Olalemi et al. *Afr. J. Clin. Exper. Microbiol.* 2023; 24 (1): xxxx https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jan 2023; Vol.24 No.1
AJCEM/2221. https://www.ajol.info/index.php/ajcem

Assessment of faecal contamination in selected concrete and earthen ponds stocked with African catfish, *Clarias gariepinus*

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Abstract:

Background: Microorganisms constitute significant fraction of the aquatic ecosystem and have been reported to be the cause of emerging novel infectious diseases in aquacultural practices. The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment. This study was conducted to assess the levels of faecal pollution markers in catfish (*Clarias gariepinus*) and their growing waters in selected earthen and concrete ponds in the teaching and research fish farm of the Federal University of Technology, Akure (FUTA), Nigeria in the dry (February-April) and wet seasons (May-July) of the year.
Methodology: Two earthen and 2 concrete ponds were randomly selected as sampling sites due to their frequent usage. A total of 120 grabs of catfishes from the earthen (n=60) and concrete (n=60) ponds, and 84 pond water samples from earthen (n=42) and concrete (n=42) ponds, were randomly collected over a 6-month period of study. Enteric bacteria count in the water and catfish samples were determined using membrane filtration and pour plate methods respectively. The physiochemical characteristics of the water samples were determined using standard methods. The rate of bioaccumulation of faecal indicator bacteria was obtained by dividing the log count of each organism in the catfish by the corresponding log count in the growing waters.
Results: Faecal coliforms count ($\log_{10}$ CFU/100ml) in the catfish from concrete and earthen ponds ranged from 1.41 to 2.28 and 1.3 to 2.47, and in the growing waters; 1.43 to 2.41 and 1.50 to 2.80 respectively. There was positive correlation of faecal coliforms with alkalinity of water samples from the earthen (r=0.61) and concrete ponds (r=0.62). *Salmonella* and faecal coliforms had the highest and least bioaccumulation in catfish raised in earthen pond while *Salmonella* and enterococci had the highest and least bioaccumulation in catfish raised in concrete pond respectively. Faecal coliforms and *Escherichia coli* had the highest and least counts in water samples from the earthen pond during the dry and wet months while *Salmonella* and *E. coli* had the highest and least counts in water samples from the concrete pond during the dry and wet months.
Conclusion: High levels of bacterial faecal pollution markers in water samples and catfishes from the earthen and concrete ponds are reported in this study. Physicochemical characteristics and seasonality played major roles in the rate of bioaccumulation of the faecal pollution markers in catfishes raised in both earthen and concrete ponds.

Keywords: Bioaccumulation; *Clarias gariepinus;* earthen; concrete; pond; coliforms; seasonality

Received Sept 10, 2022; Revised Dec 10, 2022; Accepted Dec 11, 2022

Évaluation de la contamination fécale dans des étangs en béton et en terre sélectionnés remplis de poisson-chat Africain, *Clarias gariepinus*

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Résumé:

Contexte: Les micro-organismes constituent une fraction importante de l'écosystème aquatique et ont été signalés comme étant la cause de nouvelles maladies infectieuses émergentes dans les pratiques aquacoles. Il a été observé

que la prévalence des maladies infectieuses dépend de l'interaction entre les agents pathogènes des poissons et l'environnement aquatique. Cette étude a été menée pour évaluer les niveaux de marqueurs de pollution fécale chez le poisson-chat (*Clarias gariepinus)* et leurs eaux de croissance dans des étangs en terre et en béton sélectionnés dans la pisciculture d'enseignement et de recherche de l'Université fédérale de technologie d'Akure (FUTA), au Nigeria, dans le saisons sèches (Février - Avril) et humides (Mai - Juillet) de l'année.
Méthodologie: Deux étangs en terre et 2 en béton ont été choisis au hasard comme sites d'échantillonnage en raison de leur utilisation fréquente. Un total de 120 prises de poissons-chats des étangs en terre (n=60) et en béton (n=60), et 84 échantillons d'eau des étangs en terre (n=42) et en béton (n=42), ont été prélevés au hasard sur une période d'études de 6 mois. Le nombre de bactéries entériques dans les échantillons d'eau et de poisson-chat a été déterminé en utilisant respectivement les méthodes de filtration sur membrane et de plaque de coulée. Les caractéristiques physicochimiques des échantillons d'eau ont été déterminées à l'aide de méthodes standard. Le taux de bioaccumulation des bactéries fécales indicatrices a été obtenu en divisant le nombre logarithmique de chaque organisme dans le poisson-chat par le nombre logarithmique correspondant dans les eaux de croissance.
Résultats: Le nombre de coliformes fécaux (log_{10} UFC/100ml) chez le poisson-chat des étangs en béton et en terre variait de 1,41 à 2,28 et de 1,3 à 2,47, et dans les eaux de croissance; 1,43 à 2,41 et 1,50 à 2,80 respectivement. Il y avait une corrélation positive des coliformes fécaux avec l'alcalinité des échantillons d'eau des étangs en terr (*r*=0,61) et en béton (*r*=0,62). Les *Salmonella* et les coliformes fécaux présentaient la bioaccumulation la plus élevée et la plus faible chez les poissons-chats élevés dans des étangs en terre, tandis que les *Salmonella* et les entérocoques présentaient respectivement la bioaccumulation la plus élevée et la plus faible chez les poissons-chats élevés dans des étangs en béton. Les coliformes fécaux et *Escherichia coli* présentaient les taux les plus élevés et les moins élevés dans les échantillons d'eau de l'étang en terre pendant les mois secs et humides, tandis que *Salmonella* et *E. coli* avaient les taux les plus élevés et les moins élevés dans les échantillons d'eau de l'étang en béton pendant les mois secs et humides.
Conclusion: Des niveaux élevés de marqueurs de pollution fécale bactérienne dans les échantillons d'eau et les poissons-chats des étangs en terre et en béton sont rapportés dans cette étude. Les caractéristiques physicochimiques et la saisonnalité ont joué un rôle majeur dans le taux de bioaccumulation des marqueurs de pollution fécale chez les poissons-chats élevés dans des étangs en terre et en béton.

Mots clés: Bioaccumulation; *Clarias gariepinus*; en terre; béton; étang; coliformes; saisonnalité

Introduction:

Within the genus Clarias, *Clarias gariepinus* is one of the most researched tropical catfish (1). Aquaculture has received a lot of attention recently as a fast-growing sector of global food production and a source of animal protein. Microorganisms have a key role in the aquatic ecosystem, and have been identified as one of the variables that can lead to outbreaks of infectious diseases in aquaculture (2). The interplay between fish pathogens and the aquatic environment has been shown to influence the occurrence of infectious illnesses (2). As a result, there is a need to quantify and track the microbial population in this industry.

The African catfish has been imported into at least 37 African, European, Asian, and American countries, primarily for aquaculture with economic consequences on freshwater and brackish ecosystem (3). The species have been introduced to the Caribbean for aquaculture in Cuba (4). *Clarias gariepinus* has evolved important adaptations for surviving in unsuitable environments with low oxygen levels and long periods of desiccation (5,6). According to Omeji et al., (7), water polluted with bacteria (faecal coliforms, faecal streptococci, and *Salmonella* spp) could infect fish in ponds. Bacteria can be found on the surface of decomposing materials such as leaves, metallic objects, rocks, and wood in earthen ponds. The distribution of heterotrophic bacteria and total aquatic bacteria in the earthen pond varies according to the water layers. The circulation of bacteria in fish pond, which includes predatory protozoa present in the water, is influenced by a variety of circumstances (8).

