (NLF) in Ago-Iwoye, Awa and Ijebu-Igbo ranges from 10, 22 and 28 respectively, vibrio was present only in Ijebu-Igbo fish pond water sample but was rather absent in fish pond water sample of both Ago-Iwoye and Awa. *Pseudomonas* also was found only in Awa fish pond water sample, while Ago-Iwoye and Ijebu-Igbo fish pond water sample showed the absence of *Pseudomonas*. Finally, yeast/mould were detected in all the three fish pond water sample.

Table 2 clearly explain the temperature ranged from 33.9-44.2 (oC) which does not fall within the acceptable standard of World Health Organization (WHO) (40 oC). pH of fish pond water from the three locations ranged from 6.70-7.87 which falls within the acceptable standard of WHO (pH 5.5-9.0). Other parameters such as Total Dissolved Solid (TDS), Total Suspended Solid (TSS), Total Acidity (mg/l), Total Alkalinity (mg/l), Dissolved Oxygen (DO), Chemical Oxygen Demand (COD), and Biological Oxygen Demand (BOD) does not fall within the acceptable standard of World Health Organization (WHO). The presence of Trace and Heavy metal such as Calcium, Zinc, Copper, Iron, Chromium, Sodium, Cobalt, Manganese, Lead, Cadmium, Nickel and Silver were also analyzed in the three fish pond water sample, all of which does not fall within the acceptable standard of World Health Organization (WHO).

Table 3 and 4 shows and explain the six bacterial isolates which were identified and characterized in this study. These were *Salmonella* sp, *Pseudomonas aeruginosa*, *Neisseria* sp*, Bacillus* sp, *Escherichia coli* and *Proteus* sp.

The percentage occurrence of bacteria in fish pond water sample in Ijebu-North LGA, Ogun State was shown in Figure 3. *Escherichia coli, Pseudomonas aeruginosa* and *Bacillus* species had the highest percentage occurrence of 22.2%, followed by *Neisseria* sp*, Salmonella* sp and *Proteus* sp. all of which had the least percentage occurrence of 11.11%.

The result of the microbiological characteristic in the work showed that Gram negative bacteria were dominant in the bacteria isolated from the fish pond water sample. The microorganisms isolated were *Salmonella* sp, *Pseudomonas aeruginosa*, *Neisseria* sp, *Bacillus* sp, *Escherichia coli* and *Proteus* sp. A clear indication of the presence of pathogenic organisms was provided by the presence of an indicator organism like E. coli, which was suggestive of the contamination of the pond water with fecal debris and this was in line with the work carried out by Olalemi and Oluyemi (2018) and Onajobi *et al.* (2021) showing that the high load of animal excreta in the wastewater may be responsible for the occurrence of *E. coli* and *Streptococcus faecalis*. It also serves as a sign of recent faecal contamination (Onajobi *et al.* 2021). The feces may have been secreted by the fish or may have been a result of fertilizing the ponds with animal excrement that was dumped straight into the fish ponds or through runoff and also correlate with the work of Kay *et al.* (2008).

Table 1
Microbial analyses of fish pond in Ogun State, Nigeria

S/N	Location	TBC (cfu/ml)	Coliforms	NLF	Vibrio	Pseudomonas	Yeast/mould
1.	Ago-Iwoye	4.0×10^2	TNTC	10	-	-	Yeast
2.	Awa	1.61×10^2	TNTC	22	-	+	Yeast
3.	Ijebu-Igbo	4.28×10^2	1.31×10^2	8	+	-	Yeast

Keys: TBC= Total bacteria count; LF= Lactose fermenters; NLF= Non-lactose fermenters
TNTC = Too numerous to count; - = No growth; + = Growth

Table 2
Physiochemical parameters of fish pond water sample in Ogun State, Nigeria

