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Microbiological Quality of Fish Pond Water

OMOKARO OBIRE & OVIENOVIE VINCENT
Rivers State University, Port Harcourt, Nigeria

ABSTRACT Microbial infection of farmed fish has resulted in fish diseases resulting in fish kills and huge economic loss to the farmer. The present study on the microbiological quality of fish pond water was carried out on three fish ponds stocked with catfish (*Clarias gariepinus*) located in Rivers State. Standard methods of microbiological analysis were employed in the evaluation of total heterotrophic bacteria, coliform, and fungi. The concrete pond Mgboushimini recorded the highest total heterotrophic bacterial count and lowest fungal count while the earthen pond Isua recorded the highest fungal and total coliform counts. The concrete pond of the campus had the lowest count of heterotrophic bacterial and total coliform. The bacteria isolated were *Aeromonas*, *E. coli*, *Klebsiella*, *Pseudomonas*, *Salmonella*, *Shigella*, *Staphylococcus* and *Streptococcus* species. *Enterobacter* occurred in Mgboushimini and Isua samples, *Proteus* occurred only in Mgboushimini while *Serratia* occurred only in Campus pond. The fungi isolated were *Aspergillus*, *Penicillium*, *Mucor* and *Fusarium* species. Statistical analysis showed that there were significant differences between the ponds *at* $p \geq 0.05$ confidence levels in the microbial counts and frequency of isolation of the organisms. The high microbial counts and presence of *E. coli* and other enteric bacteria indicated that the ponds were contaminated with fecal material. This is attributed to poor hygienic pond environment and their exposure to runoff water and animal manures as well as from human activities. The presence of known fish pathogens such as *Aeromonas*, *Pseudomonas*, and *Streptococcus* and other potential human pathogens calls for concern.

Keywords: Fish ponds, contamination, pathogens, *Aeromonas*, *E. coli*, *Pseudomonas*.

Introduction

Fishes are the most popular animal cultured in the pond and are among the edible food sources naturally in water, consumed by man and containing many nutrients such as protein, minerals, fats, oil etc. They provide at least 20% of animal protein to a larger percentage of the world's population (FAO, 2002). Oladipo and Bakole (2013) pointed that fish is less tough and more digestible when compared with beef, mutton, chicken and bush meat. Because of the greater digestibility of fish, it is even recommended medically to patients with digestive disorders such as cancer (Eyo, 2001). Fish has essential nutrients required for supplementing both infants and adult diet (Abdullahi *et al.,* 2011). According to Adekoya and Miller (2014), fish and its products contributes more than 60% of the total protein in adults especially in rural areas.

In Nigeria, fishes are reared in different controlled environment which could be ponds (concrete or earthen), vats (wooden or fibre glass) and plastic. The earthen pond system has been the conventional method of fish rearing but with scarcity, cost and unavailability of land for this earthen pond system, the concrete tank culture system is gaining ground (Onome and Ebinimi, 2010). Fishes cultured in these controlled environments has been found to be contaminated and this results in questionable water quality (Okpokwasili and Ogbulie, 1998).

Water is very important in fish pond. Water plays a vital role in the proper functioning of the earth ecosystem and also essential for fish and living creatures for metabolism. Fish requires water to carry out its function such as swimming, feeding, digestion, breeding, respiration and excretion (Anita *et al.,* 2013). There are various sources of water, including well water, borehole water, stream water, river water, rain water etc., which can be supplied to the fish pond.

Pond water is a body of standing water either natural or manmade which is usually smaller than a lake (Gogio and Sharma, 2013; Douglas *et al.,* 2015). Just like other water bodies do face pollution problems resulting from effluent discharged from industries, domestic waste, land and agricultural drainage, pond water is not left out in this problem as the problem results in degradation of water quality (Rajiv *et al.,* 2012).

Like other animals, fish also have some extent of water quality parameter in which they can perform optimally. According to Samir *et al.,* (2014) water quality is one of the most important limiting factors in fish production as it directly or indirectly affects the feeding efficiency, growth rate and overall health status.