Bacteriological study of fish pond water is critical in aquaculture because it might reveal potential risks to the fish, the farmers and customers (8). Allowing cattle to graze near water bodies, spreading manure as fertilizer on fields during wet periods, employing sewage sludge bio-solids, and allowing livestock to drink from streams have all been linked to faecal contamination of ponds (9). Because majority of faecal pollution comes from non-point and multiple extended sources, preventing excessive faecal contamination of ponds is difficult (10). Faeces from poultry fowl are directly released into some integrated ponds which permit the development of maggots that are then discharged into the ponds.

Microorganisms, particularly those of coliform category, have been found in fish and their aquatic habitats. Furthermore, pond water contaminated with faeces poses a major risk to human health when released into other bodies of water (11). The aim of this study is to track faecal pollution markers in catfish (*Clarias gariepinus*) and their growing waters in varieties of earthen and concrete ponds. The objectives of the study are; (i) to determine the counts of faecal indicator bacteria (FIB) in catfish and their growing waters; (ii) assess the physicochemical characteristics of the growing waters and determine their relationship with FIB; (iii) determine the rates of FIB bioaccumulation in catfish; and (iv) investigate the effect of different seasons on FIB bioaccumulation in catfish.

Materials and method:

Study setting and selection of sampling sites:

The study setting is the Teaching and Research Fish Farm, Obakekere campus of the Federal University of Technology Akure (FUTA), Nigeria, located between latitude 7° and 8°N and longitude 5° and 18°E with elevation of approximately 30.5 metres (100 feet) above sea level (Fig 1). The area contains characteristic swamp, water ways, vast plains and rain forest. The farm combines fish production and livestock production, and also operates integrated fish production with different concrete and earthen ponds stocked with tilapia, catfish and other species of fish.

Two each of the earthen and concrete ponds were randomly selected as the sampling sites because of the frequent usage of these ponds. The ponds are also prone to faecal contamination from farm animals grazing around the ponds, and the landscape encourages direct flow of erosion waters into the earthen pond during wet season.

Study design:

This was a descriptive cross-sectional study of catfishes and their growing waters from earthen and concrete ponds in fish farms of Federal University of Technology Akure (FUTA), Nigeria, conducted over a period of 6 months (February to July 2019). The schematic flowchart of the study is as shown in Fig 2.

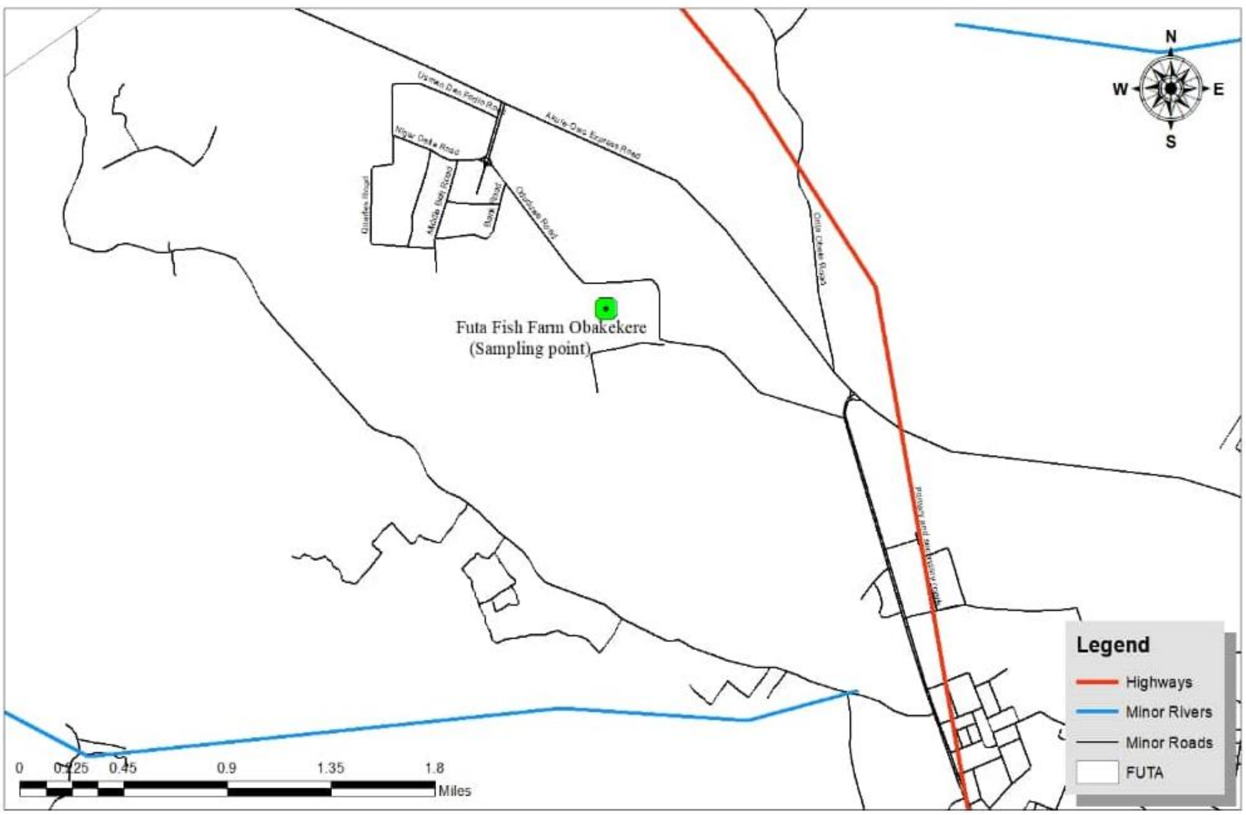


Fig 1: Map of Teaching and Research Fish Farm in Obakekere campus, FUTA, Nigeria

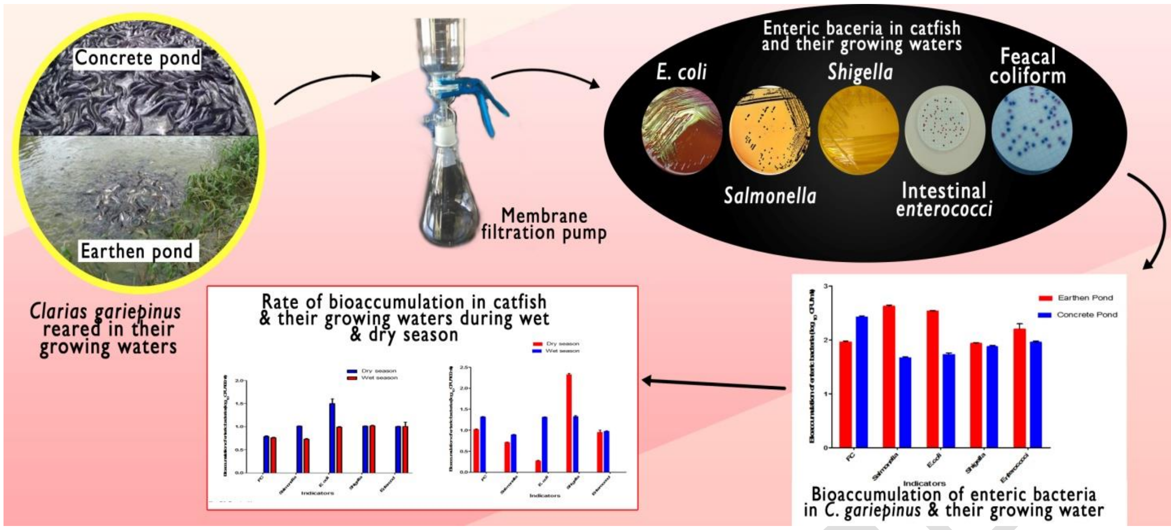


Fig 2: Schematic illustration for the assessment of faecal contamination in selected concrete and earthen ponds stocked with *Clarias gariepinus*

Collection of catfish and growing water samples from the ponds:

Water and catfish samples were collected weekly from February to July 2019. On each sampling occasion, water and catfish samples were collected from two earthen and two concrete ponds in a fish farm. The water samples were collected aseptically with sterile 800 ml screw-capped bottles labeled appropriately and the catfish samples were collected with conventional scoop net and thereafter placed in a sterile polythene bag with appropriate labeling. In total, 84 grabs of water and 120 catfish samples were collected over the study period through standard protocols. All samples were transported to the laboratory within 1 hour.

