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Physicochemical and Antibacterial Susceptibility Profile of Fish Pond Waters in Anambra State, Nigeria

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Abstract Fishes are good source of dietary protein, reared in artificial ponds in most countries. Poor sanitary condition predisposes the fishes to infestation by pathogenic microorganisms. This study was aimed at evaluating the bacteriological and physicochemical characteristics of fish pond waters in three senatorial zones in Anambra State, Nigeria and the antibiogram of the isolates determined. A total of 480 fish pond water samples collected from different fish farms during May to October 2016 (Rainy season) and November to April, 2017 (Dry season) were cultured and their physicochemical properties examined. Susceptibility of the isolates to conventional antibiotics was determined. Bacterial isolates recovered were identified based on their biochemical features. They include genera of *Vibrio, Aeromonas, Pseudomonas, Lactobacillus, Staphylococcus, Microbacterium, Serratia, Proteus, Bacillus, Streptococcus, Citrobacter*, *Micrococcus, Enterococcus, Enterobacter, Paenalcaligenes, Lysinibacillus, Acinetobacter* and *Escherichia*. The most occurring organism in the pond water in both seasons was *Staphylococcus* sp. The physicochemical parameters of the pond water samples showed that there was significant difference (p value <0.05) in temperature, pH, alkalinity, nitrite, sulphate and dissolved oxygen (DO) values during the seasons, but no significant difference (p value > 0.05) was observed in turbidity, phosphate and biological oxygen demand (BOD). Based on estimated marginal means, BOD, DO and alkalinity were higher during rainy season, while temperature, pH, conductivity, turbidity, nitrite, phosphate and sulphate were higher during dry season. Bacterial load obtained in the fish pond water samples, during dry and rainy seasons and in the three senatorial zones vary significantly (p value < 0.05). Percentage susceptibility of isolates to antibiotics was highest with chloramphenicol, vancomycin, ciprofloxacin and trimethoprim (93.3%), and least with erythromycin (66.7%). This study showed high bacterial contamination of fish pond waters, physicochemical parameters at variance with the WHO standard and presence of antibiotic resistant organisms. Therefore, proper sanitary measures are necessary to prevent disease outbreak among fish consumers.

Keywords: *fish, fish pond, antibiotic susceptibility, bacteria, physicochemical characteristics, season*

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1. Introduction

In many countries, fishes are consumed and are considered to be a good source of dietary protein [1]. Fish and fish products are important not only from a nutritional point of view, but also as an item of international trade and foreign exchange earner for a number of countries in the world [2]. The fisheries and aquaculture sector significantly expanded in the past decades and total production, trade and consumption reached an all-time record of 179 million tons in 2018 [3]. The top fish producers are China and Indonesia, accounting for almost 50 percent of total global capture production [3]. Over the past 35 years, aquaculture production in Nigeria has grown 12 percent per year (compared to the world average of 8 percent), from a little over 6,000 metric tons in 1980 to nearly 307,000 metric tons in 2016 [4]. Nigeria is the largest aquaculture fish producer in sub-Saharan Africa, accounting for 52 percent of the total farmed fish production in the region [4].

The high demand for fish has resulted in the increase in the number of fish ponds in Nigeria. Individual farmers, organized groups and institutions have developed, constructed fish ponds and started fish farm oblivious of the cost [5]. 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 [6]. 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 [7]. Water is the natural habitat of fishes and other aquatic animals; it is, therefore, of great importance to study water quality while studying fish production,

especially when done in an artificial setting [5]. Pollution of water is measured by assessing the physicochemical parameters of water. Good water quality is essential for survival and growth of fish [8]. The pollutants of fish pond water include; residual food, fecal matter, pathogenic bacteria, viruses and parasites, suspended solids, drugs and disinfectants [9]. Pond waste water if disposed untreated can, therefore, alter water quality in the receiving waters.

The microorganisms in fish and fish ponds portend grave consequences for public health [10]. Some of these microorganisms possess resistant determinant, which enhances their potentials for infecting consumers. For instance, *Escherichia coli* are known to survive well in aquatic environments, and they are highly adept at horizontal gene transfer, a notorious vehicle for antibiotic resistance dissemination [6]. The number of infections caused by antibiotic resistant bacteria is rising worldwide [1]. Resistant pathogens are capable of undermining effective health outcomes and prolonging hospitalization of patients [6]. The use of antibiotics has accompanied the growth in aquaculture. It is used for prophylaxis and for treatment in fish farming. The intensive use of antibiotics poses serious environmental and health risks. Its effects are directly linked to food safety, occupational health hazards and antimicrobial resistance. Environmental risks include residue accumulation, aquatic biodiversity toxicity, microbial community selection for antibiotic resistance and the emergence of multi-antibacterial resistant strains [11].

In this study was therefore, aimed at seasonal evaluation of bacteriological and physicochemical parameters of fish pond water samples and the antibiogram of the bacterial isolates on randomly selected antibiotics.

2. Materials and Methods

2.1. Description of Study Area

The study area is Anambra state, which is one of the five eastern states of Nigeria and covers an area of 4,416km^2 [12]. Anambra state lies at Latitude of 6°20'N and longitude 7°00'E. Twenty farms were randomly selected from the three senatorial zones of Anambra State (Anambra South, Anambra North and Anambra Central).

2.2. Description of the Sampled Fish Ponds and Fish

All the sampled fish ponds were open concrete ponds having different dimensions, with capacity to contain 100 - 1000 fishes per time. The source of water for running the pond was bore holes which were built within the farm. The spent fish pond waters are mostly removed fortnightly through an opening properly channeled to drainage system. African Catfish (*Clarias gariepinus*) was the only fish held in these ponds.

2.3. Collection of Fish Pond Water

A total of 480 fish pond water samples were collected from 20 fish farm sites. Fish pond water samples were aseptically collected using sterile screw-capped containers between the periods of May to October 2016 (Rainy season) and November to April, 2017 (Dry season). Sampling was done at two points - the surface of the pond and depth of 30cm below the water surface. The water samples were transported in a box containing ice packs to the laboratory for microbiological and physico-chemical analysis within two hours of collection.

2.4. Physico-Chemical Analysis of the Water Samples from Fish Pond

2.4.1. Temperature

The temperature of the water sample from fish pond was measured on site of collection using a mercury-in-glass thermometer calibrated in degree Celsius. The temperature probe was gently inserted into the water sample and allowed to stand for 2min. The temperature was recorded [13].

2.4.2. pH

The pH of the water sample was determined using a pH meter. The pH meter was first standardized by dipping the electrode of the meter into a buffer of pH 7.0 and then inserted into 50ml of the water sample contained in a beaker. The pH recorded accordingly [13].

2.4.3. Determination of Electrical Conductivity

Electrical conductivity was determined at 20^{0}C using a conductivity meter (Model DDS-307). The conductivity meter electrode was inserted into deionized water with specific electric conductivity to zero it before inserting into sufficient volume of the sample and allowed to stand for 2min. The electrical conductivity of the sample was read and recorded in microsiemens per centimeter (µS/cm) [13].

