



# World News of Natural Sciences

An International Scientific Journal

WNOFNS 53 (2024) 223-230

EISSN 2543-5426

---

---

## Bacteriological evaluation of fish pond waste water from different fish ponds in Owerri Imo State, Nigeria

**Maduwuba Maryjoy Chidinma**

Department of Microbiology, Faculty of Biological Sciences, Imo State University,  
P.M.B. 2000, Owerri, Imo State, Nigeria

E-mail address: [mjaychichi1@yahoo.com](mailto:mjaychichi1@yahoo.com)

### ABSTRACT

The importance of fish farming can never be over emphasized because it's a major provider of dietary protein, source of income and employment for the growing populations in Nigeria. Similarly, the importance of the bacteriological evaluation of fish pond water can never be over emphasized as it is a major indicator of the suitability and sanitary condition of the aquaculture pond and the fish in particular. This study evaluated the bacteriological profile of different fish pond water located within Owerri metropolis. A total of 10 fish pond waste water samples were collected and analyzed using different selective microbiological media and isolates identified using gram staining and biochemical reactions. Result shows that the total heterotrophic bacteria count ranged from  $6.4 \times 10^3$  CFU/mL -  $7.5 \times 10^7$  CFU/mL, total coliform count ranged from  $2.1 \times 10^4$  CFU/mL -  $5.6 \times 10^7$  CFU/mL, total vibrio count ranged from  $2.7 \times 10^3$  CFU/mL -  $5.9 \times 10^5$  CFU/mL while the *Salmonella* / *Shigella* count ranged from  $1.9 \times 10^5$  CFU/mL -  $5.9 \times 10^5$  CFU/mL. A total of nine bacterial isolates were identified which includes *Staphylococcus* spp, *Escherichia* spp, *Vibrio* spp, *Salmonella* spp, *Enterobacter* spp, *Shigella* spp and *Klebsiella* spp. The result revealed a high bacterial load in the fish pond water and therefore recommends constant water change to prevent the possible buildup of potential pathogenic bacteria in fish and introduction of these organisms into the environment.

**Keywords:** Fish pond, bacteria, pathogens, waste water, environment, fish

## 1. INTRODUCTION

Fish farming is the fastest growing farming business in Nigeria. It is a form of aquaculture which involves the breeding and rearing of fishes for commercial purposes using artificial methods. It's a lucrative sector of the countries agribusiness and the increase in the demand for fish which is a major source of protein, only help to increase its popularity [1]. Fish is less tough and more digestible when compares to beef, chicken, mutton, and bush meat. Because of this greater digestibility of fish, it is recommended medically for patients with digestive disorders such as cancer [2].

Due to high demand for fish, there has been an in the increase in the number of fish ponds in Nigeria. Fishes are reared in different water culture media or confinement such as concrete, earthen or plastic ponds. Concrete and earthen ponds have been the widely used culture system for fish [3].

The contamination of these culture systems has been attributed to poor water quality, high stocking densities and the use of animal manure and contaminated feed [4]. The most significant resource for aquaculture is water. Successful fish farming depends on the water physical, chemical and biological characteristics, and on the species nutrition management. All these factors are related and require careful and constant monitoring to avoid contamination of the aquaculture environment [5].

High quality water is an important aspect in sustaining the life of fish along with all other organism in an aquaculture environment because fish requires water to carry out its function like breeding, respiration, excretion and feeding [6]. Water has been known to be one of pathogens-transmitting components. Water of good quality is essential for the viability of aquatic lives such as fish. The contamination of fish pond water has been attributed to questionable water quality, high stocking densities and feed (animal manure) which contain organic matter and introduces a wide range of microorganism into the pond [7, 8]. Common bacterial pathogens isolated from fish feed include *Aeromonas hydrophila*, *Corynebacteria* sp., *E. coli* 0157:H7, *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Salmonella typhi*, *Serratia* sp., *Shigella dysenteriae*, *Staphylococcus aureus* [9, 10]. Since the microbial flora of a cultivated fish is a reflection of its aqueous environment [11], this paper aim to evaluate the bacteriological quality of different fish pond water located within Owerri metropolis Imo state in order to ascertain their suitability for aquaculture practice..

