Physicochemical and Microbial Evaluations of Different Fish Ponds' Wastewaters and the Antibiotics Profiles of Isolated Bacteria

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PHYSICOCHEMICAL AND MICROBIAL EVALUATIONS OF DIFFERENT FISH PONDS' WASTEWATERS AND THE ANTIBIOTICS PROFILES OF ISOLATED BACTERIA

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Abstract
Aquaculture is a global practice that entails the breeding of different fish species. It is incessant in the Nigerian community as wastewater released from pond farms into surrounding drainages and water bodies cause pollution. In this study, a comparative analysis of the physico-chemical parameters (temperature, pH, salinity, conductivity, Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD), and microbial evaluation of wastewater samples from concrete, plastic, wooden and earthen fish ponds located in Ogun and Lagos states followed with antibiotics sensitivity profile of the isolates were investigated. The values of temperature, pH, salinity, conductivity, DO and BOD of the wastewaters ranged from 23.83 – 24.67 °C, 6.57 – 9.68, 0.08 – 0.26 ppt, 65.67 – 200.7 µS/cm, 0.67 – 1.63 mg/L and 36.67 – 253.3 mg/L, respectively. Aerobic plate count and coliform count of bacteria isolated from different fish ponds wastewaters ranged from $6.40x10^5$ to $4.20x10^6$ cfu/mL and $2.80x10^3$ to $1.98x10^5$ cfu/mL, respectively. Enterococcus spp., Staphylococcus spp, Streptococcus spp., and Micrococcus spp., Aeromonas spp., Chromobacterium spp., Escherichia spp., Klebsiella spp. Listeria spp., Pseudomonas spp., Proteus spp., Providencia sp., Shigella sp., and Salmonella spp. were isolated from different ponds wastewaters. All selected bacterial isolates were susceptible to ciprofloxacin with the exception of Streptococcus sp., Chromobacterium sp. and Providencia sp. Antibiotics susceptibility pattern of isolated bacteria showed that most of the isolates had very high rate of resistance to the common antibiotics which could pose danger to the environment. The results obtained from this study suggests that control of wastewater release from fish-pond farms to environment is necessary in other to maintain a stable environment, thereby reducing pollution.

Key Words: *Fish pond, Wastewater, Physicochemical parameters, Aerobic plate count, Coliform count, Antibiotics*

Introduction

Fishery products are essential not only in terms of nutrition, but also in international trade, foreign exchange and work possibilities (Adebayo-Tayo *et al*., 2012; Akila and Kumaran, 2018). Fish farming is a profitable trade practiced throughout the globe. Fish and its products are one of the animal protein sources in the world (Emikpe *et al*., 2011). According to the Food and Agricultural Organization (2002), about 56% of the world's protein originates from fish consumption as compared to other sources of animal proteins such as chicken, beef, mutton or pork. It is relatively cheap and extremely acceptable with little or no religious preference and benefits (Philips *et al*, 2004; Emikpe *et al*., 2011).

Agriculture is an essential component of Nigeria's economy that employs more than 70% of the active workforce (FDF, 2009). Catfish productions play an important role in the aquaculture sector in Nigeria with catfish accounts for 98% of fish ponds (Ajayi and Okoh, 2014). Most catfish are grown in the southern area of Nigeria and the sector is of economic importance to some countries (Adekoya *et al*., 2006). Fishes are grown in various controlled environments that could be ponds (concrete or earthen), vats (wood or fiber glass) and plastics (Osawe, 2004). Before now, earthen pond cultivation system was the traditional technique of fish cultivation in Nigeria (Oladimeji *et al*., 2017). It has been estimated in Nigeria that 73% of fish farmers make use of concrete ponds compared to the 27% using earthen ponds (Ugwumba and Chukwuji, 2010).