Enumeration of enteric bacteria in catfish and growing water samples:

The counts of *Escherichia coli*, faecal coliforms, *Salmonella, Shigella* and intestinal enterococci in the catfish and their growing waters were determined using standard microbiological methods as described by Maheux et al., (12). Preparation of the catfish samples was carried out by dissecting the intestinal tract of the fish using a sterile knife and measuring 1g into a sterile mortar. This was macerated with about 4ml of sterile distilled water and 1ml aliquot was taken into a sterile test tube containing 9ml of sterile distilled water resulting into 1:10 dilution. Serial dilution was carried out until the fifth dilution.

Using membrane filters (0.45µm), the bacteria counts were determined by placing the filters on freshly prepared selective media; membrane lauryl sulphate agar (MLSA), eosin methylene blue (EMB), membrane faecal coliform agar (m-FC), membrane intestinal enterococci agar (m-EA) and *Salmonella–Shigella* agar (SSA). Agar plates were incubated at 37ºC for 24 hours (for MLSA, EMB and SSA), 44ºC for 24 hours (for m-FC) and 37ºC for 48 hours (for m-EA). Colonies were counted and expressed as colony forming unit (CFU) per 100ml of water or CFU per 100g of catfish using a colony counter (J-2 PEC MEDICAL, New Jersey, USA).

Determination of the physicochemical characteristics of growing waters in the ponds:

The temperature of the growing water was determined on-site during sample collection using the mercury-in-glass thermometer (ACCU-SAFE ThermoScientific, New Jersey, US). The pH, electrical conductivity, salinity, total dissolved solids, turbidity and dissolved oxygen of the water samples were determined using a multi-parameter analyzer (HI98194, PH/ORP/EC/DO). The biological oxygen demand (BOD) was determined by the Winkler's method and the chemical oxygen demand (COD) of the water samples was determined by the method described by Kolb et al., (13).

Bioaccumulation of enteric bacteria in catfish samples from the ponds:

The rate of bioaccumulation of faecal indicator bacteria was obtained by dividing the log count of each organism in catfish by the corresponding log count in the growing waters at the same point in time.

Statistical analysis:

Data were transformed into $\log_{10}$ and examined using general descriptive statistics.

The normality and distribution pattern of the enteric bacteria in the catfish and their growing waters in concrete and earthen ponds were determined using Kolmogorov-Smirnov and Shapiro-Wilk statistics. Further analyses were carried out using one-way analysis of variance (ANOVA) with significance at $p<0.05$ on GraphPad Prism version 5.0 for faecal indicator bacteria counts (mean ± standard error), and physicochemical characteristics (mean ± standard deviation) of growing water in the concrete and earthen ponds. The relationships between enteric bacteria counts and physicochemical properties of the growing waters from the earthen and concrete ponds were analyzed using the Pearson's correlation coefficient at $p<0.05$ level of significance.

Results:

Enteric bacterial counts in catfish samples and their growing waters in the ponds:

The faecal coliform counts (log_{10} CFU/ml) in catfish samples from the concrete and earthen ponds ranged from 1.41 to 2.28 and 1.3 to 2.47 respectively, while the counts ranged from 1.43 to 2.41 and 1.50 to 2.80 in the growing water samples from the concrete and earthen ponds respectively (Fig 3). In catfish samples from concrete and earthen ponds, *Salmonella* counts (log_{10} CFU/ml ranged from 1.52 to 2.56 and 1.60 to 2.70 respectively, while in the growing water samples, the counts ranged from 1.51 to 2.56 and 1.60 to 2.70 respectively (Fig 4).

Escherichia coli counts (log_{10} CFU/100 ml) in catfish samples from the concrete and earthen ponds ranged from 1.23 to 2.44 and 1.30 to 2.30 respectively, while the counts in the growing water samples from the concrete and earthen ponds ranged from 1.30 to 2.1 and 1.20 to 1.53 respectively (Fig 5). *Shigella* counts (log_{10} CFU/ml) in catfish samples from concrete and earthen ponds ranged from 1.0 to 1.2 and 1.40 to 1.50 respectively while the counts in growing water samples ranged from 1.46 to 1.60 and 1.40 to 1.50 respectively (Fig 6). Enterococci counts (log_{10} CFU/ml) in catfish samples from concrete and earthen ponds ranged from 1.30 to 1.40 and 1.30 to 1.70 respectively, while the counts in growing water samples ranged from 1.40 to 1.50 and 1.32 to 1.82 respectively (Fig 7).

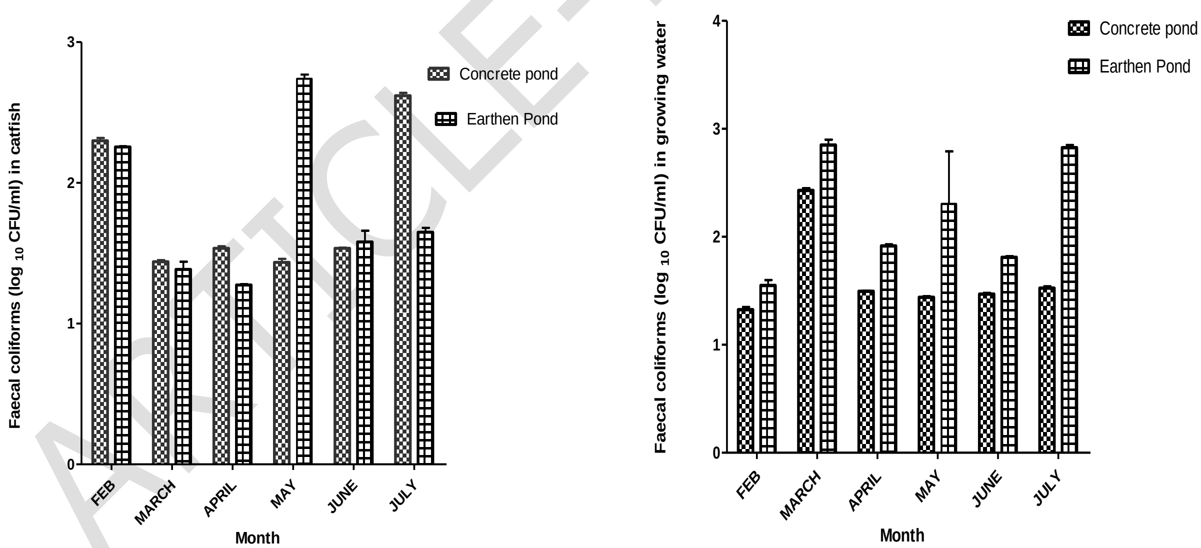


Fig 3: Mean concentration of faecal coliforms in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