## 2. MATERIALS AND METHODS

### 2. 1. Study Area

The study area is the fish farms located within Owerri metropolis. The areas have a tropical climate and the average humidity is about 70%. The coordinates lies between 5.5096°N and 7.0391°E. Owerri is one of the most populous cities in Imo State South- Eastern Nigeria. The area is inhabited by civil servants, traders, casual workers and small scale farmers.

### 2. 2. Sample collection

Water samples were collected from ten (10) different fish farms located within the owerri metropolis and labeled sample A - J. The waste water from each of the fish ponds were sampled in duplicate. The waste water from each fish farm was collected at about 10 – 15 cm depth

aseptically from the ponds using a sterile 500 ml screw cap glass jar. The containers were properly labeled and transported to the laboratory in an ice chest [12].

### 2. 3. Bacteriological analysis of fish pond water

Total culturable heterotrophic bacterial counts, total culturable coliform counts, total culturable *Salmonella/Shigella* counts and total culturable vibrio counts were evaluated in the samples microbiologically as follows:

#### 2. 3. 1. Isolation and enumeration of total culturable bacterial population

Collected fish ponds effluent samples were subjected to a tenfold serial dilution using physiological saline. A serial diluted sample of 100  $\mu\text{L}$  ranging from  $10^{-3}$  –  $10^{-6}$  dilutions of each sample was inoculated on nutrient agar for total heterotrophic bacterial count, *Salmonella/Shigella* agar (SSA) for total *Salmonella/Shigella* count and thiosulphate citrate bile salt agar (TCBS) for total vibrio count in duplicates using the spread plate technique. All agar plates were incubated at 37 °C for 24 – 48 hours under aerobic condition.

The most probable number (MPN) was adopted in determining the total coliform bacteria using MacConkey broth and five tubes technique [8]. All positive tubes from the MPN tubes were sub-cultured on EMB agar plates in duplicate and incubated at 35 °C for 24 hrs.

Microbial load of the incubated plates were determined. Distinct colonies were sub-cultured severally until pure cultures were obtained. Different pure cultures were sub-cultured to different agar slant, incubated appropriately and stored in refrigerator at 4 °C for further analyses.

#### 2. 4. Identification of isolates

The cultural, morphological and biochemical characteristics of the individual isolates were monitored. The biochemical test used in the identification and characterization of the isolates includes Gram staining, motility, indole production, methyl red, citrate utilization, oxidase, catalase, coagulase, and sugar fermentation test. The results were compared with the Bergey's manual of determinative bacteriology [13].

### 3. RESULTS

The total culturable bacterial population comprising of total heterotrophic bacterial counts, total coliform counts, total vibrio counts and total *Salmonella Shigella* counts are represented in Table 1. The total heterotrophic bacterial counts (THBC) revealed that sample A had a THBC of  $4.3 \times 10^7$  cfu/ml, sample B  $5.11 \times 10^5$  cfu/ml, sample C  $7.3 \times 10^7$  cfu/ml, sample D  $6.5 \times 10^5$  cfu/ml, sample E  $7.4 \times 10^5$  cfu/ml, sample F  $6.4 \times 10^5$  cfu/ml, sample G  $6.3 \times 10^5$  cfu/ml, sample H  $7.2 \times 10^5$  cfu/ml while sample I and J had a THBC of  $6.5 \times 10^5$  cfu/ml and  $7.4 \times 10^5$  cfu/ml respectively. The highest THBC of  $7.3 \times 10^7$  cfu/ml was observed in sample C while sample B had the lowest THBC of  $5.11 \times 10^5$  cfu/ml.

