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Investigative study on the bacteriological, physical and chemical profiles of aquaculture waters: insights into health hazards for fish and human

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Abstract

Bacteriological and physic-chemical analysis of fish pond water is very important in aquaculture as this gives insights into likely threats to aquaculture and associated personnels. Bacteriological and physico-chemical profiles of selected fish ponds in the Ilorin West area of Kwara State, Nigeria were investigated to evaluate the water quality of rearing enclosures. Physico-chemical analyses revealed quality parameters were within the recommended range for aquaculture. Following bacteriological analyses of static water pond culture, the TVC and TCC showed temporal variations with concentration increasing with sampling time. However, the FCC showed fluctuation. Totally, 8 bacteria groups were isolated from both rearing enclosures. Of these, Gram negative bacteria showed dominance. In which 5 Gram negative (*Escherichia coli, Proteus spp, Serratia spp, Enterobacter spp* and *Pseudomonas spp*) and 3 Gram positive (*Staphylococcus spp, Streptococcus spp,* and *Bacillus spp*) were encountered. Estimates of bacteria occurrence in both rearing facilities respectively gave : *Staphylococcus spp* (20%), *Streptococcus spp* (12%) *Proteus spp* (8%) *Enterobacter spp* (20%) *Serratia spp* (16%), *Bacillus spp* (9%), *Escherichia coli* (8%), *Pseudomonas spp* (7%) from earthen pond water sampled. While *Staphylococcus spp* (18%), *Streptococcus spp* (16%), *Proteus spp* (8%), *Enterobacter spp* (22)%, *Serratia spp* (8%), *Bacillus spp* (15%), *Escherichia coli* (8%), *Pseudomonas spp* (6%) from concrete water sampled. Conclusively, although there is the presence of bacteria groups of public health concern, the static water exchange provides benefits of natural processing of wastes and restoration of the pond ecosystem. Notably, the presence of *Escherichia coli* gives indication of presence of pathogenic organisms of enteric origin. The presence of these organisms has been associated with a lack of tentative pond management and effective biosecurity procedures. One recommendation to this culture system (static water aquaculture) is the consideration of adaptation of concepts in biomimicry or biofloc technology which operates on similar principles.

Keywords: pathogens, water quality, biofloc, stagnant aquaculture, bacteria, public health

Introduction

One single most significant factor devastating the aquaculture industries is disease. For example, in the shellfish industry, an estimate revealed US$8-15 billion shellfish value was lost globally to a single disease agent, white spot syndrome virus (WSSV) (Lightner et al., 2012). Likewise global losses associated with other OIE listed viruses were documented; infectious hypodermal and haematopoietic virus, IHHV (US$1billion); Yellow head virus (YHV), US$0.5billion; Taura syndrome virus, (TSV), US$3billion (Stentiford et al., 2012). Yet, unlike other livestock production, the aquatic environment presents a limitation to biosecurity. That is, due to the open nature of this environment— sometimes with exception of closed systems, such as recirculatory aquaculture— total exclusion of pathogens may be impractical (Georgiades et al., 2016; Hasimuna et al., 2020). And in the presence of stressor(s), the health of the stock may be under jeopardy (Eissa & Wang, 2016) which can devastate the economical, ethical and environmental sustainability of aquaculture. For instance, in Ecuador, loss due to WSSV outbreaks was around 63,000mt valued at US$280.5million, and it was estimated that up to 23,000people had been laid off (Alday de Graindorge & Griffith, 2001). In addition to this concern, aquatic environments have been implicated to represent reservoirs of pathogenic organisms which may pose risks to human health (Islam et al., 1993; Dekić et al., 2018). A good indication of presence of pathogenic bacteria are common attributes to faecal coliform bacteria, for example, the *Escherichia coli* and enterococci (Tyagi *et a*l., 2006).

Moreover, since pathogenic colonisation of aquatic organisms can depend on concentration of pathogens (Dekić et al., 2018), of particular concern is the aquaculture practicing zero-water exchange. In which present pathogenic challenge may increase to the level capable of overwhelming the stock defense system (Arias-Moscoso *et al.*, 2018) thereby leading to the establishment of pathogens. Subsequently, this water is discharged to the surrounding environment which further poses risk to the wild population or threats to human use (Vouga & Greub, 2016). In Nigeria, for example, a majority of the aquaculture production is pond-based (Akankali *et al.,* 2011) and due to limited water supply, these aquaculture usually involve nearly/zero water exchange. For example, according to a study in Nigeria, 35.7% surveyed farms practiced stagnant water aquaculture, with 48.2% practicing throughflow systems and only 16.1% adopting water recirculatory systems (Emmanuel *et al.,* 2014). Several lines of evidence have demonstrated increased pathogens may pose risks to the surrounding environments (Alaliyat *et al.,* 2019; Shea *et al.,* 2020). For example, the probability of detecting pathogen environmental DNA was 2.72 times greater at active farms compared to inactive farms (Shea *et al.*, 2020). To the best of the authors' knowledge, the majority of studies have based their exploration on the bacteria profiles and the antibiotic susceptibility tests (e.g., Nzeh & Udeze, 2012; Njoku *et al.*, 2015; Fakorede *et al.,* 2019) however disregarding the comparisons of sampling times and changes to bacteria profiles. Therefore, in order to give insight into the status of health of aquaculture settings practicing stagnant water aquaculture, this study set out to investigate the microbial profile, particularly bacteria, and quality of pond waters used for aquaculture in the study area. With consideration of temporal variations in bacteria concentration. The outcomes of which may serve to extrapolate conditions of other regions— especially those adopting similar management practices.

