integrity Research Journals

Global Journal of Fisheries Science
Volume 4(4), pages 40-45, December 2022
Article Number: 8F1156991
ISSN: 2782-750X
https://doi.org/10.31248/GJFS2022.039
https://integrityresjournals.org/journal/GJFS

Full Length Research

Comparative study of physico-chemical parameters and microbial load of aqua media of different receptacles used in culturing *Clarias gariepinus*

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Received 27th October 2022; Accepted 12th December, 2022

ABSTRACT: The physico-chemical parameters cum microbial loads of water from earthen, concrete and collapsible plastic tank, tagged A, B and C respectively, were used to culture *Clarias gariepinus* at the Integrated Organic Fish Farm of the Department of Fisheries and Aquaculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria, which lasted for sixteen (16) weeks. Water samples were collected aseptically every week throughout the duration of the research. The mean values of physico-chemical parameters using analysis of variance (ANOVA), showed that there was no significant difference ($p>0.05$) in treatments A, B and C. The results showed that earthen pond recorded the highest in Biological Dissolved Oxygen (BOD) 3.67±0.417 mg/L, Sulphate 4.55±0.016 mg/L, Nitrate 3.12±0.023mg/L and Magnesium 1.28±0.042 mg/L, followed by B and C, while concrete pond recorded highest mean values in temperature 28.17±0.234°C, pH 7.10±0.252, alkalinity 31.17±0.189 mg/L, calcium 0.53±0.O27 mg/L, and potassium 11.68±0.016 mg/L followed by A and C. and collapsible plastic tank recorded highest mean values in dissolved oxygen DO 9.83±0.240 mg/L, and transparency 60.67±0.667 cm/L. The physico-chemical parameters were all within the accepted standard range by the World Health Organization (WHO) and Federal Emergency Protection Agency for culturing fish. The Least Significant Difference (LSD) showed that there were significant differences ($p<0.05$) between the bacteria load of water samples from A and C receptacles. It also, showed that there was no significant difference ($p>0.05$) between bacteria load of water samples from A and B receptacles. Water sample from A, had the highest CFU count of *Escherichia coli, Staphylococcus sp., Streptococcus faecalis, Proteus sp*, *Clostridium perfringens* and *Pseudomonas aeruginosa* of 0.62×10^7, followed by B water samples with *Streptococcus faecalis*, *Staphylococcus sp.* and *Escherichia coli* of 1.16×10^7 and C water samples *E. coli* and *Staphylococcus sp*. with count of 1.78×10^7. Proper management of culturing media in different receptacles should be monitored to reduce microbial load in *Clarias gariepinus*, to avoid fish mortality and ill-health to human being when consumed.

Keywords: African catfish, culture media, microbial load, receptacles, water quality.

INTRODUCTION

Aquaculture is presently the only sure means for increasing fish production on earth. This is the consequence of the dwindling rate of wild fish captured from the fresh, brackish, and marine waters, which reason, is directly linked to; pollution (sewage dumping, oil spillage, use of chemicals (inorganic fertilizer, pesticides, insecticides, herbicides, fungicides, effluents discharge), overfishing, flood, erosion, siltation, tsunami, earthquake and landslide, obnoxious fishing methods (dynamites, explosives and bombs) (Okeke *et al.,* 2016). More than 50% of the world population derives 20% of its animal protein intake from fish and aquaculture contributes about 10% of this (FAO, 2002). Fish is comparatively cheaper, more acceptable with little or no religious stigma, rich in minerals with less saturated fatty acid and it is easily digestible (Phillips *et al*., 2004).

Aquaculture has been practiced in the past, using different types of receptacles such as earthen and concrete ponds, plastic tanks, collapsible plastic tanks, wooden troughs, vats, and fibre or glass tanks (Osawe, 2004). Among these culturing receptacles, concrete and earthen ponds are widely in use. Although the earthen pond culture system had been the conventional practice before the advent of the concrete pond system and has been gaining ground and popularity in Sub-saharan Africa (Ezenwa, 2006). The earthen pond culture system has been the conventional method of fish culture in Nigeria, until recently when concrete and other types of receptacles started to gain ground because of scarcity and high cost of land (Onome and Ebinmi 2010). Due to improvements in technology, there is an increase in the use of concrete and collapsible plastic tanks over earthen ponds.