The water quality of a fish farm is determined by its physicochemical and biological properties (Ehiagbonare and Ogundiran, 2010). The determinants of water quality may include the sources of water and the feed used for fish generated from animal manure that can serve as an appropriate substratum for a broad range of microbial development in the pond (Egbere *et al*., 2010; Ampofo and Clerk, 2010). In the Nigeria community, water sources for earthen ponds are usually untreated surface water, runoffs from streams, rivers, lakes, stored waters, while most concrete ponds use underground water sources. In these ponds, the feed used for fish contains organic cow dung materials and introduces a broad range of microorganisms into the ponds (Onianwah *et al*., 2018). Common bacterial pathogens isolated from fish feed include *Aeromonas hydrophila, Corynobacteria* sp., *E. coli* 0157:H7, *Enterobacter* sp., *Klebsiella* sp., *Proteus* sp., *Pseudomonas* sp., *Acinetobacter* sp., *Salmonella typhi*, *Serratia* sp., *Shigella dysenteriae, Staphylococcus aureus,* (Omojowo and Omojasola, 2013; Onianwah *et al*., 2018). The microbial flora of a cultivated fish is a reflection of its aqueous environment (Oni *et al*., 2013).

Most often, wastewaters are released from these ponds into the environment where they cause pollution and contamination in the surroundings as they contain offensive and potentially hazardous substances (Ikpi and Offem, 2011). Moreover, heavy metals such as zinc, cadmium, chromium, mercury, cobalt, copper, nickel, manganese, vanadium, iron, and molybdenum from industries cause heavy pollution of water bodies and increases the Biological Oxygen Demand (BOD) (Nzomo, 2005). In determining water quality, the use of indicator bacteria such as faecal coliforms is commonly used. Coliforms and *Escherichia coli* are of excellent significance for bacterial indices used in water quality evaluation (Torimiro *et al*., 2014). Physical chemical parameters such as dissolved oxygen, pH, temperature, conductivity, total alkalinity, total hardness, total solids, transparency values, carbon dioxide, nitrite nitrogen, sulphates, carbonates and ammonia are some of the important variables for fish health (Fafioye, 2011; Onianwah *et al*., 2018). Discharge from fish farms of untreated wastewater containing contaminants pollutes the soil and surface water promoting eutrophication and increases BOD (Onyango *et al*., 2009; Onianwah *et al*., 2018).

Moreover, the occurrence of multi-drug resistant pathogens associated with aquaculture and discharged fish pond wastewaters has been on the rise (Abu and Wondikom, 2018). The greatest risk is however the transfer of the resistant genes to other pathogenic bacteria which is eventually transfer to man through consumption has been a major public health concern (Njoku *et al.,* 2015b; Abu and Wondikom, 2018). In this study, the quality of waste water from different fish-ponds were assessed to elucidate their physicochemical parameters, the microbial contaminations, and the antibiotics susceptibility patterns of the isolated bacteria.

Materials and Methods

Sample Collection

Wastewater samples from four varieties of fish ponds – earthen, concrete, plastic and wooden ponds – used in this study were obtained from Sunny Fish farm, Baruwa, Egbeda, Lagos (6.5916^o N, 3.2911^o E) and First Global Bethel Farms, Glorious Avenue, Orimerunmu, Ogun State (6.5514^o N, 3.3768^o E). The wastewater from each of the fish ponds′ type were sampled in duplicate from the two locations at 1 month interval for a period for 3 months (May – July). The wastewaters were aseptically collected into sterile 500 mL screwed plastic containers at about 10 – 15 cm depth at which the caps were opened and closed after filled. The containers were properly labeled and transported to the laboratory on ice for analyses within 8 hours (Abu and Wondikom, 2018).

Physiochemical Analysis of Water Samples

Physical and chemical parameters of the wastewater samples such pH, temperature, total suspended solids, salinity, conductivity, dissolved oxygen and biological oxygen demand were investigated. The pH was determined using a pH meter (HI9813-6, HANNA); the temperature was measured using a thermometer (ACCD370PWFC, THERMCO); the total suspended solids was determined using spectrophotometer (SQ2802, UNICO) as described by Ehiagbonare and Ogunrinde (2010); the salinity was determined using salinity tester (H198319, HANNA); the conductivity was determined using a conductivity meter (HI9813-6, HANNA); dissolved oxygen and biological oxygen demand were determined according to the method described by Olukunle and Oyewumi, (2017).

