

BACTERIOLOGICAL AND PHYSICOCHEMICAL ANALYSIS OF FISH POND EFFLUENTS IN WARRI AND ITS ENVIRONS, NIGERIA

ABSTRACT

Aims: This study was to ascertain the bacteriological and physicochemical characteristics of fish pond effluents.

Study design: Waste water samples were collected from six (6) different ponds, three (3) earthen ponds (A, B & C) and three (3) concrete ponds (D, E & F).

Place and Duration of Study: The study was conducted in a laboratory in the Department of Environmental Management and toxicology, Federal University of Petroleum Resources, Effurun, Delta State, Nigeria. The research lasted for six months.

Methodology: Standard procedures were adopted for sample collection, microbiological and physicochemical analyses. Waste water samples were analyzed for total bacterial count, total coliform count and Escherichia coli (E. coli) count. Physicochemical parameters measured were pH, electrical conductivity (EC), dissolved oxygen (DO) and temperature and many others.

Results: Pond C which was an earthen pond was found with the highest bacterial, coliform and E. coli. counts ($2.8 \times 10^5 \pm 0.01$, $1.2 \times 10^3 \pm 0.10$ and $0.5 \times 10^2 \pm 0.04$ cfu/ml), and pond F, a concrete pond, recorded the lowest bacterial, coliform and E. coli. counts of ($2.3 \times 10^4 \pm 0.05$, $0.2 \times 10^3 \pm 0.00$ and $0.1 \times 10^2 \pm 0.01$ cfu/ml) respectively. The isolated bacteria species were *Lactobacillus sp.*, *E. coli*, *Klebsiella sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Enterobacter sp.* and *Proteus sp.* The occurrence of the isolated bacteria was highest in pond C with 71.43%. The values of turbidity and total hardness were above allowable limits, while values of other physicochemical characteristics complied with WHO and FEPA standards. The antibiotics susceptibility test of the bacterial isolates revealed multiple antibiotics resistance.

Conclusion: The study revealed that these ponds were disgustingly infected with pathogenic bacteria that could affect cultured fishes by causing diseases, lowering the fish yield and resulting into economic loss, threatening human's health and contaminating the environment where the effluents are discharged into.

Keywords: Fishpond, bacteriological, physicochemical, effluents, antibiotics, microbes.

1. INTRODUCTION

Researches have shown that all fish pond effluents are not friendly to the environment as some of them contain a vast number of pathogenic and multiple antibiotics resistant bacteria. These effluents, also known as waste water, are not treated before they are

being discharged into the environment thereby resulting to environmental pollution and degradation. Thus, an analysis of bacteriological and physicochemical characteristics of fish pond effluents becomes imperative to control this ugly trend.

Statistics has also shown that a vast majority of the world's population derives at least 20 percent of its animal protein intake from fish. Fish is the favored source of much wanted animal protein compared to other animal sources. It is relatively cheaper and greatly suitable without religious prejudice which gives it an added benefit over other sources of proteins (1, 2). Fishes are found mostly in aquatic environments and can be reared for consumption, domestication or commercialization in different culture media or controlled environment such as ponds (concrete or earthen) . Amid these culture systems, concrete tanks and earthen ponds are extensively used due to the ease of control by farmers, cost-effectiveness and convenience. Concrete ponds have been developed and can be easily installed even in an area where it is difficult to dig the soil for earthen ponds. In Nigeria, earthen pond culture system has been the usual means of fish culture. Until now, concrete tanks culture system takes over as land becomes expensive, rare and not readily available (3). Studies have shown that a large number of fish farmers use concrete ponds more than earthen ponds (4). Fishes cultured in these restricted environments have been found to be polluted by microbes (5, 6). The feed used for the fish in these ponds harbor organic matters which initiate a large array of microbes into the ponds (7). Due to increase rate of feeding, animal manure has been an unconventional feed used to complement or completely substitute conventional feeds. Nevertheless, introduction of organic manure leads to discharge of large volume of microbes into the ponds. Fish in ponds commonly suffer from bacterial diseases such as various kinds of skin ulcerations, albinoderma, erythroderma, furunculosis, and verticle-scale disease, primarily caused by bacteria (8). Some of these diseases were reported to be most severe during the dry season,

when there is deterioration in water quality (9). The presence of these microbes in fish ponds is of great public health implication. The pond effluents are also being discharged into the surrounding drains thereby polluting the environment and nearby household drinking water sources. Thus, it is imperative to evaluate and compare the microbial quality of the different fish ponds within the Niger Delta region of Nigeria.