2.4.4. Turbidity Determination

Turbidity of the water sample was determined by photometric method using HACH DR/2010 Spectrophotometer. A 25ml aliquot of deionized water was used as blank to zero the spectrophotometer prior to sample reading. The fish pond water sample was vigorously shaken and 25ml of it read on the spectrophotometer at a wavelength of 860nm [13].

2.4.5. Determination of Alkalinity

A 50ml volume of water sample was placed in a volumetric flask and 3 drops of phenolphthalein indicator added to it. The mixture was titrated against 0.02N sulfuric acid at pH 8.3. Positive result was indicated by clearing of the pink color of the phenolphthalein indicator [14].

Phenolphthalein Alkalinity (mg/L) = (A1 × N × 50,000)/V
Where A1 = Volume of Sulfuric acid used in ml;
N= Normality of acid used to titrate; V = Volume of Sample used in ml.

2.4.6. Nitrite Determination

The nitrite content of the water sample was estimated by sulphanilamide method [13]. A 50ml water sample in a 250ml conical flask was mixed with 1ml of sulphanilamide solution and allowed to stand for 3min. 1ml of N-(1-Naphthyl) ethylenediamine hydrochloride solution was added and the optical density of the solution measured in a spectrophotometer at 543nm.

2.4.7. Phosphate Determination

A 50ml of the water sample placed in a 100ml polyethylene bottle was added 5ml of the mixed reagent (250ml sulfuric acid solution, 100ml ascorbic acid solution, 50ml potassium antimonyl- tartarate solution and 100ml ammonium molybdate solution). The mixture was shaken properly and the absorbance read in a spectrophotometer at a wave length of 885nm. A reagent blank was similarly prepared using de-ionized water [15].

2.4.8. Sulphate Determination

A 250ml water sample in a conical flask was adjusted to pH 5 with few drops of 1N HCl. The solution was heated to boiling while stirring slowly and 4.0% (w/v) of Barium chloride solution containing 4.0% (v/v) ethanol, and 2.4% (v/v) nonyl phenol polyethyleneoxy ether added slowly until precipitation was complete. After precipitation, 2ml of $BaCl_2$ solution was added further and the precipitate digested at 80°C overnight. The precipitate was filtered through an ashless filter paper and washed with warm distilled water until it is free of chloride as indicated by testing with silver nitrate-nitric acid reagent. The precipitate was placed in a pre-weighed crucible along with the filter paper, and then dried. The crucible was kept in a muffle furnace and ignited at 800^0C for 1h, cooled in a desiccator and the Barium sulphate precipitate weighed [16].

$S0_4$ in mg/L =(Weight (mg) of $BaS0_4$ x 411.6)/ volume (ml) of sample

2.4.9. Biological Oxygen Demand (BOD) Determination

A 10ml aliquot of water sample diluted in 90ml distilled water and dispensed into BOD volumetric flask was added a mixture of 22.5g/l $MgSO_4.7H_2O$ and 2ml alkali-iodide-azide reagent. 2ml of $CaCl_2$ and $FeCl_3.6H_2O$ were also added in the same manner. The sample was mixed by inverting the BOD flask many times producing a brownish cloudy solution indicating presence of oxygen. A brown precipitate which settles at the bottom of the flask was dissolved by the addition of 2ml of concentrated H_2S0_4. The flask was kept in the incubator for 5 days and 50ml of the water sample titrated against 0.025N sodium thiosulphate to a pale yellow color. Addition of 2ml of starch solution to the titrate gives a blue color and the titration continued until a clear solution was formed. Concentration of dissolved oxygen in the sample is equivalent to the number of milliliters of titrant used [17]. The dissolved oxygen is calculated from the formula:

DO_1 = (f x titrant volume × 8000)/ Volume of sample

BOD = (DO_1 - DO_5)/ 0.05

Where, DO_1 is dissolved oxygen for day 1; DO_5 is dissolved oxygen for day 5

2.5. Bacteriological Examination

2.5.1. Enumeration and Isolation of Bacterial Isolates in Water Samples from Fish Ponds

A-1 ml aliquot of fish pond water sample was serially diluted tenfold, and 0.1ml of 10^{-4} dilution spread inoculated on sterile Nutrient agar (Oxoid) plate. The plate was incubated at 37°C for 24h, and thereafter colony counts of the bacterial growth were recorded. Colonies of the bacteria were sub-cultured, and pure cultures obtained were inoculated into sterile Nutrient agar slant and stored at 4°C for further tests.

2.5.2. Identification of Bacterial Isolates from Fish Ponds Water Samples

The bacterial isolates were identified based on their morphological and biochemical characteristics as described by Bergey's Manual of Determinative Bacteriology [18]. Biochemical tests carried out include Gram reaction, motility, indole, methyl red, Voges-Proskauer, citrate utilization, catalase, oxidase and sugar fermentation.

2.6. Antibiotic Susceptibility Test of the Bacterial Isolates from Fish Ponds Water Samples

Susceptibility of the test isolates to conventional antibiotics was carried out following disc diffusion method on Muller-Hinton agar using 0.1ml of standardized bacterial suspension (1.0 McFarland standard) [19]. The plate was spread inoculated, allowed to dry and the antibiotic discs of various concentrations placed on it. The antibiotics tested were Vancomycin (30µg), Trimethoprim (75µg), Ciprofloxacin (5µg), Gentamycin (10µg), Streptomycin (10µg), Oxytetracycline (10µg), Ampicillin (10µg) and Erythromycin (15µg). The plate was incubated at 37°C, the zones of growth inhibition around the antibiotic discs measured after 24h. The test was carried out in triplicate. The result was interpreted as resistant or susceptible according to the Clinical and Laboratory Standards Institute guidelines [20].

2.7. Statistical Analysis

Univariate analysis of variance was used to analyze the data obtained using SPSS version 21.

3. Results

3.1. Physicochemical Analysis of the Water Samples from the Fish pond

The seasonal variations in physicochemical characteristics of the fish pond water samples are presented in Table 1 and Table 2. The temperature and pH values of the samples during the seasons ranged from 27.1°C-32.1°C and 6.4-8.0 respectively (Table 1).

It can be observed from Table 1 that temperature range of 27.1°C - 28.2°C occurred during the rainy season while temperature range of 28.5°C - 32.1°C was observed in dry season. pH values were higher in rainy season than in dry season (Table 1). There were notable variations in the conductivity, turbidity (Table 1), alkalinity, nitrite, phosphate and sulphate values (Table 2). Farm A had the highest conductivity of 61 µS/cm, Farm K the highest turbidity of 10.9NTU, Farm O the highest alkalinity of 137mg/L, Farm E the highest nitrite value of 2.9mg/L, Farm L the highest phosphate value of 2.3 mg/L while Farm D had the highest sulphate value of 12.1mg/L. BOD and DO values of the pond waters ranged from 1.1mg/L -3.0mg/L and 6.2- 12.0mg/L respectively (Table 2).