Materials and Methods

The study area is within Kwara state, Nigeria, and is characterised with varieties of economic activities. Aquaculture operations is one major agricultural activity conducted in this area, with most adopting pond water aquaculture with some incorporating concrete culture during production stages. The most cultured species is the African catfish (*Clarias gariepinus*). Notably, the majority of aquaculture operations are aggregated into farm 'estates' with different people operating on the average 2 ponds/ individuals. Meaning different 'estates' adopt similar management practices and are exposed to limited variation in environmental conditions.

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Sample collection

Water samples were collected from five fish farms (two water holding facilities, an earthen pond and a concrete tank from each farm) using sterile bottles from below water level approximately 10-15cm below the surface (Njoku *et al.*, 2015). The water was transported to the Microbiology laboratory in the department of Microbiology, University of Ilorin in an ice-packed container for bacteriological analysis. While the other important information about the ponds such as source of water supply, period or length of use was obtained by interview.

Determination of water quality parameters

The physical and chemical parameters of the pond water were measured within the holding time of each parameter following standard methods.

Determination of pH, temperature, electrical conductivity, DO and TDS

Water temperature (°C), Total dissolved solids (TDS, mg/l), pH and Electrical conductivity (ms/cm) were measured in-situ using digital HANNA metre model HI 9813-5 between 8-11 GMT. Similarly the Dissolved Oxygen was measured in-situ using Digital DO meter model JPB-607A Power:6V.

Bacteriological analysis

Sterilization of Material Used: the materials used for this study were sterilized by appropriate technique. All glass wares such as Petri dishes, conical flasks, test tubes, beakers, McCartney bottles, etc., were thoroughly washed and sterilized in the hot air oven at 160°C for about 1hour. The media used for these were Nutrient Agar (NA), MacConkey Agar (MA) and Eosin Methylene Blue (EMB). 28g, 48.5g, and 37.5g of NA, MA and EMB were weighed and suspended in 1 litre of distilled water respectively. The mixture was then mixed properly and heated to dissolve the powder completely. After heating, the mouth of the flasks were plugged with cotton wool and wrapped with aluminium foil. The media were sterilized at 121°C for about 15 min in autoclave and then allowed to cool for about 45°C before pouring aseptically into the sterile Petri-dish.

Bacteriological procedures: serial dilution method was used for the enumeration of the bacterial count. 1ml of each of the samples were transferred into 9ml of sterile distilled water in the test tube. Serially, 1ml was taken from the 10^{-1} tube to another which makes 10^{-2}. This was done up to 10^{-4} dilution. Then 1m was taken from the 10^{-4} respectively into sterile Petri dishes and the molten agars that have been prepared earlier for bacterial growth were allowed to cool to about 45°C before pouring into each of the sterile Petri-dishes and allowed to solidify. After solidification, each of the plates were incubated at 37°C for the bacteria to grow between 24hrs-48hrs. After the incubation period each of the plates were examined and counted using Bacteria Colony Counter in terms of colony forming units.

Characterization and Identification of Bacterial Isolates

The isolates were characterized d andientified after obtaining pure culture of isolates through repeated sub-culturing by using their Colonial morphology, Cellular morphology and Biochemical reaction and then identified by making reference to Bergey's manual of determinative bacteriology. Colonial and Cellular morphology; Gram staining; motility test; biochemical tests (including catalase, coagulase, oxidation, indole, Urease, Voges-Proskauer, methyl red, starch hydrolysis and sugar fermentation tests) were all conducted as described by Fawole and Oso (2007).

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Data analysis

The data were analyzed using IBM Statistical Package for Social Science (SPSS) version 20. All data are the mean of three replicates. The mean and standard deviation of each parameter were determined. The mean data were compared using one way Analysis of variance (ANOVA) and separated using Duncan Multiple Range Test (DMRT) at α=0.05.

Results

This study helps to determine some ecological parameters and the bacteriological properties of fish pond water of two different kinds of rearing facilities (earthen ponds and concrete tanks). Earthen pond and concrete tank water samples were collected from different fish farms in the study area at the different sampling time (3days interval three times each).

