Microbiological and Physicochemical Properties of Effluents (Waste) From Fish Farm

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ABSTRACT

Rearing catfish is very common in many communities in Nigeria and the wastewater from these fish ponds are often discharged into the surrounding drains. Assessment of the antimicrobial and physicochemical characteristics of three fish farms stocked with African catfish (*Clarias gariepinus*) was conducted using standard microbiological techniques. The results of physicochemical properties of the water samples showed that the pH, electrical conductivity, dissolved oxygen, biochemical oxygen demand, total dissolved solid, calcium ranged from 5.8 – 5.81, 123 µ/cm – 130 µ/cm, 4.8 mg/l – 6 mg/l, 3 mg/l -5 mg/l, 330 mg/l – 500 mg/l, 19.34 mg/l – 32.3 mg/l respectively. The total bacterial count, total coliform, and f*ungal* count of the wastewater ranged from 6.5 x 10^5 – 7.4 x 10^5cfu/ml, 2 x 10^5 – 3.3 x 10^5 cfu/ml, and 1.9 x 10^5 – 2.11 x 10^5cfu/ml respectively. Some bacterial species isolated from the ponds were *Staphylococcus sp., Bacillus sp., Pseudomonas sp., Salmonella sp.,* and some fungi includes *Aspergillus sp., Penicillium sp., and Cladosporium sp.* It can be concluded from this study that there is need to monitor the quality of wastewater from the fish ponds before being discharged into the environment since potential pathogens were isolated. Good quality water such as well or borehole should be used in the fish pond rather than water from questionable sources such as river, stream and surface runoff. The fish feed should be sourced from reputable manufacturers and water from the pond changed regularly.

Keyword: Aquaculture, effluent, biochemical oxygen demand.

INTRODUCTION

The aquaculture industry is one of the fastest growing agricultural sectors globally. The 2024 edition of "The State of World Fisheries and Aquaculture (SOFIA) said global fisheries and aquaculture production in 2022 surged to 223.2 million tonnes, a 4.4 percent increase from 2020 (FAO, 2024). However, the long-term sustainability of aquatic environment has raised concerns over the environmental impact of this vital sector, due to its negative impact on aquatic ecology and systems (Fernandes *et al.,* 2001; Hasan, 2001). This is because intensification of aquaculture involves the use of highly nutritious feeds and other chemical products, which generate wastes that, in most cases, are difficult to curtail and toxic to aquatic lives (Pandey and Satoh, 2006). Effluent water containing wastes are discharged in all aquaculture systems (Tacon and Forster, 2003). The number of wastes generated from aquaculture practices depends on the culture system characteristics, choice of species, feed quality and management practices (Wang *et al.,* 2005). The discharge of wastewater in the form of effluents into the surrounding soil surfaces could lead to the alterations of the receiving environments.

High organic load in aquaculture wastewater can result in the eutrophication of receiving water bodies, which causes a lot of havoc on the biodiversity in aquatic ecosystems (Hardy and Gatlin, 2002; Lazzari and Baldisserotto, 2008). Nitrogenous wastes, which are the major component of aquaculture waste, are highly toxic to macro-fauna in the open water body. Stephen and Farris (2004) reported that an increase in ammonia concentrations could elevate blood ammonia, which is highly toxic to fish. Suspended solids in aquaculture wastes in receiving water bodies cause interstitial clogging and substrate embeddedness (Magni *et al.,* 2008). The deposition of solids and sediments could enhance the growth of heterotrophic bacteria and increase the

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formation of colony-forming units, leading to additional interstitial clogging and deoxygenation (Carr and Goulder, 1990).

Fish farming is an agricultural activity with economic importance. Production occurs intensively with the daily use of rations and high cultivation densities. Consequently, this promotes an increase in nitrogen and phosphorus in the water as the result of fish excretion and feed leftovers. Thus, the release of nutrient-rich effluents that occur during cultivation (due to water renewal rates), at the moment of fish removal, and at the end of cultivation, produce impacts on the environment. Increasing income and urbanization shall be responsible for increasing demand for fish and meat by 2020 in the developing countries. There is an increasing demand for high-value fish species in developed countries where urbanization is high. Thus, the demand for high-value species may increase in developing countries as urbanization increases (Bhaskar *et al.*, 2010).

