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Physicochemical Properties, Isolation and Identification of *Pseudomonas Aeruginosa* from the Skin of Some Selected Pond Grown Catfish from Gwagwalada Area Council Abuja

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ABSTRACT

This study aimed to investigate the physicochemical characterizations such as the PH, temperature, alkalinity, hardness, biological oxygen demand, turbidity and the isolation of bacteria using the spread plate technique in pond water in Abuja, Nigeria using major pilot farms in the region. The results obtained for physicochemical characterization showed that pH ranged from 6.25 6.73, with the highest pH of 6.73 from Andrew Azazi Barack Pond. The temperature ranged from 25.3-25.8, ammonia (0.26-0.55), alkalinity (46.8-53.6), hardness (19.6-25.7), dissolved oxygen (2.8-5.5), biochemical oxygen demand (2.9-4.4) and the turbidity (34.1-52.2) respectively. The swab sticks were inoculated on the MacConkey agar, Cetrimide agar and Cledagar. The plates were allowed to stand undisturbed for 15 minutes and then the plates were inverted and incubated at 37°C for 24 hours. After 24 hours, the plates were examined, discrete colonies were picked and streaked on Nutrient agar, and incubated for 24 hours. The plates were observed for growth. Biochemical test such as; catalase, oxidase, citrate, indole and methyl red test were carried out. The presence of blue-green colouration on a Cetrimide agar, Cledagar and Nutrient agar signifies the presence of *P. aeruginosa*. Finally, the organism was identified as; P. fluorescence strain PF-5 and *P. aeruginosa*, strain PAO.

1. Introduction

It has been estimated that the current world population is over seven billion people and it has also been projected to increase to above nine billion by 2050, therefore this presents challenges to continue feeding an ever-increasing population. Fish is an integral part of human diet but while the demand is increasing, the wild fish stocks have remained static over the past three decades (FAO 2016).

Fish take a large number of bacteria into their gut from water sediment and food (Adedeji *et al*., 2011). It has been well known that both freshwater and brackish water fishes can harbour human pathogenic bacteria particularly the coliform group (Adedeji *et al*., 2011). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (Adedeji *et al*., 2011). According to Guzman *et al.,* (2010). Fish living in natural environment are known to harbour pathogenic enterobacteriaceae. More than 140 invasive bacteria species have been identified in great lakes and other water bodies (Udeze *et al.*, 2012). Invasion of fish muscles due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish is raised in ponds with faecal coliform, *E. coli* and *Salmonella* of greater than 103 per ml in pond water respectively (Adedeji *et al*., 2011). It has been shown that pathogens like *Escherichia coli, Enterobacter* spp*, Klebsiella pneumonia* and *Aeromonas* are frequently isolated from fish in pisciculture (Yagoub, 2009). Fish take a large number of bacteria into their gut from water sediment and food (Adedeji *et al*., 2011). It has been well known that both freshwater and brackish water fishes can harbour human pathogenic bacteria particularly the coliform group (Adedeji *et al*., 2011). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform is not named flora of bacteria in fish (Adedeji *et al*., 2011). According to Guzman *et al.,* (2010). Fish living in natural environment are known to harbour pathogenic

enterobacteriaceae. More than 140 invasive bacteria species have been identified in great lakes and other water bodies (Udeze *et al.*, 2012).

2. Methodology

2.1 Study Area

This research work was carried out in Gwagwalada Area Council Abuja, FCT, North-Central Zone of Nigeria. The town is located at Longitude 8 ºN and Latitude 7 ºE with a land mass of 7, 315 sq km of which Gwagwalada occupies 1, 043 sq km. It is situated within the Savannah region with moderate climatic conditions (Polgreen, 2006).