This study is designed to evaluate the population bacteria and fungi, total coliform and fungi and types of microorganisms and hence the water quality of fish pond water. Objective of study is to isolate, characterize, and identify the types of micro-organisms present in fish pond water. This will unravel the type of microorganisms and their effect on fish or fish diseases associated with the microorganisms.

Materials and Methods

Study Area and Sample Collection

The study area of the fish ponds were three different locations viz; Mgboushimini community, Isua community in Ahoada west L.G.A and Rivers State university campus all in Rivers State which is within the Niger Delta region of Nigeria. The region in addition to other practice also practices fish production having both concrete and earthen fish pond stocked with catfish (*Clarias gariepinus*).

Water samples were aseptically collected from the different fish ponds using sterile 50cl plastic bottles. Samples were collected biweekly for a period of three (3) months. A total of thirty-six (36) water samples were collected. Upon collection, the water samples were immediately transported to the laboratory in an ice packed cool box for microbiological analysis within two hours of collection.

Enumeration of Microbial Count

A serial dilution of each pond water was made up to $x10^{-6}$ and 0.1ml aliquot of each dilution was inoculated on freshly prepared sterile nutrient agar and Sabouraud dextrose agar plates (SDA), using the spread plate technique. The plates were then incubated at 37°C for 24h for the nutrient agar plates while the SDA plates were incubated for five days under room temperature. Colonies that developed on the nutrient agar plates were counted and the number of colony forming unit (CFU/ml) was calculated for total heterotrophic bacteria. While colonies/spores that developed on the SDA plates were counted and the number of spore forming unit (SFU/ml) was calculated for total fungi. The most probable number (MPN) technique which included the presumptive test, confirmation test and completed test was used for the estimation of total coliform. It was performed as described by Verma *et al*., (1999).

Characterization and Identification of Bacterial isolates

Discrete colonies were all sub cultured to obtain pure colonies. This was done by streaking a loop full of a particular isolate on an already prepared NA and SDA plate for bacteria and fungi respectively. The sub cultured NA plates were incubated at 37°C for 24 hours while the SDA plate was for four days. The pure cultures were accordingly stored in NA slant (bacteria) at 4°C and SDA (fungi) slants in the refrigerator for further studies.

The pure cultures that grew on the nutrient agar plates were further purified and characterized based on the following standard bacteriological tests; Gram Staining, Motility Test, Biochemical Tests, Catalase Test, Oxidase Test, Coagulase Test, Citrate Test, Starch Hydrolysis, Urease Production, Methyl Red-Vogues Proskauer (MRVP), and Sugar Fermentation Test. The isolates were presumptively identified based on the results of their cultural, microscopic and biochemical characteristics, and with comparison with those of known taxa of Bergey's manual of determinative Bacteriology (Holt *et al*., 1994).

Characterization and identification of fungal isolates

Routine macroscopic and microscopic procedures were carried out on the pure fungal isolates. In the macroscopic examination, the colony structure was observed, nature of growth, aerial and substratum region were also observed for colour. In the microscopic examination, two drops of lactophenol cotton blue was used placed in the center of a sterilized clean grease free glass slide. Using a sterile inoculating needle, a small piece of the fungal isolate was transferred and teased onto the slide with lactophenol cotton blue and covered with a cover slip. The preparation was then viewed and examined under the microscope first at low power (×10) and then at high power (×40).

The fungal features noted were the; surface appearance, structure of colony margin, colony colour, structure of hyphae, vegetative structure, somatic structure, and reproductive structure. The fungal isolates were identified based on the results of their cultural, macroscopic, and microscopic characteristics such as sporangia, conidia, arthrospores, and vegetative mycelium, septate and non-septate hyphae according to Barnett and Hunter (1972).

Statistical analysis

The results obtained in the course of the study was subjected to statistical analysis using the computer based program SPSS. Analysis of variance (ANOVA) and the Duncan's multiple range tests were used to test for significance and mean separation respectively at 5% level of confidence.