Fishes reared in these various water receptacles have been found to be contaminated by microorganisms (Fafioye, 2011). The microorganisms' build-up in water is caused by many reasons; type of substrates, pond bottom, "fish seeds", feeds, and water sources. The current use of organic manure in fish production has led to a high build-up of opportunistic and pathogenic microorganisms in the ponds which are of public health concern (Okonkwo *et al.,* 2012). Fishes cultured in these contaminated pond water are affected by these pathogenic organisms, which if consumed by man have the tendency of causing disease and transferring the zoonotic infection to man (Omojowo and Omojosola, 2013). Also, the physico-chemical parameters (temperature (T^o), biological dissolved oxygen (BOD), alkalinity, transparency, total dissolved solids (TDS), nitrate (NO_3), potassium (K), magnesium (Mg), calcium (Ca)) are very vital for optimum, rapid growth and survival of fish when present at the right recommended levels. This is because fish carry out all its physiological (movement, feeding, excretion, growth, respiration, irritability, reproduction, death) functions in the water (Ugwu *et al.,* 2006). The dependency on the water by fish is crucial. Hence, the source, volume, and quality of the physisco-chemical parameters are some of the salient factors to consider in relation to fish health (Njoku, 2015). Therefore, this study intends to assess the water qualities and microbial loads of culture water used in different receptacles (ponds) in rearing African catfish-*Clarias gariepinus* in Anambra State of Nigeria.

MATERIALS AND METHODS

The study was carried out for a period of sixteen weeks (16), between the months of June and September, in the Fish Farm of the Department of Fisheries and Aquaculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The global positioning system (GPS) coordinates of the fish farm (earthen ponds, concrete ponds, and collapsible plastic tanks), is at latitude 6.245° and 6.283° N and longitude 7.115° and 7.121° E. The earthen and concrete ponds and the collapsible plastic tank were located in the fish farm and tagged A, B, and C respectively, and were triplicate. The culturing medium measured 2 m × 2 m × 1 m each in dimension. The source of water was from the borehole dug in the farm, except for the earthen pond that was constructed in a hydromorphic area. The water depth was maintained at a 1 m level throughout the duration of the research. Water was changed every week, on days of sample collection for data analysis. The cultured fish (African catfish – *Clarias gariepinus*) were stocked at a density of sixty (60) juveniles per pond, making it one hundred and eighty (180) juveniles for A, B, and C receptacles. The fish were fed with the same branded commercial feed 'Vital-feed' at 5% body weight throughout the entire experiment.

Data collection

Sample collection

Water samples were aseptically collected between 8.00 am and 10 am on weekly basis from the earthen pond, concrete pond, and collapsible plastic tank receptacles using sterilized 500 ml and 250 ml universal containers. The containers were dipped 30 cm inside the aqua media and were filled to remove air completely and the sample bottles were closed with rubber corks. The samples were immediately taken to the laboratory for analysis, of those physicochemical parameters that cannot be taken *in-situ* at fish ponds.

Sample analysis

Physical analysis: The physical parameters of (temperature (T°), hydrogen ion concentration (pH), and transparency) were done *in-situ* in the A, B, and C culturing water using a mercury glass thermometer, Pen type pH meter (pH-009-111) and Secchi disc respectively. The turbidity was measured with the help of a turbidity meter. The HACH conductivity/TDS meter version was used in analyzing the total dissolved solids (TDS) and electric conductivity, using the recommended procedure in APHA (2005).

Chemical analysis: The dissolved oxygen (DO) and biological oxygen demand (BOD) of culturing water, were analyzed using water collected with DO bottles and fixed with Winkler solution using Winkler titration methods as described by Sterlings (1985) method. The sulphate, calcium, nitrate, magnesium, and phosphate were all determined by titration method.