2.2 Physicochemical characteristics of fish pond water

The physicochemical characterizations carried out in this research are: Temperature, pH, Ammonia, Alkalinity, Total hardness, Dissolve oxygen (DO) and Biological oxygen demand (BOD)

Water temperature was measured at the pond sites using a mercury-in-glass thermometer graduated in degree Celsius (0-100^{o}C). The pH, ammonia, total alkalinity and total hardness were determined with Hach's Model FF-2 Aquaculture test kit. Dissolved Oxygen (DO) and Biological Oxygen Demand (BOD) were determined by Winkler's method (Welch, 1948). Turbidity of samples was analyzed using a Hach ratio Turbidimeter as in APHA (1992). The electrical conductivity was measured with a conductivity meter (Lovibond US meter, type CM-21).

2.3 Isolation and characterization of bacterial isolates

The isolation of bacteria was done using the spread plate technique described by Chesebrough (2006). Some drops of normal saline were introduced into the swab sticks, the swab sticks were spread on an already solidified MacConckey agar, Cetrimide agar and Cled agar. The plates were allowed to stay undisturbed for 15 minutes. After 15 minutes, the plates were inverted and incubated at 37^{o}C for 24 hours. After 24 hours of incubation, the plates were examined, discrete colonies were picked with a sterile wireloop and streaked on Nutrient agar, and the plates were inverted and incubated for another 24 hours. The plates were observed for growth. Colonial morphology such as; size, shape, optical characteristics, elevation and colour change were observed on the plates. The presence of blue-green colouration on a Cetrimide agar, Cled agar and Nutrient agar signifies the presence of *P.aeruginosa*. Additionally, *P. aeruginosa* was isolated and identified by using conventional biochemical tests and Api system (Biomeraux, France) (Forbes *et al*., 2007) and cultivated in pure culture, at 176 Guards Battalion brigade Medical Laboratory Dukpa Gwagwalada Abuja.

The isolates were characterized and identify using the conventional methods discussed below;

2.4 Colonial Morphology

The bacterial colonies were described and characterized by their morphological appearances (i.e. colony shape, edge or margin, pigmentation, elevation, colony surface, consistency and optical characteristics) on the plate. In addition to the colonial characterization, cellular morphologies and biochemical characteristics as described in the Laboratory manual of microbiology by Fawole and Oso (2007) were used to characterize the bacteria. The isolates were subsequently identified using the Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

2.5 Motility test

The hanging drop method was used. Loop full of sterile distilled water was placed on a cover slip. A small portion of each bacterial isolate from 24 hours old culture was transferred to the drop of water on the cover slip using a sterile inoculating loop and a smooth suspension was made by thoroughly mixing it. Vaseline was applied around the edges of the cover slip to disallow air and it was carefully covered with a clean cavity glass slide. Cover-slip was pressed down to make an air tight seal. The cover slip was subsequently observed and was inverted upon the cavity slide under the ×40 objective lens. Motile bacterial cells were seen moving rapidly in the field (Fawole and Oso, 2007).

2.6 Resuscitation of pure Isolates

The obtained pure isolates were resuscitated on agar slant by aseptically streaking a loopful of inoculum onto nutrient agar slant and incubated at 37^{o}C for 24 hours. Resultant fresh cultures of isolates were stored at 4^{o}C for further studies. Characteristic blue green colonies were used for the biochemical test.

2.7 Biochemical test

This was used to characterize the microorganisms based on their reactions to various biochemical tests. The test include; catalase test, triple sugar ion test, methyl red, citrate utilization test, and Voges-Proskauer were carried out.

2.8 Gram staining and microscopy of isolates

Sterile grease-free slides was labeled accordingly and bacteria smear of discrete colony was made on a clean glass slide and allow to air dry. They were heat by passing the smeared slide over the flame and allow cooling. The slides were then covered with crystal violet for 1mins and were rinsed under running tap for 5sec.

Slides were covered with Grams iodine and flood with same reagent for 1minute and were rinsed in slow running tap. Alcohol reagent was added and allowed to stayed for 30sec and was rinsed slowly under tap running water.