The total coliform count (TCC) revealed a TCC of  $5.6 \times 10^7$  cfu/ml in sample A,  $7.7 \times 10^5$  cfu/ml in sample B,  $4.37 \times 10^6$  cfu/ml in sample C,  $5.5 \times 10^5$  cfu/ml in sample D,  $2.1 \times 10^5$  cfu/ml in sample E,  $2.3 \times 10^5$  cfu/ml in sample F,  $7.0 \times 10^5$  cfu/ml in sample G,  $5.8 \times 10^5$  cfu/ml in sample

H,  $5.9 \times 10^4$  cfu/ml in sample I and  $2.1 \times 10^4$  cfu/ml in sample J. Sample A had the highest TCC of  $5.6 \times 10^7$  cfu/ml followed by sample C while sample J had the lowest TCC of  $2.1 \times 10^4$  cfu/ml. The total vibrio count (TVC) showed that sample A had a TVC of  $5.3 \times 10^4$  cfu/ml, sample B  $2.7 \times 10^3$  cfu/ml, sample C  $4.31 \times 10^3$  cfu/ml, sample D  $4.9 \times 10^5$  cfu/ml, sample E  $5.9 \times 10^5$  cfu/ml, sample F  $5.1 \times 10^5$  cfu/ml, sample G  $4.9 \times 10^5$  cfu/ml, sample H  $3.7 \times 10^5$  cfu/ml, sample I  $4.9 \times 10^4$  cfu/ml while sample J had  $5.9 \times 10^4$  cfu/ml. The highest TVC was observed in sample E ( $5.9 \times 10^5$  cfu/ml) while the lowest TVC was observed in sample B ( $2.7 \times 10^3$  cfu/ml).

The total *Salmonella Shigella* counts (TSSC) revealed a TSSC of  $6.3 \times 10^7$  cfu/ml in sample A,  $4.3 \times 10^6$  cfu/ml in sample B,  $2.31 \times 10^4$  cfu/ml in sample C,  $3.2 \times 10^5$  cfu/ml in sample D,  $1.9 \times 10^5$  cfu/ml in sample E,  $3.1 \times 10^5$  cfu/ml in sample F,  $1.7 \times 10^5$  cfu/ml in sample G,  $2.5 \times 10^5$  cfu/ml in sample H,  $3.2 \times 10^5$  cfu/ml in sample I and  $1.9 \times 10^5$  cfu/ml in sample J. the peak TSSC of  $6.3 \times 10^7$  cfu/ml was observed in sample A while the lowest TSSC of  $1.7 \times 10^5$  cfu/ml was observed in sample G.

The gram staining technique and other biochemical tests carried out to characterize the resulting bacterial isolates revealed the presence of *Staphylococcus* spp., *Escherichia* spp., *Pseudomonas* spp., *Vibrio* spp., *Salmonella* spp., *Enterobacter* spp., *Shigella* spp. and *Klebsiella* spp. as probable bacterial genera present in the fish pond waste water sampled. This is represented in Table 2.

**Table 1.** Total bacterial counts of fishpond waste water.

Samples	(THBC) (CFU/mL)	(TCC) (CFU/mL)	(TVC) (CFU/mL)	(TSSC) (CFU/mL)
A	$4.3 \times 10^7$	$5.6 \times 10^7$	$5.3 \times 10^4$	$6.3 \times 10^7$
B	$5.11 \times 10^5$	$7.7 \times 10^5$	$2.7 \times 10^3$	$4.3 \times 10^6$
C	$7.3 \times 10^7$	$4.37 \times 10^6$	$4.31 \times 10^3$	$2.31 \times 10^4$
D	$6.5 \times 10^5$	$5.5 \times 10^5$	$4.9 \times 10^5$	$3.2 \times 10^5$
E	$7.4 \times 10^5$	$2.1 \times 10^5$	$5.9 \times 10^5$	$1.9 \times 10^5$
F	$6.4 \times 10^5$	$2.3 \times 10^5$	$5.1 \times 10^5$	$3.1 \times 10^5$
G	$6.3 \times 10^5$	$7.0 \times 10^5$	$4.9 \times 10^5$	$1.7 \times 10^5$
H	$7.2 \times 10^5$	$5.8 \times 10^5$	$3.7 \times 10^5$	$2.5 \times 10^5$
I	$6.5 \times 10^5$	$5.9 \times 10^4$	$4.9 \times 10^4$	$3.2 \times 10^5$
J	$7.4 \times 10^5$	$2.1 \times 10^4$	$5.9 \times 10^4$	$1.9 \times 10^5$