Microbial Analysis of the Water Sample

Collected ponds wastewaters were serially diluted separately and appropriate dilutions were inoculated into Nutrient agar and MacConkey agar using pour plate method. Nutrient agar and MacConkey agar plates were incubated at

37 °C for 24 – 48 hours under aerobic condition. Microbial load (Aerobic Plate count and Coliform count) of the incubated plates were determined (Njoku *et al*., 2015a). 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. Morphology and biochemical properties of the isolates were used to identify isolated bacteria (Sneath *et al*. 1986; Olutiola *et al*., 2000).

Antibiotics Susceptibility Test

One bacterium from each Genus was subjected to antibiotics susceptibility test. The susceptibility of selected bacteria to antibiotics were determined using Disc Diffusion method on Mueller Hinton agar (Bauer *et al*., 1966). Commercially prepared antibiotics sensitivity disc containing ampicillin (30 µg), augmentin (30 µg), chloramphenicol (30 µg), ciprofloxacin (15 µg), cotrimoxazole (25 µg), erythromycin (15 µg), gentamicin (10 µg), pefloxacin (5 µg), rifampin (5 µg), streptomycin (10 µg), and vancomycin (30 µg) was used to carry out sensitivity test. Set of plates of sterile Mueller-Hinton agar were inoculated with the standardized test organisms using sterile swab sticks and the antibiotic disc was placed on the inoculated medium. The plates were incubated at 37 °C for 24 hrs. After incubation, the plates were observed for zones of inhibition around each of the antibiotics. The diameter of the zone of inhibition were measured in millimeter (mm) and interpreted as sensitive (S) and resistant (R) (CLSI, 2005; Brown and MacGowan, 2010).

Statistical Analysis

The physicochemical parameters of wastewaters samples data were presented as a mean values and analyzed using a descriptive analysis (Bhat *et al*., 2014).

Results

The physicochemical properties of the collected pond water are shown in Table 1. The temperature, pH, salinity, conductivity, dissolved oxygen, and biological oxygen demand of the pond waters ranged from 23.83 - 24.67 ºC, 6.57 – 9.68, 0.08 - 0.26 ppt, 65.67 - 200.7 µS/cm, 0.67 - 1.63 mg/L and 36.67 – 253.3 mg/L respectively.

Table 2 shows aerobic plate and total coliform counts of different ponds wastewaters. Lowest aerobic plate count (6.40 x 10^5 cfu/mL) of bacteria isolated from different pond wastewater was observed in concrete pond wastewater while the highest aerobic plate count (4.20 x 10^6 cfu/mL) was recorded in plastic pond wastewater. Wastewater from concrete pond had highest total coliform count (1.98 x 10^5 cfu/mL), followed by wooden pond wastewater (6.40 x 10^4 cfu/mL) and the least (2.80 x 10^3 cfu/mL) was observed in Earthen pond wastewater.

Table 1: Physicochemical parameters with standard error values indicating pooled or integrated sampling method

Pond types	Physicochemical parameters of the wastewater					
	Temperature (°C)	pH	Salinity (ppt)	Conductivity (µS/cm)	DO (mg/L)	BOD (mg/L)
Concrete	23.83±0.44	8.23±0.12	260±50	200.7±1.45	1.30±0.12	80.00±5.77
Plastic	23.83±0.44	9.68±0.06	210±10	151.0±0.58	0.67±0.09	36.67±8.82
Wooden	24.67±0.17	6.70±0.06	90±10	65.67±0.67	0.73±0.09	50.00±5.77
Earthen	24.17±0.44	6.57±0.09	80±10	74.00±1.16	1.63±0.09	253.30±8.82

Key: DO – Dissolved Oxygen; BOD – Biological Oxygen Demand

Table 2: Bacterial counts of fish pond wastewaters sample

Pond types	Bacterial count (cfu/mL)	
	Aerobic Plate Count	Total Coliform Count
Concrete	6.40×10^5	1.98×10^5
Plastic	4.20×10^6	5.53×10^4
Wooden	7.30×10^5	6.40×10^4
Earthen	1.63×10^6	2.80×10^3

Forty six bacteria from 15 Genera were isolated from different ponds wastewater (Table 3). The highest number of isolates (5) from each Genus were recorded in *Micrococcus*, *Staphylococcus* and *Listeria* species which account for 33 % of the isolates *Enterococcus*, *Streptococcus* and *Escherichia* species had four isolates each. One isolate each of *Providencia* and *Shigella* species were among the 46 isolates.