Physicochemical parameters of Earthen ponds and concrete tanks of selected farms

Table 1a shows the physicochemical properties of the earthen pond water from the selected farms with DO range of 5.93mg/l-6.43mg/l, TDS range of 243mg./l-451mg/l, pH range of 6.47-7.47, Electrical conductivity range of 0.34mS/cm-1.36mS/cm, Temperature range of 28.07°C-28.60°C, Alkalinity range of 43.43mg/l-53.17mg/l, Nitrate range of 2.11mg/l-2.93mg/l and Water Hardness range of 15.81mg/l-26.25mg/l. Whereas Table 1b shows the physicochemical properties of the concrete tank water from the selected farms with DO range of 6.30 mg/l-6.77mg/l, TDS range of 182.33mg/l- 306.67mg./l, pH range of 6.80-8.27, Electrical conductivity range of 0.25mS/cm-1.62mS/cm, Temperature range of 28.10°C-28.90°C, Alkalinity range of 48.40mg/l-70.11mg/l, Nitrate range of 1.73mg/l-2.18mg/l and Water Hardness range of 20.44mg/l-29.24mg/l.

Table 1a : Physicochemical characteristics of earthen pond water from selected farms

Farms	Earthen ponds Physicochemical parameters							
	DO (mg/l)	TDS (mg/l)	pH	EC (mS/cm)	T (°C)	ALK (mg/l)	NIT (mg/l)	WH (mg/l)
A	$6.17^{b}\pm0.06$	$243.33^{a}\pm0.58$	$6.83^{b}\pm0.06$	$0.34^{b}\pm0.00$	$28.60^{b}\pm0.00$	$53.17^{e}\pm0.15$	$2.12^{a}\pm0.01$	$19.53^{b}\pm0.03$
B	$5.93^{a}\pm0.06$	$322.67^{a}\pm0.58$	$7.47^{c}\pm0.06$	$0.45^{c}\pm0.00$	$28.43^{a}\pm0.06$	$44.07^{b}\pm0.06$	$2.43^{b}\pm0.01$	$26.25^{c}\pm0.01$
C	$6.43^{c}\pm0.11$	$451.00^{e}\pm5.29$	$6.47^{a}\pm0.15$	$0.62^{d}\pm0.01$	$28.07^{a}\pm0.06$	$43.43^{a}\pm0.32$	$2.11^{a}\pm0.01$	$16.42^{a}\pm0.01$
D	$6.10^{b}\pm0.10$	$265.33^{b}\pm1.16$	$7.33^{c}\pm0.06$	$1.36^{e}\pm0.04$	$28.27^{a}\pm0.55$	$45.17^{c}\pm0.06$	$2.93^{d}\pm0.01$	$16.47^{a}\pm0.01$
E	$6.23^{b}\pm0.06$	$281.61^{c}\pm1.16$	$6.63^{a}\pm0.15$	$0.14^{a}\pm0.02$	$29.87^{b}\pm0.70$	$46.37^{d}\pm0.15$	$2.73^{c}\pm0.01$	$15.81^{a}\pm0.96$

Table 1b: Physicochemical parameters of Concrete tanks water of selected farms

Farms	Concrete tanks physicochemical parameters							
	DO (mg/l)	TDS (mg/l)	pH	EC (mS/cm)	TEM (°C)	ALK (mg/l)	NIT (mg/l)	WH (mg/l)
A	$6.43^{a}\pm0.06$	$218.33^{a}\pm3.79$	$7.70^{b}\pm0.10$	$0.25^{a}\pm0.01$	$28.60^{c}\pm0.00$	$59.37^{c}\pm0.06$	$1.73^{a}\pm0.29$	$22.17^{c}\pm0.12$

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B	6.30^{a}±0.00	306.67^{e}±0.58	6.80^{a}±0.00	0.43^{d}±0.00	28.10^{a}±0.10	48.83^{b}±0.23	1.73^{a}±0.06	29.24^{d}±0.09
C	6.67^{b}±0.06	231.33^{d}±0.58	7.70^{b}±0.10	0.32^{c}±0.00	28.37^{b}±0.06	48.40^{a}±0.20	1.77^{a}±0.15	20.44^{a}±0.01
D	6.67^{b}±0.15	219.67^{b}±1.53	8.10^{c}±0.10	1.62^{e}±0.00	28.90^{d}±0.00	68.43^{d}±0.02	2.15^{b}±0.06	22.13^{c}±0.01
E	6.77^{b}±0.06	221.33^{c}±15.7	8.27^{c}±0.23	0.29^{b}±0.02	28.17^{a}±0.12	70.11^{e}±0.01	2.18^{b}±0.06	21.18^{b}±0.01

Table 1a & 1b Note: mean followed by the same superscript within the same column are not significantly different at α= 0.05 based on Duncan Multiple Range Test (DMRT). DO (Dissolved oxygen); TDS (Total dissolved solids); EC (Electrical conductivity); T (Temperature), ALK (Alkalinity); NIT (Nitrate); WH (Water hardness).