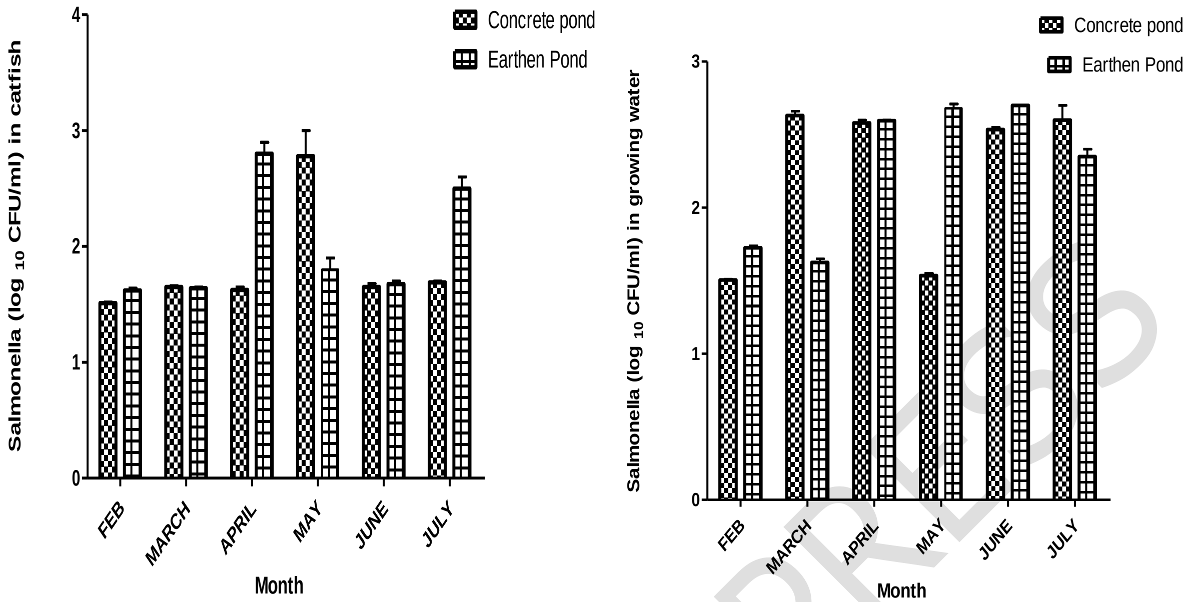


Fig 4: Mean concentration of *Salmonella* in catfish and their growing water samples in concrete and earthen pond. Values are expressed as mean ± standard error of mean (SEM) of $\log_{10}$ CFU/100 ml

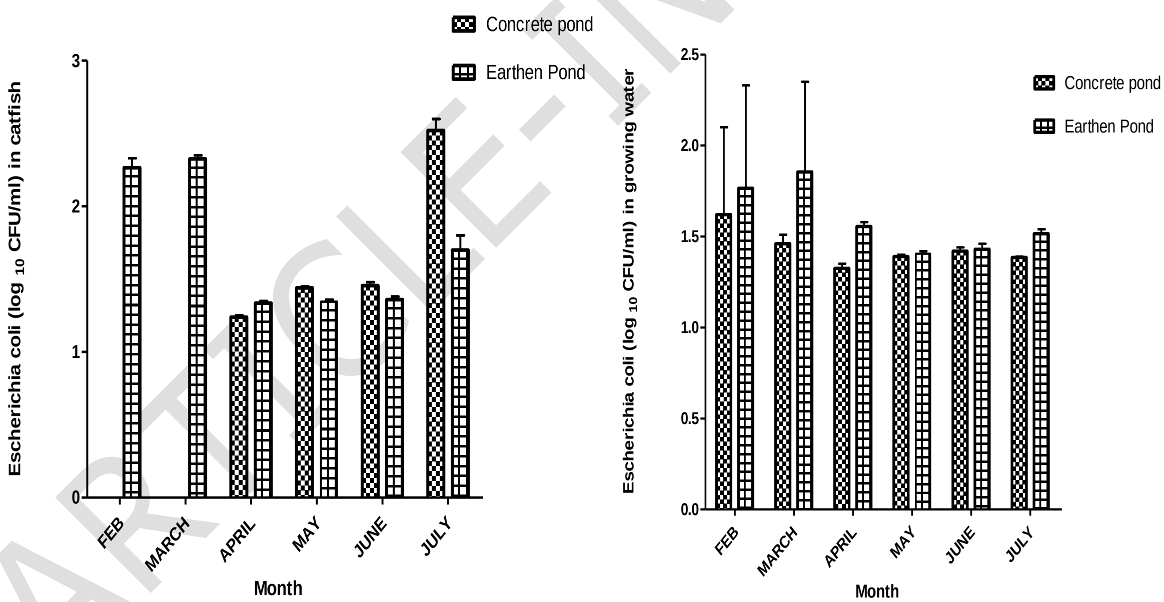


Fig. 5: Mean concentration of *E. coli* in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of $\log_{10}$ CFU/100 ml

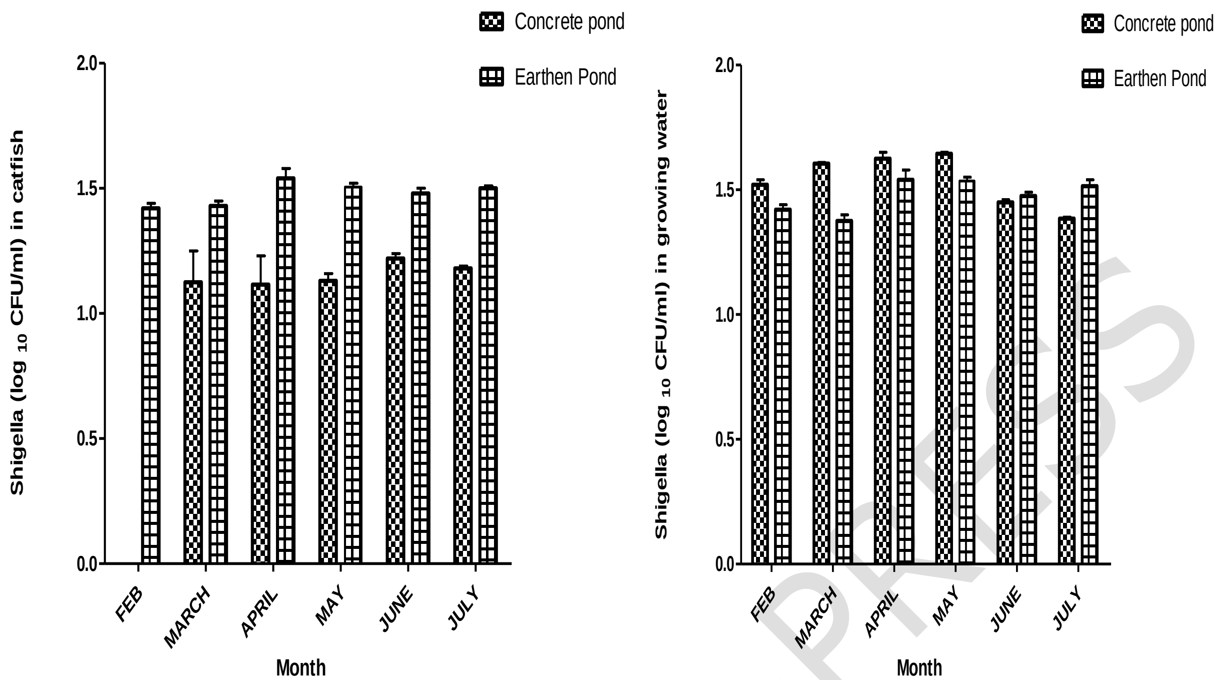


Fig 6: Mean concentration of *Shigella* in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