In both seasons, there were significant differences (p value <0.05) in temperature, pH, alkalinity, nitrite, sulphate and DO values. Turbidity, phosphate and BOD values of the pond water samples were statistically similar (p value > 0.05). The values of pH, BOD, DO and alkalinity were higher during rainy season while temperature, conductivity, turbidity, nitrite, phosphate and sulphate were higher during dry season.

3.2. Enumeration and Isolation of Bacterial Isolates in Water Samples from Fish Ponds

Table 3, shows the total bacterial count of organisms in the water samples in the fish ponds, from three senatorial zones in Anambra state during the dry and rainy season. During the dry season, the mean bacterial count obtained in the fish pond water ranged from $2.1\pm0.95 \times 10^6$ to $22.6\pm 0.81\times10^6$ cfu/ml and $20.6\pm0.81 \times 10^6$ to $44.8\pm0.49 \times10^6$ cfu/ml during the rainy season. The highest bacterial load in the water samples from the fish ponds in the three senatorial zones was observed during the rainy season than in dry season. Also the bacterial loads during the rainy and dry seasons were higher in the water samples obtained from Anambra South than in the other two senatorial zones. The univariate analysis of the bacterial counts obtained in the fish ponds in the three senatorial zones shows that there are significant differences (p value < 0.05) in the counts obtained during the dry and rainy season in all the ponds sampled and in the three senatorial zones.

Table 1. Physical characteristics of the water samples from the fish ponds

	Names of the fish pond	Temp (^{0}C)		pH		Conductivity (µS/cm)		Turbidity (NTU)	
		Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Anambra North	Farm A	27.1±0.1	29.0±0.1	8.0±0.2	7.7±0.2	54.0±2.0	61.0±3.1	8.3±0.3	9.7±0.5
	Farm B	27.3±0.3	28.1±0.1	7.7±0.4	6.6±0.4	51.0±3.6	59.7±1.5	6.0±0.3	9.6±0.4
	Farm C	28.0±0.2	30.3±0.6	7.7±0.7	6.4±0.2	47.3±3.1	56.7±5.0	7.0±0.2	9.3±0.6
	Farm D	27.4±0.3	29.1±1.1	7.8±0.2	6.5±0.2	41.3±4.2	50.7±8.3	6.0±0.2	7.4±1.1
	Farm E	28.2±0.3	29.6±0.5	7.7±0.4	6.8±0.5	45.3±4.2	57.3±4.2	9.7±0.1	9.7±0.5
	Farm F	28.2±0.2	29.6±0.8	7.5±0.6	6.9±0.1	42.0±5.3	49.0±2.6	6.4±0.2	7.7±1.3
Anambra South	Farm G	28.2±0.3	31.3±0.6	7.8±0.2	6.8±0.1	40.0±9.2	43.3±6.5	5.5±0.6	6.3±0.9
	Farm H	28.2±0.1	31.9±1.7	7.9±0.1	6.9±0.2	45.3±4.2	43.3±1.5	6.7±0.4	7.6±0.4
	Farm I	28.5±0.1	30.4±1.4	7.6±0.5	6.7±0.3	47.0±5.6	50.7±3.1	5.5±0.3	7.0±0.2
	Farm J	28.5±0.2	31.4±1.5	7.7±0.2	6.9±0.3	52.7±11.0	54.7±4.2	8.4±0.6	8.3±1.1
	Farm K	28.3±0.1	29.9±1.8	7.3±0.2	7.0±0.2	52.0±5.3	57.0±4.6	9.8±0.7	10.9±1.0
	Farm L	28.1±0.1	29.3±0.6	7.3±0.4	6.7±0.3	44.3±5.1	47.3±3.1	8.0±0.1	8.7±0.2
	Farm M	28.1±0.1	30.4±1.6	7.5±0.5	6.6±0.3	43.3±4.7	46.7±4.2	8.2±0.2	9.7±0.4
	Farm N	28.0±0.2	32.1±0.9	7.4±0.2	6.4±0.4	52.0±5.3	55.3±4.7	7.9±0.3	8.4±1.8
Anambra Central	Farm O	28.2±0.1	30.1±0.9	8.0±0.1	6.9±0.8	44.7±7.6	43.0±4.6	7.9±0.9	9.6±0.5
	Farm P	27.2±0.3	29.9±1.8	7.5±0.1	6.9±0.2	42.7±6.4	47.3±3.1	8.7±0.4	8.9±1.1
	Farm Q	27.2±0.4	28.9±0.9	7.4±0.4	6.9±0.4	46.0±7.2	40.7±3.2	7.1±0.3	7.1±0.3
	Farm R	27.6±0.3	31.4±2.1	7.4±0.2	6.5±0.2	44.7±11.0	50.0±2.0	8.1±0.2	9.3±0.6
	Farm S	27.4±0.4	29.3±1.1	7.3±0.4	6.8±0.4	46.0±10.6	58.3±2.5	8.3±1.5	9.6±0.7
	Farm T	28.2±0.2	31.0±1.0	7.3±0.4	6.8±0.2	40.0±2.0	40.3±2.5	7.6±0.7	8.6±0.6