Bacteriological counts of earthen ponds and Concrete tanks in different sampling times

Table 2 shows the total viable counts, total coliform counts and fecal coliform counts of the earthen pond water in the selected areas. The TVC, TCC and FCC of all the earthen pond water samples range from 5.10±0.12 LogCFU/ml to 5.83±0.04 LogCFU/ml, 4.46±0.15 LogCFU/ml to 5.48±0.03 LogCFU/ml and nil to 4.83±0.13 LogCFU/ml respectively. Moreover, in figure 1 (a, b and c) earthen pond water sampled from Farm C had the highest TVC 5.64 ±0.10 LogCFU/ml while that of Farm D had the least TVC 5.26±0.21 LogCFU/ml. Similar is the case with TCC and FCC with TCC value of Farm C reaching 5.34 ±0.10 LogCFU/ml while that of Farm D had the least TCC 4.83±0.41 LogCFU/ml; and FCC value from Farm C recorded at 4.83±0.13 LogCFU/ml and Farm D had least FCC 3.58±0.81 LogCFU/ml. However, no FCC was recorded for Farm B. Furthermore, results recorded for bacteria counts in concrete ponds are presented in table 3. This shows variation with time of sampling. In table 3, the TVC, TCC and FCC of all the concrete tanks water samples ranged from 5.08±0.07 LogCFU/ml to 5.67±0.06 LogCFU/ml; 4.26±0.24 LogCFu/ml to 5.36±0.26 LogCFU/ml; and 0.00 to 4,77±0.07 LogCFU/ml respectively. And grossly, Figure 2 (a, b and c) shows that the concrete tank water sample from Farm C had the highest TVC 5.39±0.14 LogCFU/ml while that of Farm B had the least TVC 5.17±0.25 LogCFU/ml. Where Farm E recorded the highest TCC 5.23±0.11 LogCFU/ml while that of Farm B had the least TCC 4.81±0.48 LogCFU/ml. Concrete water sampled from Farm E had the highest FCC 4.57±0.11 Log CFU/ml while that of Farm A, Farm B and Farm C showed no value FCC counts.

Presented in table 4a and 4b are the distribution of bacteria occurrence in farm waters in 3 different sampling occasions. Altogether, total 8 bacteria were isolated and their occurence showed variation between farms, culturing facilities as well as temporal variation. Finally, in table 5, the percentage occurrence of this bacteria group was estimated for the two exclosure systems. In the case of earthen pond waters, *Staphylococcus spp* and *Enterobacter* group showed highest occurence (20%) while *Proteus spp* and *Esherichia coli* showed lowest percentage occurrence of 8%. Similarly, in the concrete water sampled, *Staphylococcus spp* and *Enterobacter* group showed highest occurence approximately 22% and 18% respectively. While lower occurrence was attributed to *Escherichia coli and Proteus spp* (8%), with lowest occurrence recorded for *Pseudomonas spp* (approx. 6%).

Table 2: Bacteriological counts of Farm A-E earthen pond water of different sampling time

	Sampling time	**TVC (Log cfu/ml)**	**TCC(Log cfu/ml)**	**FCC(Log cfu/ml)**
Farm A	1st sampling	5.41^{a}±0.08	5.04^{a}±0.07	4.54^{b}±0.28
	2nd sampling	5.61^{a}±0.15	5.28^{b}±0.05	2.77^{ab}±2.40
	3rd sampling	5.83^{b}±0.04	5.48^{c}±0.03	0.00^{a}±0.00
Farm B	1st sampling	5.11^{a}±0.10	4.83^{a}±0.16	0.00±0.00
	2nd sampling	5.46^{b}±0.13	5.03^{ab}±0.12	0.00±0.00

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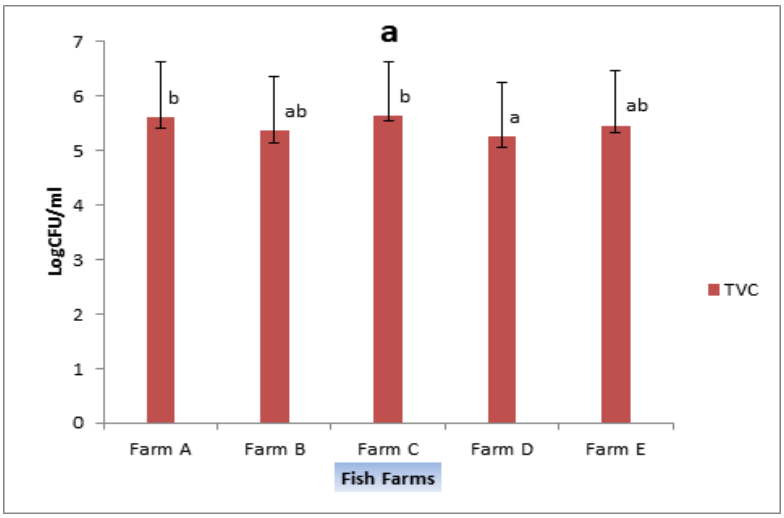
	3rd sampling	5.52^{b}±0.13	5.14^{b}±0.10	0.00±0.00
Farm C	1st sampling	5.56^{a}±0.14	5.25^{a}±0.11	0.00^{a}±0.00
	2nd sampling	5.61^{a}±0.09	5.33^{a}±0.14	4.83^{b}±0.13
	3rd sampling	5.76^{a}±0.09	5.44^{a}±0.12	0.00^{a}±0.00
Farm D	1st sampling	5.10^{a}±0.12	4.46^{a}±0.15	4.39^{b}±0.36
	2nd sampling	5.17^{a}±0.09	4.75^{a}±0.18	0.00^{a}±0.00
	3rd sampling	5.50^{a}±0.09	5.27^{b}±0.14	2.77^{ab}±2.40
Farm E	1st sampling	5.26^{a}±0.15	4.59^{a}±0.11	0.00^{a}±0.00
	2nd sampling	5.41^{a}±0.07	4.97^{b}±0.14	4.26^{b}±0.24
	3rd sampling	5.62^{b}±0.07	5.38^{c}±0.06	0.00^{a}±0.00

Means with the same superscript in columns are not significantly different at α = 0.05 based on the Duncan Multiple Range Test (DMRT). TVC (Total Viable Count); TCC (Total Coliform Count); FCC (Fecal Coliform Count) Note: Values presented as mean ± standard deviation of triplicates.