Microbial count/load: The microbial counts were analyzed using a direct plate colony count. All assays were carried out in treatments A, B, and C. The Mack Conkey

Table 1. Mean values of physico-chemical parameters of water from different culturing receptacles A, B and C.

Parameters	Pond earthen	Concrete pond	Collapsible plastic tank	Range	Mean ± SD
Temperature ^{0}C	23.53±0.41^a	28.17±0.234^a	27.10±0.101^a	23.53 – 28.17	26.267±1.984
pH	6.48±0.0 23^a	7.10±0.252^b	7.05±0.041^b	6.48 – 7.10	6.88±0.281
Dissolved O_2 mg/L	8.72±0.019^a	9.25±0.016^b	9.83±0.240^b	8.72 – 9.83	9.27±1.040
Conductivity µ/cm	14.39±0.238^a	14.33±0.056^a	17.10±0.013^b	14.33 – 17.10	15.27±0.411
BOD mg/L	3.67±0.417^a	3.21±0.056^a	3.08±0.095^a	3.08 - 3.67	3.32±0.460
TDS mg/L	25.25±0.370^a	19.29±0.153^b	15.02±0.133^c	15.02 – 25.25	19.85±4.195
Transparency cm/L	37.67±2.055^a	55.33±0.471^b	60.67±0.667^b	37.67 – 60.67	51.22±0.667
Alkalinity mg/L	30.27±0.040^a	31.17±0.189^a	25.60±0.481^b	25.60 – 31.17	29.01±2.441
Sulphate mg/L	4.55±0.016^a	2.90±0.001^b	2.50±0.016^b	2.50 – 4.55	3.00±0.887
Nitrate mg/L	3.12±0.023^a	1.98±0.001^b	1.85±0.095^b	1.65 – 3.12	2.32±0.597
Magnesium mg/L	1.28±0.042^a	1.17±0.036^a	1.09±0.013^a	1.09 – 1.28	1.18±0.259
Calcium mg/L	0.35±0.016^a	0.53±0.027^b	0.27±0.197^a	0.27 – 0.53	0.38±0.225
Potassium mg/L	10.26±0.066^a	11.68±0.016^a	10.10±0.026^a	10.10 – 11.68	10.68±0.707

Means values in the same row with the same superscript are not significantly different (p>0.05).

nutrient agar was used for staining. The stained agar was put in an incubation which triggers the growth of specific bacterium to grow in colonies on the plated stained agar. This was done repeatedly until the nutrient medium became visible to the naked eye and the number of colonies on the plate can be counted. A series of biological tests and morphological characteristics of the various microbial isolates were identified as described by Slabyj *et al*. (1981) and Olayemi and Opaleye (1990).

Statistical analysis

The physicochemical parameters and microbial data were subjected to Analysis of Variance (ANOVA) at 5% level of significance. The Least Significance Difference (LSD) test was used to separate the difference between means at the same level of probability using SPSS (Version 22.0) statistical package (APHA, 2005)

RESULTS

Physicochemical parameters

The physico-chemical parameters recorded in this study are represented in Table 1. The biological oxygen demand (BOD), total dissolved solid (TDS), sulphate, nitrate and magnesium was highest in the earthen pond (A) with mean values of 3.67±0.417 mg/L, 25.25±0.370 mg/L, 4.55±0.016 mg/L, 3.12±0.023 mg/L and 1.28±0.042 mg/L respectively, followed by concrete pond (B). Also, concrete pond recorded the highest in temperature (T°), ion concentration (pH), alkaline, calcium (Ca), potassium (K), with the mean values of 28.17±0.234°C, 7.10±0.252, 31.17±0.189 mg/L, 0.53±0.027 mg/L and 11.68±0.016 mg/L respectively, while collapsible plastic tank (C) recorded the highest mean values of dissolved oxygen (DO), conductivity and transparency with the mean values of 9.83±0.240 mg/L, 17.19±0.013 µ/cm and 60.68±0.667 cm/L. Table 2 shows the comparisons of various values of the physic-chemical parameters obtained in this research and the accepted established world standard limits for fish culture.

