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Physicochemical, Bacteriological and Parasitological Examination of Selected Fish Pond Water Samples in Awka and Its Environment Anambra State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author UOR designed the study, did the statistical analysis, wrote the protocol and wrote the manuscript. The three authors managed the analysis of the study. Author UOR did the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A fish pond with recommended water quality will produce healthy fishes. Fish ponds with poor water quality will cause fish mortality and outbreak of diseases to fish consumers. Physicochemical analysis was done using standard analytical methods, the total bacterial count was determined by dilution and membrane filtration techniques. Parasitological analysis was done using the centrifugation method. A total of fifteen well waters were sampled during wet season. Results showed that the temperature ranged from 27°C to 29°C, p^H, 6.21 to 8.15; dissolved oxygen, 4.28 mg/l to 5.78 mg/l, electrical conductivity, 166.36 µs/cm to 394.00 µs/cm; total dissolved solids, 41 mg/l to 121 mg/l; total suspended solids, 1.00 mg/l to 19.40 mg/l; total solids, 42.00 mg/l to 140.4 mg/l; turbidity values, 7.01 NTU to 10.36 NTU; nitrate, 3.10 mg/l to 28.00 mg/l; total alkalinity, 36 mg/l to 91 mg/l; phosphate, 1.26 mg/l to 13.11 mg/l; sulphate, 0.39 mg/l to 4.37 mg/l; total chloride, 7.08 mg/l to 14.19 mg/l; carbonates, 1.33 mg/l to 2.35 mg/l; bicarbonates, 34.59 mg/l to 89.38 mg/l;

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total hardness, 25.31 mg/l to 53.04 mg/l; calcium hardness, 23.94 mg/l to 51.96 mg/l; magnesium hardness, 1.08 mg/l to 4.20 mg/l; total acidity, 2 mg/l to 22 mg/l; potassium, 0.04 mg/l to 2.23 mg/l; cadmium, 0.00 mg/l to 0.04 mg/l; lead, 0.01 mg/l - 0.16 mg/l; chromium, 0.00 mg/l - 0.03 mg/l; mercury was not detected, copper, 0.00 mg/l - 0.04 mg/l; arsenic, 0.00 mg/l - 0.02 mg/l; zinc, 0.00 mg/l to 0.02 mg/l; iron, 0.01 mg/l - 1.19 mg/l. The total bacterial counts ranged from 3.60-4.12 log cfu/ml; total coliforms, 14-46 cfu/100ml, *Vibrio cholerae*, 0-11 cfu/100ml; *Vibrio parahaemolyticus*, 0-15 cfu/100ml; faecal coliform, 1-9 cfu/100 ml; *Acinetobacter calcoaceticus*, 0-8 cfu/100 ml; *Bacillus subtilis*, 0-9 cfu/ml; *Staphylococcus aureus*, 0-5 cfu/ml; *Pseudomonas aeruginosa*, 0-12 cfu/100 ml; *Pseudomonas fluorescens*, 0-12 cfu/100 ml and *Clostridium perfringens* were not detected in any of the samples. Twelve bacterial species namely *Klebsiella pneumoniae, Acinetobacter calcoaceticus, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Pseudomonas fluorescens, Pseudomonas aeruginosa, Proteus mirabilis, Vibrio parahaemolyticus, Bacillus subtilis, Shigella flexineri* and *Salmonella typhi* were isolated and identified using standard analytical and molecular procedures. Parasites identified were *Ichthyobodo* species, *Diplostomum* species, *Myxobolus* species, *Chilodonella* species, *Bothriocephalus* species, *Ambiphrya* species and *Leech* species. *Salmonella typhi* had the highest frequency of isolation (20.63%) while *Acinetobacter calcoaceticus* and *Staphylococcus aureus* had the lowest frequency of isolation (2.83%). *Ichthyobodo* species had the highest frequency of isolation (21.43%) while *Leech* species had the lowest frequency of isolation (5.71%). Some of the physicochemical, bacteriological and parasitological parameters had values above World Health Organization admissible limits and therefore proper sanitary practices and water treatments must be employed to prevent epidemic among fish consumers.

Keywords: Sanitary risk assessment; physicochemical; bacteriological; parasitological and fish pond waters.

1. INTRODUCTION

Fish farming is the principal form of aquaculture which involves raising fish commercially in ponds, tanks or enclosures, usually for food [1]. Water is the home of fish but its quality is one of the most overlooked aspect of fish pond management until it affects fish production.

Fish is one of the staple foods and contributes about 60% of the world's source of protein. However, 60% of the developing countries derives more than 30% of their protein from fish [2]. Fish protein improves nutrition, in that it has a high biological value in terms of high protein retention in the body, low cholesterol level and presence of essential amino acids [2]. Fish is an important part of a healthy diet due to its high quality protein, other essential nutrients and omega 3-fatty acids, and its low fat content as compared to other meats [3]. Consumption of fishes may cause diseases due to infection or intoxication and some of these diseases have been specifically associated with pathogens which are resistant to antibiotics [4].

The high demand for fish has resulted to the increase in the number of fish ponds in Awka and its environment, Anambra State. Individual farmers, organized groups and institutions have developed constructed fish ponds and started fish farming oblivious of the cost. Due to lack of knowledge and failure to consult the Department of fisheries in Anambra State, most of the farmers are carrying out fish farming in a non-standard environment.

Heavy metals such as cadmium, zinc, mercury, chromium, copper, cobalt, nickel, manganese, iron, vanadium and molybdenum cause heavy pollution particularly in fish ponds [3]. Micro-organisms contribute a significant fraction of importance in the aquatic ecosystem and they have been observed to be among the factors that can cause the emergence of infectious diseases in the practice of aquaculture [5,6]. The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment [7,5].

These bacteria and heavy metals may enter the fish through the gills, penetration of egg membrane, ingestion, and rupture of skin, wounds or through the digestive tract. Symptoms of bacterial infections include: loss of appetite, fin and tail rot, pale gills and fluid in the abdomen. Behavioural signs of diseases include decreased feeding, weakness, lazy or erratic swimming; floating on water with the belly up, gathering or crowding at the inlet. Physical signs include gaping mouth, cloudy or distended eyes, open sores, lesions, loss of scales, pale, eroded, swollen, bloody or brownish gills. *Escherichia coli* (*E. coli*) is a frequent contaminant of food and

water and a well-recognized food-borne pathogen [8].

Lack of knowledge on good hygiene practices has also directly contributed to the degradation of fish ponds water quality for habitats thereby resulting to death of fish and outbreak of diseases among fish consumers. Hence, the need for rapid development and proper management of fishery is becoming a necessity in view of the high demand for fish at a relatively cheap rate.

1.1 Aim

Physicochemical, bacteriological and parasitelogical examination of selected fish pond water samples in Awka and its environment Anambra State, Nigeria.

1.2 Specific Objectives

The specific objectives of this research were to:

I. Evaluate the sanitary risk of the sampled points.
II. Determine the physicochemical parameters such as temperature, pH, electrical conductivity, total dissolved solids, total suspended solids, total solids, turbidity, nitrate, total alkalinity, phosphate, sulphate, total chloride, dissolved oxygen, $B.O.D_5$, total hardness, calcium hardness, magnesium hardness, carbonates, bicarbonates, total acidity, potassium, cadmium, lead, chromium, mercury, copper, arsenic, zinc and iron on the selected fish pond water samples and comparing with World Health Standard.
III. Enumerate the total bacterial load, total and faecal coliforms and other pathogenic bacteria present (*Staphylococcus aureus*, *Acinetobacter calcoaceticus* and *Bacillus subtilis*) in the selected fish pond water samples and comparing with World Health Organization Standard.
IV. Determine the parasites present in the selected fish pond water samples.
V. Characterize the bacteria.

2. METHODS

2.1 Study Area

The study area for this research is selected fish pond waters in Awka and its environment, Anambra State, Nigeria. Awka is the capital of Anambra State, Nigeria. Awka is made up of two local government areas, namely: Awka South and Awka North.

2.2 Sanitary Risk Inspection of the Fish Ponds

Vital information about the fish ponds such as source of water supply, period or length of use of water, size of the ponds, feed formulation, challenges, fingerlings production, proximity of fish ponds to farm lands, Proximity of fish ponds to septic tanks, stocking density, age of fishes were obtained by oral interview and visual analysis.

2.3 Sample Collection

Fifteen water samples were collected from fifteen major commercial concrete fish ponds, stocked with cat fishes in Awka environment, Anambra State during the month of August, 2018. These water samples were collected in the morning period (7 am – 9 am). One liter of composite water samples were collected in one liter sterile containers with stoppers, from the outlets of each pond. Prior to sample collection, all the sampling bottles were rinsed with the same water to be collected from the ponds. The sampling bottles were labeled with dates and collection sites. The water samples were kept at 4°C in an ice box and transported to the laboratory within 2 hours for immediate sample analysis. These water samples were sampled three times each for bacteriological analysis.