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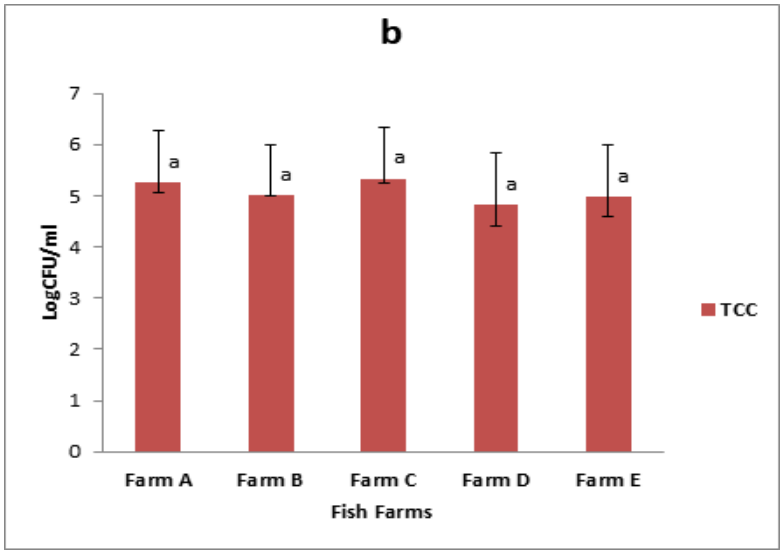

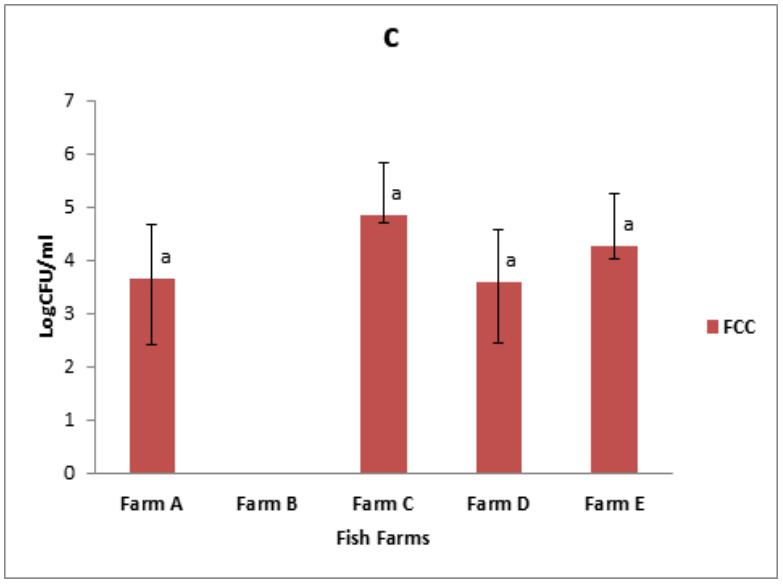

Figure 1a,b and c: gross TVC, TCC and FCC in earthen pond water of fish farm. Bars with the same alphabets are not significantly different at α = 0.05 based on the Duncan Multiple Range Test (DMRT). Notes: Farms not barred shows no recorded value; Values presented as mean ± standard deviation of replicates.TVC (Total Viable Count); TCC (Total Coliform Count); FCC (Fecal Coliform Count).

Bacteriological counts of Farm A-E Concrete tank waters in different sampling time

Table 3: Bacteriological counts of Farm A-E concrete tank water of different sampling time

	Sampling time	TVC (Log cfu/ml)	TCC(Log cfu/ml)	FCC(Log cfu/ml)
Farm A	1st sampling	5.16^{a}±0.15	4.88^{a}±0.16	0.00±0.00
	2nd sampling	5.11^{a}±0.07	4.97^{a}±0.20	0.00±0.00