METHODOLOGY

Study Area

Umuahia is the capital city of Abia State in south-eastern Nigeria. Umuahia is located along the rail road that lies between Port Harcourt to its south and Enugu city to its north. Umuahia has a population of 359,230 according to the 2006 Nigerian census. Umuahia is renowned for being a railway and agricultural market center, which attracts traders and farmers from neighbouring towns to sell their produce, such as yams, cassava, corn (maize), taro, citrus fruits, and palm oil and kernels. Uzuakoli is a city in Bende LGA of Abia State. It is located at latitude 5° 37' 39" N and longitude 7° 33' 18" E

Sampling

Six samples of water (2 samples from each farm) were collected from three different fish farms (1 farm in Umuahia and 2 farms at Uzuakoli) which was obtained by pumping water from the effluent water through a tap into a sterile labelled bottle (farm 1 is A and B, farm 2 is C and D while farm 3 is E and F) and transported to Microbiology laboratory.

Media Preparation

All media was prepared according to manufacturer's instructions autoclaved at 121°C at 15 PSI for 15 minutes, then allowed to cool to about 45°C before dispensing into a sterile plate. The plates will be incubated at 37°C for 24 hours to test for sterility (Cappuccino and Sherman, 2014).

Microbiological Analysis

Tenfold serial dilution was done and the 6^{th} tube was used for the analysis. Total heterotrophic bacterial counts was determined using nutrient agar. Total coliform counts were determined using MacConkey agar. Total fungal counts were determined using Sabrouad dextrose agar supplemented with chloramphenicol. Total fecal coliform counts were determined using Eosin Methylene Blue agar. Potential pathogenic bacterial counts were determined using blood agar and organisms observed was subjected to coagulase test too. SDA for fungal growth was at 28 ± 2°C for 3-5 days. MaConkey and Nutrient agar plates was at 37°C for 24-48 hours. EMB agar plates was incubated at 44°C for 48 hours. Blood agar plates was incubated at 35 ± 2°C for 18-72 hours. Plates was observed for visible growth and microbial counts and the load is calculated using this

$$\frac{\text{number of colonies X dilution factor}}{\text{volume}}$$

Discrete colonies were sub cultured to obtain pure isolates on fresh plate (Onyeagba, 2015).

Determination Of The Physicochemical Properties Of The Various Water Samples.

This involved the analyses of the following parameters; pH, conductivity, temperature, total hardness,

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VIII August 2025

carbonate, bicarbonates, biochemical oxygen demand, dissolved oxygen, turbidity and presence of mineral. They were carried out following the standard protocols and methods AOAC, (2016).

Determination Of Ph

The water pH was determined using Jenway multipurpose tester (Jenway HANA 1910 Jenway HANA Instruments, Woonsuket Rhode Island USA). The probe of the instrument was rinsed in a portion of the water sample to be analyzed and the water discarded. The probe was dipped into 100ml of the different water samples (spring, stream, well and borehole water) and read after 3-5 minutes. The probe was left in the water sample for 3-5 minutes to stabilize. The probe was rinsed with deionized water to avoid cross contamination after testing each sample.

Determination Of Temperature

Temperature was determined using Jenway multipurpose tester (Jenway HANA 1910 Jenway HANA Instruments, Woonsuket Rhode Island USA). The probe of the instrument was rinsed in a portion of the water sample to be analyzed. Then the probe was immersed in 100ml of the test water sample and read after 3-5 minutes.

Determination Of Total Dissolved Solids (Tds)

Total dissolved solids (TDS) were measured with a Lovibond cm-21 Tintometer. The meter was immersed in the water, ensuring a continuous flow of water past the membrane to obtain a steady response on the meter. The values were read off while still in the water, (mg/l). The instrument was rinsed with a portion of the water sample to be analyzed and the rinse water was discarded. The meter was immersed in 100ml of the water sample, and the average of the reading was taken. TDS of the water samples was determined by gravimetric method. After the filtration for TSS analysis, the filtrate was heated in oven at 100^0C until all the water was completely evaporated. The remaining mass of the residue represents the amount of TDS in the sample. This was carried out according to the standard of (ASTM, 2004).