2.4 Physicochemical Analysis

The physicochemical parameters evaluated were temperature, pH, electrical conductivity, total dissolved solids, total suspended solids, total solids, turbidity, nitrate, total alkalinity, phosphate, sulphate, total chloride, dissolved oxygen, biological oxygen demand, total hardness, calcium hardness, magnesium hardness, carbonates, bicarbonates, total acidity, potassium, cadmium, lead, chromium, mercury, copper, arsenic, zinc and iron. The evaluation was carried out as contained in APHA [9]. Temperature, p^H, dissolved oxygen, turbidity and electrical conductivity were measured *in-situ* because of low stability.

2.5 Bacteriological Analysis

Bacterial isolation was done according to the method described by Cheesbrough [10]. The media used were prepared according to manufacturer's instruction.

The glass wares such as Petri dishes, conical flasks, test tubes, beakers and bijou bottles were thoroughly washed and sterilized in a hot air oven at 160°C for an hour. The inoculating loop was sterilized by flaming in the Bunsen burner until it turns red hot. Similarly, microbial load on the working surfaces were reduced by the application of disinfectant solution (70% ethanol).

2.6 Determination of Total Bacterial Count

Composite water samples collected from the outlets of each pond were homogenized by shaking them for 25 times, beside a bunsen burner. The bacterial load of the water samples from the fish ponds were determined by performing ten-fold serial dilution in test tubes containing sterile water up to 10^{-5}. Nine millilitres (mls) of sterile water was transferred aseptically into 5 sterile test tubes labelled 10^{-1} to 10^{-5}, one ml of the water samples were also aseptically transferred into the first tube (10^{-1}) with a sterile pipette then serial dilution. This was repeated until the 5th tube. The total viable count (Total plate count) was determined using the pour plate technique, cultured in triplicates. 1 ml of the samples from 10^{-1} to 10^{-3} of the dilution test tubes were aseptically transferred into the Petri plates. The plates were labelled before inoculation and the culture medium was Nutrient Agar. The medium was prepared according to the manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes at 15psi and then allowed to cool to 45°C before dispensing about twenty milliliters into sterile Petri-dishes and allowed to solidify, inverted to prevent condensation droppings from the lid into the agar and incubated in the incubator at 37°C for 24 hours. A control was equally prepared without adding the sample. The bacterial colonies ranging from 30 to 300 were counted using a colony counter and expressed in colony forming unit per ml (CFU/ml).

$$\text{Colony forming unit / ml} = \frac{N}{V \times D}$$

N = Average number of colonies
V = Aliqout volume
D = Dilution factor

The bacteria isolates were counted using a colony counter and sub-cultured on a freshly prepared nutrient agar for characterization and identification.

2.7 Examination of Total and Faecal Coliform by Membrane Filtration Method

A sterile filtration apparatus was placed in position and connected to a vacuum pump. The apparatus was rinsed by passing small amount of sterile water and the water sample through the funnel and applying pressure through the vacuum pump. The water samples were thoroughly mixed by shaking for 25 times beside a Bunsen flame and one hundred milliliters of the water samples were measured and dispensed into the funnel and slowly filtered through the membrane filter consisting of a cellulose compound with a uniform pore diameter of 0.2 µm by applying pressure through the vacuum pump. After filtration, the membrane filter containing the bacteria was carefully unscrewed and picked up using sterile forceps and placed upright in a Petri-plate ensuring that there was no air bubbles trapped under the membrane paper, incubated at an appropriate temperature with a selective and differential culture medium, characteristic colonies of total coliforms/ faecal coliforms developed and were counted using a colony counter. The sterile funnel was carefully and accurately replaced on the filter base and then screwed for another filtration. Eosine methylene blue agar at 44.5°C incubation for 24 hours was used for faecal coliforms, while Mac Conkey agar medium at 37°C incubation for 48 hours was used for total coliforms.

2.8 Detection of *Vibrio cholerae and Vibrio parahaemolyticus*

Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar was weighed and prepared based on the manufacturer's instruction. A given volume of sterile water was dispensed into the weighed medium, shaked, heated using a Bunsen flame, cooled, aseptically dispensed into the Petri dishes and allowed to gel. The membrane filter paper containing the bacteria was carefully placed with the grid-side uppermost on the prepared Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar. Duplicate plates were prepared and labeled for the water samples. Incubation was carried out in an inverted position at 37°C for 24 hours. The presence of yellow colonies were suspected to be *Vibrio cholerae* and green colonies were suspected to be *Vibrio parahaemolyticus*. The colonies that developed were counted using a

colony counter and the result recorded. Each colony was sub-cultured and stored on a sterile nutrient agar slant for characterization and identification.

2.9 Detection of *Salmonella typhi* and *Shigella flexineri*

Salmonella-Shigella agar was weighed and prepared based on the manufacturer's instruction. A given volume of sterile water was dispensed into the weighed medium, shaked, heated using a Bunsen flame, cooled, aseptically dispensed into the petri dishes and allowed to gel. The membrane filter paper containing the bacteria was carefully placed with the grid-side uppermost on the prepared Salmonella-Shigella agar. Duplicate plates were prepared and labeled for the water samples. Incubation was carried out in an inverted position at 37°C for 24 hours. The presence of colourless colonies with black centers were suspected to be *Salmonella* species while colourless colonies without black centers were suspected to be *Shigella* species. The colonies that developed were counted using a colony counter and the result recorded. Each different colony was sub-cultured and stored on a sterile nutrient agar slant for characterization and identification using Gram stain and biochemical tests.

2.10 Detection of *Pseudomonas aeruginosa and Pseudomonas fluorescens*

Cetrimide agar was weighed and prepared based on the manufacturer's instruction. It was sterilized in an autoclave at 15 psi (121°C) for 15 minutes, allowed to cool and aseptically dispensed into Petri dishes. The membrane filter paper containing the bacteria was carefully placed with the grid-side uppermost on the cetrimide agar. Duplicate plates were prepared and labeled for the water samples. Incubation was carried out in an inverted position at 37°C for 24 hours. The presence of green discrete colonies on the agar were suspected to be *Pseudomonas* species. The colonies that developed were counted using a colony counter and result recorded. Each different colony was sub-cultured and stored on a sterile nutrient agar slant for characterization and identification using Gram stain and biochemical tests.

2.11 Detection of *Clostridium perfringens*

The water samples (1 milliliter) were inoculated into labelled Petri-plates. Sterile Reinforced Differential Clostridial medium with nystatin at45°C was dispensed into the plates, gently swirled and incubated anaerobically using an anaerobic jar at 25°C for 72 hours. No growth or black colonies were seen.

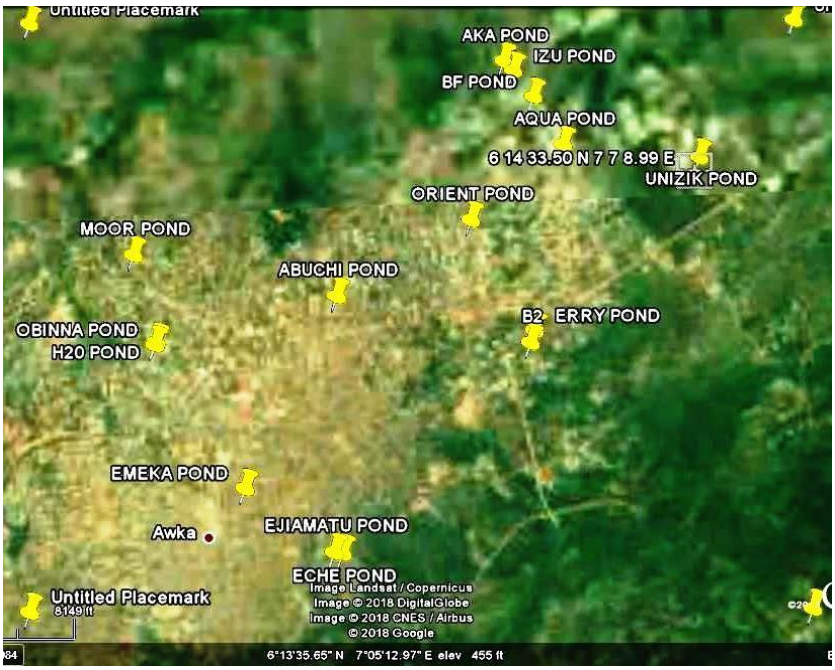

Fig. 1. Satellite imagery of the study area

2.11.1 Detection of parasites

The sample cans were shaken for 25 times and 10 millilitres of the samples were dispensed into a 10 ml centrifuge tube, capped and cleaned using a sterile cotton wool. These tubes were placed inside a centrifuge machine for centrifugation at 360 revolutions per second for 10 minutes. The tubes were allowed to settle properly for five minutes and the supernatants were removed. The resulting residue was poured onto a clean grease-free glass slide. Two drops of Lugol's iodine were poured (to intensify reactions between cells and stain) and covered with a cover slide. Excess water was blotted using a sterile filter paper and viewed at ×10 and ×40 objective lens for the presence of parasite ova and cysts. The parasites were counted, identified and recorded per fish pond water sample.

2.12 Characterization and Identification of the Bacterial Isolates was done According to the Method of Cheesbrough [10]

The cultural characteristic of the respective isolates were examined and recorded.

2.12.1 Gram-staining and microscopic examination

This was done according to the procedure described by Cheesbrough [10].

2.12.2 Biochemical tests

These biochemical tests were carried out according to Cheesbrough [10].