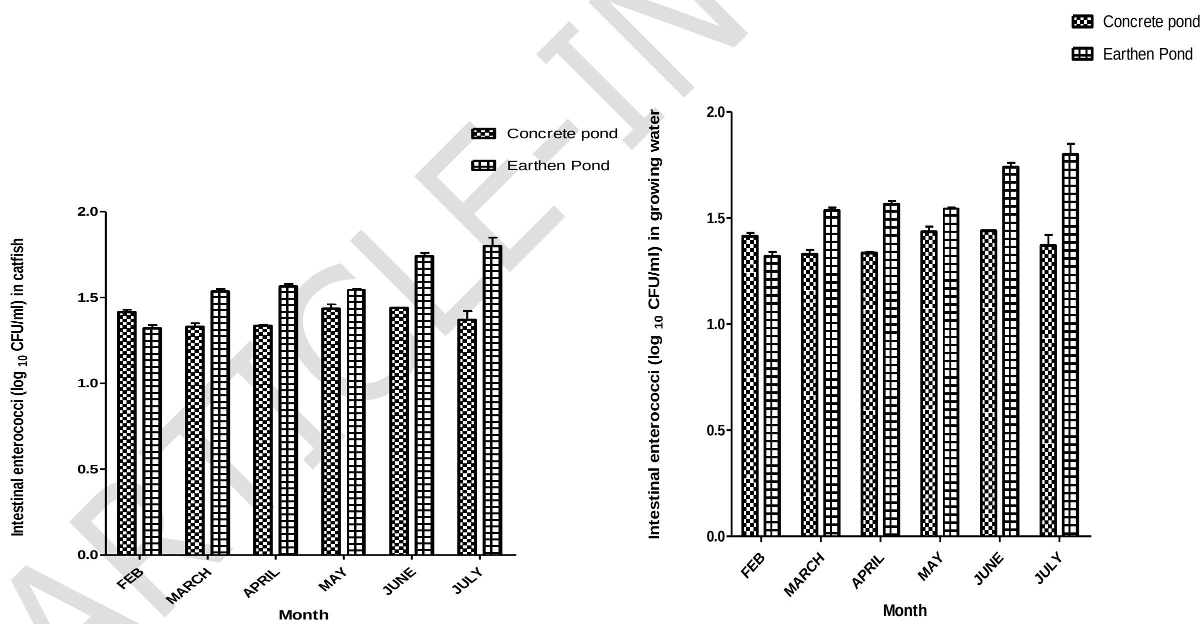


Fig. 7: Mean concentration of intestinal enterococci in catfish and their growing water samples in concrete and earthen ponds. Values are expressed as mean ± standard error of mean (SEM) of log_{10} CFU/100 ml

Distribution pattern of enteric bacteria in catfish and growing waters from the ponds:

The frequency distribution patterns of the enteric bacteria showed that faecal coliforms (Sig.=0.757) and *Salmonella* (Sig.= 0.843) in catfish samples from the earthen ponds were normally distributed while enterococci (Sig.= 0.001), *Shigella* (Sig.=0.029) and *E. coli* (Sig.= 0.028) were not normally distributed. Furthermore, *Salmonella* (Sig.=0.761) and *Shigella* (Sig.=0.761) counts in water samples from the earthen pond were normally distributed, while faecal coliforms (Sig.=0.000), enterococci (Sig. =0.038) and *E. coli* (Sig.=0.038) were not normally distributed (Table 1).

On the other hand, faecal coliforms (Sig.=0.527) and *Salmonella* (Sig.=0.643) in catfish samples from the concrete ponds were normally distributed while enterococci (Sig.= 0.011), *Shigella* (Sig.=0.029) and *E. coli* (Sig. =0.018) significantly deviated from normal distribution. In addition, *Salmonella* (Sig.=0.461) and *Shigella* (Sig. =0.751) in water samples from the concrete ponds were normally distributed while faecal coliforms (Sig.=0.000), enterococci (Sig.=0.038) and *E. coli* (Sig.=0.028) significantly deviated from normal distribution (Table 2).

Table 1: Normality and distribution pattern of enteric bacteria in *Clarias gariepinus* and their growing water in earthen ponds

Test of Normality	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Catfish samples						
Faecal coliforms	0.130	20	0.200*	0.970	20	0.757
Salmonella	0.120	20	0.200*	0.974	20	0.843
Enterococci	0.258	20	0.001	0.795	20	0.001
Shigella	0.197	20	0.040	0.891	20	0.029
Escherichia coli	0.141	20	0.200*	0.891	20	0.028
Water samples						
Faecal coliforms	0.345	20	0	0.717	20	0
Salmonella	0.096	20	0.200*	0.970	20	0.761
Enterococci	0.208	20	0.024	0.898	20	0.038
Shigella	0.096	20	0.200*	0.970	20	0.761
Escherichia coli	0.208	20	0.024	0.898	20	0.038

Df = Difference; Sig. = Significance

Table 2: Normality and distribution pattern of enteric bacteria in *Clarias gariepinus* and their growing water in concrete pond

Tests of Normality	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	Df	Sig.
Catfish samples						
Faecal coliforms	0.130	20	0.200*	0.970	20	0.757
Salmonella	0.120	20	0.200*	0.974	20	0.843
Enterococci	0.258	20	0.001	0.795	20	0.001
Shigella	0.197	20	0.040	0.891	20	0.029
Escherichia coli	0.141	20	0.200*	0.891	20	0.028
Water samples						
Faecal coliforms	0.345	20	0	0.717	20	0
Salmonella	0.096	20	0.200*	0.970	20	0.761
Enterococci	0.208	20	0.024	0.898	20	0.038
Shigella	0.096	20	0.200*	0.970	20	0.761
Escherichia coli	0.208	20	0.024	0.898	20	0.038

Df = Difference; Sig. = Significance

Table 3: Physicochemical characteristics of growing water samples in earthen and concrete ponds

Parameters	Earthen pond	Concrete pond
Temperature (°C)	26.7±0.28	29.3±0.35
pH	7.0±0.19	6.95±0.07
EC (μs/cm)	20.8±1.06	26.8±0.35
Salinity (PSU)	149.3±1.06	148.0±0.71
Turbidity (NTU)	43.5±0.25	44.3±0.29
TDS (mg/L)	23.5±0.38	22.3±0.35
Alkalinity (meq/L)	109.8±2.89	107.8±3.89
DO (mg/L)	11.95±2.13	14.6±6.29
BOD (mg/L)	2.53±1.23	1.53±0.03
COD (mg/L)	199.6±2.55	202.5±3.54

Values presented are expressed as mean values ± standard deviation (n=6). Temp = Temperature; EC = Electrical conductivity; TDS = Total dissolved solids; DO = Dissolved oxygen; BOD = Biological oxygen demand; COD = Chemical oxygen demand.