DISCUSSION

Fish as aquatic organisms lives in water and carry out all their physiological activities in the water. Their growth and survival depend a lot on the biotic (animal) and abiotic (physico-chemical) factors of the water (Ugwu *et al*., 2006). As a result of improved knowledge in fish production, this has necessitated the culturing of fish in different types of aqua receptacles, and this study shows the physico-chemical parameters and microbiological level of waters housed in these receptacles A, B, and C. The study showed that the physico-chemical parameters of cultured media (water) from A, B, and C ponds analyzed were within the desirable and acceptable limits. The pH mean values of 6.48±0.023, 7.10±0.252, and 7.05±0.041 were obtained for A, B, and C, respectively, with A showing highest acidic level. These values were similar to those obtained by Stone and Thomforde (2003) and Fafioye *et al*. (2005) in their respective research with culturing fish in similar receptacles. The slight mean water temperature difference recorded may be a result of the type of receptacle used, and the frequency of changing water. This is in agreement with the difference in temperature recorded by King (1998), which he opined might be due to differences in light penetration and rate of absorption of light by culturing water. This study shows that the differences in the values of the physico-chemical parameters can be related to the environment; such as geological characteristics of the watershed, rainfall, and

Table 2. Mean Physico-chemical parameters of A, B, and C and established world standard.

Parameters	Mean±SD	S.O.N.	W.H.O.	FMEnv.
Temperature ^{0}C	26.27±1.984	Ambient	<35	27
Ph	6.88±0.281	6.50 – 8.50	6.50 – 8.50	6.00 – 9.00
DO mg/L	9.27±1.0 40	-	4.00 – 6.00	8.00 – 9.00
Conductivity µs/cm	15.27±0.411	1000	300	-
BOD mg/L	3.32±0.460	-	6.00	10.00
TDS mg/L	19.85±4.195	-	500	500
Transparency cm/L	51.22±0.667	5.00	10.00	< 7.00
Alkalinity mg/L	29.01±2.441	-	600	-
Sulphate mg/L	3.00±o.887	-	500	500
Nitrate mg/L	2.32±0.597	-	20.00	50.00
Magnesium mg/L	1.18±o.259	-	-	-
Calcium mg/L	0.38±0.225	-	200	-
Potassium mg/L	10.68±0.707	-	-	-

W.H.O.: World Health Organization (2005); F.E.P.A.: Federal Emergency Protection Agency (1991); S.O. N.: Standard Organization of Nigeria (1970).

Table 3. Bacteria isolates from water samples of different culturing receptacles A, B and C.

Receptacles	Appearance	Observation	Bacteria count CFU
Earthen pond	Brown, cloudy water	Profuse growth of *Escherichia coli, Streptococcus faecalis, Proteus sp., Clostridium perfringes, Staphylococcus sp. Pseudomonas aeruginosa*	0.74×10^7
Concrete pond	Light brown, cloudy water sample	Heavy growth of *Streptococcus faecalis, Staphylococcus sp*. and *Escherichia coli* seen	1.18×10^7
Collapsible plastic tank	Light greenish, cloudy water sample	Growth of *Escherichia coli* and*Staphylococcus sp.* seen	1.66×10^7