Catalase test, coagulase test, citrate utilization test, oxidase test, urease test, indole test, motility test, voges-proskauer test, methyl red test, sugar fermentation and hydrogen Sulphide Test.

3. RESULTS

3.1 The Physical Parameters of the Fish Pond Waters (Table 1)

The temperature values ranged from 27°C to 29°C, ph ranged from 6.21 to 8.15 and 20% of the ph values were below W.H.O. standard, electrical conductivity ranged from 166.36 µs/cm to 394.00 µs/cm, total dissolved solids ranged from 41 mg/l to 121 mg/l, total suspended solids ranged from 1.00 mg/l to 19.40 mg/l, total solids values ranged from 42.00 mg/l to 140.4 mg/l, turbidity values ranged from 7.01 NTU to 10.36 NTU, 100% of the turbidity values were above W.H.O. Standard.

3.2 The Chemical Parameters of the Fish Pond Waters (Table 2)

The values for nitrate ranged from 3.10 mg/l to 28.00 mg/l and 53.33% of the nitrate values were above W.H.O. standard, total alkalinity values ranged from 36 mg/l to 91 mg/l, phosphate ranged from 1.26 mg/l to 13.11 mg/l and 100% of the phosphate values were above W.H.O. standard, sulphate ranged from 0.39 mg/l to 4.37 mg/l, total chloride ranged from 7.08 mg/l to 14.19 mg/l, dissolved oxygen (1^{st} day) ranged from 4.28 mg/l to 5.78 mg/l and 46.67% of the dissolved oxygen values were below W.H.O (2006) Standard of > 5, dissolved oxygen (5^{th} day) ranged from 0.04 mg/l to 3.18 mg/l, biochemical oxygen demand (BOD_5) ranged from 2.25 mg/l to 4.27 mg/l, total hardness ranged from 25.31 mg/l to 53.04 mg/l, calcium hardness ranged from 23.94 mg/l to 51.96 mg/l, magnesium hardness ranged from 1.08 mg/l to 4.20 mg/l, carbonates ranged from 1.33 mg/l to 2.35 mg/l, bicarbonates ranged from 34.59 mg/l to 89.38 mg/l, total acidity ranged from 2 mg/l to 22 mg/l and potassium values ranged from 0.04 mg/l to 2.23 mg/l.

3.3 The Heavy Metal Parameters of the Fish Pond Waters (Table 3)

The values for cadmium ranged from 0.00 mg/l to 0.04 mg/l and 6.67% of the cadmium values were above W.H.O. standard, lead values ranged from 0.01 mg/l to 0.16 mg/l and 73.33% of the lead values were above W.H.O. standard, chromium ranged from 0.00 mg/l to 0.03 mg/l, mercury was not detected, copper ranged 0.00 mg/l to 0.04 mg/l, arsenic ranged from 0.00 mg/l to 0.02 mg/l and 26.67% of the arsenic values were above W.H.O. standard, zinc values ranged from 0.00 mg/l to 0.02 mg/l and iron values ranged from 0.01 mg/l – 1.19 mg/l and 20% of the iron values were above W.H.O. standard.

3.4 The Bacteriological Characteristics of the Fish Pond Waters (Table 4)

The mean total bacterial count for 10^{-2} dilution tube ranged from 40 cfu/ml to 133 cfu/ml with 40% having values above 100 cfu/ml World Health Standard. The logarithmic values for 10^{-2} dilution tube ranged from 3.60 cfu/ml to 4.12

cfu/ml. The faecal coliform count ranged from 1 cfu/100 ml to 9 cfu/100 ml where all the examined fish ponds had values above 0cfu/ml W.H.O Standard. *Staphylococcus aureus* count ranged from 0 cfu/ml to 5 cfu/ml where 66.67% had values above 0cfu/ml W.H.O Standard. *Bacillus subtilis* count ranged from 0 cfu/ml to 9 cfu/ml where 73.33% had values above 0 cfu/ml W.H.O Standard. *Pseudomonas aeruginosa* count ranged from 0 cfu/100ml to 12 cfu/100 ml where 93.33% had values above 0 cfu/ml W.H.O Standard. *Vibrio cholerae* count ranged from 0 cfu/100 ml to 11 cfu/100 ml where 80% had values above 0cfu/ml W.H.O Standard. *Vibrio parahaemolyticus* count ranged from 0 cfu/100ml to 15 cfu/100 ml where 60% had values above 0 cfu/ml W.H.O Standard. Total coliform count ranged from 14 cfu/100 ml to 46 cfu/100 ml and 100% were above 0 cfu/ml W.H.O Standard. *Clostridium perfringens* count ranged from 0 cfu/100ml to 0 cfu/100 ml and were within W.H.O Standard. *Pseudomonas fluorescens* count ranged from 0 cfu/100 ml to 12 cfu/100 ml where 53.33% had values above 0 cfu/ml W.H.O Standard. *Acinetobacter calcoaceticus* count ranged from 0 cfu/ml to 8 cfu/ml where 33.33% had values above 0cfu/ml W.H.O Standard.

3.5 Distribution of Bacteria Present in the Fish Pond Waters (Table 5)

The bacteria isolated from the fish pond water samples were denoted using a positive (+) sign while those bacteria not found in some fish pond water samples were shown using a negative (-) sign.

3.6 The Frequency of Occurrence and Percentage Frequency of Bacteria Present in the Fish Pond Waters (Table 6)

One hundred and sixty-three colonies of *Klebsiella pneumonia*e were isolated from the whole fish pond water samples with a percentage frequency of 16.48%. Twenty-eight colonies of *Acinetobacter calcoaceticus* were isolated from the whole fish pond water samples with a percentage frequency of 2.83%. Fifty-six colonies of *Escherichia coli* were isolated from the whole fish pond water samples with a percentage frequency of 5.66%. Twenty-eight colonies of *Staphylococcus aureus* were isolated from the whole fish pond water samples with a percentage frequency of 2.83%. Eight-two colonies of *Vibrio cholerae* were isolated from the whole fish pond water samples with a percentage frequency of 8.29%. Fifty-six colonies of *Pseudomonas fluorescens* were isolated from the whole fish pond water samples with a percentage frequency of 5.66%. Ninety colonies of *Pseudomonas aeruginosa* were isolated from the whole fish pond water samples with a percentage frequency of 9.10%. Seventy-five colonies of *Proteus mirabilis* were isolated from the whole fish pond water samples with a percentage frequency of 7.58%. Sixty-eight colonies of *Vibrio parahaemolyticus* were isolated from the whole fish pond water samples with a percentage frequency of 6.88%. Forty colonies of *Bacillus subtilis* were isolated from the whole fish pond water samples with a percentage frequency of 4.05%. Ninety-nine colonies of *Shigella flexineri* were isolated from the whole fish pond water samples with a percentage frequency of 10.01%. Two hundred and four colonies of *Salmonella typhi* were isolated from the whole fish pond water samples with a percentage frequency of 20.63%.

3.7 The Distribution of Parasites in the Fish Pond Water Samples (Table 7)

The parasites isolated from the fish pond water samples were denoted using a positive (+) sign while those parasites not found in some fish pond water samples were shown using a negative (-) sign.

3.8 The Frequency of Occurrence and Percentage Frequency of Parasites in the Fish Pond Water Samples (Table 8)

Fifteen species of *Ichthyobodo* were isolated from the whole fish pond water samples with a percentage frequency of 21.43%. Fourteen species of *Diplostomum* were isolated from the whole fish pond water samples with a percentage frequency of 20%. Seven species of *Myxobolus* were isolated from the whole fish pond water samples with a percentage frequency of 10%. Ten species of *Chilodonella* were isolated from the whole fish pond water samples with a percentage frequency of 14.29%. Eleven species of *Bothriocephalus* were isolated from the whole fish pond water samples with a percentage frequency of 15.71%. Nine species of *Ambiphrya* were isolated from the whole fish pond water samples with a percentage frequency of 12.86%. Four species of *Leech* were isolated from the whole fish pond water samples with a percentage frequency of 5.71%.

Table 1. The physical parameters of the fish pond waters

Pond names	Temperature (°C)	pH	E.C (µs/cm)	TDS (mg/l)	TSS (mg/l)	TS (mg/l)	Turbidity (NTU)
Morr	29	6.73	258.19	56	02.47	58.47	08.07
Aka	29	8.15	394.00	121	19.40	140.4	10.36
Erry	27	7.00	191.61	43	01.16	44.16	07.13
Unizik	29	7.91	376.11	95	13.00	108.0	09.44
H_2O	28	6.88	258.94	57	02.13	59.13	07.69
Eche	27	7.71	344.05	82	05.96	87.96	08.81
Ejiamatu	28	6.94	299.23	77	03.10	80.10	08.24
Abuchi	28	6.52	221.77	51	01.28	52.28	07.21
Aqua	28	6.42	209.05	50	01.02	51.02	07.09
Emeka	27	6.21	166.36	41	01.00	42.00	07.01
B.F.	28	7.19	290.07	66	02.19	68.19	07.92
Orient	27	6.82	237.85	54	01.51	55.51	07.50
Obinna	28	7.50	325.14	80	05.53	85.53	08.33
B_2	28	6.45	260.64	61	02.15	63.15	07.71
Izu	28	7.05	264.32	65	02.17	67.17	07.85
W.H.O (2006)	**25-32**	**6.5-8.5**	**1000**	**500**	**30**	**500**	**5**

Key: B.F pond = Book foundation pond; TDS = Total dissolved solids; TS = Total solids; NTU = Nephelometric turbidity unit; mg/l = Milligram per milliliter; E.C = Electrical conductivity; TSS = Total suspended solids; M = Meters; °C = Degree centigrade; µs/cm =Microsiemens per centimeter

3.9 The Morphological and Biochemical Characteristics of Bacteria Isolated from the Fish Pond Water Samples (Table 9)

Salmonella typhi, Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Vibrio parahaemolyticus, Klebsiella pneumoniae, Shigella flexineri, Proteus mirabilis, and *Acinetobacter calcoaceticus* were identified.