Physicochemical characteristics of the growing waters in the earthen and concrete ponds:

In the earthen and concrete ponds, the mean temperatures of the growing waters were 26.70 ± 0.28°C and 29.30 ± 35.5°C respectively (Table 3). In the earthen and concrete ponds, the mean salinity of the growing waters was 149.31.06 PSU (practical salinity unit) and 148.00.71 PSU (practical salinity unit), respectively. Furthermore, the mean values of turbidity of the growing waters in the earthen and concrete ponds were 43 and 44 (NTU) (Nephelometric turbidity unit), respectively, whereas the mean values of total dissolved solids of the growing waters in the earthen and concrete ponds were 23 and 22 mg/L. The alkalinity levels of the growing waters in the earthen and concrete ponds were respectively 109 and 107 meq/L.

Relationship between enteric bacteria in catfish and physicochemical characteristics of the growing waters in the ponds:

In the earthen pond, alkalinity correlated positively with enterococci (r=0.600, p< 0.05), faecal coliforms (r=0.610, p<0.05), and *E. coli* counts (r=0.650, p<0.01). The total dissolved solids positively correlated with *E. coli* (r=0.51, p<0.05). Turbidity showed a positive correlation with faecal coliforms (r=0.54, p< 0.05). Biological oxygen demand had positive correlation with enterococci (r=0.52, p<0.05) and *E. coli* (r=0.75, p<0.01). Chemical oxygen demand also positively correlated with enterococci (r=0.500, p<0.05), faecal coliforms (r= 0.530, p<0.05), and *E. coli* (r=0.750, p<0.01) (Table 4).

In the concrete ponds, alkalinity had a positive correlation with faecal coliforms (r= 0.620, p<0.01) and *E. coli* (r=0.600, p<0.01). Total dissolved solids had a positive correlation with *E. coli* (r=0.650, p<0.05). Biological oxygen demand had a positive correlation with enterococci (r=0.580, p<0.05) and *E. coli* (r= 0.820, p<0.01). Chemical oxygen demand had positive correlation with enterococci (r=0.540, p<0.05) and faecal coliforms (r=0.580, p< 0.05). Salinity had a positive correlation with enterococci (r=0.51, p<0.05) and *E. coli* (r= 0.50, p<0.05) (Table 5).

Table 4: Correlation of enteric bacteria and physicochemical characteristics of growing waters in earthen pond

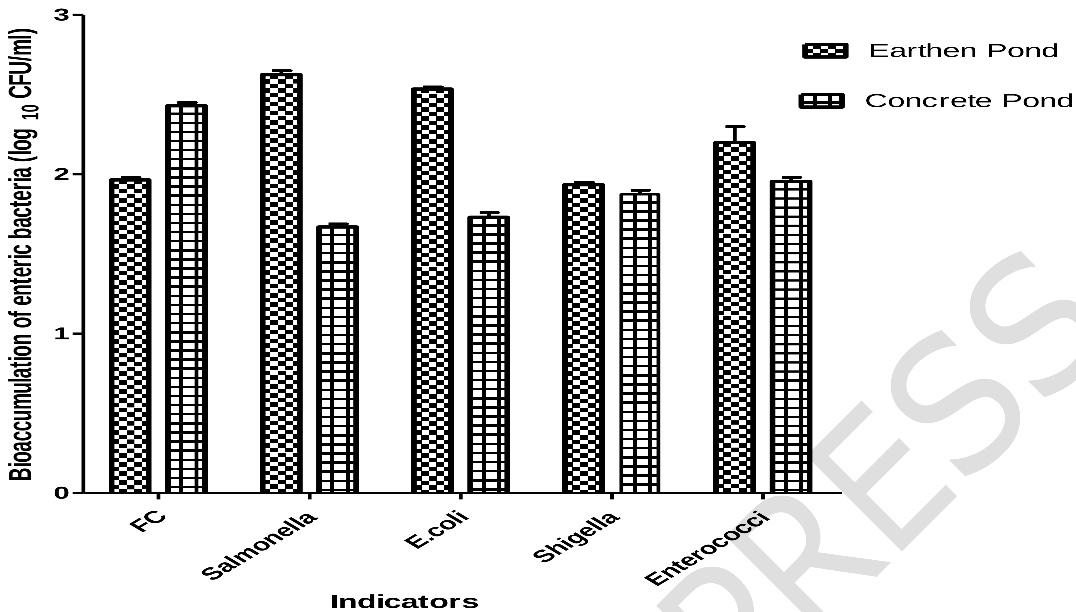
Physicochemical parameters	Enterococci	Faecal coliforms	*Escherichia coli*	*Salmonella*	*Shigella*
Temp (°C)	0.03	0.04	0.08	-0.10	-0.05
pH	-0.32	0.08	0.05	-0.28	-0.29
EC (µs/cm)	0.05	0.43	0.08	0.48	0.49
Turbidity (NTU)	0.10	**0.54***	0.34	0.29	0.22
Alkalinity (meq/L)	**0.60**	**0.61**	**0.65**	-0.18	-0.08
DO (mg/l)	-0.10	-0.13	0.29	-0.02	-0.01
Salinity (PSU)	0.31	-0.03	0.30	0.16	0.16
TDS (mg/L)	0.34	0.11	**0.51***	-0.14	-0.14
BOD (mg/L)	**0.52***	0.44	**0.75**	-0.18	-0.18
COD (mg/L)	**0.50***	**0.53***	**0.73**	-0.14	-0.14

Correlation is significant at the 0.05 level (2-tailed) *; Correlation is significant at the 0.01 level (2-tailed) **; Temp = Temperature; EC=Electrical conductivity; DO=Dissolved oxygen; TDS= Total dissolved solids; BOD=Biological oxygen demand; COD=Chemical oxygen demand.
Values in bold figures indicate significant correlation

Table 5: Correlation of enteric bacteria and physicochemical characteristics of growing waters in concrete pond

Physicochemical parameters	Enterococci	Faecal coliforms	*Escherichia coli*	*Salmonella*	*Shigella*
Temp (°C)	-0.11	-0.20	-0.22	-0.11	-0.12
pH	-0.42	-0.08	-0.15	-0.25	-0.25
EC (µs/cm)	-0.05	0.33	0.04	0.29	0.29
Turbidity (NTU)	0.10	0.37	0.21	0.19	0.19
Alkalinity (meq/L)	0.45	**0.62**	**0.60**	-0.28	-0.28
DO (mg/l)	-0.16	-0.23	0.19	-0.04	-0.04
Salinity (PSU)	**0.51***	0.03	**0.50***	0.36	0.26
TDS (mg/l)	0.44	0.21	**0.65**	0.18	-0.10
BOD (mg/l)	**0.58***	0.49	**0.82**	0.19	-0.12
COD (mg/l)	**0.54***	**0.53***	**0.77**	0.04	0.04

Correlation is significant at the 0.05 level (2-tailed) *; Correlation is significant at the 0.01 level (2-tailed) **; Temp=Temperature; EC=Electrical conductivity; DO=Dissolved oxygen; TDS=Total dissolved solids; BOD=Biological oxygen demand; COD=Chemical oxygen demand.
Values in bold figures indicate significant correlation



FC- Faecal coliforms

Fig 8: The bioaccumulation of enteric bacteria in catfish from their growing waters in earthen and concrete ponds

Bioaccumulation of enteric bacteria in catfish samples from the ponds:

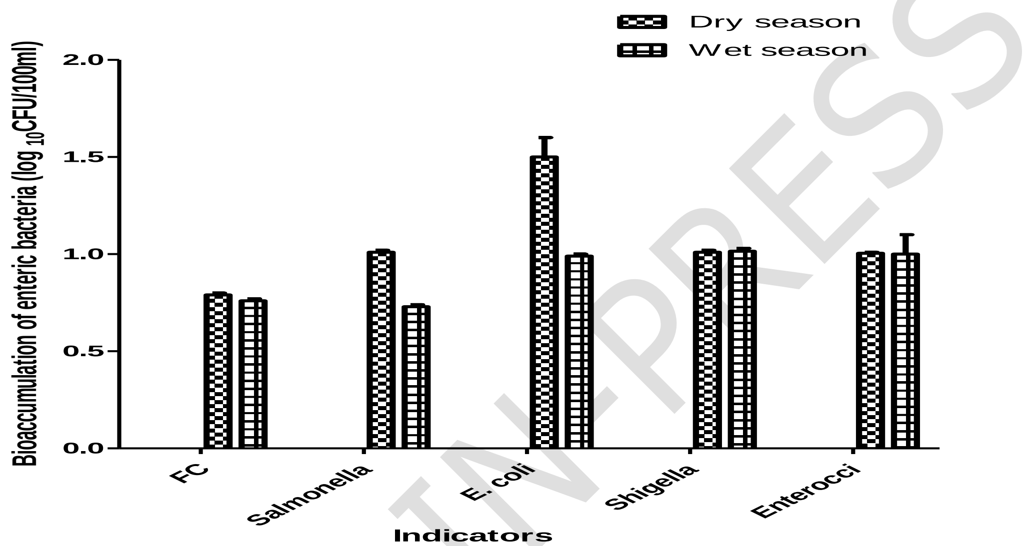
Enterococci bioaccumulation ($\log_{10}$ CFU /ml) in catfish from their growing waters in earthen and concrete ponds ranged from 0.90 to 2.20 and 0.92 to 2.19 respectively. Faecal coliform bioaccumulation in catfish from earthen and concrete ponds ranged from 0.79 to 2.00 and 0.70 to 2.40 respectively. The bioaccumulation of enteric bacteria in catfish reared in concrete pond had a positive correlation with alkalinity, total dissolved solids, biological oxygen demand, chemical oxygen demand and salinity, and a negative correlation with temperature, pH, dissolved oxygen, and turbidity.

Escherichia coli bioaccumulation ($\log_{10}$ CFU/ml) in catfish from their growing waters in earthen and concrete ponds ranged from 0.94 to 2.65 and 0.00 to 1.89 respectively, while *Salmonella* bioaccumulation in catfish from their growing waters in earthen and concrete ponds ranged from 0.97 to 2.70 and 0.95 to 1.75 respectively. In earthen ponds, alkalinity, total dissolved solids, turbidity, biological oxygen demand, and chemical oxygen demand all had positive correlation with enteric bacteria in cat fish with the exception of *Salmonella* and *Shigella*, while salinity, temperature, pH, electrical conductivity, and dissolved oxygen had negative correlation. Furthermore, *Shigella* bioaccumulation ($\log_{10}$ CFU/ml) in catfish from their growing waters in earthen and concrete ponds was 0.97 to 1.89 and 0.95 to 1.86 respectively (Fig 8).

Effects of seasonality on bioaccumulation of enteric bacteria in catfish from their growing waters:

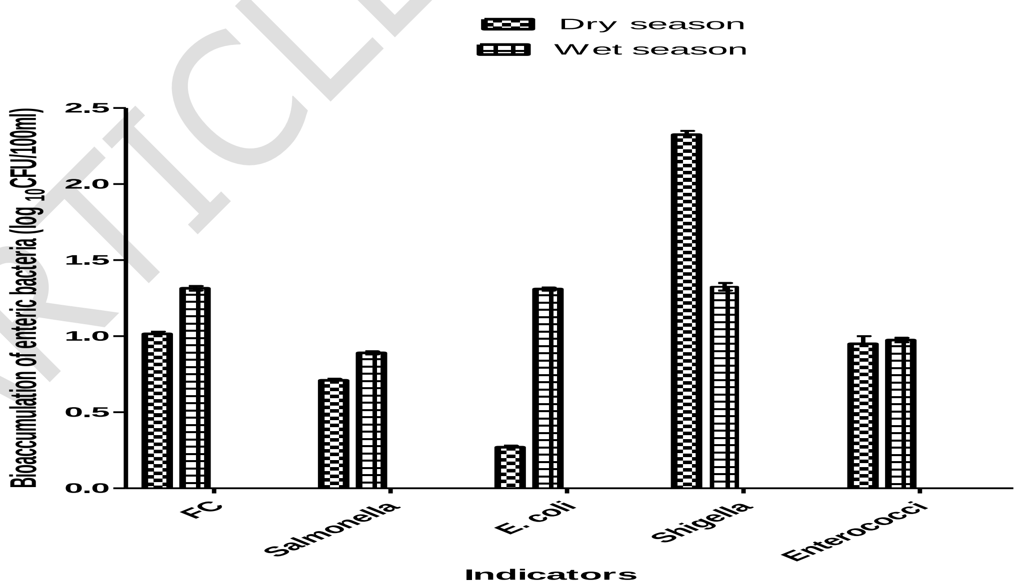
In the earthen ponds, the mean values of bioaccumulation ($\log_{10}$ CFU/ml) of enterococci in catfish samples in dry and wet periods were 0.99 and 0.62 respectively, while the mean values of bioaccumulation of faecal coliforms in catfish samples in dry (February - April) and wet periods (May - July) were 0.50 and 0.51 respectively. The mean values of bioaccumulation ($\log_{10}$ CFU/100ml) of *E. coli* in catfish samples in dry and wet periods were 1.02 and 0.89 respectively. The mean values of bioaccumulation ($\log_{10}$ CFU/100ml) of *Salmonella* in catfish samples in dry and wet periods were 0.60 and 0.45 respectively while the mean values of bioaccumulation of *Shigella* in catfish samples in dry and wet periods were 0.55 and 0.54 respectively. *Escherichia coli* had the highest mean value of bioaccumulation during the dry period while *Shigella* had the highest mean value of bioaccumulation during the wet period (Fig 9).

In the concrete ponds, the mean values of bioaccumulation ($\log_{10}$ CFU/100ml) of enterococci in catfish samples in dry and wet periods were 0.5 and 0.93 respectively while the mean values of bioaccumulation of faecal coliforms in catfish samples in dry and wet periods were 0.85 and 1.06 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *E. coli* in catfish samples in dry and wet periods were 0.21 and 1.07 respectively. The mean values of bioaccumulation (log_{10} CFU/100ml) of *Salmonella* in catfish samples in dry and wet periods were 0.52 and 0.80 respectively while the mean values of bioaccumulation of *Shigella* in catfish samples in dry and wet periods were 1.77 and 1.07 respectively (Fig 10). The relationship of seasonality and bioaccumulation showed that seasonality played major roles in the rate of bioaccumulation of enteric bacteria in catfish samples from the earthen and concrete ponds.