Result

The result of the microbial count showed that the total heterotrophic bacterial count for pond water samples A, B, and C ranged from 9.2×10^8 CFU/ml to 1.20×10^9 CFU/ml, from 5.7×10^8 CFU/ml to 6.3×10^8 CFU/ml and from 2.1×10^8 CFU/ml to 4.3×10^8 CFU/ml respectively. While the fungal count ranged from 1.1×10^8 SFU/ml to 2.5×10^8 SFU/ml, from 1.2×10^8 SFU/ml to 3.0×10^8 SFU/ml and from 9.0×10^8 SFU/ml to 2.1×10^8 SFU/ml respectively. On the other hand, the most probable number index was for total coliform of the ponds was 26 MPN index/100ml, 70 MPN index/100ml and 9 MPN index/100ml respectively. Sample B which is earthen pond water from Isua had the highest fungal and total coliform counts while Sample C which is concrete pond water from the campus had the lowest total heterotrophic bacterial count and lowest total coliform counts. Sample A which is also concrete pond water from Mgboushimini recorded the highest heterotrophic bacterial count and lowest fungal count.

The following bacterial isolates; *Aeromonas spp*, *Escherichia coli*, *Klebsiella*, *Pseudomonas* sp, *Salmonella* sp., *Shigella* sp, *Staphylococcus* sp, and *Streptococcus* sp occurred in all the pond water samples. *Enterobacter sp*. occurred in samples A and B, *Proteus sp* occurred only in sample A, while *Serratia sp* occurred only in sample C. The frequency of isolation of bacteria in the fish pond water samples is shown in Figure 1. *E. coli* had the highest frequency, immediately followed by *Staphylococcus* sp in the Mgboushimini and Isua (A and B) pond waters, while *Staphylococcus sp* had the highest frequency immediately followed by *E. coli* in the Campus (C) pond water. On the other hand, *Salmonella sp* had the lowest frequency in sample A while *Klebsiella sp* had the lowest frequency in samples B and C.

Statistical analysis showed that there were significant differences between the ponds *at* $p \geq 0.05$ confidence levels in the microbial counts and frequency of isolation of the organisms.

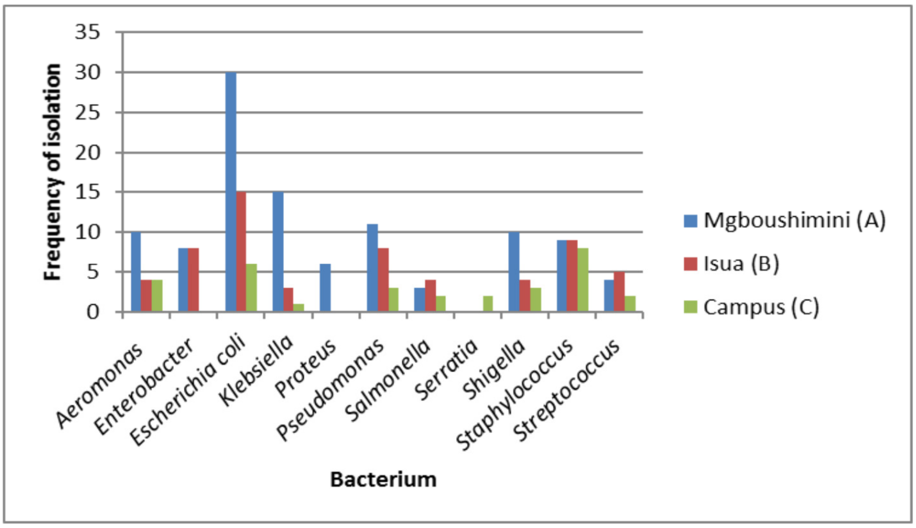

Fig. 1: Frequency of isolation of bacteria in the fish pond water samples

The frequency of isolation of fungi in the fish pond water samples is shown in Figure 2. The fungi isolated were; *Aspergillus*, *Fusarium*, *Mucor* and *Penicillium*. *Aspergillus* and *Penicillium* occurred in all the pond water samples. *Mucor* occurred in samples A and B while *Fusarium* occurred in samples B and C. *Aspergillus* recorded the highest frequency of isolation while *Fusarium* recorded the lowest.