4. DISCUSSION

Results of sanitary risk assessment of the fish ponds revealed poor sanitary practices among fish farmers.

The physicochemical characteristics of the fish pond waters varied and this may be attributed to different levels of sanitary practice among fish farmers, fish metabolism, fish feeds, stocking density, pond size and organic manure used in pond fertilization. The variations observed in the physicochemical properties of the fish ponds carried out in Nigeria could be attributed to the influences of the micro-climatic, topographic and edaphic conditions of fish ponds in the areas.

Temperature of an organism is defined as the level of hotness or coldness in the body of a living organism either in water or land. Temperature, an important parameter in this study influences fish growth and biological oxygen demand in ponds. Fish is a poikilothermic animal, so its temperature is dependent on the temperature of its environment. It changes with the temperature of the surroundings. The temperature changes affect the metabolism and physiology of fishes and so its productivity. As water temperature increases, it holds less oxygen. Also plants and animals use more oxygen due to increased respiration. These factors commonly result in less available oxygen for fishes in water. The temperature values observed in the fish ponds ranged from 27°C to 29°C (Table 1) respectively and are within W.H.O (2006) maximum containment level goal (25°C-32°C). The values were similar to the report by (43) who recorded a temperature range of 27°C-29°C from the physicochemical analysis of freshwater fish ponds at Ile-ife, (18) who recorded temperature range of 24.66°C – 28.70°C from concrete fish ponds in Okada and its environments, Nigeria and [11] who observed a temperature of 27°C-28°C in the water characteristics of Kojalo fish ponds. The observed water temperatures in the fish ponds are considered normal for fishes.

Table 2. The chemical parameters of the fish pond waters in milligram per liter (mg/l)

Pond Names	Ni.	T.A.	Pho.	Sul.	T.C.	DO_1	DO_5	$B.O.D_5$	T.H.	C.H.	M.H.	Car.	Bic.	T.A.	Po.
Morr	13.49	72	3.07	1.12	14.00	4.28	0.76	3.52	51.39	47.36	04.03	1.77	70.23	16	0.89
Aka	28.00	91	13.11	1.10	08.74	4.31	0.04	4.27	47.00	44.15	02.85	1.62	89.38	02	2.23
Erry	05.01	46	1.37	0.92	10.30	5.78	2.40	3.38	50.06	47.59	02.47	1.44	44.56	10	0.17
Unizik	25.03	85	3.95	0.97	09.17	4.39	0.15	4.24	43.25	40.62	02.63	1.89	83.11	05	1.84
H_2O	07.88	54	2.14	4.37	14.19	5.36	2.25	3.11	40.28	36.09	04.19	2.35	51.65	11	0.75
Eche	22.69	83	3.77	1.30	07.10	5.21	1.27	3.94	33.15	29.54	03.61	1.86	81.14	13	0.91
Ejiamatu	16.33	76	3.30	1.18	07.15	4.85	0.74	4.11	44.71	43.23	01.48	2.00	74.00	19	0.80
Abuchi	06.22	47	1.55	1.03	07.08	5.00	2.01	2.99	35.90	34.18	01.72	1.71	45.29	20	0.66
Aqua	03.63	41	1.31	0.50	12.52	5.43	3.18	2.25	33.87	32.33	01.54	1.50	39.50	14	0.10
Emeka	03.10	36	1.26	0.39	07.33	5.09	2.11	2.98	28.64	24.84	03.80	1.41	34.59	22	0.04
B.F.	12.47	68	2.83	2.00	13.75	4.82	0.73	4.09	29.17	24.97	04.20	2.03	65.97	07	1.42
Orient	06.27	50	1.86	1.24	09.11	5.16	1.33	3.83	31.08	29.87	01.21	1.75	48.25	17	0.63
Obinna	19.04	79	3.49	2.17	14.19	4.75	0.63	4.12	37.22	35.52	01.70	1.46	77.54	09	1.76
B_2	08.51	61	2.29	1.03	12.37	5.37	1.79	3.58	53.04	51.96	01.08	1.33	59.67	12	0.59
Izu	10.60	68	2.80	2.03	12.87	4.41	0.49	3.92	25.31	23.94	01.37	2.17	65.83	14	1.12
W.H.O (2006)	**10**	**250**	**0.5**	**250**	**250**	**5**	**5**	**6**	**250**	**75**	**50**	**-**	**-**	**50**	**5**

Key: Ni = Nitrate; Sul = Sulphate; B.O.D = Biological oxygen demand; M.H = Magnesium hardness; T.A = Total acidity; T.A = Total alkalinity; T.C = Total chloride; T.H = Total hardness; Car = Carbonates; Po = Potassium; Pho = Phosphate; DO = Dissolved oxygen; C.H = Calcium hardness; Bic = Bicarbonates

Table 3. The heavy metal parameters of the fish pond waters

Pond Names	Cadmium (mg/l)	Lead (mg/l)	Chromium (mg/l)	Mercury (mg/l)	Copper (mg/l)	Arsenic (mg/l)	Zinc (mg/l)	Iron (mg/l)
Morr	0.01	0.02	0.00	0.00	0.02	0.02	0.01	0.02
Aka	0.03	0.16	0.03	0.00	0.02	0.02	0.02	1.08
Erry	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Unizik	0.03	0.10	0.02	0.00	0.02	0.00	0.02	1.19
H_2O	0.00	0.02	0.00	0.00	0.01	0.00	0.02	0.03
Eche	0.02	0.03	0.02	0.00	0.03	0.01	0.01	0.24
Ejiamatu	0.02	0.02	0.02	0.00	0.04	0.01	0.01	0.10
Abuchi	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.02
Aqua	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.01
Emeka	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
B.F.	0.01	0.02	0.02	0.00	0.01	0.01	0.02	0.02
Orient	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.01
Obinna	0.04	0.02	0.03	0.00	0.02	0.02	0.01	1.15
B_2	0.01	0.03	0.00	0.00	0.00	0.01	0.02	0.07
Izu	0.02	0.06	0.01	0.00	0.02	0.02	0.02	0.03
W.H.O (2006)	**0.03**	**0.01**	**0.05**	**0.001**	**2.0**	**0.01**	**3.0**	**0.3**

Table 4. Bacteriological characteristics of the fish pond waters

Pond names	Log cfu/ml (10^{-2})	F. C. C. (cfu/100 ml)	S. a. C cfu/ml)	B. s. C. (cfu/ml)	P. a. C. (cfu/100 ml)	V. c. C. (cfu/100 ml)	V. p. C. (cfu/100 ml)	T. C. C. (cfu/100 ml)	*C. p. C.* (cfu/100 ml)	*P. f. C.* (cfu/100 ml)	*A. c. C.* (cfu/ml)
Morr	4.03	4	2	4	10	5	5	44	0	4	0
Aka	4.12	9	5	9	6	11	10	43	0	0	0
Erry	3.77	2	1	2	2	4	0	22	0	0	0
Unizik	4.11	7	4	7	6	9	9	40	0	0	0
H_2O	3.92	2	0	1	0	0	5	46	0	0	0
Eche	4.07	8	5	3	3	11	0	41	0	6	4
Ejiamatu	4.06	4	2	3	8	7	15	40	0	0	0
Abuchi	3.83	1	0	0	7	0	0	30	0	11	5
Aqua	3.74	1	3	0	3	3	4	19	0	4	8
Emeka	3.60	1	0	0	5	0	0	14	0	0	0
B.F.	3.97	4	1	3	8	8	4	45	0	4	3
Orient	3.85	1	0	0	3	4	0	35	0	12	0
Obinna	4.05	5	3	5	10	10	7	40	0	0	0
B_2	3.94	2	0	1	7	5	0	45	0	12	8
Izu	3.95	5	2	2	12	5	9	37	0	3	0
W.H.O (2006)	**100**	**0**	**0**	**0**	**0**	**0**	**0**	**10**	**0**	**0**	**0**

Key: Log cfu/ml Logarithm colony forming unit/ml; F.C.C. Faecal coliform count on Eosin methylene blue agar; S.a.C Staphylococcus aureus count on Nutrient agar; B. s. C. Bacillus subtilis count on Nutrient agar; P. a. C Pseudomonas aeruginosa count on Cetrimide agar; V. c. C. Vibrio cholerae count on Thiosulphate citrate bile salt sucrose agar; V. p. C; Vibrio parahaemolyticus; count on Thiosulphate citrate bile salt sucrose agar; T. C. C. Total coliform count on MacConkey agar; C. p. C. Clostridium perfringens count Reinforced differential clostridial medium; P. f. C. Pseudomonas fluorescens count on Cetrimide agar; A. c. C. Acinetobacter calcoaceticus Count on Nutrient agar