Table 2. Chemical characteristics of the water samples from the fish ponds

	Names of the fish pond	Alkalinity (mg/l)		Nitrite (mg/l)		Phosphate (mg/l)		Sulphate (mg/l)		BOD (mg/l)		DO (mg/l)	
		Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry
Anambra North	Farm A	118.3±15.5	112.7±7.0	1.9±0.2	2.1±0.2	2.1±0.2	2.2±0.2	8.1±0.3	9.7±0.6	2.2±0.3	3.0±0.2	8.5±0.4	8.6±0.1
	Farm B	117.3±17.5	118.0±7.0	2.2±0.1	2.6±0.2	1.8±0.2	2.0±0.2	9.1±0.5	10.4±0.4	1.9±0.2	1.8±0.1	7.3±0.5	7.7±0.2
	Farm C	104.0±7.2	94.0±8.7	2.5±0.2	2.9±0.1	1.4±0.2	1.2±0.2	9.4±0.4	9.2±0.4	2.7±0.2	2.4±0.3	7.4±0.2	7.2±0.1
	Farm D	118.7±13.8	92.0±5.3	2.2±0.3	2.4±0.2	1.1±0.3	1.0±0.2	10.0±0.5	12.1±0.3	2.4±0.4	2.7±0.1	6.2±0.1	6.2±0.1
	Farm E	127.0±23.1	132.0±2.0	2.9±0.2	2.9±0.1	1.6±0.1	1.6±0.3	8.4±0.2	9.0±0.2	1.4±0.2	1.2±0.2	10.1±0.3	8.3±0.1
	Farm F	124.7±5.5	123.3±4.9	2.1±0.5	1.8±0.2	1.2±0.7	0.9±0.1	6.2±0.2	6.5±0.1	2.1±0.2	2.3±0.3	8.5±0.3	7.5±0.1
Anambra South	Farm G	120.3±4.5	101.3±3.1	2.2±0.5	1.9±0.8	1.7±0.1	1.5±0.3	6.9±0.3	8.1±0.3	1.8±0.1	1.6±0.1	9.3±0.3	8.5±0.2
	Farm H	123±10.1	120.0±8.5	1.6±0.3	1.9±0.1	1.8±0.2	1.9±0.1	9.9±0.3	10.1±0.5	1.4±0.1	1.1±0.1	9.4±0.3	8.5±0.5
	Farm I	108.7±6.7	100.0±2.0	1.7±0.2	2.3±0.4	1.0±0.1	1.1±0.1	8.0±0.21	8.2±0.3	1.4±0.1	1.2±0.2	9.3±0.2	9.2±0.1
	Farm J	129.7±2.5	122.7±5.0	2.0±0.2	2.4±0.5	1.0±0.2	1.2±0.2	6.3±0.1	8.5±0.3	2.3±0.2	2.2±0.2	12.0±0.4	10.4±0.2
	Farm K	121.7±17.2	120.0±1.0	1.8±0.1	2.0±0.2	1.7±0.1	1.8±0.1	9.9±0.3	11.2±0.4	3.2±0.1	2.6±0.3	7.3±0.6	6.9±0.4
	Farm L	100.3±2.5	104.7±6.4	1.4±0.3	1.4±0.1	2.1±0.5	2.3±0.3	8.9±0.2	10.6±0.4	2.5±0.5	2.6±0.3	9.9±0.6	9.6±0.2
	Farm M	110.0±9.2	106.7±4.2	1.6±0.2	2.0±0.3	0.8±0.3	0.9±0.3	6.1±0.1	7.4±1.0	2.4±0.5	2.3±0.2	9.4±0.4	8.6±0.1
	Farm N	107.0±12.3	101.3±6.1	1.1±0.1	1.3±0.4	1.8±0.2	2.1±0.1	6.8±0.2	7.1±0.2	1.9±0.1	1.7±0.1	6.3±0.2	6.6±0.3
Anambra Central	Farm O	137.3±7.4	130.0±2.0	1.6±0.1	1.6±0.1	1.9±0.1	1.8±0.2	9.9±0.2	10.0±0.3	2.1±0.2	2.1±0.1	6.7±0.5	6.8±0.1
	Farm P	135.3±3.1	133.7±5.7	1.5±0.1	1.6±0.1	1.6±0.1	1.7±0.2	8.5±0.7	9.5±0.5	2.4±0.3	2.4±0.2	10.1±0.1	10.1±0.2
	Farm Q	134.0±4.6	120.0±14.8	2.0±0.2	2.3±0.2	0.9±0.3	1.1±0.6	8.0±0.1	8.8±0.2	1.6±0.1	1.6±0.1	6.9±0.3	6.7±0.4
	Farm R	105.3±7.2	109.3±16.3	1.4±0.1	1.8±0.2	0.9±0.2	1.2±0.1	9.1±0.2	10.3±0.6	1.7±0.1	1.7±0.1	9.7±0.6	9.5±0.3
	Farm S	114.7±5.7	108.0±6.0	1.2±0.1	1.3±0.1	1.3±0.1	1.4±0.2	8.9±0.3	10.1±0.5	2.7±0.2	2.6±0.1	6.6±0.4	6.3±0.3
	Farm T	127.0±4.4	118.7±9.1	1.3±0.8	1.2±0.4	1.2±0.1	1.2±0.1	7.2±0.4	8.3±0.8	2.4±0.3	2.2±0.1	8.3±0.3	8.2±0.1

Table 3. Mean bacterial counts of organisms isolated from fish ponds water samples

Senatorial Zone	Pond	Mean Bacteria counts ($\times 10^6$cfu/ml) Dry Season	Rainy Season
Anambra North	A	17.3±0.70	27.9±0.61
	B	21.1±0.66	22.4±0.36
	C	13.1±1.25	32.1±1.85
	D	2.1±0.95	20.6±0.81
	E	20.8±0.75	24.8±1.04
	F	11.3±0.61	23.7±0.61
Anambra South	G	14.2±3.33	19.2±1.04
	H	19.5±0.50	37.6±0.35
	I	13.3±0.29	40.5±0.50
	J	13.5±0.50	31.2±1.08
	K	21.0±1.32	35.9±0.36
	L	22.6±0.81	30.9±0.26
	M	22.0±0.50	44.8±0.49
	N	19.9±0.78	36.8±0.70
Anambra Central	O	11.8±0.26	30.0±0.45
	P	12.1±0.15	38.9±0.25
	Q	15.0±1.00	22.0±0.50
	R	13.5±0.45	23.9±0.32
	S	10.9±0.36	36.2±0.76
	T	10.3±0.42	38.3±0.42

3.3. Identification of the Bacterial Isolates from Fish Ponds Water Samples

Of the 480 water samples from the fish ponds, a total of 15 genera of bacterial isolates were recovered. They include Enterococcus, Pseudomonas, Bacillus, Staphylococcus, Enterobacter, Paenalcaligenes, Lysinibacillus, Serratia, Streptococcus, Citrobacter, Micrococcus, Proteus, Lactobacillus, Acinetobacter and Escherichia. Micrococcus sp was isolated only during the rainy season while Lysinibacillus and Paenalcaligenes sp were isolated only during the dry season (Table 4). Staphylococcus sp. had the highest percentage abundance (14.0%) while Escherichia. sp had the least (1.7%) [Table 5].

3.4. Antibiotic Susceptibility Tests of the Bacterial Isolates from Fish Ponds Water Samples

Table 6, shows the antibiotic susceptibility tests of the bacterial isolates. *Staphylococcus* sp, *Enterobacter* sp, *Micrococcus* sp and *Escherichia* sp showed 100% susceptibility to the antibiotics tested, while *Serratia* sp had the least susceptibility (62.5%). Percentage susceptibility of the bacterial isolates to antibiotics was highest with chloramphenicol, vancomycin, ciprofloxacin and trimethoprim (93.3%), and least with erythromycin (66.7%).