Determination Of Electrical Conductivity

The conductivity was determined using Jenway multipurpose tester (Jenway HANNA 1910, Jenway HANA Instruments, Woonsuket Rhode Island USA). The probe of the instrument was then rinsed with a portion of the water sample to be measured and the rinse water was discarded. Then the probe was immersed in a beaker containing the water sample. The reading was taken after 3 - 5 minutes, once the beaker was free from bubbles.

Determination Of Metals

Determination of Calcium: The EDTA titration method (AOAC, 2016) was used in determining calcium. To a 50-ml sample or portion diluted to 50-ml in a conical flask, 2 ml of NaOH was added to raise pH ranging from 12 to 13. About 0.1g murexide (i.e. mixture of 200mg ammonium purpurate and 100g NaCl) indicator was added. The treated sample was titrated with 0.01m EDTA to the purple color end point. Calcium hardness as mg/1 $CaCO_3$: $\frac{\mathbf{A\ xB\ x1000}}{\boldsymbol{Volume\ of\ sample}}$ *Where:* A is ml titrant for sample and B mg CaCC>*3* equivalent to 1.00 ml EDTA.

Determination of Phosphate: Phosphate was determined using the stannous chloride method (AOAC, 2016). *2.0*ml ammonium molybdate reagent and *0.2*ml stannous chloride reagent was mixed and added to 50 ml of the sample. The stannous chloride stayed for 12 minutes, and then at another 10 minutes the absorption of the treated sample was read on Spectronic 21d at 690nm. Phosphate level was obtained by reading off absorption level from standard curve of known standards treated as the samples. The detection level is 0.05 mg/1.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VIII August 2025

Determination of Nitrate: Nitrate measurement was determined by the Brucine method (AOAC, 2016). To a 2.5 ml sample contained in test tube (immersed in ice-cold water), 2.5 ml of H_2SO_4 solution was added and mixed by gentle swirling. After cooling, the absorption of the resulting yellow color was read on Spectronic 2ID at 410nm. The nitrate-nitrogen was estimated from calibration curve treated in the same way as the samples. Limit of detection was 0.05 mg/1.

RESULTS

Table 1: Physicochemical Analysis Of The Effluent From The Three Fish Farms.

Farm Parameter	A	B	C	D	E	F	WHO	FWS
EC (µs/cm)	123	111	130	127	132	130	NS	100-100
pH	5.81	5.85	5.8	5.79	5.87	5.8	6.5-8.5	65-9.5
DO (Mg/L)	5	6	5.2	4.8	5	5	6.5-8.5	<5
BOD (Mg/L)	4	3.5	3.3	3	5	5	6	6-Mar
Temp. (°C)	29	29	28	28	28	28	Ambient	25-30
TDS (Mg/L)	330	500	458	552	407	409	500	-
Turbidity (NTU)	120	260	290	276	146	139	10	-
Calcium (Mg/L)	24.2	19.34	20.41	20.5	23.64	32.3	200	25-100
Phosphate (Mg/L)	60.73	80.32	77.57	78.21	61.1	60.9	-	0.03-2.0
Nitrate (Mg/L)	2.53	3.1	3.21	3.45	3.50	3.49	50	0.1-4.0

Keys: NS: Not Stated. EC: Electrical Conductivity WHO : World Health Organization DO: Dissolved Oxygen

FWS: Fish Water Standard BOD: Biochemical Oxygen Demand TDS: Total Dissolved Solid.

Table 2: Microbial Count From The Effluent From The Three Fish Farms.

	A	B	C	D	E	F
THBC	6.5×10^5	6.3×10^5	6.4×10^5	7.4×10^5	6.8×10^5	6.9×10^5
TCC	3.3×10^5	3.0×10^5	3.3×10^5	2.6×10^5	3.0×10^5	3.0×10^5
TFC	2.11×10^5	2.5×10^5	1.8×10^5	2.4×10^5	2.2×10^5	1.9×10^5
TFCC	1.9×10^5	1.7×10^5	2.0×10^5	1.5×10^5	1.7×10^5	1.7×10^5
TPBC	1.5×10^5	1.4×10^5	1.3×10^5	1.29×10^5	1.3×10^5	1.4×10^5

Keys: THBC: Total Heterotrophic Bacterial Count. TCC: Total Coliform Count. TFC: Total Fungal Count.