Table 5. Distribution of bacteria in the fish pond waters

Pond Names	*Salmonella typhi*	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Vibrio cholerae*	*Pseudomonas aeruginosa*	*Clostridium perfringens*	*Pseudomonas fluorescens*	*Vibrio parahaemolyticus*	*Klebsiella pneumoniae*	*Shigella flexineri*	*Proteus mirabilis*	*Acinetobacter calcoaceticus*
Morr	+	+	+	+	+	+	-	+	+	+	-	+	-
Aka	+	+	+	+	+	+	-	-	+	+	+	+	-
Erry	+	+	+	+	+	+	-	-	-	+	+	+	-
Unizik	+	+	+	+	+	+	-	-	+	+	+	+	-
H_2O	+	+	+	-	-	-	-	-	+	-	+	+	-
Eche	+	+	+	+	+	+	-	+	-	+	-	-	+
Ejiamatu	-	+	+	+	+	+	-	-	+	+	+	-	-
Abuchi	+	-	+	-	-	+	-	+	-	-	+	-	+
Aqua	-	-	+	+	+	+	-	+	+	+	-	+	+
Emeka	+	-	+	-	-	+	-	-	-	+	+	-	-
B.F.	+	+	+	+	+	+	-	+	+	-	+	-	+
Orient	+	-	+	-	+	+	-	+	-	-	-	+	-
Obinna	+	+	+	+	+	+	-	-	+	+	+	-	-
B_2	-	+	+	-	+	+	-	+	-	-	+	+	+
Izu	+	+	+	+	+	+	-	+	+	+	-	-	-

Table 6. Frequency of occurrence and percentage frequency of bacteria in the fish pond waters

Bacterial isolates	Number of colonies isolated	Frequency of isolation (%)
Klebsiella pneumoniae	163	16.48
Acinetobacter calcoaceticus	28	2.83
Escherichia coli	56	5.66
Staphylococcus aureus	28	2.83
Vibrio cholerae	82	8.29
Pseudomonas fluorescens	56	5.66
Pseudomonas aeruginosa	90	9.10
Proteus mirabilis	75	7.58
Vibrio parahaemolyticus	68	6.88
Bacillus subtilis	40	4.05
Shigella flexineri	99	10.01
Salmonella typhi	204	20.63
Total	**989**	**100**

pH value is an indication of the level of acidity or alkalinity of a solution. The pH values obtained for fish ponds ranged from 6.21 to 8.15 (Table 1) and 20% of the water samples were below W.H.O (2006) maximum containment level goal of 6-9. The outlet values were similar to the report by Torimiro et al. [12] who recorded pH range of 6.65-7.80 from the physicochemical analysis of freshwater fish ponds at Ile-ife. The values were slightly different from the report of [13] who recorded pH range of 6.97-7.12 from concrete fish ponds in Okada and its environments, Nigeria. The pH values were also slightly different from the results of Babatunde Woke and [14] who observed values of 6.41-7.75 from physicochemical burden of Oyo State fish pond, Ibadan, South-west Nigeria. This may be attributed to high level of fish wastes, composition of fish feeds, stocking density, organic manure in my study areas. The values were similar to the report by Ogeneogaga and Solomon [15] who recorded a pH range of 7.00 to 8.00 from the physicochemical analysis of some selected fish Pond in Kuje Area Council, Nigeria.

Electrical conductivity is a measure of the ability of water to conduct electricity. It is dependent on the ionic concentration and water temperature. The total load of salts in a water body is directly related to its conductivity. Conductivity values reported in the fish ponds ranged from 166.36 µS/cm to 394.00 µS/cm (Table 1). These values fall within the WHO [16] limits of 1000 µS/cm, and disagreed with the report of [14] who observed higher values of 78 µS/cm – 1428.50 µS/cm from physicochemical burden of Oyo State fish pond, Ibadan, South-West Nigeria, so the studied fish pond waters would be regarded as safe for fish production. The excellent (low) conductivity values gotten from my analysis can be attributed to the rainy season in which the samples were collected. Previous studies have shown that dilution of water during the rainy season lowers the levels of electrical conductivity and it increases during dry season [17].

Total Dissolved Solids (TDS) is an indication of the amount of dissolved substances. The TDS values ranged from 41 mg/l to 121 mg/l (Table 1). These values were within W.H.O [16] maximum containment level goal of 500mg/l. The values were above findings (19.91 to 24.25 mg/l) recorded by [18]. This can be attributed to presence of organic manure and composition of fish feeds. It has been reported that fish farmers use artificial animal feeds to supplement pond which has been reported to increase total dissolved solids [18].

The total suspended solids (TSS) are made up of carbonates, bicarbonates, chlorides, phosphates and nitrates of metals such as calcium, magnesium sodium, potassium, magnesium as well as other particles. TSS affects the turbidity of water bodies [19]. The values obtained from the fish pond waters ranged from 1.00 mg/l to 19.40 mg/l (Table 1). The values obtained were within the W.H.O permissible limit of 30 mg/l which is good for optimum fish productivity. The TSS values were below 80 mg/l to 180 mg/l recorded by [12] from fish pond water samples at Ile-Ife. This may be due to better sanitary practices.

Total solids (TS) is a combination of dissolved solids and total suspended solids. The values obtained from the fish pond waters ranged from

42.00 mg/l to 140.4 mg/l (Table 1). The values obtained are within the W.H.O permissible limit of 500mg/l which is good for optimum fish productivity. The TS values were below 230 mg/l to 340 mg/l recorded by Torimiro et al. [12] from fish pond water samples at Ile-Ife. This may be due to better sanitary practices.

Turbidity is an indication of the clarity of a water or a measure of the ability of water to transmit the light that restricts light penetration and limit photosynthesis [19]. Turbidity consists of suspended particles in water and is usually affected by factors such as clay particles, dispersion of plankton organism, particulate organic matters as well as pigments caused by decomposition of organic matter. Turbidity values reported in the fish pond waters ranged from 7.01 NTU to 10.36 NTU (Table 1) and were within the W.H.O. [16] permissible limit of 10 NTU except Aka pond and therefore only 6.67 % of the turbidity values were above the permissible limit. The turbidity results were below [20] findings, who reported higher turbidity values of 16 NTU to 118.0 NTU, which he attributed to rains which cause flooding and surface run off hence depositing nutrients, silt, and domestic wastes into the ponds. The turbidity results were similar to the values (7.73 NTU to 11.33 NTU) recorded by [21]. Turbidity affects the appearance of water. Water with high turbidity is normally associated with high microbiological contamination which is in congruence with the bacterial population in Aka fish pond which had the highest value of turbidity in this study. This high turbidity in Aka pond may be as a result of poor sanitary risks such as low or absence of apron, fissured interior linings in the ponds, stocking density, time frame for removal/exchange of pond water, organic matter. Emeka pond had the lowest value of turbidity due to better sanitary practice. The well depth (source of water) ranged from 11 meters to 14 meters and are classified as shallow wells according to WHO [16].

Nitrate represents the final product of the biochemical oxidation of ammonia [19]. It is important that the level of nitrate in a pond is controlled to avoid eutrophication. Nitrates are however not harmful to fish but cause methaemoglobinemia in humans when it exceeds the maximum containment level goal. Nitrate concentrations in the fish ponds ranged from 3.10 mg/l to 28.00 mg/l (Table 2) and 53.33% of the nitrate values were above W.H.O. permissible limit of 10 mg/l and therefore not good for fish farming. This may be as a result of proximity of well water (which serves as the source of water) to farmlands (fertilizers), septic tanks and the use of organic manure for pond fertilization.

Water alkalinity is a measure of its capacity to neutralize acids. It can be referred to as the buffering capacity of water. Waters with high alkalinity are undesirables. The obtained alkalinity values for fish pond water samples ranged from 36 mg/l to 91 mg/l (Table 2). According to [22], optimum alkalinity for fish productivity is between 25 mg/l to 100 mg/l. The values obtained were within [22] and [16] permissible limit of 250 mg/l, which makes these ponds suitable for fish farming. The values obtained are also similar to the values (18 mg/l to74 mg/l) recorded by [12].

Phosphate is the chemical term for the various combinations of phosphorous and the element oxygen. Phosphate is the main nutrient for algae. The values observed from the fish ponds ranged from 1.26 mg/l to 4.11 mg/l (Table 2) and 100% of the phosphate values were above W.H.O. permissible limit of 0.5 mg/l and therefore not good for fish farming. High concentration of phosphates permits rapid multiplication of algae leading to algal bloom which is poisonous to humans (fish consumers). These values are above the acceptable range of 0.03 mg/l to 2.00 mg/l as recommended by [22]. This may be as a result of proximity of well water (which serves as the source of water) to farmlands (fertilizers), septic tanks, geologic formations, the use of organic manure for pond fertilization, domestic (human wastes, synthetic detergents) and industrial waste waters. Higher values could lead to eutrophication.