Table 3: Occurrence of bacterial genera isolated from fish ponds wastewaters

Genera	Number of isolates	Percentage of occurrence
Micrococcus spp.	5	11
Staphylococcus spp.	5	11
Listeria spp.	5	11
Enterococcus spp.	4	9
Streptococcus spp.	4	9
Escherichia spp.	4	9
Chromobacterium spp.	3	7
Enterobacter spp.	3	7
Pseudomonas spp.	3	7
Aeromonas spp.	2	4
Klebsiella spp.	2	4
Proteus spp.	2	4
Salmonella spp.	2	4
Providencia sp.	1	2
Shigella sp.	1	2
Total	46	100

Antibiotics sensitivity profile of selected bacteria is as shown in Table 4. All the selected bacteria were resistant to at least three of the 11 used antibiotics. Highest resistance to antibiotics was observed in *Enterococcus* sp. and *Staphylococcus* sp. that were susceptible to just two (ciprofloxacin and ampicillin) of the 11 antibiotics used. Twelve out of the fifteen selected bacteria screened for antibiotic sensitivity were susceptible to ciprofloxacin. All selected bacteria were resistant to Pefloxacin with the exception of *Micrococcus* sp., *Escherichia* sp. and *Pseudomonas* sp. All used antibiotics were able to inhibit *Listeria* sp. except augmentin, pefloxacin and cotrimoxazole.

Table 4: Antibiotics sensitivity profile of selected bacteria isolated from fish ponds wastewater

Bacterial genera	Antibiotics/Reaction status										
	AUG	GEN	ERY	CPX	PEF	CXT	STR	RFP	CHL	AMP	VAN
Enterococcus sp.	R	R	R	S	R	R	R	R	R	S	R
Micrococcus sp.	R	S	S	S	S	R	R	R	S	R	S
Staphylococcus sp.	R	R	R	S	R	R	R	R	R	S	R
Streptococcus sp.	R	S	R	R	R	S	S	S	S	R	R
Aeromonas sp.	S	S	R	S	R	S	S	S	S	R	R
Chromobacterium sp.	S	R	R	R	R	R	R	S	R	S	R
Enterobacter sp.	R	R	R	S	R	S	S	S	R	R	R
Escherichia sp.	R	S	R	S	S	S	R	R	S	R	R
Klebsiella sp.	R	S	S	S	R	S	R	R	R	R	S
Listeria sp.	R	S	S	S	R	R	S	S	S	S	S
Providencia sp.	R	R	S	R	R	S	S	R	R	R	R
Pseudomonas sp.	S	S	S	S	S	S	R	R	R	R	S
Proteus sp.	S	R	S	S	R	S	S	R	S	R	S
Salmonella sp.	R	R	S	S	R	S	S	R	S	R	S
Shigella sp.	R	R	S	S	R	S	S	R	S	R	R

Key: S - Sensitive; R - Resistant; AMP - Ampicillin; AUG - Augmentin; CHL - Chloramphenicol; CPX - Ciprofloxacin; CXT - Cotrimoxazole; ERY - Erythromycin; GEN - Gentamicin; PEF - Pefloxacin; RFP - Rifampin; STR - Streptomycin; VAN – Vancomycin.

Discussion

The physicochemical characteristics of wastewater that are of special concern are pH, dissolved oxygen (DO), oxygen demand (chemical and biological), solids (suspended and dissolved), nitrogen (nitrite, nitrate and ammonia), phosphate, and metals (Akpor and Muchie, 2011). Also, the major microorganisms found in wastewater effluents are viruses, bacteria, fungi, protozoa and helminthes. Although numerous microorganisms in water are considered to be critical factors in contributing to numerous waterborne outbreaks like pneumonia, diarrhea, meningitis, degenerative heart disease and stomach ulcer, they play many valuable roles in waste effluents (Kris, 2007). Moreover, the wastewater used in this study was collected from fish ponds located in South-western Nigeria. Wastes in water reduces the quality of water used in fish farming and abhorred some pathogenic microbes as well as toxic metals which decreases the quality of the fishes and increases the risk of death. It also resort to health complications and eventual death to humans that consume them (Ogbonna and Inana, 2018).