Table 4. Morphological and biochemical characteristics of bacterial organisms isolated from the fish ponds water samples

Biochemical tests	Isolates														
	$1W^{dr}$	$2W^{dr}$	$3W^{dr}$	$4W^{dr}$	$5W^{dr}$	$6W^{d}$	$7W^{d}$	$8W^{dr}$	$9W^{dr}$	$10W^{dr}$	$11W^{r}$	$12W^{dr}$	$13W^{dr}$	$14W^{dr}$	$15W^{dr}$
GR	+ve	-ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve
Shape	Cocci	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Cocci	Rod	Cocci	Rod	Rod	Rod	Rod
Motility	+	+	+	-	+	+	+	+	-	+	-	+	-	-	+
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
M R	-	-	-	+	-	-	-	-	-	+	-	+	-	-	+
V P	+	-	+	+	+	-	+	+	-	-	+	-	-	-	-
Citrate	-	+	+	+	+	-	+	+	-	+	+	+	-	+	
Catalase	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+
Oxidase	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-
Coagulase	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Sugar fermentation															
Lactose	+	-	+	+	+	-	-	-	+	+	-	-	+	-	+
Mannitol	+	+	+	+	+	-	-	+	-	+	-	-	-	-	+
Sucrose	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
Arabinose	-	-	+	-	+	+	-	-	-	+	-	-	+	+	+
Maltose	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+
Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Key: GR = Gram reaction, MR = Methyl red, VP = Voges Proskaeur, 1W= *Enterococcus* sp., 2W = *Pseudomonas* sp., 3W = *Bacillus* sp., 4W = *Staphylococcus* sp., 5W = *Enterobacter* sp., 6W = *Paenalcaligenes* sp., 7W = *Lysinibacillus* sp., 8W = *Serratia* sp.,9W = *Streptococcus* sp., 10W = *Citrobacter* sp., 11W = *Micrococcus* sp.,12W = *Proteus* sp., 13W = *Lactobacillus* sp.,14W = *Acinetobacter* sp. and 15W = *Escherichia* sp. Isolates with superscript 'dr' were isolated during the dry and rainy season, isolate with superscript 'r' was isolated only during rainy season, isolates with superscript 'd' were isolated only during dry season.

Table 5. Relative abundance of the bacterial organisms isolated from the fish ponds water samples during dry and rainy seasons

Organism	Frequency of abundance	Percentage abundance (%)
Enterococcus sp	15	5.2
Pseudomonas sp	32	11.2
Bacillus sp	38	13.3
Staphylococcus sp	40	14.0
Enterobacter sp	28	9.8
Paenalcaligenes sp	8	2.8
Lysinibacillus sp	10	3.5
Serratia sp	20	7.0
Streptococcus sp	15	5.2
Citrobacter sp	16	5.6
Micrococcus sp	12	4.2
Proteus sp	10	3.5
Lactobacillus sp	22	7.7
Acinetobacter sp	15	5.2
Escherichia sp	5	1.7
Total	286	100

$$Relative\ abundance\ (\%) = \frac{frequency\ of\ occurrence}{Total\ no\ of\ occurrence} \times 100$$

Table 6. Antibiotic susceptibility test of the bacterial isolates from fish ponds water samples

	Zones of inhibition (mm)								
Organism	**Ampicillin**	**Erythromycin**	**Chloramphenicol**	**Gentamicin**	**Vancomycin**	**Ciprofloxacin**	**Trimethoprim**	**Oxytetracyclin**	**% susceptibility**
***Enterococcus* sp**	6	12	50	18	0	15	22	16	87.5
***Pseudomonas* sp**	8	0	32	0	28	20	CI	13	75
***Bacillus* sp**	32	2	32	21	CI	0	0	28	75
***Staphylococcus* sp**	45	36	10	25	16	25	21	10	100
***Enterobacter* sp**	16	18	30	12	30	50	20	CI	100
***Paenalcaligenes* sp**	CI	0	35	8	50	13	20	0	75
***Lysinibacillus* sp**	8	0	12	0	13	50	22	CI	75
***Serratia* sp**	0	0	45	0	50	38	26	CI	62.5
***Streptococcus* sp**	16	28	0	45	CI	12	16	50	87.5
***Citrobacter* sp**	38	0	56	20	37	22	24	16	87.5
***Micrococcus* sp**	26	12	28	6	12	10	16	8	100
***Proteus* sp**	0	28	37	48	CI	26	20	18	87.5
***Lactobacillus* sp**	16	CI	15	27	40	10	26	0	87.5
***Acinetobacter* sp**	22	26	12	58	66	19	28	0	87.5
***Escherichia* sp**	18	14	26	30	10	22	16	20	100
% Susceptibility	88.7	66.7	93.3	80.0	93.3	93.3	93.3	80.0	

Key: CI = complete inhibition; 0= No inhibition.

4. Discussion

Fish has become increasingly important source of protein and other elements necessary for the nourishing of the body, and fish aquaculture is a practice in some parts of Anambra state which serves as a means of ensuring all year supply of fishes.

Physicochemical characteristics of water contribute to quality of water, and fish pond water is known to affect the activities and well-being of the fishes [21]. Table 1 and Table 2, show the results for the physico-chemical analysis of the different pond water samples.

The temperature recorded in this study ranged from 29.0-31.9°C in dry season and 27.1 - 28.5°C in rainy season. The values obtained in both seasons were well within WHO permissible limit for aquaculture. The WHO standard for aquaculture ranges from 25-32°C [22]. This result corroborates with the report of [23], who also observed a temperature range of 27-28°C in the preliminary studies and water characteristics of bacterial population in Kojalo fish pond. Reference [24], also reported a temperature range of 26-29^{0}C and opined that such temperature range supports fish productivity. Contrary to our findings [25], reported much lower temperature, below 25°C, in their study on occurrence, temporal variations, and ecological risks of use of antibiotics in crab ponds of Lake Guchenghu Basin, China.

Optimum pH directly stabilizes the physicochemical parameters of pond water, enhancing fish health and productivity, and the maintenance of a proper balance of

the microbial ecology in pond water [21]. Fluctuations in pH have been reported to cause extreme stress in fish [6], since they are known to have an average blood pH of 7.4 [8]. It has been confirmed that the pH between 6.5 and 9 is most appropriate for maintenance and increased fish production [26,27]. The pH recorded in this study ranged from 6.4 - 8.0 (Table 1). These values are within the range required for aquaculture and are similar to the work of [28], who reported a pH range of 6.7- 7.4, in the assessment of water quality characteristics for aquaculture uses in Abeokuta, Nigeria. Similarly our result is in agreement with the works of [8,9,29], who recorded a pH range of 7.3 -7.9.

Conductivity is an indicator of the freshness of a water body, and high values of conductivity are indicative of pollution [8]. High level of nutrient content of fish feed is known to contribute to the high conductivity values observed in most pond water [8]. The electrical conductivities of the pond water samples in our studies varied from 40 - 61µs/cm throughout the period of study, but significantly higher ($p < 0.05$) during the dry season (Table 1). This result obtained is within the permissible limit stated by FAO, which is 20 -1500 µs/cm and quite suitable for fish production [30]. A similar study in Busia County recorded electrical conductivity (EC) levels of 34.67 - 86.67µs/cm [21]. South East Brazil ponds had a wider range of 24 - 610 µs/cm, while analysis carried out in a fish pond in Ghana recorded higher EC levels of 102.2 - 132.30 µs/cm [31].