The sulphate values observed in the fish pond water samples ranged from 0.39 mg/l to 4.37 mg/l (Table 2) which is within W.H.O permissible limit of 250 mg/l and also similar to the values (2.25 mg/l to 4.50 mg/l) reported by [21] and 0.00 mg/l to 10.03 mg/l observed by [12]. Sulphate is known as one of the least toxic anions [23]. The main natural sources of sulphate in water is the process of chemical weathering and dissolution of sulfur containing minerals, predominantly gypsum ($CaS0_42H_20$), oxidation of sulfides and elemental sulfur, and the decomposition of animal and plant residues. Direct anthropogenic sources of sulphates include industrial and municipal wastes, agricultural drainage and runoff.

Table 7. Distribution of parasites in the fish pond waters

Pond names	*Ichthyobodo* species	*Diplostomum* species	*Myxobolus* species	*Chilodonella* species	*Bothriocephalus* species	*Ambiphrya* species	*Leech* species
Morr	+	-	-	+	-	-	-
Aka	+	+	-	+	-	-	-
Erry	-	-	+	-	+	-	-
Unizik	+	+	-	+	-	-	-
H_2O	-	-	-	+	-	+	-
Eche	-	+	-	-	+	-	-
Ejiamatu	+	-	-	-	+	-	-
Abuchi	-	-	-	-	-	-	+
Aqua	+	-	-	-	+	-	-
Emeka	-	-	-	-	-	+	-
B.F.	-	-	+	-	-	-	+
Orient	-	+	-	-	-	-	-
Obinna	-	+	-	-	-	+	-
B_2	-	+	-	-	-	-	+
Izu	+	-	+	-	-	+	-

Table 8. Frequency of occurrence and percentage frequency of the parasites in the fish pond waters

Parasite species	Number of parasites isolated from the fish pond waters	Frequency of isolation (%)
Ichthyobodo	15	21.43
Diplostomum	14	20
Myxobolus	07	10
Chilodonella	10	14.29
Bothriocephalus	11	15.71
Ambiphrya	09	12.86
Leech	04	5.71
Total	**70**	**100**

Table 9. Morphological and biochemical characteristics of the bacteria from fish pond waters

Isolates	Colony morphology	Microscopy	Gram stain	Catalase test	Coagulase Test	Citrate utilization test	Oxidase test	Urease test	Indole test
51	Raised mucoid pink colonies	Rods	-	+	-	+	-	+	-
24	Small, mucoid yellow-greenish colonies	Rods	-	+	ND	+	-	-	-
12	Green metallic sheen	Rods	-	+	-	-	-	-	+
48	Round golden yellow	Cocci in clusters	+	+	+	+	-	+	-
42	Small, round yellowish colonies	Curved rods	-	+	-	+	+	-	+
63	Circular, lemon green colonies	Rods	-	+	-	+	+	+	-
21	Circular, lemon green colonies	Rods	-	+	-	+	+	-	-
30	Colourless mucoid colonies	Rods	-	+	-	+	-	+	-
31	Small, round greenish colonies	Curved rods	-	+	-	+	+	+	+
57	Large, circular and jagged colonies	Rods in chain/pairs	+	+	-	+	-	-	-
25	Round colourless colonies	Rods	-	+	-	-	-	-	-
44	Round colourless colonies with black centers	Rods	-	+	-	+	-	-	-

Table 9. Morphological and biochemical characteristics of the bacteria from fish ponds (continued)

Isolates	Motility	Voges proskauer test	Methyl red test	Glucose fermentation test	Sucrose fermentation test	Lactose fermentation test	Maltose fermentation test	Hydrogen sulphide test	Bacterial identity
51	-	+	-	A/G	A/G	A/G	A/G	-	*Klebsiella pneumoniae*
24	-	+	-	A/G	A/G	-	-	-	*Acinetobacter calcoaceticus*
12	+	-	+	A/G	A	A/G	A/G	-	*Escherichia coli*
48	-	+	+	A	A	A	A	-	*Staphylococcus aureus*
42	+	-	+	A/G	A/G	-	A/G	-	*Vibrio cholerae*
63	+	-	-	-	-	-	-	+	*Pseudomonas fluorescens*
21	+	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*
30	+	-	+	A/G	-	-	-	+	*Proteus mirabilis*
31	+	-	-	A/G	-	-	A/G	-	*Vibrio parahaemolyticus*
57	+	+	-	A	A	-	A/G	-	*Bacillus subtilis*
25	-	-	+	A	-	-	A	+	*Shigella flexineri*
44	+	-	+	A	-	-	A	+	*Salmonella typhi*

Key: A: Acid- : No acid and gas; A/G: Acid and gas; ND: Not done

Chloride ion is a common constituent of all natural water and it's generally regarded as a non-harmful constituent [23]. Though chloride is present in all natural water bodies, high concentration is an indication of pollution from sewage, industrial or intrusion of seawater or saline water into fresh water aquifer [23]. Chloride content obtained from the fish pond water samples ranged from 7.08 mg/l to 14.19 mg/l (Table 2). These values are reasonable and are within [16] permissible limits of 250 mg/l and similar to values (7.22 mg/l to 10.74 mg/l) obtained by [21]. Higher concentrations may be harmful to aquatic life. At smallest concentration, it burns the edges of the gills with long term after effects [22].

Dissolved Oxygen (DO) is defined as the measure of the amount of gaseous oxygen dissolved in an aqueous solution [24]. It has been reported that natural waters are saturated with dissolved oxygen in equilibrium with air. The concentration at this saturation is known to decrease as the temperature of water increases [18]. The low DO obtained may be attributed to the small size of the ponds and eutrophication due to over fertilization with manure [25]. This may also be due to the presence of microbes and plants as fish are not the only consumer of oxygen in aquaculture system. Low concentration of dissolved oxygen in water causes suffocation in fish while its super saturation may result into the gas bubble disease leading to the mass mortality of fish in both the cases. Oxygen depletion in water leads to poor feeding of fish, starvation, poor distribution of fishes, reduced growth and more fish mortality, either directly or indirectly. The solubility of oxygen in water decreases as the water temperature increases. The DO values obtained from the fish pond water samples ranged between 4.28 mg/l to 5.78 mg/l (Table 4). These values are within the W.H.O limit of >5 mg/l except Morr, Aka, Unizik, Ejiamatu, Book foundation, Obinna and Izu ponds with DO values less than 5 mg/l. 46.67% of the outlet values were below recommended value by W.H.O [16] and therefore not suitable for high fish productivity. The DO values were below 9.33 to 10.74 mg/l and 9.3 to 16.2 mg/l recorded by [21] and [13] respectively. This may be as a result of higher microbial load, high stocking density and phytoplankton's in the studied ponds. Oxygen is needed for the body activities of the fish. It can be introduced into the ponds mainly through photosynthesis by aquatic green plants and dissolved oxygen from air because these fish ponds are surrounded with trees.

Biochemical Oxygen Demand (BOD) is a measure of the amount of dissolved oxygen required by microorganisms to degrade the organic matter present in a body of water over a 5-day period of incubation in the dark at 20°C. In the study, the B.O.D values obtained ranged from 2.98 mg/l to 4.27 mg/l (Table 2). These values are within the W.H.O [16] value of 6mg/l. These BOD values are therefore within the values for optimum fish activities. The values were similar to the values (2.01 mg/l to 3.88 mg/l) reported by [21]. The values were below values (13.86 mg/l to 78.40 mg/l) recorded by (7) which he related to poor sanitary quality of the pond and can cause anoxic conditions capable of killing living organisms in the pond, fish produced in such a pond may be under oxidative stress and this can affect the flesh quality of the fish for human consumption.

Total hardness of water is used to describe the effect of dissolved minerals (mainly Ca. and Mg.) suitable for domestic and industrial purposes which is attributed to the presence of bicarbonates, sulphates, chlorides and nitrates. Calcium and Magnesium are essential for bone and scale formation [26]. [27] recommended a range of 30-180 mg/l for good fish productivity. Total hardness of the water samples ranged from 25.31 mg/l to 53.04 mg/l (Table 2). These values are within the W.H.O (2006) permissible limit of 250 mg/l and higher than values (3.84 mg/l to 5.80 mg/l) reported by [21] which he attributed to low calcium and magnesium content. [22] opined that the total hardness value of less 20 mg/l would cause stress due to lack of calcium and magnesium needed for bone and scale formation. It might therefore be necessary to add some calcium, and magnesium supplements since these are necessary for bone and scale formation.

The calcium hardness observed in the fish pond water samples ranged from 23.94 mg/l to 51.96 mg/l (Table 2). The values are in agreement with the W.H.O [16] permissible limit of 75 mg/l and therefore suitable for fish farming. The values were similar to the values (6.19 mg/l to 37.58 mg/l) reported by [12].

The magnesium hardness observed in the fish pond water samples ranged from 1.08 mg/l to 4.20 mg/l (Table 2). The values are in agreement with the W.H.O permissible limit of 50 mg/l and

therefore suitable for fish farming. The values were similar to the values (1.04 mg/l to 1.36 mg/l) obtained by [21].

The carbonate values observed in the fish pond water samples ranged from 1.33 mg/l to 2.35 mg/l (Table 2) with B_2 pond having the least value of 1.33 mg/l while H_2O pond had the highest value of 2.35 mg/l.