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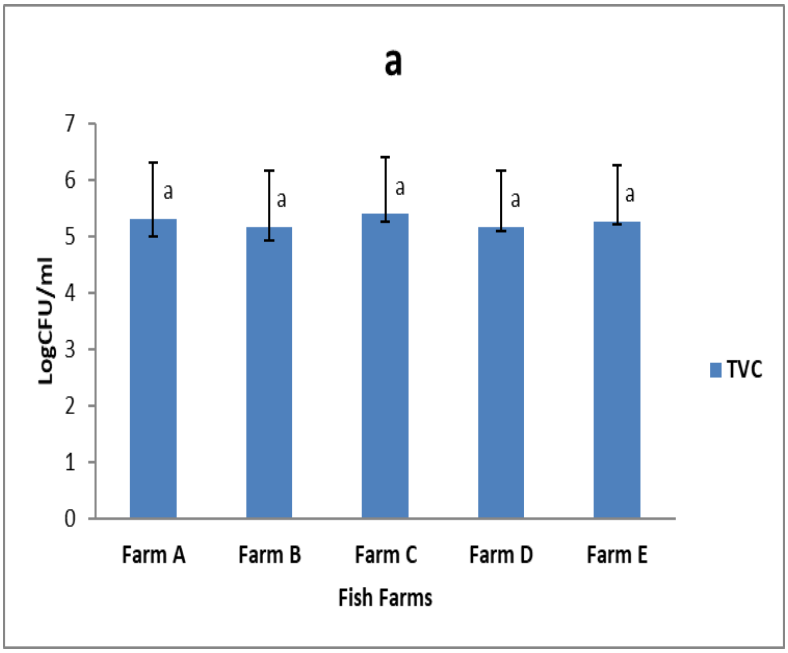
	3rd sampling	5.67^{b}±0.06	5.36^{b}±0.26	0.00±0.00
Farm B	1st sampling	5.46^{a}±0.09	5.19^{a}±0.10	0.00±0.00
	2nd sampling	4.99^{a}±0.14	4.26^{a}±0.24	0.00±0.00
	3rd sampling	5.09^{b}±0.13	4.99^{b}±0.14	0.00±0.00
Farm C	1st sampling	5.23^{a}±0.08	4.92^{a}±0.21	0.00±0.00
	2nd sampling	5.44^{b}±0.06	5.20^{ab}±0.10	0.00±0.00
	3rd sampling	5.50^{b}±0.04	5.29^{b}±0.11	0.00±0.00
Farm D	1st sampling	5.17^{ab}±0.06	5.03^{b}±0.12	4.77^{b}±0.07
	2nd sampling	5.08^{a}±0.07	4.63^{a}±0.31	4.00^{a}±0.00
	3rd sampling	5.25^{b}±0.07	5.08^{b}±0.07	4.26^{a}±0.24
Farm E	1st sampling	5.22^{a}±0.10	5.11^{a}±0.10	0.00^{a}±0.00
	2nd sampling	5.29^{a}±0.10	5.27^{ab}±0.11	4.46^{b}±0.15
	3rd sampling	5.53^{b}±0.06	5.32^{b}±0.07	4.67^{b}±0.19

Means with the same superscript in columns are not significantly different at α = 0.05 based on the Duncan Multiple Range Test (DMRT). TVC (Total Viable Count); TCC (Total Coliform Count); FCC (Fecal Coliform Count) Note: Values presented as mean ± standard deviation of triplicates.



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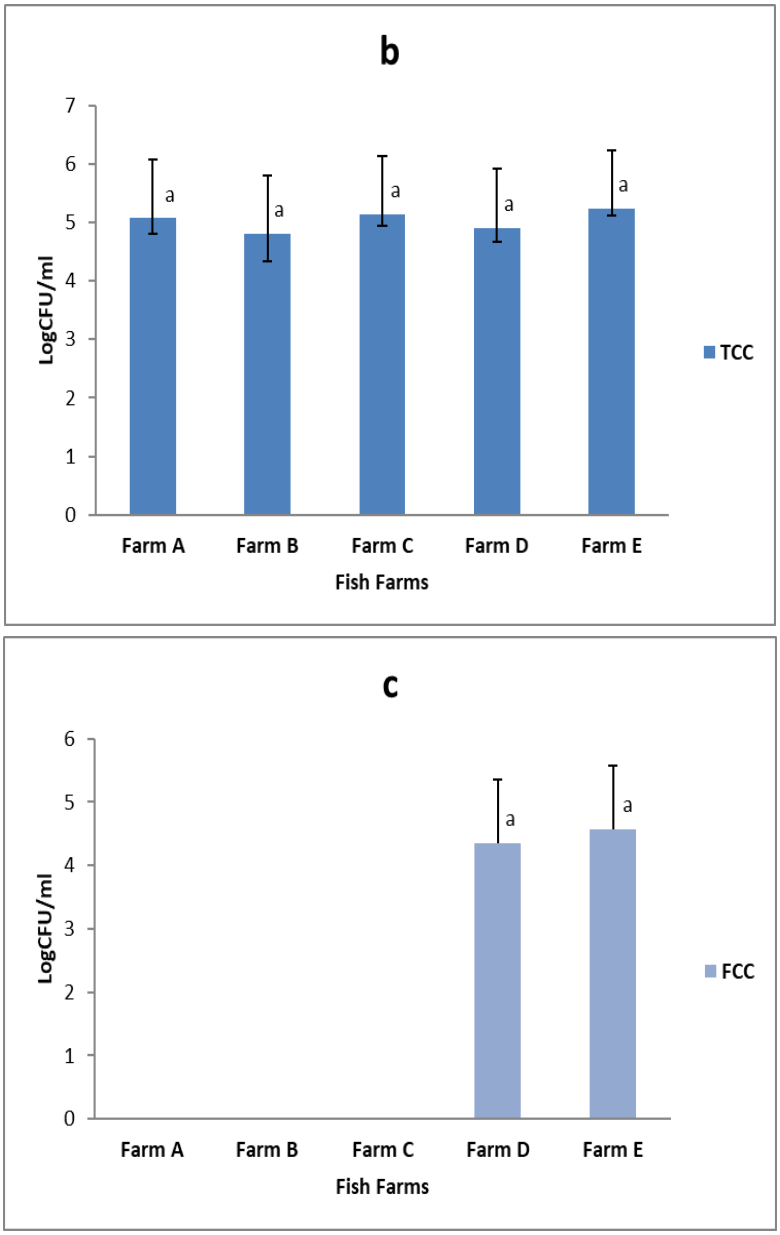