Turbidity in water is caused by presence of suspended particles, and at increased turbidity of pond water penetration of light is greatly impeded and absorption of nutrient by fishes is slowed down [3]. In this study, turbidity values of the pond water samples were significantly lower ($p > 0.05$) in the rainy season. This could be attributed to periodic dilution of the open pond water with rain water. However, contrary to WHO's standard, the turbidity values 6.0 -10.9 NTU obtained from the ponds investigated, were not within the range that supports aquatic life (Table 1). The WHO standard for turbidity of pond water is 5NTU [26]. The high turbidity values obtained could be attributed to the types of feeds introduced into the ponds.

The alkalinity contents of the water samples obtained from some of the farms used for this study (92 - 137mg/L) were not within the WHO limit for fish farming. The WHO standard for alkalinity of pond water is 25-120mg/L [32]. The result obtained is similar to that reported by [33], who worked on physico-chemical analysis of fish pond water in Okada and its environs, and obtained the alkalinity value ranging from 35 to 135mg/L. Higher alkalinity values of 148.33 - 210.5 mg/L was however, reported by [34].

It has been shown that nitrite is an invisible fish killer, and even at low concentration of 0.25ppm is deadly to the smallest fish. Excess nitrite concentration in fish pond water has been reported to prevent blood cells from absorbing oxygen from water, thus making fish blood to appear dull brown in color, which is significant of a nitrite poisoning popularly called "brown blood disease" [24]. Nitrite concentrations of 1.1 - 2.9mg/L obtained in this study were within the maximum desirable limit of 0.1-4.0mg/L [27]. Reference [33], recorded a range of 2.21-4.91 mg/L.

In this study phosphate levels was found to vary from 0.62 - 2.41mg/L (Table 2),which are within WHO limits for aquaculture (0.03-3mg/L) [26]. It has been reported that use of fertilizers and phosphorous-rich fish feeds impact phosphate concentrations in fish ponds significantly, and increased phosphate concentration of the pond encouraged algal growth [9,26]. Phosphate levels in pond water in this study support the range of 0.69 - 2.41mg/L reported by [9]. Reference [35], reported a range of 0.51- 1.28 in fish ponds at Karnataka while [36], recorded phosphate level ranging from 0.36 - 2.86mg/L. However [33], recorded a higher range of 1.40 - 4.51mg/L.

Fishes can survive wide range of sulphate in water, which is known to be one of the least toxic anions. The usual sources of sulphate in fish pond are the borehole water used for rearing the fishes and the sulphate-containing organic feeds. The concentration of sulphate in the ponds as shown in Table 2, varied from 6.1 - 12.1mg/L. Reference [37], recorded a sulphate concentration of 10mg/l for fresh water fish pond while [38], reported sulphate values ranging from 0.39 mg/l to 4.37 mg/l in the fish pond water samples.

Biochemical Oxygen Demand (BOD) is the measurement of total dissolved oxygen consumed by microorganism for biodegradation of organic matter [39]. High BOD affects the oxygen cycle and oxygen equilibrium in the water. In Nigeria, the water quality criterion for BOD for the protection of aquatic life is 4-6 mg O_2 l^{-1} [40]. Reference [41] stated, that a BOD level above 5mg/L is an indication of water pollution. In this study, the BOD obtained was not significantly different (p value > 0.05) between dry and rainy season, the values ranged from 1.1 - 3.2mg/L (Table 2), which is within the range given by Federal Environmental Protection Agency of Nigeria [40]. Reference [5], noted a higher value of BOD in their study of cat fish ponds in Ozoro town, Nigeria, and reported that the fish ponds were grossly polluted.

The Dissolved Oxygen (DO) obtained from this study was significantly higher ($p < 0.05$) in the rainy season and ranged from 6.2 to 12.0mg/L. Reference [33], recorded DO range of 9.3 - 16.2mg/L in their work on physicochemical analysis of fish pond water in Okada and its environs, Nigeria, while [9], reported a DO range of 4.72 - 5.02mg/L in their study on impact of aquaculture development on water quality of fish ponds in Gatundu North and South sub-countries, Kenya. Dissolved Oxygen is known to affect attributes such as growth, survival distribution, behavior and physiology of aquatic organism [42]. WHO standard for DO concentration for the support of aquatic life ranges from 5 - 9.5mg/L [26]. At concentration below 5mg/L, the functions and survival of biological organisms may be adversely affected, while DO concentration below 3mg/L leads to death of most fish [43].

The seasonal variation of the bacterial load in the water samples from fish pond obtained from different fish farms in the three senatorial zones in Anambra state (Table 3), showed that bacterial load was higher during rainy season than during the dry season. The bacterial counts obtained

in all the pond waters in the three senatorial zones during rainy and dry seasons were significantly different (p value < 0.05).

The high bacterial load of the pond waters observed during the rainy season in this study could be due to conducive temperature for microbial growth in the pond water during rainy season as against the dry season (Table 3). The mean bacterial counts obtained in Anambra south senatorial zone were higher than those obtained in other zones in both the rainy and dry seasons. As can be observed (Table 3) the mean bacterial counts in the pond waters in the three zones during the dry and rainy seasons range from 2.1±0.95 $\times 10^6$ to 44.8±0.49 $\times 10^6$cfu/ml. Reference [44], reported bacterial counts ranging from 7.983×10^6 cfu/ml to 15.483×10^6 cfu/ml while working on the physicochemical and bacteriological investigation of selected fish ponds in Kuje Area Council, Nigeria, although there was no seasonal indication. On the contrary [38], recorded quite low bacterial count (3.981×10^3 - 1.32×10^4cfu/ml) during their work on physicochemical, bacteriological and parasitological examination of selected fish pond water samples in Awka and its environment in Anambra state, Nigeria. The unhygienic condition of the fish pond is likely to be contributory to the microbial load of pond water irrespective of the season.

The bacterial organisms isolated from the fish pond water (Table 4) are similar to the organisms isolated by many researchers. [24,38,45,46] *Micrococcus* was isolated only during rainy season while *Paenalcaligenes* and *Lysinibacillus* were isolated only during dry season (Table 4). *Staphylococcus* spp was observed as the most prevalent bacteria in the pond water in both seasons while *Escherichia* sp was the least (Table 5). This is contrary to the reports of [45] and [46], who reported *E. coli* as the most occurring bacteria in fish pond water.