The smears were counter stained with safranin reagent for 1min, was rinsed under slow running tap and were blot dry with filter paper. Oil immersion was applied and was examined under the microscope using oil immersion lens.

2.9 Catalase test

As described by (Boon *et al*., 2007). A drop of 3% hydrogen peroxide (H_2O_2) was placed on a free grease slide and a bit of the growth on the slant (isolate) was picked with nichrome wire loop, then a drop of H_2O_2 was added to the slide. A positive test indicated by bubbling and frothing.

2.10 Oxidase test

This was carried out as described by (Isenberg, 2004) to determine whether a bacterium produces an enzyme cytochrome oxidase. The procedures are described as follows:

i. Each disk was wet with about four inoculating loops of deionized water.
ii. A loop was used to aseptically transfer a large mass of pure bacteria to the disk.
iii. The disk was observed for up to three minutes. The area of inoculation turns dark-blue to maroon to almost black; this indicates that the result was positive.

2.11 Citrate test

Citrate test was carried out according to Forbes *et al*. (2010), by Inoculating on Simmons citrate agar (not heavily) by using straight sterile wireloop from an 18 to 24 hours old colony and incubate at 35°C for up to seven days. The growth and development of a deep blue colour indicates a positive result.

2.12 Indole test

A pure isolate of *P.aeruginosa* was grown in 5mls of peptone water for 24 hours, then kovac`s indole reagent of about 3 drops was added. The mixture was carefully agitated. A negative test was indicated by absence of colour change in the reagent layers above the broth within 1 minute.

2.13 Methyl Red / Voges-Proskauer (MR/VP)

Methyl Red Test.

4-5 drops of Methyl Red Reagent was added to the culture and shake in order to homogenize. Colour development was observed in the medium. The test is considered negative as it maintains it yellow colouration.

2.14 Voges Proskauer Test

Barrit's Reagent was added to the medium until it gets a milky appearance and then O'Meara's Reagent was added until milky appearance disappears and was shaked vigorously. The medium acquires a pink-violet colour, forming at the top of the tube indicates a positive. Though, a negative result was recorded.

2.15 Preparation of MacFarland Standard

The suspensions of the test microorganisms were made in comparison with 0.5 MacFarland standards to give an approximate cell density of $1.5X10^8$ cells/ml.

It is prepared by;

- 1% $^{w}/_{v}$ solution of barium chloride ($BaCl_2$) was dissolved in 1.17g of barium chloride dihydrate ($BaCl_2.2H_2O$) and 98.83ml of distilled water (H_2O).
- 1% $^{v}/_{v}$ solution of sulfuric acid (H_2SO_4) was added to 1ml of concentrated H_2SO_4 and 99ml of water (H_2O) and mix properly.
- 0.5ml of the barium chloride ($BaCl_2$) was added to 99.5ml of sulfuric acid (H_2SO_4).

- Lastly, a small volume of the turbid solution was transferred to a capped tube of the same type as used for preparing the test and control inocula (Brantner *et al.,* 1996).

2.16 Statistical analysis

SPSS was used to analyze the data into simple percentage and standard deviation of the mean at $P > 0.05$ level of significant.

3. Results and Discussion

Results

Physicochemical characteristics of fish pond waters

The physicochemical characteristics of fish pond waters collected from three different ponds each; Pond A, (Andrew Azazi Barack Earthen Pond Dukpa) Pond B, (Muhammad Salihu's Concrete pond phase 3) and Pond C, (Abdullahi's Earthen Pond Dagiri). The pH ranges from 6.25-6.73, with the highest pH of 6.73 from Andrew Azazi Barack pond. The temperature of the ponds are approximately equal with the range of 25.3-25.8, the ammonia value ranges from 0.26-0.55, the alkalinity value is within 46.8-53.6, the hardness ranges from 19.6-25.7, the dissolved oxygen ranges from 2.8-5.5, the B.O.D ranges from 2.9-4.4, while the turbidity is within the range of 34.1-52.2 as shown in Table 1 and Figure 1 respectively.