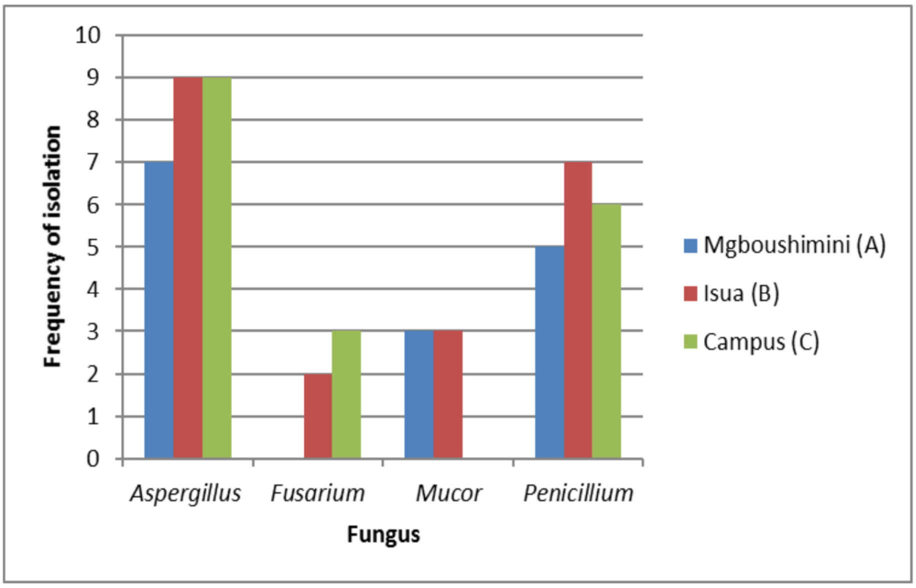

Fig. 2: Frequency of isolation of fungi in the fish pond water samples

Discussion

The present study has revealed the microbial counts of both concrete and earthen fish ponds in Rivers State. The counts of total heterotrophic bacteria, coliforms and fungi vary significantly between the various ponds. The concrete pond water from Mgboushimini (sample A) recorded the highest total heterotrophic bacterial count and lowest fungal count. Isua (B) earthen pond water recorded the highest fungal and total coliform counts, while campus (C) concrete pond recorded the lowest total heterotrophic bacterial and total coliform counts.

The bacteria isolated from the three pond waters *Aeromonas*, *Enterobacter*, *E. coli*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Salmonella*, *Serratia*, Shigella, *Staphylococcus* and *Streptococcus*. Bacteria are naturally present in fish pond water and sediment, being regarded as integral to the biological structure acting upon the metabolic ecosystem and bearing basic role to water quality (Macedo *et al.,* 2011).

A number of these organisms are found in the feces of animals. Faeces contaminated water used for fish pond fertilization may pose sanitary risk to the fish itself and in general to humans who consumes these fish or their products. Fish pond system with their sanitary condition may deteriorate along time when food waste, feces and other manure are constantly added to fish ponds (Macedo *et al.,* 2011). Examples of bacteria that are known to cause disease are the *Salmonella typhi* and pathogenic *E. coli*, which causes typhoid fever and gastroenteritis respectively.

The presence of coliforms and *E. coli* in particular is an indication of the presence of fecal contamination of the fish pond waters. These fecal materials may have resulted through runoff, fertilization of the fish ponds with manures from animal origin which is discharged into the ponds, or possibly excreted by the fish into the ponds (Kay *et al.,* 2008). *E. coli* dominated the bacterial species isolated in terms of frequency of occurrence immediately followed by *Staphylococcus sp* as they occurred virtually in all the water samples. The dominance of *E. coli* in occurrence is naturally expected because of its high association with faeces of animals and human origin since the fish in the pond would naturally defecate into ponds. This is in conformity with the study of Kay *et al.,* (2008), Obire and Minimah (2013) and Njoku *et al*., (2015). The different groups of bacteria isolated from these ponds are in line with the report of Dabbor (2008) and Danba *et al.,* (2015) who reported similar organisms.