The occurrence of antibiotic resistance among bacteria from livestock has raised considerable concern due to the potential for transfer of resistant pathogens to the human consumers [47]. The bacterial isolates showed varying susceptibility and resistance to the antibiotics tested (Table 6). The susceptibility of the isolated bacterial organisms to conventional antibiotics revealed that the organisms had highest percentage susceptibility (93.3%) to chloramphenicol, vancomycin, ciprofloxacin and trimethoprim and and highest resistance (66.7%) to erythromycin (Table 6). Highest resistance and least susceptibility to the antibiotics tested were observed with *Serratia*. The resistance of the isolates to antibiotics could be attributed to indiscriminate use of these antibiotics in aquaculture. The high susceptibility of the bacterial isolates to chloramphenicol observed in this study, is similar to the work of [48]. They observed that all strains tested were susceptible to chloramphenicol. Contrary to our result [49], reported high resistance of *Escherichia coli* to antibiotics. However [50], noted that bacterial isolates from fish ponds were most susceptible to erythromycin. High resistance to lincomycin (100%) and least resistance to sulphamethoxazole (13%) by bacterial isolates recovered from fish were reported by [47]. Reference [51], reported that bacterial organisms isolated from fish farm showed highest resistance to penicillin and least to gentamicin (1.7 - 5.6%).

5. Conclusion

The seasonal evaluation of fish pond waters in Anambra state, Nigeria showed that conductivity, turbidity, phosphate and sulphate concentration were higher during the dry season, while pH, alkalinity, BOD and DO were higher during the rainy season. Rearing of fishes in artificial ponds was observed to contain large population of bacterial organisms. The bacterial load in the pond waters varied with season and among the senatorial zones. Susceptibility tests indicate that all the bacterial isolates were susceptible to chloramphenicol with exception of *Streptococcus* sp. *Pseudomonas* sp, *Paenalcaligenes* sp, *Lysinibacillus* sp, *Serratia* sp and *Citrobacter* sp were resistant to erythromycin.

Monitoring of physicochemical parameters of fish pond water is of essence, in order to prevent health defect on the fishes and also the microbial load periodically checked to avoid public health hazards. Controlled use of antibiotics in fish farming is very important, to avoid occurrence of antibiotic resistance.

Competing Interests

Authors have declared that no competing interests exist.

References

[1] Claudious G., Tinashe, C.H., Bernar, M., Shuvai, M., Gilbert, J., Pious, V.M. and Jairus, M., "Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe," *International Journal of Microbiology*, 2019. 1-7. May. 2019.

[2] Danba, E.P., Bichi, A.H., Ishaku, S., Ahmad, M.K., Buba, U., Bingari, M.S., Barau, B.W. and Fidelis, U.F., "Occurrence of pathogenic bacteria associated with *Clarias gariepinus* selected fish farms of Kumbotso Local Government Area of Kano State, Nigeria," *Bayero Journal of Pure and Applied Sciences*, 7(2). 145-149. Jan. 2014.

[3] Food and Agricultural Organization (FAO)," The state of world fisheries and aquaculture," sustainability in action. Food and Agriculture Organization of the United Nations. Rome, 2020

[4] WorldFish. WorldFish Nigeria Strategy: 2018-2022. http://pubs.iclarm.net/resource_centre/2018-09.pdf. [Assessed June 11, 2020].

[5] Sawere, B. T. and Oghenekowhoyan, O.C, "Determination of some physicochemical parameters and selected heavy metals from water collected from concrete fish ponds in Ozoro town," *International Journal of Advances in Scientific Research and Engineering*, 5(5). 1-7. May. 2019.

[6] 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," *Preprints*, https://www.preprints.org/manuscipt/201904.0108/v1. [Assessed April 18, 2019].

[7] 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*, 7(1). 190-197. Jan. 2019.

[8] Kumar, D.G., Karthik, M. and Rajakumar, R., "Study of seasonal water quality assessment and fish pond conservation in Thanjavur, Tamil Nadu, India," *Journal of Entomology and Zoology Studies*, 5(4). 1232-1238. June. 2017.

[9] Wairimu, T.O., Kitur, E., Ngugi, C.C., Mwangi, B.M. and Nzere, J.K., "Impact of aquaculture development on water quality of fish ponds in Gatundu North and South sub-countries, Kenya".

International Journal of Fisheries and Aquatic Studies, 4. 01-06. June. 2019.

[10] Li, Y., Pei, X., Yan, J., Liu, D., Zhang, H., Yu, B., Li, N. and Yang, D. "Prevalence of food borne pathogens isolated from retail fresh water fish and shell fish in China". *Food Control*, 99. 131-136. Dec. 2019.

[11] Lulijwa, R., Rupia, E.J. and Alfaro, A.C. "Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers". *Review in Aquaculture*, 12(2). 640-663. Apr. 2019.

[12] Ojiako, J. C., Okafor, C.M. and Igbokwe, E.C. "Geospatial mapping of fish farms in animal state using GIS Approach". *International Journal of Scientific and Engineering Research*, 6(11). 1-12. Nov. 2015.

[13] American Public Health Association APHA. Standard methods for the Examination of Water, 22nd edn. American Public Health Association, Washington, D.C., USA. 2012.

[14] Sawyer, C. N., Mc Carty, P.L., and Parkin, G.F. "Chemistry for environmental engineering," 4th Edn. Tata Mc Graw-Hill Publishing Company Limited, New Delhi. 2000.

[15] Nawar, F. "Determination of phosphorus: Ascorbic acid procedure protocols," https://www.protocols.io/view/determination-of-phosphorus-ascorbic-acid-procedure-ggcbtsw. [Accessed April 18, 2019].

[16] Zarate, N., Perez-Olmos, R. and dos Reis, B. F. "Turbidimetric determination of sulfate in rainwater a LED based photometer and multicommuted flow analysis system with in-line preconcentration". *Journal of the Brazilian Chemical Society*, 22(6). 1009-1014. June. 2011.

[17] Vlab, "Biological Oxygen Demand," Virtual Amrita Laboratories Universalizing Education. www.Vlab.amrita.edu. 2012[Online] [Accessed Sept. 25, 2018].

[18] Noel, K.R. and John, H.G,." Bergey's Manual of Systematic Bacteriology," Barbara T. edition. William and Wilkins publishers, Baltimore, USA. pp 85-115. 1984.

[19] Bauer,A.W., Kirby, W.M.M., Sherris. J.C. and Turck, M. "Antibiotic Susceptibility testing by a standardized single disk method." *Americal Journal of Clinical Pathology*. 36. 493-496.

[20] Clinical and Laboratory Standards Institute (CLSI). (2017). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, 27th edition, Wayne, USA.

[21] Makori, A.J., Abuom, P.O., Kapiyo, R., Anyona, D.N. and Dida, G.O. "Effects of water physicochemical parameters on tilapia (*Oreochromis niloticus*) growth in Teso North sub County, Busia County". *Fisheries and Aquatic Sciences*, 20(30).1-10. Nov. 2017.

[22] World Health Organization (WHO), "Guidelines for drinking water quality," Recommendation WHO, Geneva. 569-571, 2006.

[23] Fafioye, O.O. "Preliminary studies on water characteristics and bacterial population in high yield kajola fish ponds". *Journal of Agricultural Extension and Rural Development*, 3.68-71. Mar. 2011.