S/N	PARAMETER	RESULT			WHO. STANDARD
		AGO-IWOYE	AWA	IJEBU-IGBO	
A	**PHYSICAL**				
	COLOUR	102	138	31	250Pt.Co.APHA
	APPEARANCE	Cloudy	Slightly cloudy	Clear	Clear
	Temperature	34.2	34.0	33.9	<35
	Ph	6.70	6.77	7.87	6.5-8.5
	Turbidity	53.9	79.8	4.73	NTU
	Conductivity	196	163	164	20-1500
	Total Suspended Solids	85	38	2	100mg/l
	Total Dissolved Solids	113	394	212	2100mg/l
	Total Solids	198	432	214	2200mg/l
B	**CHEMICAL**				
	Total acidity (mg/l)	32	140	16	NS
	Total alkalinity (mg/l)	240	600	160	NS
	Choride	45.36	30.62	22.68	250mg/l
	Nitrate	0.0	23.30	3.20	
	Phosphate	0.44	2.27	0.38	
	Sulphate	10	7	11	
	Phenol	NA	NA	NA	1.0mg/l
	Oil and Grease	4.7	NA	6.2	10.0mg/l
	Dissolved Oxygen	4.95	0.11	4.08	< 2mg/l
	Chemical Oxygen Demand	176	104	54	200mg/l
	Biological Oxygen Demand	44	26	13.5	50mg/l
C	**TRACE/TOXIC HEAVY METAL**				
	Calcium	0.4757	1.2943	1.327	200mg/l
	Magnesium	NA	NA	NA	5.0mg/l
	Zinc	0.0005	0.0490	0.0014	5.0mg/l
	Copper	0.0052	0.0169	0.0038	3.0mg/l
	Iron	0.0491	0.3837	0.0048	10.0mg/l
	Chronium	0.0004	0.0002	0.0047	0.1mg/l
	Sobium	0.7048	1.2807	O.0900	
	Cobalt	NA	NA	NA	
	Manganese	0	0.0372	0.0005	5.0mg
	Lead	0	0.0010	0.0033	0.1mg/l
	Cadmium	0	0.0014	-0.0018C	2.0mg/l
	Potassium	NA	NA	NA	200.00mg/l
	Nickel	0.0205	0.0006	-0.0035	3.0mg/l
	Silver	0.0068	0.0071	0.0058	<0.10mg/l
	Mecury	NA	NA	NA	0.01mg/l

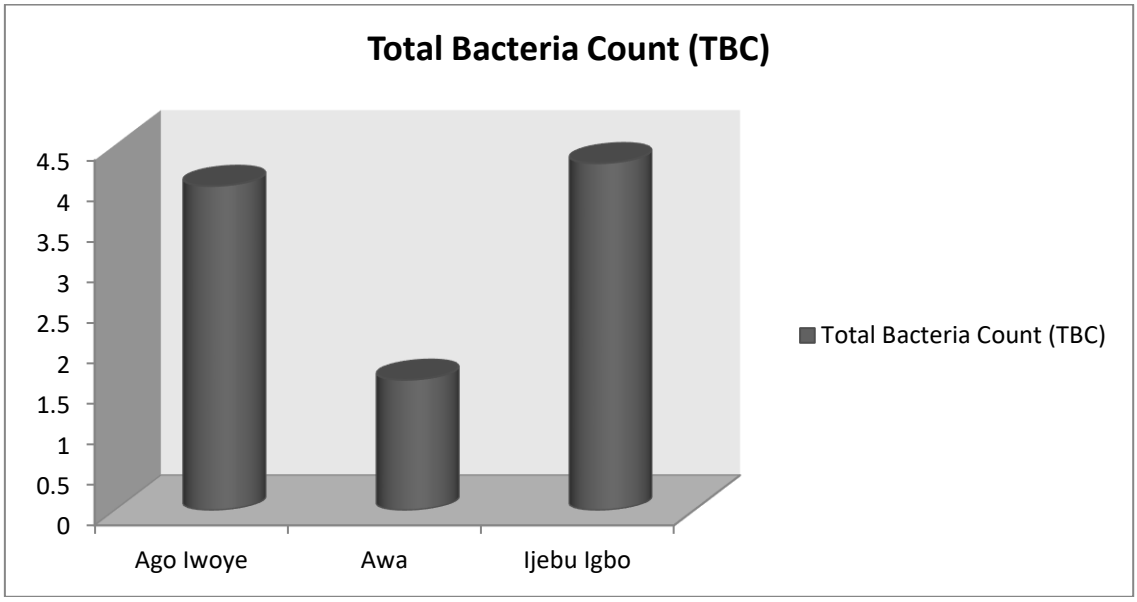
KEYS: NA= NOT ANALYSED NS= NOT SPECIFIED ND= NOT DETECTED
Pt. Co. APHA= Platinum Cobalt APHA Method

The many bacterial species that have been identified from these ponds are consistent with the study of Olulemi and Oluyemi, (2018) on pond water, which claims that allochthonous bacteria from feed that has been introduced to the ponds is the

primary source of germs that are significant for human health. Additionally, identical species were found in the microbiological analysis of the fish pond at El-Quater by Dorota *et al.* (2002).