Results represent mean values of duplicate counts

**Table 2.** Biochemical characteristics of isolate from all the samples

S/N	Gram	Catalase	Coagulase	Citrate	Indole	Methyl red	Motility	Oxidase	Glucose	Fructose	Lactose	Sucrose	Manitol	Sorbitol	Probable organism
1	+	+	+	+	-	+	-	+	+	+	+	+	+	+	<i>Staphylococcus</i> spp.
2	-	+	-	-	+	+	+	-	+	+	+	+	-	A/G	<i>Escherichia</i> spp.
3	-	-	-	+	-	-	+	+	+	-	-	-	A/G	A/G	<i>Pseudomonas</i> spp.
4	-	-	-	+	+	-	+	+	+	+	-	+	-	-	<i>Vibrio</i> spp.
5	-	=	-	+	-	-	+	-	+	+	-	-	+	+	<i>Salmonella</i> spp.
6	-	+	-	+	-	-	+	-	+	A/G	A/G	A/G	A/G	A/G	<i>Enterobacter</i> spp.
7	-	+	-	+	+	+	+	-	A/G	A/G	-	+	A/G	A/G	<i>Shigella</i> spp.
8	-	+	-	+	-	-	-	-	A/G	A/G	A/G	A/G	A/G	A/G	<i>Klebsiella</i> spp.

#### 4. DISCUSSIONS

The current study sought to determine the acceptability of pond water for fish culture by evaluating various bacteriological characteristics of water from ten selected fish farms in Owerri. The total heterotrophic bacteria count revealed a relatively high bacterial concentration in the fish pond waste water and varied within the ponds in this investigation. The bacterial load were high because of the ideal environmental circumstances for bacterial development, as well as the organic matter content present in fish pond water as a result of the meal utilized to feed the fishes. Adebami *et al.*, [14] reported similar high heterotrophic bacterial counts ranging from  $7.30 \times 10^5$  cfu/ml to  $1.6 \times 10^6$  cfu/ml within their investigation period. The high bacterial load could also have resulted from the duration of the fish pond water. Also the quality and physico-chemical properties of the water used in feeding the ponds affect the bacterial load of the waste water. The results revealed that gram negative bacteria were the dominant bacteria isolated from the ponds which include *Escherichia* spp, *Salmonella* spp, *Vibro* spp, *Shigella* spp, *Pseudomonas* spp, *Klebsiella* spp and *Enterobacter* spp, while *Staphylococcus* spp is the only Gram positive bacteria present in all the samples. This finding is in line with species recovered in previous research on the microbiological profile of concrete and earthen fish ponds conducted by Njoku *et al.*, [8]. A number of these species, particularly *Escherichia* spp. and *Enterobacter* spp., have been identified in animal faeces [14]. The faecal material could be the consequence of pond fertilization with animal dung that is dumped directly into the fish ponds

or fish excretion into the ponds [15]. Water polluted with faeces used for fish pond fertilization may constitute a health risk to the fish and, more broadly, to humans who consume these fish or their products. Given that the microbial flora of a cultivated fish is a reflection of its aquatic environment. The presence of coliforms in the pond water is also an indication of pond water pollution with faecal materials, which may result in the presence of pathogenic microorganisms in the fish [11]. Organisms isolated from fish or the aquatic environment can cause infectious diseases in people depending on the season, the patients' interaction with fish and related environments, dietary patterns, and the strength of the immune system of the exposed individual [16, 17]. *Salmonella* spp. is the causative agent of salmonellosis, which can cause severe typhoid fever (enteric fever) or salmonella fever in humans, as well as bacteremia. *Salmonella enterica* has been identified as the causative agent of the greatest number of enteric infections worldwide [18]. Waterborne diseases such as Cholera and food poisoning, gastroenteric disease such as diarrhoea (traveller's disease), dysentery, vomiting, fever, colitis hemolytic ureamic syndrome with renal failure can be transmitted by consuming improperly cooked fish cultivated in these ponds. *Enterobacter* species have been linked to urinary tract infections, wound infections, and septicemia, particularly in the elderly. Food sickness has also been linked to *Staphylococcus* spp. For example, *Staphylococcus aureus*, a typical human flora, has been implicated in food poisoning outbreaks of some food items. The presence of *Staphylococcus* is a sign of contamination from fish pond water handlers because 80% of them are harboured by people [19, 20].