amount of run-off (Fafioye *et al*., 2005). The dissolved oxygen (DO) mean values of 8.72±0.019, 9.25±0.016, and 9.83±0.240 for A, B, and C water samples respectively were in agreement and slightly above those obtained by Saloom and Duncan (2005), who reported that the minimum dissolved oxygen should not be less than 3.00 mg/L for tropical fishes like *Clarias gariepinus*. The mean values and standard deviation of alkalinity were 29.01±2.441, and according to Bhatnagar and Devi (2013), the optimum alkalinity for fish culturing is between 20 to 100 mg/L. The values obtained in this research were within the accepted range of WHO (2009), FEPA (1991), and SON (1970), which makes these receptacles suitable for fish culture. The presence and intensity of infestation of organisms (fish) reared in water by microbial organisms (pathogens) are dependent on the water quality (Poonkundran, 2019). Nitrate concentration in this study had a mean value of 2.32±0.597 mg/L. It is important that the ammonia level in the pond is controlled because some forms of ammonia are very harmful to fish, except in nitrate form, which if produced in abundance can lead to algal bloom (eutrophication), which might result in depletion of oxygen in water and decline in water quality. Although nitrate form is not however harmful to cultured organisms. The total dissolved solid (TDS) mean values obtained in this study compared reasonably with the results obtained by Eze and Ogbaran (2010).

The microbial load of water and foodstuff is very important, and this is due to an increase in awareness among consumers for the safety of the food products they consume. The result of the microbial organisms that showed gram negative -ve bacteria were dominant isolates from earthen > concrete > collapsible plastic tank with bacteria count (CFU) of 0.74×10^7, 1.18×10^7, and 1.66×10^7 respectively). Which was found to be growing profusely in earthen, heavily in concrete, and not heavily in collapsible plastic tanks, as shown in Tables 3 and 4. This observation is supported by Chukwura (2001), who stated that pathogens in water are usually out numbered by bacteria because they are easier to detect and that they are not found, does not mean that the water is free from pathogens. The bacteria status of culturing water depends on a wide variety of factors influencing the environment. The most important being faecal, sewages, and organic matter content (Zmysłowska *et el*., 2003). This study showed that there was a significant difference ($p< 0.05$) in

Table 4. Biochemical characterization of isolates from samples of A, B, and C receptacles.

Shape	Edge	C	P	E	GS	IT	CT	MT	CO.T	OJ	Organism
Cocci cluster	Dente	Butyrous	Creamy	Raised	+ve	-ve	+ve	-ve	+ve	-ve	*Staphylococcus sp.*
Cocci chain	Lobate	Butyrous	Creamy	Raised	+ve	-ve	-ve	-ve	-ve	-ve	*Streptococcus faecalis*
Cocci short	Lobate	Filament	Smooth	Curved	-ve	-ve	+ve	+ve	+ve	-ve	*Proteus sp*
Rod chain	Dente	Mucoid	Smooth	Raised	-ve	-ve	+ve	+ve	-ve	-ve	*P. qeruginosa*
Rod	Rough	Mucoid	white	Raised	-ve	-ve	+ve	+ ve	-ve	-ve	*Escherichia coli*
Rod	Rough	Mucoid	Purple	Raised	-ve	-ve	-ve	-ve	-ve	-ve	*C. perfrigens*

Key: -ve = Negative, +ve = Positive, C = Consistency, P = Pigmentation, E = Elevation, GS = Gram stain, IT =Indole Test, CO.T = Coagulase Test, OT = Oxidase Test.

the bacteria count of treatments A, B, and C culturing waters of the isolates; *Escherichia coli*, *Streptococcus faecalis*, *Proteus sp*., *Clostridium perfringes*, *Staphylococcus sp.,* and *Pseudomonas aeroginosa*, were highest in earthen pond followed by the concrete pond and collapsible plastic tank in that order as shown in Table 3. However, the relatively high occurrence of outbreaks of food-borne diseases in many countries, including developed ones, has resulted in increased concern and intensive investigation of sources of food-borne pathogens (Monaghen and Hutchison, 2010).

Conclusion

The result of this research shows that fish can successfully be cultured in different types of receptacles under right physico-chemical parameters, and the microbial load of the culturing water is dependent on the type of receptacle housing it. It is advocated that proper management of water qualities of culturing water should be monitored, in order to reduce the level of microbial load, which can lead to fish disease, death, reduced fish quality, and cause ill-health when consumed by human being.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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