The finding on the temperature range (23.83 – 24.67 ^oC) of pond water in this study is consistent with the report of Kumar (2004) where he recorded temperature range of 20 - 30 ºC which were within the recommended limits of WHO. Ajayi and Okoh (2014), Omofunmi *et al*. (2016) and Olukunle and Oyewumi (2017) however recorded higher temperature range of 26 - 27 ^oC, 27 – 31 ^oC and 26 – 29 ^oC for pond waters, sampled from Lagos, Araromi and Akure Nigeria respectively.

The hydrogen-ion concentration is an important quality parameter of both natural and wastewater, as it is used to describe the acid or base properties. The pH of Earthen and Wooden pond wastewaters (6.57 and 6.70) were slightly acidic while those of Concrete and Plastic ponds were alkaline (8.23 and 9.68). These pH values were within the acceptable range of 6.5 – 9.0 for healthy fish productions (Njoku *et al*., 2015a). The report of Sule *et al*. (2016) is consistent and within the range of these findings for wastewater samples from Earthen (6.83) and Concrete (8.04) ponds. Olukunle and Oyewumi (2017) also reported a pH range of 7.0 – 10.0 for concrete pond water. The acidic nature of Earthen pond was due to solubilization of ions from the soil into the pond. The report of Ajayi and Okoh (2014) was in disparity with this findings where alkaline pH range of 7.82 - 8.15 were recorded for waste water from Earthen ponds. Low pH in the earthen pond may also be due to the production of carbon dioxide by zooplankton during respiration which negatively affect the breathing of the fish (Onianwah *et al*., 2018).

Salinity is among the most important factor in assessing the quality of water, as it exerts various effects on the vitality of microorganisms. Differences in the salinity of fish ponds are encouraged by marginal changes in the evaporation rate (Sule *et al*., 2016). The optimum salinity range for maintenance of fish health has been reported to be in the range of 15 – 32 ppm (Andrew, 2007). The result from this present study suggests that the salinity content in earthen ponds were slightly higher owing to increased evaporation rate compared to that in concrete pond. Moreover, the water in earthen ponds infiltrate into the soil resulting into water loss.

Electrical conductivity of water is a factor of the ion concentration in it; hence, the more concentrated the ions, the higher the conductivity. The World Health Organization permissible limit for conductivity of water is 100 µS/cm (WHO, 2006). Values of electrical conductivity gotten were within the permissible limit set of WHO. Moreover, the lower range of conductivity obtained could be due to the dilution of the ions in wastewaters, which is often the case during wet season. Previous studies have shown that dilution of water during the rainy seasons lowers the levels of electrical conductivity (Sipaúba-Tavares *et al*., 2007).

Oxygen demand, which may be in the form of BOD or COD, is the amount of oxygen used by microorganisms as they feed upon the organic solids in wastewater (Gray, 2002; FAO, 2007; Akpor and Muchie, 2011). It is necessary for the respiration of aerobic microorganisms as well as all other aerobic life forms. The BOD measures biodegradable organics and needs comparatively lengthy test outcomes (Gray, 2002; Metcalf and Eddy, 2003). The amount of oxygen that can be present in wastewater has been reported to be governed by other inherent factors such as solubility, temperature, partial pressure of the atmosphere and the concentration of impurities such as salinity and suspended solids in the water (Metcalf and Eddy, 2003). The level of dissolved oxygen (DO) should be maintained at 5 ppm for better production in the tropical aquaculture ponds (Saloom and Duncan, 2005). The values obtained in this study were below the recommended value, confirming the poor quality of the water. The low DO obtained may be attributed to the small size of the ponds as well as occurrences of eutrophication due to the presence of manure or inorganic fertilizer used by farmers (Thilza and Muhammad, 2010). Moreover, the presence of microbes and plants have been reported to contribute to the low DO in some aquaculture system (Thilza and Muhammad, 2010).