The bicarbonate values observed in the fish pond water samples ranged from 34.59 mg/l to 89.38 mg/l (Table 2) with Emeka pond having the least value of 34.59 mg/l while Aka pond had the highest value of 89.38 mg/l.

The total acidity observed in the fish pond water samples ranged from 2 mg/l to 22 mg/l (Table 2). 100% of the values exceeded the W.H.O [16] permissible limit of 0.3 mg/l and therefore not suitable for fish farming. The values were similar to the values (10 mg/l to 22 mg/l) reported by [12].

The potassium content observed in the fish pond water samples ranged from 0.04 mg/l to 2.23 mg/l (Table 4). The values are in agreement with the W.H.O permissible limit of 5 mg/l and therefore suitable for fish farming.

Heavy metals are chemical elements with a specific gravity that is at least four to five times the specific gravity of water at the same temperature and pressure [28]. Heavy metals refer to metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations [29]. Fishes and other aquatic organisms are constantly immersed in water containing pollutants. They absorb the pollutants through skin, gut (from food) and respiratory surfaces [30]. The heavy metals: lead, chromium, mercury, copper, arsenic, iron, cadmium and zinc concentrations in ponds water in all the sampling sites were compared with W.H.O [16] standard. The obtained results showed that, with the exception of lead, arsenic, iron and cadmium, the heavy metal concentrations in the pond water did not exceed WHO, [16] standard. Heavy metals pollutants after entering into aquatic environment accumulate in tissues and organs of aquatic organisms. The cadmium content observed in the fish pond water samples ranged from 0.00 mg/l to 0.04 mg/l (Table 3). 6.67% of the fish pond water samples exceeded the WHO recommended standard of 0.03 mg/l and therefore not fit for fish farming. Cadmium is a metal with no known beneficial properties that supports life. [31] stated that the source of contamination may be attributed to the interaction of the groundwater and the rock layers or soil minerals. Contamination of groundwater with cadmium can also be possible through the application of fertilizer that is common in the study area. According to [32], cadmium may also enter drinking water through weathering of soil and bedrock, corrosion of galvanized pipes, atmospheric decomposition of direct discharge from industrial operation, burning of coal and house hold wastes, volcanic eruptions, leakages from landfills and from the use of fertilizers. Therefore the presence of cadmium in the water could be attributed to any of the above factors. At low concentrations, it is toxic to plants, fishes, birds, humans etc. Fishes absorb cadmium through gills, liver, kidney which can be transferred to humans causing cancer, birth defects and genetic mutations [32].

The lead content observed in the fish pond water samples ranged from 0.01 mg/l to 0.22 mg/l (Table 3). 73.33% were above the WHO recommended standard of 0.01 mg/l and therefore not fit for fish farming. This result exceeded values recorded by Peter, [20] who reported a lead concentration of 0.035 mg/l to 0.068 mg/l in fish pond water in Abothuguchi Central, Meru County, Kenya which may be as a result of fissured water pipes, sewage effluents, automobile exhaust fumes, run off wastes and atmospheric depositions. Lead rarely occurs naturally in water, it usually gets into drinking water through the delivery systems. Materials that contain lead have frequently been used in the construction of water supply distribution and plumbing systems in private homes and other buildings. Lead in these materials can contaminate drinking water as a result of corrosion that takes place when water comes into contact with these materials for a long time. The above facts could offer explanation for the presence of high lead content in some inlets and outlets water samples analyzed. High level of lead in water can lead to cancer, interference in vitamin D metabolism, adverse effects in mental development in infants and toxicity to the central and peripheral nervous systems. Acute intoxication of fish with lead can be recognized by erratic swimming, damaged gills epithelium, erythrocytes, leucocytes, nervous system and death of fishes. In human beings, it binds with SH group of proteins, apart from that, lead damages blood circulation, central nervous system, liver and kidneys [33]. In addition, lead can delay embryonic development, suppress

reproduction, and inhibit growth, increase mucus formation, neurological problem, enzyme inhalation and kidney dysfunction in fishes and feeders [33].

The chromium content observed in the fish pond water samples study ranged from 0.00 mg/l to 0.03 mg/l (Table 3). These values were within the WHO recommended standard of 0.05 mg/l and therefore fit for fish farming. Chromate compounds are used at homes and school laboratories. Chromium therefore may have entered the groundwater through leaching. Again some chemical operation like fossil fuel combustion and waste incineration, might have contributed by releasing chromium to the atmosphere. The analysis shows that inlet and outlet water samples contain very low concentrations of chromium, which are within the acceptable limit of [16].

Mercury was totally absent in the fish pond water samples and were within the WHO recommended standard of 0.001 mg/l and therefore fit for fish farming (Table 3).

The copper content observed in the fish pond water samples ranged from 0.00 mg/l to 0.04 mg/l (Table 3). These values were within the WHO recommended standard of 2.0 mg/l and therefore fit for fish farming. The results were similar to values recorded by [20] who reported a copper concentration of 0.0691 mg/l to 0.1272 mg/l in fish pond water in Abothuguchi Central, Meru County, Kenya. Copper is often used to plumb residential and commercial structures that are connected to water distribution systems. Copper contaminates drinking water as a result of the corrosion of copper pipes that remain in contact with water for a prolonged period. Copper toxicity in natural water arising from pollutants may cause severe damage in gills and necrotic changes in the liver and kidneys of fish. Long term exposure to copper, higher than normal levels can cause nausea, vomiting, stomach cramps, or diarrhea when ingested by humans from the fish [34].

The arsenic content observed in the fish pond water samples ranged from 0.00 mg/l to 0.02 mg/l (Table 3). 26.67% of the fish pond waters exceeded the WHO recommended standard of 0.01 mg/l and therefore not fit for fish farming.

The zinc content observed in the fish pond water samples ranged 0.00 mg/l to 0.02 mg/l are within the set values by WHO [16] of 3.0 mg/l (Table 3). These values are similar to the findings by [35]. The main source of zinc into fish ponds is dissolved zinc from zinc related appliances such as galvanized pipes. Low levels can be attributed to less zinc load from industrial, agricultural, domestic and urban waste waters [36]. Zinc accumulation results in several dysfunctions in fish. It exerts adverse effects by accruing structural damage which affects the growth, development and survival. Sub lethal levels adversely affect hatchability, survival and hematological parameters of fish [33].

The iron content observed in the fish pond water samples ranged from 0.01 mg/l to 1.19 mg/l (Table 3). 20% of the results exceeded the [16] recommended standard of 0.3 mg/l and therefore not fit for fish farming. These values are similar the values reported in USA [35]. These results were also similar to values recorded by [20] who reported an iron concentration of 0.3174 mg/l to 0.3537 mg/l in fish pond water in Abothuguchi Central, Meru County, Kenya. The high amount of iron which exceeded the limits [16] may be attributed to the use of iron sheets as pond shelters, high density of people with buildings having iron sheets roofs. Due to corrosion, the iron ions find their way into the fish ponds.

The result of the bacteriological characteristics showed that Gram negative bacteria were dominant in the studied fish pond waters. The bacterial identification revealed the presence of twelve isolates; *Escherichia coli, Salmonella typhi, Shigella flexineri, Pseudomonas aeruginosa, Pseudomonas fluorescens, Proteus mirabilis, Klebsiella pneumoniae, Vibrio cholerae, Vibrio parahaemolyticus, Enterobacter aerogenes, Staphylococcus aureus* and *Bacillus subtilis* (Tables 9, 10). This may be attributed to contamination from fish feeds, pond fertilization, personnels, and fingerlings. The coliforms isolated were an indication of the contamination of the pond water with faecal materials. The faecal material may be as a result of source of water used for fish farming, composition of fish feeds, personnel's working therein, poor sanitary level of the farms such as proximity to septic tanks, poultry droppings and fertilization of the ponds with organic manure which is inoculated directly into the fish ponds. The diverse groups of bacteria isolated from these ponds are in line with the report of [37] who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principle source of bacteria and [38] who reported similar organisms

in the microbiological study of El-quanter fish pond. The presence of pathogenic microorganisms especially *Salmonella typhi, Shigella flexineri, Escherichia coli* and *Vibrio cholerae* can lead to the transmission of water borne diseases such as, Diarrhea, Typhoid fever, Cholera. *Salmonella typhi* was the most dominant organism occurring in the studied fish pond waters (Table 6). The presence of *Salmonella typhi and Escherichia coli* in water or food indicates the possible presence of causative agents of many gastrointestinal diseases [39].

The analysis of the total bacterial count in the water samples revealed the presence of heterotrophic bacteria in all the fish pond waters (Table 4). The W.H.O standard for heterotrophic bacteria in potable water states that the total heterotrophic bacteria count should not be more than 100 cfu/ml [16]. The presence of counts exceeding the W.H.O limits indicates that the water samples contain high concentration of bacteria that could make the water unsafe for domestic purposes. Result shows that the values of total bacterial count ranged from 3.60 log cfu/ml to 4.12 log cfu/ml (Table 4). Emeka pond water had the least bacterial load while Aka pond water had the highest value. 40% of the pond water values exceeded the WHO permissible limit for domestic water (100 cfu/ml). This result agrees with the findings of [40] who recorded 6.5 x 10^5 cfu/ml to 7.4 x 10^5 cfu/ml for concrete fish pond water samples in Niger Delta, Nigeria and stated that the fish pond water samples had bacterial count above the admissible limit of 100 cfu/ml for optimum fish productivity.