The many bacterial species that have been identified from these ponds are in agreement with the study of Olalemi and Oluyemi, [21] on fish pond waste water, which claims that allochthonous bacteria from feed that has been introduced to the ponds is the primary source of pathogenic contamination that are significant for human health. Additionally, identical species were found in the microbiological analysis of the fish pond at El-Quater by Dorota *et al.* [22] and Ismail *et al.*, [23] who worked on microbiological and physiochemical assessment of selected fish pond water sample in southwest Nigeria.

## 5. CONCLUSION

The bacteriological evaluation of the fish pond water indicates a high bacterial load in all ponds. These bacteria are possible pathogens and opportunistic microbes capable of causing serious impact on the 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. The presence of these organisms could causes diseases to the fish, resulting in economic loss and eventually affects the final consumer of the fish if it is improperly cooked. It is therefore recommended that fish pond water be constantly changed and monitored to reduce the buildup of these organisms in the ponds before discharging into the environment.

## References

- [1] S.A. Adewuyi, B.B. Phillip, I.A. Ayinde & D. Akerele (2010) Analysis of Profitability of Fish Farming in Ogun State, Nigeria. *Journal of Human Ecology*, 31:3, 179-184, DOI: 10.1080/09709274.2010.11906313

- [2] Musa, J., Dada, P. , Adewumi, J., Akpoebidimiyen, O., Musa, E., Otache, M. and Yusuf, S. (2020) Fish Pond Effluent Effect on Physicochemical Properties of Soils in Southern Guinea Savanna, Nigeria. *Open Access Library Journal*, 7, 1-15. doi: 10.4236/oalib.1105990
- [3] Fakorede, C.N., Fatokun, E. N., Philip-kantiok, B., Iwu, C.J. and Jaja, I.F., Bacteriological assessment and antibiotics susceptibility profile of bacteria recovered from pond water, fish skin and gut in Ile-Ife, Osun State, Nigeria. *Notulae Scientia Biologicae*, (2019), 5(1): 15-19
- [4] Mukwabi, D. M., Okemo, P. O., Otieno, S. O., Oduor, R.O. and Macharia, C. M. Temperature and pH influence on bacterial pathogens infecting farmed Nile tilapia in aquaculture systems in Bungoma County Kenya. *International Journal of Fisheries and Aquatic Studies*, (2019), 7(1). 190-197
- [5] Gorlach-diva, K., Pacheco, C., Carvalho, L.C., Melo-Junior, H.N., and Crispim, M.C. The influence of fish culture in floating net cages on microbial indicators of water quality. *Brazillian Journal of Biology*, (2013), 73(3): 457-463
- [6] Anita, A. and Pooja, D. Water quality guidelines for the management of pond fish culture. *International Journal of Environmental Science*, (2019), 5(2)
- [7] Okpokwasili G. C. and Ogbulie, J.N. Bacterial and metal quality of tilapia (*Oreochromia nilotica*) in aquaculture system. *International Journal of Enviromental Health Research*. (1998), 3: 190-202
- [8] Njoku, O.E., Agwa, O.K. and Ibiene, A.A. An Investigation of the microbiological and physiochemical profile of some fish pond water within Niger Delta Region of Nigeria. *European Journal of Food Science and Technolgy*. (2015), 3: 4, 20-30
- [9] Omojowo, F.S. and Omojasola, P.F. Antibiotic resistance pattern of bacterial pathogens isolated from poultry manure used to fertilize fish ponds in New Bussa, Nigeria. *Albanian Journal of Agricultural Science*, (2013), 12: 81-85
- [10] Onianwah, I.F, Stanley, H.O. and Oyakhire, M.O. Microorganism in aquaculture development. *Global Advanced Research Journal of Microbiology*. (2018), 7(8): 127-131
- [11] Oni, T.A., Olaleye, V.F. and Omafuvbe, B.O. Preliminary studies on associated bacterial and fungal load of artificially cultured *Clarias gariepinus*. Burchell 1822 fingerlings. *Ife Journal of Science*, (2013), 15(1): 9-16
- [12] Abu, G.O. and Wondikom, A.C. Isolation, characterization and antibiotic resistance profile studies of bacteria from an excavated pond in Port Harcourt Metropolis, Nigeria. *Journal of Appl Sci Environ Manag*, (2018), 22(8): 1177-1184.
- [13] Shils ME, Hermann MG. Unproved dietary claims in the treatment of patients with cancer. *Bull N Y Acad Med*. 1982 Apr; 58(3): 323-40
- [14] Adebami, G.E., Fasiku, S.A., Solomon, O.D. and Babalola, B.A. Physicochemical and microbial evaluation of different fish ponds waste waters and the antibiotic profile of isolated bacteria. *Ethiopian Journal of Environmental Studies and Management*, (2010), 13(4): 509-521. doi:<http://ejesm.org/doi/v/34.11>