The BOD of wastewaters is normally measured to estimate the amount of oxygen consumed by microorganisms during the decomposition of suspended waste materials (Olukunle and Oyewumi, 2017). There is a reciprocal relationship between DO and BOD. Ehiagbonare and Ogurinde (2010) in their study observed that the higher the BOD levels, the lower the DO, as the amount of oxygen available in the wastewater is being consumed by bacteria. Moreover, Olukunle and Oyewumi (2017) reported a very low BOD of 12.60 and 12.63 mg/L respectively from Oda Road pond and FUTA pond located in Ondo State, Nigeria contrary to the present finding. Ehiagbonare and Ogurinde (2010) also reported a very low BOD of 2.4 and 1.6 mg/L in Concrete and Earthen ponds respectively. From this study, the level of BOD which range from 36.67±8.82 g/mL to 253.3±8.82 g/mL of the samples from the sampling locations were very high as compared to the standard limit (30 mg/L) according to Federal Environmental Protection Agency, FEPA (FEPA, 1991). Increase in BOD might be due to seasonal changes, type of feed use, high organic matter or fish excretions (Kay *et al.,* 2008).

The persistence of pathogens in the water environment is considered as one of the crucial factors for infection transmission in terms of acute outbreaks of disease. This study reveals the presence of *Aeromonas* sp., *Escherichia coli*, *Enterobacter* sp., *Enterococcus* sp., *Micrococcus* sp., *Staphylococcus aureus*, *Listeria* sp., *Chromobacterium* sp., *Salmonella* sp., *Shigella* sp., *Pseudomonas* sp. and *Providencia* sp. in the fish ponds wastewaters, as this may pose threat to the health of the fishes and consumers. It was found that Gram negative bacterial genera were predominant and this is in agreement with findings of Molokwu and Okpokwasili (2002) and Njoku *et al.* (2015a). The recovery of *Escherichia coli* and Enterobacteriacea groups in the fish ponds suggest faecal contamination of water. The faecal material may be as a result of fertilization of the ponds with animal manure which is discharged directly into the fish ponds or excretion by the fish into the ponds (Sule *et al*., 2016).

Antibiotics sensitivity result showed that selected *Staphylococcus* sp. was resistance to gentamicin and augmentin, a result similar to the work of Fasiku *et al*. (2020) who observed some *Staphylococcus aureus* that were resistant to gentamicin and augmentin. The most effective antibiotics against all selected bacteria was ciprofloxacin followed by cotrimoxazole. Broad zone of inhibition of ciprofloxacin against bacteria isolated from Ekenwan River was recorded by Imarhiagbe and Oshoma (2017). Most of the bacteria showed resistance to the antibiotics, while the others were sensitive indicating the level of pathogenicity of the bacteria. The result from the test infers that bacteria isolates from wastewater have some level of resistance to antibiotics, which could lead to prevalence of infections, prolonged sickness and ultimately death if not treated effectively before being released to the environment.

Conclusion

The physico-chemical parameters and microbial evaluation of the wastewater from fish ponds shows the level of its toxicity to the natural ecosystem, which could lead to further complications and death. Moreover, this study suggests that there is need to monitor the quality of wastewater that is being discharged from the fish ponds into the environment. Hence, its release and use should be with caution and subjected to closer regulation by environmental, agricultural and health protection agencies. It is therefore recommended that microbiological examination and physicochemical analysis of the discharged wastewaters from fish ponds be carried out regularly for proper monitoring so as to prevent avoidable epidemics in the country. Good quality waters 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 installation of waste water management techniques should be approved and enforced by the government on all fish farm owners. Moreover, public awareness on the effects caused by the indiscriminate release of fish ponds' effluents into the surroundings should be organized for farmers especially those in the rural areas. And Fish feeds should be obtained from reliable manufacturers' to reduce the level of contamination associated with feeds. Antibiotics susceptibility pattern of isolated bacteria showed that most of the isolates had very high rate of resistance to the common antibiotics which could pose danger to the environment as the likely occurrence of antibiotics resistance observed will be present in the fish and its consumers. This invariably complicate the management of diseases contracted from fish consumption and limit potent and effective therapeutics to a very few options.

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