TFCC: Total Fecal Coliform Count. TPBC: Total Pathogenic Bacterial Count

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VIII August 2025

Table 3: Occurrence Of Bacterial Isolates From The Effluent From The Fish Farms.

	A	**B**	**C**	**D**	**E**	**F**
Staphylococcus species	+	+	+	+	+	+
Bacillus species	+	+	+	+	+	+
Pseudomonas species	-	+	-	-	-	+
Escherichia coli	+	-	+	-	+	-
Enterobacter species	+	+	+	+	+	+
Salmonella species	+	-	+	-	-	-
Shigella species	+	-	+	-	-	-
Aeromonas species	+	+	+	+	+	+
Klebsiella species	+	+	+	+	+	+

Keys: +: Present -: Absent

Table 4: Occurrence Of Fungal Isolates From The Effluent From The Fish Farms.

	A	**B**	**C**	**D**	**E**	**F**
Aspergillus species	+	+	+	+	+	+
Penicillum species	+	+	+	+	+	+
Fusarium species	+	+	+	+	+	+
Mucor species	+	+	+	+	+	+
Clasdosporium species	+	+	+	+	+	+

Keys: +: Present -: Absent

Table 5: Percentage Prevalence Of Bacterial Isolates From The Effluent From The Fish Farms.

	A	**B**	**C**	**D**	**E**	**F**
Staphylococcus species	4(33.33)	5(41.67)	4(33.33)	4(33.33)	5(41.67)	5(41.67)
Bacillus species	4(33.33)	3(25)	3(25)	3(25)	4(33.33)	3(25)
Pseudomonas species	-	6(50)	-	-	-	6(50)
Escherichia coli	6(50)		5(41.67)	-	5(41.67)	-
Enterobacter species	2(16.67)	2(16.67)	3(25)	3(25)	3(25)	3(25)
Salmonella species	6(50)	-	6(50)	-	-	-

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VIII August 2025

Shigella species	6(50)	-	6(50)	-	-	-
Aeromonas species	3(25)	3(25)	4(33.33)	4(33.33)	4(33.33)	4(33.33)
Klebsiella species	3(25)	3(25)	4(33.33)	4(33.33)	4(33.33)	4(33.33)

Keys: -: Absent. N: 9

Table 6: Percentage Prevalence Of Fungal Isolates From The Effluent From The Fish Farms.

	A	**B**	**C**	**D**	**E**	**F**
Aspergillus species	4(80)	3(60)	3(60)	3(60)	3(60)	3(60)
Penicillum species	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)
Fusarium species	3(60)	4(80)	4(80)	4(80)	5(100)	5(100)
Mucor species	4(80)	3(60)	3(60)	3(60)	3(60)	3(60)
Clasdosporium species	5(100)	4(80)	4(80)	4(80)	4(80)	4(80)

Keys: N: 5

DISCUSSION

Total Heterotrophic Bacterial Count (THBC), Total Fungal Count (TFC), Total Pathogenic Bacterial Count (TPBC), Total Coliform Count (TCC), Total Fecal Coliform Count (TFCC) were assessed in the three fish farms. Microbial count was recorded at CFU/ml. it was observed that microbial count did not vary significantly between ponds. THBC was highest at D (7.4 X 10^5 CFU/ml). The load was high due to the temperature which was optimum for bacterial growth and also due to the organic matter load from within the fish pond resulting from the diet used in feeding the fishes. This makes the pond water an ideal culture medium for the proliferation of bacterial pathogens causing bacterial infections in fish and an important cause of fish poisoning (Njoku *et al.,* 2015).

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The diverse groups of bacteria isolated from these ponds are in line with the report of Okpokwasili and Ogbulie (1993) who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principal source of bacteria of health importance and Daboor (2008) also reported similar organisms in the microbiological study of El-quanter fish pond.