[24] Njoku, O. E., Agwa, O. K. and Ibiene, A. A., "An investigation of the microbiological and physicochemical profile of some fish pond water within the Niger Delta region of Nigeria," *African Journal of Food Science*, 9(3).1-12. Mar. 2015a.

[25] Wang, W., Gu, X., Zhou, L., Chen, H., Zeng, Q. and Mao, Z. "Antibiotics in crab ponds of Lake Guchenghu Basin, China. Occurrence, temporal variations, and ecological risks". *International Journal of Environmental Research and Public Health*, 15(3). 548. Mar. 2018.

[26] World Health Organization (WHO), "Water Quality Requirements https://www.who.int/water_sanitation_health/resourcesquality/wpcchap2.pdf. 2020 [Online] [Assessed June 11, 2020].

[27] Bhatnagar, A. and Devi, P. "Water quality guidance for the management of pond fish culture". *International Journal of Environmental Sciences*, 3(6). 1980-1993. 2013.

[28] Olaniyi, O. "Assessment of water quality characteristics for aquaculture uses in Abeokuta Nigeria". *Lakes, Reservoirs and Ponds*, 7(1). 9-19. Jan. 2013.

[29] Kamal, D., Khan, A.N., Raham, M.A. and Ahamed, A. "Study on physicochemical properties of water of Mouri River, Khulna, Bangladesh". *Pakistan Journal of Biological Sciences*, 10(5). 710-717. Apr. 2007.

[30] Food and Agricultural Organization (FAO), "Aquaculture production," *In: The state of world fisheries and aquaculture.* Food and Agriculture Organization of the United Nations: Rome, 18-19. 2016.

[31] Ansa, Y.B., Frimpong, E.A. and Amisah, S. "Characterization of potential aquaculture pond effluents and physicochemical and microbial assessment of effluent receiving waters in Central Ghana". *African Journal of Aquatic Science*, 38(2). 185-192. May. 2013.

[32] Atiku, S., Ogbaga, C. C., Alonge, O.O. and Nwagbara, O, F. "Comparative study of the physicochemical and bacteriological qualities of some drinking water sources in Abuja, Nigeria". *Global Journal of Pure and Applied Sciences*, 24(1) 91-98. June. 2018.

[33] Ehiagbonare, J.E. and Ogunrinde, Y.O. "Physicochemical analysis of fish pond water in Okada and its environs, Nigeria". *African Journal of Biotechnology*, 9(36). 5922-5928. Sept. 2010.

[34] Manjare, S.A., Vhanalakar, S.A. and Muley, D.V. "Water quality assessment of Vadgaon tank of Kolhapur (Maharashtra) with special reference to zooplankton". *International Journal of Advanced Biotechnology and Research*, 1.91-95. Dec. 2010.

[35] Kiran, B.R. "Physicochemical characteristics of fish ponds of Bhadra project at Karnataka". *Rasayar Journal*, 3(4). 671-676. 2010.

[36] Raj, J. A. and Sevarkodiyone, S.P. "A Study on physico-chemical parameters of Urinjikulam pond, Thiruthangal (Virudhungar District, Tamil Nadu)". *International Journal of Aquaculture and Fishery Sciences*, 4(1). 10-12. Feb. 2018.

[37] Torimiro, N., Bebe, P.T., Ogundipe, F.E., Esan, D.M.and Aduwo, A.I. "The bacteriology and physicochemical analysis of freshwater fish ponds". *International Research Journal of Microbiology*, 5(3). 28-32. Apr. 2014.

[38] Umeh, O. R., Chukwura, E. I., and Ibo, E. M., "Physicochemical, bacteriological and parasitological examination of selected fish pond water samples in Awka and its environment Anambra State, Nigeria". *Journal of Advances in Microbiology*, 20(3). 27-48. Mar. 2020.

[39] Stevens, C. "Dissolved Oxygen Science and Technology". *Aquaculture Networks*, 1.14-16. 2007.

[40] Federal Environmental Protection Agency (FEPA), Guidelines and standards for environmental pollution control. Federal Environmental Protection Agency, 27. 20-21. 1991.

[41] Prasanna, M. and Ranjan, P.C. "Physicochemical properties of water collected from Dhamra estuary". *International Journal of Environmental science*, 1(3). 334 - 342. 2010.

[42] Nduka, J.K., Orisakwe, O.E. and Ezenweke, L.O. "Some physicochemical parameter of portable water supply in Warri, Niger Delta Area of Nigeria". *Scientific Research and Essay*, 3(11). 547-551. Nov. 2008.

[43] Swann, L, "A fish farmer's guide to understanding water quality," http://aquatic.org/publicat/il-in/as-530htlm. 2006 [Online] [Assessed June 19, 2019].

[44] Ogeneogaga, O.I. and Solomon, R. J. "Physico-chemical and bacteriological investigation of selected fish pond in Kuje Area Council, Nigeria". *Researcher*, 9(4).31 - 45. Jan. 2017.

[45] Okafor, U.C., Ezeanochie, P. E. and Obubu, M. "Microbial assessment of some selected fish ponds in Awka, Anambra State: Comparative study and modeling". *Agricultural and Biological Sciences Journal*, 6(2).91-99. May. 2020.

[46] Douglas, S. I. and Isor, F.N. "Bacteriological investigation of pond water quality from Ogoniland, Nigeria". *Journal of Environmental Science, Toxicology and Food Technology*, 9(2).36-41. Feb. 2015.

[47] Gufe, C., Hodobo,T.C., Mbonjani, B., Majonga, O., Marumure, J., Musari, S., Jongi, G., Makaya, P.V. and Machakwa, J. "Antimicrobial profiling of bacteria isolated from fish sold at informal market in Mufakose, Zimbabwe". *Hindawi International Journal of Microbiology*, 2019: 1 - 7. May. 2019.

[48] Hua,L.M. and Apun, K., "Antimicrobial susceptibilities of *Vibrio parahaemolyticus* isolates from Tiger Shrimps (Penaeus monodon) aquaculture in Kuching, Sarawak," *Research Journal of Microbiology*. 8(1). 55-62. Jan. 2013.

[49] Ogbukagu, C.M., Anakwenze, V.N., Ekwealor, C.C., Ezemba, C.C. and Ekwealor, I.A., "Incidence of urinary tract infections (UTI) amongst patients attending primary health centres in Anambra state," *Advances in Microbiology*, 6. 537-547. Jan. 2016.

[50] Njoku, O. E., Agwa, O. K. and Ibiene, A. A. "Antibiotic susceptibility profile of bacteria isolates from some fishponds in Niger Delta Region of Nigeria". *British Microbiology Research Journal*, 7(4). 167-173. Mar. 2015b.

[51] Agoba, E.E., Adu, F., Agyare, C., Boamah, V.E. and Boakye, Y.D. "Antibiotic resistance patterns of bacterial isolates from hatcheries and selected fish farms in the Ashanti region of Ghana". *Journal of Microbiology and Antimicrobials*, 9(4). 35-46. Aug. 2017.