Figure 2

Total bacteria count (TBC) of fish ponds water in Ijebu North, Ogun State



The existence of harmful microbes like *E. coli*, *Salmonella* spp., and *Bacillus* spp. on ingestion of inadequately prepared fish raised in these ponds might result in the transfer of water borne illnesses such Typhoid fever, cholera, and food poisoning (Ashbolt *et al.* 2001; Sarker *et al.,* 2021). The most prevalent organism in both concrete and earthen ponds was *E. coli* in this study and these results were consistent with that of Onajobi *et al.* (2021) explaining food poisoning that has been linked to *Proteus* and *Pseudomonas* species (Hastein *et al.,* 2006; Prosun *et al.,* 2018). In the pond, *Neisseria* spp. was also detected. One of the most opportunistic pathogens for freshwater fish, this bacterium is also the primary causative agent of illness outbreaks that have resulted in numerous fatalities (Das and Mukheyee, 1999; Onajobi *et al.,* 2015). These organisms may affect the biological balance of the soil and are a sign of potential contamination.

Both the coliform count and the overall viable bacterial count (Table 1 and figure 2) were high and varied among the ponds. The ideals could be due to variation in optimum temperature in the ponds which could encourage bacterial growth in one over the other. It could also be because of the variations in organic matter loads in the pond brought on by the fish's diet. Thus, the pond water turns into an excellent environment for the growth of bacterial pathogens that cause bacterial infections in fish and are a significant source of food poisoning and this also confirms the study carried out by (Eze and Ogbaran, 2010; Olalemi and Oluyemi, 2018). Additionally, *Neisseria* species have been discovered to be essential in fish culture systems (Amand *et*

al., 2010; Anon, 2014). As they degrade the water quality, they introduce secondary and primary diseases that affect fish health and cause fish death. An indication of the public health danger of water and food intended for human consumption might be the presence of this opportunistic human pathogenic (Ganesh *et al.,* 2009)

Table 3
Morphological Characteristics of Bacteria Isolates

S/N	Isolate Code	Cellular morphology	Growth on Salmonella/ Shigella agar	Growth on MacConkey agar	Growth on Nutrient agar	Probable organism
1.	FPA1	Rod	Dark	Pale	Cream	*Salmonella* sp
2.	FPA2	Rods	N/A	Pink	Pink	*Escherichia coli*
3.	FPA3	Rods	N/A	Pale	Bluish green	*P. aeruginosa*
4.	FPI1	Rods	N/A	N/A	Cream	*Neisseria* sp
5.	FPI2	Rods	N/A	N/A	Cream	*Bacillus* sp
6.	FPI3	Rods	N/A	Grey	Cream	*Proteus* sp
7.	FPIJ1	Rods	N/A	N/A	Cream	*Bacillus* sp
8.	FPIJ2	Rods	N/A	Pale	Bluish green	*P. aeruginosa*
9.	FPIJ3	Rods	N/A	Pink	Cream	*Escherichia coli*

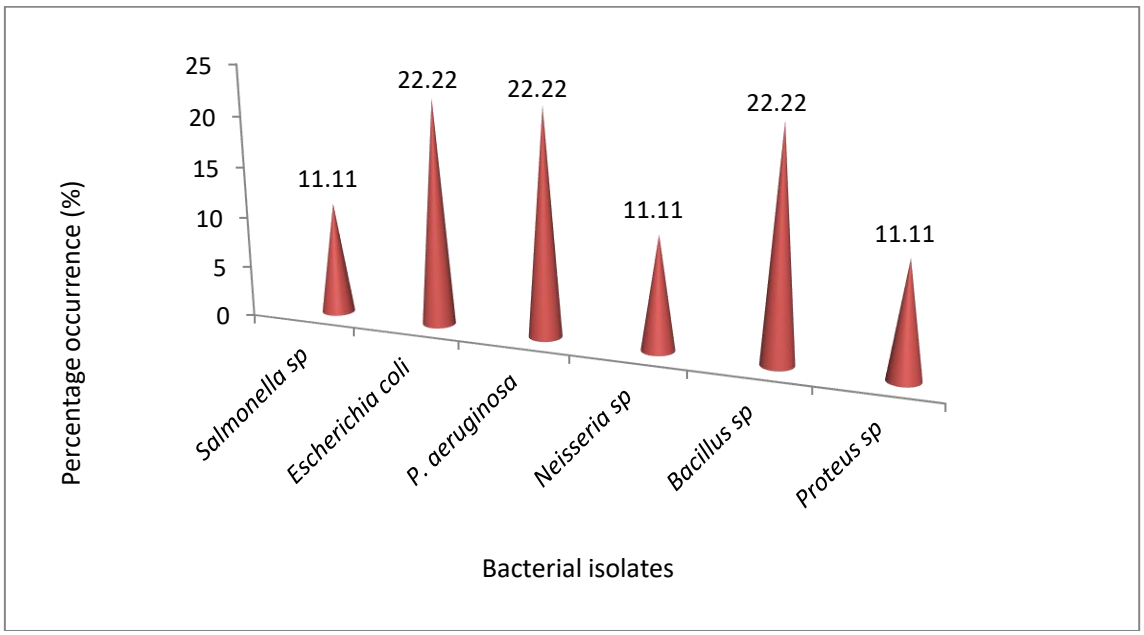
Keys: - = No reaction; + = Reaction; N/A = Not applicable