Table 1: Physicochemical characteristics of fish pond waters

S/No.	Parameters	Pond A	Pond B	Pond C
1.	pH	6.73± 0.10	6.25± 0.05	6.5± 0.20
2.	Temperature (℃)	25.8	25.3	25.5
3.	Ammonia (NH_3) ppm	0.55± 0.23	0.48±0.10	0.26± 0.23
4.	Alkalinity	46.8± 19.35	49.2± 18.0	53.6± 9.2
5.	Hardness	19.6±4.60	25.7± 3.80	24.3±3.70
6.	Dissolved oxygen	5.5±0.5	5.1± 0.8	2.8±0.20
7.	Biological oxygen demand	2.9± 0.60	3.0± 0.50	4.42± 0.90
8.	Turbidity (ppm)	49.1± 4.7	34.12± 0.5	52.2± 40.3

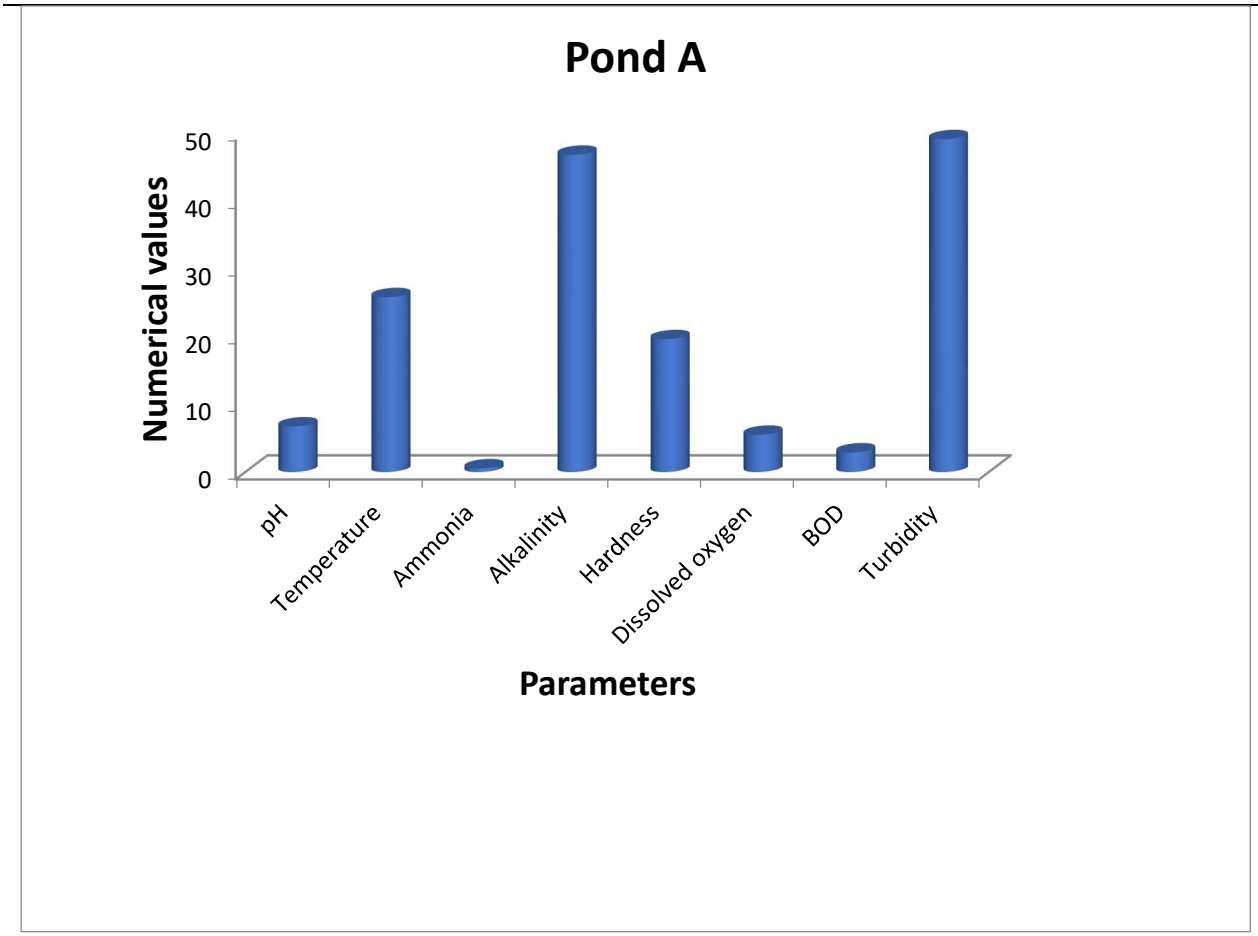

Plate 3: Physicochemical Characteristics of Pond A water (Andrew Azazi Barack Earthen Pond Dukpa)