FC- Faecal coliforms

Fig 9: The rate of bioaccumulation of enteric bacteria in the catfish samples from the earthen pond during wet and dry periods



FC- Faecal coliforms

Fig 10: The rate of bioaccumulation of enteric bacteria in the catfish samples from the concrete pond during wet and dry periods

Discussion:

The assessment of faecal contamination in selected concrete and earthen ponds stocked with *Clarias gariepinus* in a fish farm was investigated in this study. The load of enteric bacteria in water and the catfish samples were in agreement with Njoku (14) who observed that the load of heterotrophic bacteria in pond water fluctuated between 0.01 and 8.7×10^5 CFU/ml, which was consistent with the load of enteric bacteria in water and catfish samples. The earthen pond had a higher repertoire of faecal contamination than the concrete pond, which could be due to faecal coliforms in generally consisting part of *Enterobacteriaceae* group that thrive well at temperature ranges of between 26ºC and 29ºC observed in earthen and concrete ponds respectively in this study.

Increased rainfall, as well as subsequent contamination from both direct and diffuse sources, may have contributed to the high amounts of faecal coliforms found in the earthen pond (15). This is consistent with the study of Ajayi and Okoh (16), who reported that microbial load is typically higher in earthen ponds as a result of natural nutrients in soils that promote microbial development. The load of faecal coliforms in catfish samples and their growing waters from the earthen and concrete ponds was found to be high, which could be due to increased temperature (26-30°C) of the developing water and increased dissolved nutrient in the growing water following catfish feeding. This is consistent with the findings of Wyatt et al., (17) in which the increased temperature and dissolved nutrients in catfish growing waters influenced the load of faecal coliforms in the catfish samples and their growing waters. Furthermore, the higher load of faecal coliforms in catfish samples and their growing waters in the earthen pond compared to the concrete pond could be attributed to the natural habitat of the earthen pond, which provides vital nutrients and minerals that may support microbial growth better than the concrete pond. This is consistent with the findings of Njoku et al., (14), who attributed the high load of faecal coliforms in the growing water samples of the earthen ponds to the natural propensity of the environment to foster development of microbes.

The load of *Salmonella* in the catfish samples and their growing waters from the earthen and concrete pond was also high in our study. This is in line with the findings of Olalemi (15), who concluded that indigenous and non-indigenous bacterial infections could be linked to fresh fish or their environment. *Salmonella* is not a recognized typical bacterial flora of catfish, and its prevalence is usually associated to its breeding, poor hygiene measures and inappropriate handling as reported by Ajayi and Okoh (16), who also confirmed that *Salmonella* can survive transitorily in the gastrointestinal system of fish. The load of *Shigella* in catfish samples and their growing waters from the earthen and concrete pond was equally high in our study. According to Salome and Faith (17), the greater load is potentially sustained by amplified nutrient load as a result of flood and runoffs into the ponds. The load of intestinal enterococci in catfish samples in the earthen and concrete ponds that were high may be as a result of the bacteriological profile of *C. gariepinus* as reported by Omeji et al., (18) where water contaminated with faecal streptococci have the tendency to infect fish in ponds.

The distribution pattern of the faecal coliforms and *Salmonella* in catfish samples of earthen and concrete ponds may be the result of use of maggots from chicken dungs as feeds and these may serve as reservoir for these bacteria, while the deviation in the distribution pattern of enterococci, *Shigella* and *E. coli* in the catfish samples in earthen and concrete ponds may be as a result of change in varying environmental conditions. The distribution pattern of faecal indicator bacteria in earthen and concrete ponds in our study agrees with the study of Ganesh et al., (19), who reported that divergence in the distribution of heterotrophic and total aquatic bacteria within the water layers could be linked to various factors affecting the distribution pattern of bacteria in fish ponds.

In our study, the mean temperature values varied from 25°C to 30°C, which is remarkably comparable to the findings of Ntengwe and Edema (20). The water temperature reading could have been impacted by the weather conditions during the sam pling process (21). In the earthen and concrete ponds, the pH of the growing waters was 7.00.19 and 6.950.07, respectively. These pH levels are consistent with the findings of Ehiagbonare and Ogunrinde (22), who reported that pH 6 to 9 is a basic prerequisite for improved fish production. The ideal pH range for ponds was also reported by Stone and Thomforde (23) to be 5.5 - 10.0 in order to maintain good pond productivity and fish health. It is worth noting that the results of this study are consistent with those of Ntengwe and Edema (20), who studied the physicochemical and microbiological features of water for fish production in tiny ponds. The electrical conductivity of the pond water was influenced by climatic conditions such as rainfall that occurred during the sample period, since surface runoff that contained substantial nutrients during the wet season was probably one of the variables that raised the pond water conductivity (21). The electrical conductivity values, on the other hand, were still within the normal range for fish rearing (10–1000μS/cm) (22). Dissolved oxygen condenses as a result of boost in water temperature, respiration and organic matter breakdown by aerobic aquatic organisms (23).

Within the allowed limit of 200 meq/L, the mean alkalinity values of earthen and concrete ponds were 109 and 107 meq/L respectively (24). Alkalinity levels of 75 to 200 meq/L have been reported by Rana and Jain (25), but not less than 20 meq/L is optimum in an aquaculture pond. For catfish development and good pond productivity, Rana and Jain (25) advised total alkalinity values of at least 20 meq/L. The high mean alkalinity of pond water in our study implies that even a small amount of acid will not cause a pH change. In the growing waters of the earthen and concrete ponds, dissolved oxygen (DO) was 11.952.13 and 4.66. 29 mg/L respectively. Despite the fact that the minimum amount of dissolved oxygen for tropical fish should be 5mg/L (26), the high amounts of dissolved oxygen found in this study could be due to photosynthetic activities of primary producers that comprise an elevated bio-assortment of plants especially in the earthen pond. The mean total dissolved solids (TDS) in this study were below the standard permissible limit of 1000 mg/L (27).

The biological oxygen demand (BOD) of the growing waters in the earthen and concrete ponds respectively were 2.531. 23% and 1.530.03% mg/L. The high BOD depletes oxygen levels to dangerous levels, indicating that the water is polluted. According to Bhatnagar and Singh (28), only BOD values between 3.0-6.0 mg/L are most advantageous for normal activities of catfishes while level of 6.0-12.0 mg/L is toxic to catfishes and >12.0 mg/L could effectively cause their death through suffocation. The chemical oxygen demand (COD) was 199. 62.55 mg/L in one case and 202.53.54 mg/L in another. The average chemical oxygen demand in the ponds in the current study exceeded the standard allowable limit of 10 mg/L (29).

The negative correlation of *Salmonella* with physicochemical parameters in this study was similarly reported by Olalemi and Oluyemi (30) in their study of incidence and existence of faecal pollution markers in an earthen fish pond in Akure, Nigeria. The positive correlation between turbidity and faecal coliforms seen in this study agrees with the finding of Olalemi and Oluyemi (30). Previous studies have reported that the progressive bioaccumulation of microbes in marine animals like *C. gariepinus* is influenced by an array of ecological factors (31) as evident in our study.

Conclusion:

The findings of this study revealed high levels of faecal pollution in water and African catfish (*C. gariepinus*) from earthen and concrete ponds. Physicochemical characteristics of the pond water and seasonality influenced the rate of bioaccumulation of enteric bacteria in *C. gariepinus* raised in these earthen and concrete ponds.

Acknowledgements:

Authors appreciate the technical staff at Federal University of Technology Akure (FUTA) fish farm for their assistance during the course of sample collection.

Contributions of authors:

MB deduced and analyzed the study inference, wrote the original draft and edited the manuscript; OO conducted the study and collected the samples; AO conceived the study idea and reviewed the original draft. All authors agreed to the content of the final manuscript.

Source of funding:

Authors received no funding

Conflict of interest:

No conflict of interest is declared.

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