The presence of pathogenic organisms like *Salmonella* and *Shigella* leads to the transmission of water borne diseases such as typhoid fever, cholera, and gastroenteritis (Okafor *et al.,* 2020) on the consumption of improperly cooked fish cultivated from these ponds. The presence of *E. coli* in the water indicates the possible presence of causative agents of many gastro intestinal diseases (Ampofo and Clark, 2010).

Pseudomonas and *Staphylococcus* species have been implicated in food poisoning (Oni *et. al.,* 2013). *Aeromonas* species were also predominantly present in these ponds. This according to Das and Mukheyee (1999) shows that *Aeromonas* species are one of the most opportunistic pathogens for fresh water fish and the main etiological agents in disease outbreak where several mortalities were recorded.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue VIII August 2025

Fungal infections are an important economic and limiting factor in intensive fish production. This is consistent with the work of Obire and Anyanwu (2009) who noted that *Aspergillus* and *Mucor* species are believed to penetrate into the environment through dead plant materials and remains for a long period of time. The occurrence of *Fusarium* and *Mucor* species could be attributed to the fact that there was a conducive environment for their growth and proliferation due to the presence of soil and plants in the ponds.

The pH recorded in almost all the ponds were below the range required for aquaculture. pH measurement helps to determine if the water is a proper environment for fishes although most fishes can tolerate pH as low as 5.0 (Njoku *et al.,* 2015). Ntengwe and Mojisola (2008) observed that the appropriate pH for increased fish production is 6.0 - 9.0.

Temperature is a factor of great importance for aquatic ecosystem, as it affects the organisms as well as the chemical and physicochemical parameters of water. The optimum condition for increased fish productivity were found to be at 20 – 30 °C (Ntengwe and Mojisola, 2008) and the temperature obtain was within the range and was within the range to support fish productivity. This corroborates with the report of Fatioye (2011) who observed a temperature of 27 – 28 °C in the preliminary studies and water characteristics and bacterial population in Kojalo pond.

Phosphate maybe introduced into the pond through fish feed or through surface runoff and could also be from the material used in the construction of the bonds. These fishes can also store phosphate in their organs and when they die, they release the previously absorbed into the water which triggers the growth of new algae (Njoku *et al.,* 2015).

Higher electrical conductivities of the water samples generally varied and ranged from 111 – 130 µs/cm for the ponds. Electrical conductivity is a useful indicator of mineralization and salinity or total salt in a water sample. The FAO acceptable limit for conductivity in aquaculture is 20 – 1500 µs/cm (DWAF, 1998). This limit was not exceeded in these ponds. Thus, the parameter is suitable for fishes.

The pond effluents had an offensive and unusual odor which might be attributed to microbial decomposition of organic matters in the water. Turbidity is the measure of relative clarity of a liquid. It is an optical characteristic of water and is an expression of the amount of light that is scattered by materials in the water when a light is shined through the water sample. This is due to fine particles suspended in the water, causing cloudiness (Odesiri-Eruteyan & Uribo, 2015). The turbidity values were high in all samples and did not comply with WHO standard. The high turbidity values of the effluents were due to presence of suspended solid particles, planktonic organisms, microbial activities and decomposition of organic matter. This elevated turbidity can obstruct the access of sunlight in the pond making it intricate for aquatic habitat to obtain the positive consequence of light. Dissolved Oxygen (DO) level of the effluents was below FEPA allowable limits. The low values may be due to increase in microbial metabolism and decomposition involving utilization of oxygen and releasing of carbon dioxide (Odesiri-Eruteyan *et al.,* 2017)

CONCLUSION

The study revealed that the ponds were grossly contaminated with pathogenic bacteria that could affect fish cultivated since the microbial quality of any fish pond water is a reflection of the microbial flora of the fish itself. These organisms could lower fish yield, cause diseases and economic loss and equally endanger the ultimate consumers particularly if the fish harvested from the ponds are under processed. The Ministry of Agriculture should ensure that the fish farmers are supplied with healthy fry for their stock. Good quality water such as well or borehole should be used in the fish pond rather than water from questionable sources such as river, stream and surface runoff. The fish feed should be sourced from reputable manufacturers and water from the pond changed regularly.

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