- [15] Sule, I.O., Agbabiaka, T.O., Ahmed, R.N., Saliu, B.K. and Olayinka, K.J. Bacteriological and physicochemical analysis of waste water from fish ponds. *Ethi. J. Technology* (2016), 8(4): 87-95
- [16] Onyango DM, Wandili S, Kakai R, Waindi EN. Isolation of Salmonella and Shigella from fish harvested from the Winam Gulf of Lake Victoria, Kenya. *J Infect Dev Ctries.* 2009 Mar 1; 3(2): 99-104
- [17] Karl McDonald, Da-Wen Sun. Predictive food microbiology for the meat industry: a review. *International Journal of Food Microbiology*, Volume 52, Issues 1–2, 1999, Pages 1-27, [https://doi.org/10.1016/S0168-1605\(99\)00126-9](https://doi.org/10.1016/S0168-1605(99)00126-9)
- [18] Bell, C. and Kyriakides A. Salmonella: a practical approach to the organism and its control in foods. Publisher: Oxford; Malden, Mass. Blackwell Science Ltd. (2002), 59: 269
- [19] Obire, O and Minimah, T. R. Mycoflora of fresh shrimps (*Penaeus aztecus*) from different markets in Port Harcourt, Nigeria. *e- Journal of Science and Technology* (2013), 8(4): 87-95
- [20] Ogbonna, D.N. and Inana, M.E. Characterization and Multiple Antibiotic Resistance of Bacterial Isolates Associated with Fish Aquaculture in Ponds and Rivers in Port Harcourt, Nigeria. *J. Adv. Microbiol.* (2018), 10(4): 1-14
- [21] Olalemi, A. and Oluyemi B.. Environmental Assessment of Bacterial faecal Pollution Markers In Overlying Waters from an Earthen Fish Pond. *J Microbiol Biotech Food Sci.* (2018), 7 (6), 566-570
- [22] Dorota, L; Iwona. G, Izabella; Z. and Mariusz T. Sanitary and Bacteriological studies of water and European Catfish (*Silurus Glanis L*) During Wintering Wintering. *Environ. Studies Manag.* (2002), 9(2): 167-178
- [23] Ismail, B. O., Oyindamola, J. S., Sulaimon, A. A., Emmanuel, O. O., and Abdulrazaq, O. O. Microbiological And Physicochemical Assessments of Selected Fish Pond Water Sample in South-West, Nigeria. *Al-Hayat: Journal of Biology and Applied Biology*, (2023), 6(1): 1-13 DOI. 10.21580/ah.v6i1.14166