The aim of this study was to carry out physicochemical and bacteriological analyses of fish pond effluents in Warri and its environments.

2. METHODOLOGY

2.1 Sample collection

Waste water samples were collected from six (6) different ponds, three (3) earthen ponds (A, B & C) from a multipurpose fish farm located at Ekpan, Effurun/Warri, Delta State and three (3) concrete ponds (D, E & F) located at Ogbomro Community, Effurun, Delta State. Samples were collected from both concrete and earthen ponds for purpose of comparism.

Waste water samples were obtained with sterile containers. Samples were collected at a depth of 10 – 30 cm below the water surface. Collected samples were preserved in an ice chest and moved to the laboratory for analysis.

2.2 Physicochemical analyses

Measurement of some parameters was carried out on site. Parameters measured were pH, electrical conductivity (EC), dissolved oxygen (DO) and temperature. Physical parameters such as colour and odour were determined by physical examination. Standard methods were adopted for the various in-situ and

laboratory analyses of the effluent samples according to APHA (1998) and as adopted by (11).

2.3 Microbiological Analyses

Water samples were analyzed for total bacterial count, total coliform count and Escherichia coli (E. coli) count. All cultured media were prepared according to manufacturer's specification and autoclaved at 121°C for 15 minutes. Samples were analyzed for total bacterial count using nutrient agar with standard pour plate method. Total coliform Count was analyzed using MacConkey agar and incubated at 37°C for 24 hours. Escherichia coli count (Faecal Coliform) was analyzed using Eosine methylene blue agar (12). These bacteria were identified by both macroscopic and microscopic examinations. Biochemical analyses were carried out for further identification and characterization with reference to Bergey's Manual of Determinative Bacteriology (13)

2.3.1 Susceptibility test

The susceptibility of the bacterial isolates to various antibiotics was determined by the standard disk diffusion method. This was done by placing the antibiotics discs (Gram-positive to Gram-positive bacteria and Gram-negative to Gram-negative bacteria) on the prepared peptone plate that were spread with the test isolates (12-18 hours of

3. RESULTS AND DISCUSSION

Table 1 shows physicochemical characteristics of the pond effluents. pH values ranged from 6.33 to 8.24. The samples were slightly acidic to slightly alkaline. The odour was highly offensive. The values of the BOD and DO of effluents from different ponds were 1.20±0.10mg/l and 1.70±0.01mg/l and 3.0±0.02mg/l and 4.85±0.01mg/l

the test isolates that were diluted to a 0.5% McFarland standard), incubated at 37°C for 24 hours. After 24 hours of incubation, the zones of inhibition were measured to check the susceptibility. Antibiotics used in the analysis were Gram positive: Ciprofloxacin, Norfloxacin, Gentamycin, Amoxil, Streptomycin, Rifampicin, Erythromycin, Chloramphenicol, Ampiclox, Levofloxacin. Gram negative: Tarivid, Reflacine, Ciproflox, Augmentin, Gentamycin, Streptomycin, Ceporex, Nalidixic Acid, Septrin, Amplicin.

2.3.1.1 Multiple Antibiotics Resistance (MAR) Indexing of Isolates

The multidrug resistance of the bacterial isolates were analysed using the multiple antibiotic resistance (MAR) index. The multiple antibiotic resistance (MAR) index was defined as a/b where 'a' represents the number of antibiotics to which the isolate is resistant to, and 'b' the number of antibiotics to which the isolate is exposed to (14, 15).