The total coliform obtained from the pond water samples ranged from 14 cfu/100 ml to 46 cfu/100ml (Table 4) and 100% of the values exceeded [16] Standard of 10 cfu/100 ml. This indicated that the water samples were not fit for fish farming or other domestic purposes. This result corroborates with the findings of [40] who recorded high total coliform values of 21 cfu/100 ml to 70 cfu/100 ml in concrete fish pond water samples in Niger Delta, Nigeria and stated that the fish pond water samples had total coliform count above the permissible limit of 10 cfu/100 ml for optimum fish growth.

The faecal coliform obtained from the pond water samples ranged from 1 cfu/100 ml to 9 cfu/100 ml (Table 4) and 100% of the values exceeded [16] Standard of 0 cfu/100 ml. This indicated that the pond water samples are not fit for fish farming or other domestic purposes. This result is similar to the findings of [40]. This may be as a result of poor sanitary result such as proximity to septic tanks, agricultural farms, and poultry houses which were against the [16]. WHO recommends that boreholes should be located at least 30 m away from latrines and 17 m from septic tank.

This fish pond waters revealed the presence of *Klebsiella pneumoniae* (16.48%)*, Acinetobacter calcoaceticus* (2.83%)*, Escherichia coli* (5.66%)*, Staphylococcus aureus* (2.83%)*, Vibrio cholerae* (8.29%)*, Pseudomonas fluorescens* (5.66%)*, Pseudomonas aeruginosa* (9.10%)*, Proteus mirabilis* (7.58%)*, Vibrio parahaemolyticus* (6.88%)*, Bacillus subtilis* (4.05%)*, Shigella* flexineri (10.01%) and *Salmonella typhi* (20.63%) (Table 6). These findings were similar to the values obtained by [15,12,40] and [41] below. The slight differences in results may be due to collection methods, sanitary quality of the different studied areas and geographical location.

Ogeneogaga and Solomon [15] reported a total of eight bacteria species isolated from the fish ponds with the following percentage occurrence; *Escherichia coli* (25%), *Flavobacterium* spp. (16.7%), *Psuedomonas* spp. (8%), *Samonella* spp. (8%), *Bacillus* spp. (16.7%), *Staphylococcus* spp. (16.7%), and *Bacillus cereus* (8%).

Torimiro et al. [12] revealed the presence of *Escherichia coli, Aeromonas sp. Klebsiella sp., Staphylococcus aureus* and *Shigella spp* in the fish pond water samples stocked with *Clarias gariepinus* in Ile-Ife. He stated that this may pose a threat to the health of the fishes and consumers.

Njoku et al. [40] isolated *Escherichia coli, Salmonella sp. Shigella sp. Pseudomonas sp. Proteus sp. Klebsiella sp. Vibrio sp. Enterobacter sp, Serratia sp, Aeromonas sp, Staphylococcus sp,* and *Streptococcus sp* from some fish pond water samples within the Niger Delta region of Nigeria. The coliforms isolated were an indication of the contamination of the pond water with fecal materials.

Adedayo and Anthony [41] revealed the presence of *Staphyloccocus sp, Streptococcus sp, Bacillus sp, Pseudomonas sp, Escherichia coli, Enterobacter sp, Proteus sp, Citrobacter sp, Salmonella sp,* and *Klebsiella sp* in the selected fish pond water samples from Akungba Akoko, Ondo State.

The distribution of the bacterial isolates in the fish pond water samples showed that *Salmonella typhi* was present in all the inlets except

Ejiamatu, Aqua and B_2 pond outlets. *Bacillus subtilis* was not detected in Abuchi, Aqua, Emeka and Orient pond outlets, *Escherichia coli* was found in all the outlet ponds. *Staphylococcus aureus* was present in Morr, Aka, Erry, Unizik, Eche, Ejiamatu, Aqua, B.F, Obinna and Izu ponds. *Vibrio cholerae* was not detected in H_20, Abuchi and Emeka ponds, *Pseudomonas aeruginosa* was not found in H_20 pond. *Clostridium perfringens* was completely absent in all the ponds. *Pseudomonas fluorescens* was only present in Morr, Eche, Abuchi, Aqua, B.F, Orient, B_2 and Izu pond outlets. *Vibrio parahaemolyticus* was present in Morr, Aka, Unizik, H_20, Ejiamatu, Aqua, B.F, Obinna and Izu pond outlets. *Klebsiella pneumoniae* was not detected in H_20, Abuchi, Book foundation (B.F), Orient and B_2 ponds. *Shigella flexineri* was not present in Morr, Eche, Aqua, Orient and Izu outlets. *Proteus mirabilis* was not detected in Eche, Ejiamatu, Abuchi, Emeka, Book foundation (B.F), Obinna and Izu ponds. *Acinetobacter calcoaceticus* was only detected in Eche, Abuchi, Aqua, B.F and B_2 ponds (Table 5).

Parasites are common in most ecological system and all free living organisms can be potential hosts to parasites; parasitism in itself is one of the most common lifestyles on earth [42]. One reason of concern is parasites not only impact other animals but humans also. Fish parasites and the diseases that they cause are found in both wild settings and in the aquaculture industry. Parasites have been known to cause illnesses, deformations and even prove fatal for the parasite's host [43]. In small numbers of each of these parasites, there may be little or no harm caused to the fish. But in larger numbers, the parasites identified have been known to cause anemia, lethargy, ulcers, lesions, shedding skin, erratic behavior and death [43].

The distribution of parasites in the outlet water samples showed that *Ichthyobodo* species was present in Morr, Aka, Unizik, Ejiamatu, Aqua and Izu pond inlets. *Diplostomum* species was present in Aka, Unizik, Eche, Orient, Obinna and B_2 pond outlets. *Myxobolus* species was detected in Erry, B.F and Izu pond outlets. *Chilodonella* species was only present in Morr, Aka, Unizik and H_2O pond outlets. *Bothriocephalus* species was present in Erry, Eche, Ejiamatu and Aqua pond outlets. *Ambiphrya* species was present in H_2O, Emeka, Obinna and Izu pond outlets. *Leech* species was found in Abuchi, B.F and B_2 pond outlets (Table 7).

The findings obtained from the fish pond waters showed the presence of *Ichthybodo sp* (21.43%), *Diplostomum sp* (20%), *Myxobolus sp* (10%) *Chilodonella sp* (14.29%), *Bothrioephalus sp* (15.71%), *Ambiphrya sp* (12.86%) and *Leech* (5.71%) (Table 8). These findings were similar to [44] and [45] below. The high concentration of these parasites in Aka and Unizik may be attributed to worst sanitary result they possess. The differences in results may be due to collection methods, sanitary quality of the different studied areas and geographical location.

Sigei [44] isolated five species of parasites comprising 29.12% trematodes, 12.36% crustacean, 4.12% acanthocephalans, 37.91% nematodes and 16.48% cestodes from economically important fish species of which *Clarias gariepinus* (cat fish) was among. The parasites species were *Contracecum* sp. *Capillaria* sp. *Camallanus* sp. *Bothriocephalus* sp. *Ligula intestinalis, Proteocephalus* sp. *Diplostomum* sp. *Clinostomum* sp. *Acanthocephala* sp and *Diplostomum* sp.

Bichi and Dawaki [45] observed in their study, two protozoan parasites namely *Ichthyophthirius spp* and *Myxobolus spp*, and two trematodes identified as *Clinostomum spp* and *Euclinostomum spp* on the gills, skin and fins of *Oreochromis niloticus* at Bagauda fish farm, Kano, Nigeria.

5. CONCLUSION

The assessment of the physicochemical parameters of selected fish pond water samples in Awka and its environment Anambra State, Nigeria showed serious contamination and may not be suitable for fish production for human consumption. The fish pond water quality did not compare well with stipulated standards for fish farming [16]. Therefore, health defects due to consumption of fishes containing high levels of these parameters may occur among fish feeders in the study area especially in the cases of bacteria, nitrate, lead, phosphate, arsenic and cadmium if they accumulate beyond the tolerable concentrations in the body. The fish pond water samples examined in Awka and its environment were of poor quality with regards to bacteriological parameters. The detection of total bacteria, total coliforms, faecal coliforms, parasites and other pathogenic bacteria in significant numbers indicated that the water samples are not potable for fish farming and this may be attributed to bad sanitary practices by

fish farmers. Proper sanitary practices should be employed. Samples of the fish (fingerlings) should be taken and examined in the laboratory for its microbiological quality before stocking. More studies should be carried out on the personnels, fish surface, body organs and fish feeds to determine the microbial load and heavy metal contamination. Water treatment campaigns should be organized in the studied area to enlighten the residents and fish farmers on the safety of potable water thereby enhancing economic growth, food security and maintenance of natural systems hence, there is need for treated water in fish farming. Fish farmers should avoid the use of organic manure for pond fertilization as this changes the physical and chemical properties of the water. Environmental education should be incorporated in school curricula at all levels.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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