Figure 2a,b and c: gross TVC, TCC and FCC in concrete tank water of fish farm. Bars with the same alphabets are not significantly different at α = 0.05 based on the Duncan Multiple Range Test (DMRT). Notes: Farms not barred shows no recorded value; Values presented as mean ± standard deviation of replicates.TVC (Total Viable Count); TCC (Total Coliform Count); FCC (Fecal Coliform Count).

Table 4a: Distribution of occurrence of Bacterial species of earthen pond water

Bacterial isolates	Farm A earthen pond water			Farm B earthen pond water			Farm C earthen pond water			Farm D earthen pond water			Farm E earthen pond water		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Staphylococcus spp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

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Streptococcus spp	+	-	-	-	-	+	+	+	-	-	+	+	+	+	+	
Proteus spp	-	+	+	+	+	+	-	-	-	-	-	+	-	-	-	
Enterobacter spp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Serratia spp	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	
Bacillus spp	+	-	-	-	-	-	+	-	-	-	+	+	+	+	+	
Escherichia coli	+	+	-	-	-	-	-	+	-	+	-	+	-	+	-	
Pseudomonas spp	-	-	-	-	+	-	-	+	-	-	-	+	+	+	-	

Key: (+) presence of bacteria isolate, (-) absence of bacteria isolates

Table 4a: Distribution of occurrence of Bacterial species of earthen pond water

Bacterial isolates	Farm A concrete tank water			Farm B concrete tank water			Farm C concrete tank water			Farm D concrete tank water			Farm E concrete tank water		
	1st	2nd	3rd	1s	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Staphylococcus spp	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+
Streptococcus spp	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Proteus spp	-	+	-	-	+	+	-	-	+	-	-	-	-	-	+
Enterobacter spp	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serratia spp	+	-	+	+	-	-	+	-	-	-	+	-	-	-	-
Bacillus spp	+	+	-	-	+	+	+	+	+	+	+	-	-	-	+
Escherichia coli	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+
Pseudomonas spp	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-

Key: (+) presence of bacteria isolate, (-) absence of bacteria isolates

Table 5: Percentage occurrence of bacteria in earthen pond water

Bacteria	Earthen pond water (%)	Concrete tank (%)

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Staphylococcus spp	20	18
Streptococcus spp	12	16
Proteus spp	8	8
Enterobacter spp	20	22
Serratia spp	16	8
Bacillus spp	9	15
Escherichia coli	8	8
Pseudomonas spp	7	6

Discussion

The present study was designed to investigate the health status of aquaculture settings practicing stagnant water aquaculture. It is well established that water used for the culture of fish will not give maximum production if the 'physicochemical' parameters are not optimal for fish. And subsequently presenting fish to stressor(s) experience (Santos *et al.,* 2010). One outcome of this is increased susceptibility to diseases (Fridell *et al.,* 2007; Boyd, 2017). For example, channel catfish held in water with suboptimal quality (dissolved oxygen, carbon dioxide and ammonia) experienced reduced survival when injected intraperitoneally with sublethal doses of *Aeromonas hydrophila* conversely to fish kept in favourable water condition (Walters & Plumb, 1980). In the present study area investigated, all the parameters observed (Table 1a & b), both in earthen and concrete tank rearing enclosures, fall within the recommended range in the literature for aquaculture (e.g., Dalvi *et al.,* 2009; Boyd, 2017). It is worth stating here that, the extremely reduced nitrate concentrations (Table 1a & b) recorded might be due to the increased utilisation by present bacteria (Schneider *et al.,* 2005). This is a similar principle in conventional static water aquaculture systems, biofloc system (Poh, 2014) and aquamimicry (Romano, 2017; Romano and Kumar, 2017). Where the nitrogenous wastes including ammonia is converted to bacterial biomass (Schneider *et al.,* 2005). This is especially beneficial for aquaculture performance. For example, it has been documented that freshwater fish reared at 0, 10, and 100mg/l nitrate showed increased specific growth rate and food conversion efficiency relative to higher nitrate concentration (e.g., >100-1000 mg/l) (Monsees *et al.,* 2017).