2.4 Statistical analyses

Descriptive statistics such as mean and standard deviation were used to analyse the data collected (16).

respectively. The TDS and TSS values of effluents ranged from 14±0.09mg/l to 105±0.22mg/l and 1.1±0.00mg/l to 4.7±0.01mg/l respectively. Turbidity value was recorded highest at pond B. Table 2 presents bacteriological population of the different samples. Bacterial, coliform and E. coli counts were recorded highest in pond

C ($2.8 \times 10^5 \pm 0.01$, $1.2 \times 10^3 \pm 0.10$ and $0.5 \times 10^2 \pm 0.04$ cfu/ml) and lowest at pond F ($2.3 \times 10^4 \pm 0.05$, $0.2 \times 10^3 \pm 0.00$ and $0.1 \times 10^2 \pm 0.01$ cfu/ml) respectively.

Table 3 shows the occurrence of the bacteria in the samples. The occurrence of the isolated bacteria was highest in pond C with 71.43% and lowest in ponds D and F with 42.86%. The isolated bacteria were *Lactobacillus sp.*, *E. coli*, *Klebsiella sp.*, *Staphylococcus sp.*, *Streptococcus sp.*, *Enterobacter sp.* and *Proteus sp.* Table 4 shows the biochemical characterization and identification of bacterial isolates. Gram negative bacteria were more

prevalent and the isolated bacteria were more of coliforms. Table 5 presented the multiple antibiotics resistance (MAR) of the bacterial isolates. All the isolates exhibited multiple antibiotics resistance.

Staphylococcus sp. showed the highest antibiotics resistance while *Lactobacillus sp.*, and *E. coli*, exhibited the least resistance.

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Comment [DW2]: Why you did not record species name of bacteria although you refer Biochemical analyses were carried out for further identification and characterization with reference to Bergey's Manual of Determinative Bacteriology for ex. You must say staph aureus

Table1: Physicochemical characteristics of ponds effluents.

Physicochemical Parameters	Standard (FEP A)	Effluent Samples					
		*Pond A	*Pond B	*Pond C	**Pond D	**Pond E	**Pond F
pH	6 – 9	6.35±0.02	6.44±0.01	6.33±0.02	8.34±0.05	7.99±0.02	8.24±0.03
Electrical conductivity (µS/cm)	2500	224±0.21	192±0.19	159±0.13	52±0.15	28±0.11	29±0.13
Total dissolved solids (mg/L)	2000	105±0.22	95±0.14	79±0.12	27±0.08	14±0.12	14±0.09
Nitrate (mg/L)	20	0.67±0.01	5.33±0.10	0.40±0.00	1.07±0.01	1.40±0.04	5.87±0.02
Odour (T.O.N)	Odour less	Offensive Odour	Offensive Odour	Offensive Odour	Offensive Odour	Offensive Odour	Offensive Odour
Turbidity (N.T.U)	N/A	108±0.31	186±0.22	117±0.05	21±0.03	78±0.15	35±0.06
Salinity (Mg/L)	600	24.25±0.13	16.97±0.06	12.12±0.02	4.85±0.03	7.27±0.05	7.27±0.02
Total Hardness (Mg/L)	20	24±0.03	24±0.02	48±0.14	32±0.01	58±0.15	41±0.01
Total suspended solid (Mg/L)	<10.0	2.7±0.02	4.4±0.02	1.1±0.00	1.8±0.01	4.7±0.01	4.5±0.03

Biological oxygen Demand (Mg/L)	10	1.50±0.01	1.70±0.01	1.60±0.01	1.30±0.01	1.70±0.01	1.2±0.1
Dissolved oxygen (Mg/L)	8 – 10	3.25±0.02	3.50±0.02	4.85±0.01	3.45±0.00	3.55±0.01	3.0±0.02
Temperature (°C)	<40	26.0±0.03	26.0±0.05	26.3±0.01	37.7±0.02	32.0±0.05	31.0±0.00

Source: Federal Environmental Protection Agency (FEPA) (1991). The values are expressed in mean ± standard error.

N/A: Not Applicable. * Indicates Earthen pond, ** Indicates Concrete pond.

Table 2: Bacterial population (cfu/ml) of the samples.