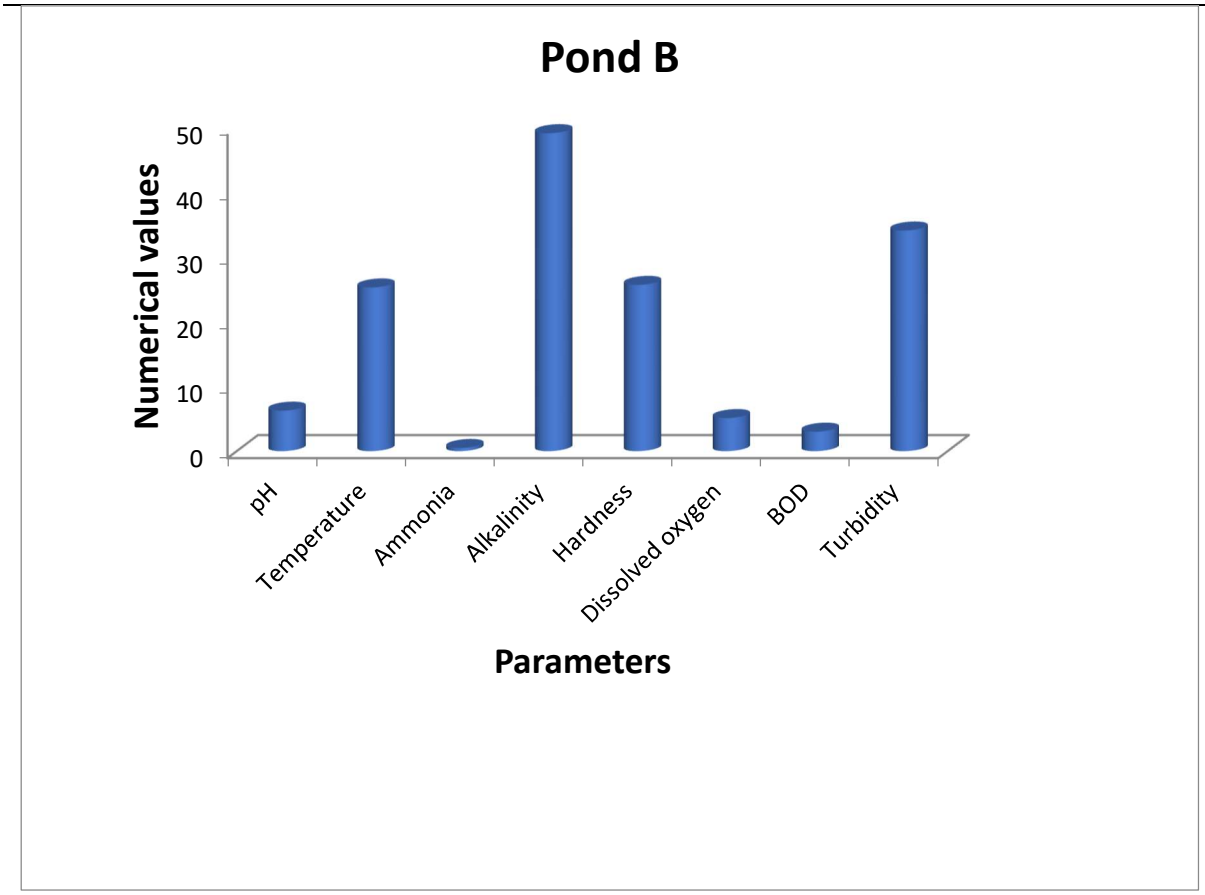

Plate 4: Physicochemical Characteristics of Pond B