The bacteria such as *Aeromonas*, *Pseudomonas*, and *Streptococcus* isolated from the fish pond water samples are known pathogens of fish. Many of these organisms have also been reported in transmission of diseases to humans. *Pseudomonas*, *Proteus*, *Staphylococcus* species have been implicated in food poisoning (Oni *et al.,* 2013).

Salmonella sp is seen to have been the cause of salmonellosis could result in severe typhoid fever (enteric fever) or salmonella fever which in humans and bacteriaemia. *Enterobacter* species are associated with urinary tract infections, wound infections, and septicaemia, especially in persons with poor health. *E. coli* and *Shigella sp* have been implicated for a number of gastroenteric disease such as diarrhea (traveller's disease), dysentery, vomiting, fever, colitis hemolytic ureamic syndrome with renal failure.

Staphylococcus sp has also been implicated in food poisoning. E.g. *Staphylococcus aureus* which have been reported in food poisoning outbreak of some food materials, the presence of *Staphylococcus* is an indication of contamination from fish handlers as 80% of them is being harbored by man as normal micro flora (Obire and Minimah, 2013).

Fungi infection is an important economic and limiting factor in intensive fish production. The fungi isolated from the pond waters were species of *Aspergillus, Fusarium, Mucor,* and *Penicillium*. *Aspergillus* dominated the fungi population of this study followed by *Penicillium*. These organisms have been reported to gain entrance into the environment through dead plant materials and are believed to remain for a long period of time (Obire and Anyanwu, 2009). *Penicillium* species also dominated fungal species reported by Eze and Ogbaran, (2010). Njoku *et al*., (2015) also reported the occurrence of *Fusarium* and *Mucor* species.

Sample C which is concrete pond water from the university campus had the lowest total heterotrophic bacterial count and lowest total coliform counts. Sample A which is also concrete pond water from Mgboushimini recorded the highest total heterotrophic bacterial count and lowest fungal count. Sample B which is earthen pond water from Isua had the highest fungal and total coliform counts. All the fungi isolated in this study were present in sample B (an earthen fish pond). This is attributed to the fact that the earthen pond being a natural environment serves as a more conducive environment for their proliferation and growth of the microorganisms. The difference in the results recorded also attributed to the sanitary conditions of the surroundings of the various fish ponds as evidenced in the Mgboushimini recording the highest total heterotrophic bacterial count and Isua recording the highest fungal and total coliform counts. While the concrete pond water from the university campus recorded the lowest total heterotrophic bacterial count and lowest total coliform counts.

Conclusion

Just as farm land is important to crops in terrestrial agriculture, so is water to fish farming aquaculture. Fishes performs all their body function in water. Source of water pollution includes effluent of untreated waste that is dumped directly into water bodies, runoff containing faecal material etc.

Pathogenic microbes cause many diseases both in wild and cultured fish. They may vary from a primary pathogen to that of an opportunistic invader of a host (Inglis *et al.,* 1994). Fish may harbor pathogens on or inside its body after exposure to contaminated water or food. Disease is a departure of the body from the normal health and may in some case lead to death. Maintaining a hygienic pond environment is the best preventive method of checking disease outbreak in cultured fish.

This study has revealed that the pond waters were contaminated with potential pathogenic bacteria that could have severe effect on fish cultivated in these ponds since the microbial quality of any fish pond water is a reflection of the microbial flora of the fish itself. These potential pathogens could lower fish yield, cause diseases, economic loss and equally endanger the life of the ultimate consumers (human) particularly if the fish harvested from the ponds are not properly processed.

Recommendations

Daily or regular drainage and replenishment of fresh water fish pond water; Earthen pond should be constructed in such a way that it prevents the inflow of runoff waste water into it as some of these microbes may have occurred as a result of runoffs; Maintaining a hygienic pond environment as to prevent disease outbreak. Fish should be properly processed before consumption.

Correspondence
Obire, Omokaro
Department of Microbiology, Rivers State University
P.M.B. 5080, Port Harcourt, Nigeria
E-mail: omokaro515@yahoo.com

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