Essentially, following the investigation of microbial profile, the most obvious finding to emerge from bacteria analyses of different sampling time is the increased concentration of total viable and coliform bacteria counts (TVC and TCC, Table 2 & 3). Contrary to expectation, unlike earthen pond waters, this trend was not experienced in concrete tank waters (Table 3). A possible explanation for this might be the fact that the majority of farm operators using concrete water aquaculture replenish the water use due to inability of the concrete rearing environment to naturally process wastes relative to earth ponds (Jha & Barat, 2005; Jha, 2019). Which otherwise could result in reduced performance of the stock (Das *et al.,* 2021). Therefore, one associable consequence of increasing concentration over time is the possible increase in pathogenic challenges over time, probably due to accumulation of organic substrates. Because it has been reported that growth of pathogenic bacteria such as *Escherichia coli, Vibrio cholerae* and *Pseudomonas aeruginosa* is affected by the composition and concentration of organic carbon in the water (Vital *et al.,* 2010). Collectively, the presence of Gram-negative bacteria provides indication of presence of pathogenic bacteria groups. Especially, the presence of *Escherichia coli* provides indication of presence of pathogens (Ampofo & Clerk, 2010). Although not isolated from this study, *Vibrio cholerae,* a pathogenic bacteria, has been isolated in other regions from aquaculture rearing facilities (Njoku *et al.,*

2015) and has been implicated to account for several diarrhea cases in Nigeria. For example, in the 2017 incident in the Ilorin metropolis, the culture positive rate of the *Vibrio cholerae* isolates was 41% (Amadu *et al.,* 2021). Compared to the 78% level of occurrence reported by Borah *et al.* (2010), *Escherichia coli* found in the present study was lower (8%) in both rearing enclosures and the regular trend recorded for other bacteria groups (TVC and TCC) was not witnessed for the fecal coliform group (Table 2 & 3). It seems appropriate to associate this irregularity recorded to the long time exposure to solar radiation (Chandran & Hatha, 2003) due to minimal water exchange. Because long term exposure to UV radiation has been reportedly claimed to inactivate *E. coli* cells (Muela *et al.,* 2000). In fact, up to 90% of *E.coli* cells have been reported to be disrupted by sunlight exposure (Chandran & Hatha, 2003).

Before proceeding to examining the favourability of isolated bacteria, it is important to mention that during the period of study, no gross sign of disorders were observed. In another study, increased tolerance of the culture species, *Clarias gariepinus,* has been reasonably attributed (Oni *et al.,* 2013). Furthermore, other isolated bacteria including *Bacillus spp* and *Staphylococcus spp* form beneficial components of the pond ecosystem. And due to constant contact and inevitable ingestion of surrounding water, farmed fish are strongly influenced by the microorganisms contained in the water (Defoirdt *et al.,* 2011; El-Saadony *et al.,* 2021). For example, previous research has established that there is similarity between intestinal and culture water microbiota (Wu *et al.,* 2012). In the study area investigated, of the bacteria group isolated, *Staphylococcus spp* appeared to show the highest occurrence (up to 18-20%). This gives an indication that cultured stock benefits, on one hand, from the antimicrobial effects of these bacteria (Sumi *et al.,* 2014; Santos *et al.,* 2018) and, on the other hand, reduced food conversion ratio (FCR). For instance, using *Bacillus sp* and photosynthetic bacteria isolated from carp ponds as probiotics, Yanbo & Zirong (2006) reported a significant increase in growth performance and FCR. However, a note of caution is due here, due to the static water aquaculture practice in the study area, there is risk of opportunistic infection, as a result of overwhelming growth of bacteria or prolonged stimulation time of immune response (Cruz *et al.,* 2012).

Conclusively, there is no doubt the open nature of aquatic environments present limitations to total exclusion of pathogens. As presented in this study, the appearance of *Escherichia coli* is an indication of possible presence of pathogens that may grow overwhelmingly to suppress the immune system of aquaculture stock (Hess *et al.,* 2021), with potential consequences of disease outbreak. Arguably, compared to other livestock zoonosis, there has not been enough evidence examining the relationships between aquaculture originated pathogens and workers health threats, however, possible contamination of aquaculture products has been evaluated. For example, it was reported that tissue of fish cultured in fertilised ponds showed *Salmonella* contamination compared to unfertilised ponds (Ampofo & Clerk, 2010). This is an area requiring attention especially with mounting concern ‘one health initiatives’ requirements. Nevertheless, from environmentalists' perspective, the practice of static water aquaculture is an approach that limits the use of freshwater and frequent interactions between aquaculture and the wild environment. This has the advantage of limiting pathogenic interactions between these artificial and natural ecosystems. Yet, there is the risk of increased pathogenicity due to continuum in pathogenic growth. Therefore it may be appropriate to recommend that aquaculture practicing zero water exchange should adapt principles from biofloc technology or aquamimicry. The latter concept being a conventional zero water exchange aquaculture, concurrently provides the advantages of increasing growth of heterotrophic organisms, maximises biosecurity, and accumulation of organics. Interestingly, a study comparing culture conditions. Biofloc systems and stagnant water renewal reported a greater performance in a biofloc system supplemented with probiotics (Mohammadi *et al.,* 2021).

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