Table 4
Biochemical Characteristics of Bacteria Isolate in Ogun State, Nigeria

S/N	Isolate code	Gram's reaction	Catalase test	Oxidase test	Indole test	Urease test	Probable Organism
1.	FPA1	-	+	-	-	-	*Salmonella* sp
2.	FPA2	-	+	-	+	-	*Escherichia coli*
3.	FPA3	-	+	+	-	-	*P. aeruginosa*
4.	FPI1	-	+	-	-	-	*Neisseria* sp
5.	FPI2	+	-	-	+	+	*Bacillus* sp
6.	FPI3	-	+	-	-	+	*Proteus* sp
7.	FPIJ1	+	+	-	-	-	*Bacillus* sp
8.	FPIJ2	-	+	+	-	-	*P. aeruginosa*
9.	FPIJ3	-	+	-	+	-	*Escherichia coli*

Keys: - = No reaction; + = Reaction.

Figure 3
Percentage occurrence of bacteria isolated from fish pond water in Ogun State



It is possible to explain the changes in the physicochemical qualities of the fish ponds studied in the current study to the effects of the microclimate and topographic circumstances in Ijebu-North Ogun State, Nigeria, and this is consistent with the work of Sarker *et al.* (2020) describing a way to gauge how much oxygen bacteria and other microbes need to stabilize decomposable organic matter over a certain amount of time. Additionally, interactions between people and animals, including livestock, may have a role. The pH results are in line with what was discovered in Nigeria by Ayanwale *et al.* (2020) pH levels in ponds should be between 6.5 and 9.0. Both the greatest and lowest recorded temperatures (Table 2) were within the advised range of 20°C to 40°C. The safe temperature for fish development and production is 27°C, according to the World Health Organization (WHO, 1995; Anon, 2012; Anon, 2014; Onajobi *et al.,* 2015; Onajobi *et al.,* 2015b; Onajobi *et al.,* 2021).

The results of fish pond water sample showed a high limit in the chemical contamination and heavy metal analysis and this could be detrimental of the state and health of the fish in the pond. These results collaborate with the study of Onajobi *et al.* (2021) which reported that as the microbiota oxidizes the organic materials in the water, a high oxygen demand signals the possibility of DO sag. This also suggests that the operations at the slaughterhouse are adding to the stream's pollution burden, which over time might have the unintended consequence of contaminating the nearby water body. The range of chloride content is 8 to 15 mg/l (Adebayo *et al.,* 2012b; Onajobi *et al.,* 2020; Onajobi *et al.,* 2023). By preventing or eradicating the microorganisms that break down the organic and inorganic substances in a sample, chlorine might interfere with BOD measurements (Ida, 2012; Onajobi *et al.,* 2019; Onajobi *et al.,* 2015a).

Conclusion

The physicochemical and microbial quality assessment of the three fish pond water samples in this study have proven to be contaminated. This work reveals that all the ponds in Ijebu-North, Ogun state, Nigeria contains pathogenic bacteria. This then confirms the pond water to be highly unsafe for use. The physicochemical assessment of the three fish pond water samples were not in correlation with the W.H.O. standard for drinking water. As a result, this could also cause a lot effect in fish rearing and cultivation. It should be mentioned that the microbial flora of the fish itself is reflected in the microbial quality evaluation of any fish pond water. Therefore, these organisms may result in sickness, decreased fish productivity, and financial loss. However, it might also put the final consumers (people) in risk, especially if the fish taken from the ponds isn't properly handled. Encouragement should be made to expert in fish cultivation on the proper treatment of water used in fish pond.

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