BACTERIAL COUNT	POND					
	A	B	C	D	E	F
Bacterial counts on nutrient agar	3.2×10^4 ±0.12	4.2×10^4 ±0.04	2.8×10^5 ±0.01	2.6×10^4 ±0.15	2.5×10^4 ±0.02	2.3×10^4 ±0.05
Total coliform count	0.8×10^3 ±0.06	1.1×10^3 ±0.01	1.2×10^3 ±0.10	0.3×10^3 ±0.05	0.5×10^3 ±0.03	0.2×10^3 ±0.02
Total faecal count	0.3×10^2 ±0.01	0.2×10^2 ±0.00	0.5×10^2 ±0.04	0.1×10^2 ±0.02	0.2×10^2 ±0.01	0.1×10^2 ±0.01

The values are expressed in mean ± standard error.

Table 3: Occurrence of the Bacteria in the samples

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Comment [DW7]: Isolation of bacteria from pond samples

Isolated samples	Pond A	Pond B	Pond C	Pond D	Pond E	Pond F
<i>Klebsiella</i>	-	-	+	-	+	-
<i>Proteus</i>	-	+	+	-	+	-
<i>Streptococcus</i>	+	+	+	+	-	+
<i>Staphylococcus</i>	+	+	+	+	+	
<i>Enterobacter</i>	-	+	-	-	+	-
<i>Lactobacillus</i>	+	-	-	-	-	+
<i>E.coli</i>	+	-	+	+	-	+
% Occurrence	57.14	57.14	71.43	42.86	57.14	42.86

Comment [DW8]: Percent of isolation

Key: + Present - Absent, * Indicates Earthen pond, ** Indicates Concrete pond.

Table 4: Biochemical Characterization and Identification of Isolates

Comment [DW9]: According to Bergey's Manual

Bacterial Isolates	Gram Stain	Shape	Motility	Oxidase	Catalase	Coagulase	Citrate	Urease	Indole	Glucose	Lactose	Gas Prod.	Acid Prod	H ₂ S	VP	MR	Nitrate Reduction	Probable isolates
1	+	Rod	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	<i>Lactobacillus sp.</i>
2	-	Rod	+	-	+	-	-	-	+	+	+	+	+	-	-	+	+	<i>E.coli</i>
3	-	Rod	-	-	+	-	+	-	+	+	+	+	+	-	+	-	+	<i>Klebsiella sp.</i>
4	+	Cocci	-	-	+	-	+	-	-	+	+	+	+	-	-	+	-	<i>Staphylococcus sp</i>
5	+	Cocci	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	<i>Streptococcus sp.</i>
6	-	Rod	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	<i>Proteus sp.</i>
7	-	Rod	+	-	+	-	+	-	-	+	+	+	+	-	+	-	+	<i>Enterobacter sp.</i>

KEY: *Sp: Specie, + Positive – Negative, H₂S: Hydrogen sulphide, MR: Methyl red, VP: Voges proskauer

Table 5: Multiple antibiotics resistance (MAR) of the bacterial isolates

Comment [DW10]: According to (MAR) index

Bacterial Isolates	Number of Antibiotics Resistance (a)	Number of Antibiotics used (b)	MAR Index (a/b)
<i>Klebsiella</i>	4	10	0.4
<i>Proteus</i>	3	10	0.3
<i>Streptococcus</i>	4	10	0.4
<i>Staphylococcus</i>	5	10	0.5
<i>Enterobacter</i>	3	10	0.3
<i>Lactobacillus</i>	2	10	0.2
<i>E.coli</i>	2	10	0.2

DISCUSSION

Water quality study is essential for setting water base line conditions and standards. The pH recorded in all the ponds were within acceptable range required for aquaculture (6.5 – 9.5). This range of pH indicates that the water is a proper environment for fish although most fish can tolerate pH as low as 5.0. The elevated pH values observed suggested that carbon dioxide, carbonate-bicarbonate balance is affected more due to changes in physico-chemical condition (18). The pH values obtained in this study fall within the FEPA permissible limits of effluent and hence, the pH values of the effluents are favorable and environmentally friendly.

From table 1, the pond effluents had an offensive and unusual odour 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 (11). 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 (19)

All samples total hardness from the different ponds was above FEPA allowable limits. The high values may be attributed to high concentration of feeds introduced into the ponds. The low total suspended solid values in the samples suggested that there was no external introduction of solid waste into the ponds. 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

carbondioxide. Temperature is a factor of great importance to aquatic ecosystem, as it affects the organisms as well as the chemical and physicochemical parameters of water (20, 21). The temperature range of the effluent samples was found to be between 26.0°C – 37-7°C and is within the limit that supports fish productivity and the approved limit set by FEPA (40°C for effluent before such effluent can be discharged into any water system).

The effluents from the ponds contained a vast number of potential contaminants. Gram positive and Gram negative bacteria were detected in the effluent samples. Gram negative bacteria were more prevalent. The bacteria population of pond C was the highest while pond F was the lowest. Water and feeds introduced into the ponds could be the sources of contaminants. Water from dirty sources and feeds from organic manure may introduce and promote proliferation of microbes in these ponds. Well water is usually used for concrete ponds while water from rivers, streams and surface runoff are used for earthen ponds, this could perhaps be responsible for higher bacterial load in earthen ponds. Well water is of good quality when used for the pond than water from river, surface runoff or stream as reported by (14). The feeds used for fish in ponds contain organic materials which introduce microbes into the ponds. Omojowo and Omojasola (2013) isolated six bacterial pathogens: E. coli, E.coli 0157:H7, Shigella dysenteriae, Staphylococcus aureus, Salmonella typhi, and Aeromonas hydrophila from Cow dung used as feed in a fish pond. The earthen ponds harboured more microbes than the concrete ponds. The mode of change of water in ponds influences the microbial load. Concrete ponds are completely discharged and refilled with fresh water while earthen ponds use a topping system where more water is added as revealed by (14). This was also

observed in a similar study by (22) that the earthen ponds contain higher microbes than those of concrete ponds.

The isolated microbes included *Klebsiella sp*, *Proteus sp*, *Streptococcus sp*, *Staphylococcus sp*, *Enterobacter sp*, *Lactobacillus sp*, and *E.coli*. The presence of these pathogenic bacteria in the effluents is of a great health implication. Their presence could be of detrimental effects to the fish, man and the receiving environment where the effluents are being discharged into. The presence of *Klebsiella sp*, *Proteus sp*, *Enterobacter sp*, *Streptococcus sp* and *E.coli* also suggested faecal contamination of the ponds from animal dung. This was earlier reported by (9). Coliforms and Escherichia coli are used as indicators in determining water quality. Their presence in water and waste water has significant environmental and health risk to man and receiving environments.

Streptococcus sp proved resistant to Ampiclox, Amoxil, Chloramphenicol and Levofloxacin but susceptible to all other antibiotics on the Gram-positive disc. *Lactobacillus* proved resistant to Amoxil and Ampiclox but susceptible to all other antibiotics on the Gram-positive disc. *E.coli* proved resistant to Reflacine and Septrin but susceptible to all other available antibiotics on the Gram-negative disc. Multiple antibiotics resistances have been reported by many researchers (14, 15). Hence, this study established their reports. Isolation and detection of pathogenic and multiple antibiotics resistant bacteria calls for proper monitoring and disposal of pond effluents.

Conclusion

The study revealed that the physicochemical characteristics and bacteriological quality of the earthen and concrete fish ponds were not significantly different, but there were slight differences in the concentration of nutrients which could be attributed to leaching of

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these substances into the soil of the earthen ponds. The study also revealed that both ponds were wholly soiled with pathogenic bacteria that could affect cultured fishes by causing diseases, thereby lowering fish yield and resulting into economic loss, and jeopardizing human's health and also fouling the environment. While endeavoring to realize monetary remunerations from fish farming, there is desirable need to examine the fish pond effluents at intervals. This is to certify that there are no lethal matters in the ponds that could lead to possible bio-accumulation and magnification. In this way the good health of the aquatic ecosystem, humans and environment can be guaranteed. As many fish farmers are not adequately educated and are not aware of the health and environmental effects of these pond effluents, education on proper ways of detoxifying the wastes and on their disposal is therefore needed.

Glossary of Abbreviations: FEPA: Federal Environmental Protection Agency; WHO: World Health Organization; Sp: species; cfu/ml: colony forming unit/millimeter; APHA: American Public Health Association; BOD: Biological oxygen demand; DO: Dissolved oxygen. H₂S: Hydrogen sulphide, MR: Methyl red, VP: Voges proskauer.

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UNDER PEER REVIEW