water (Muhammad Salihu's Concrete pond phase 3)

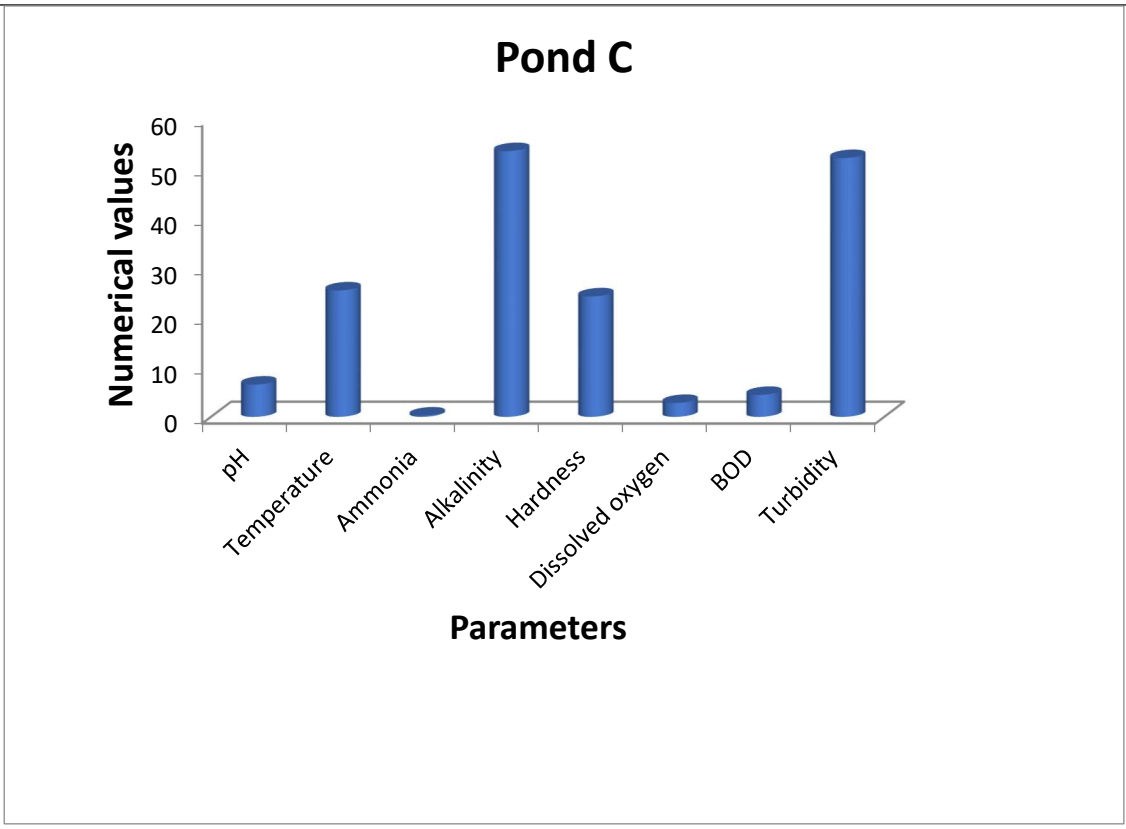

Plate 5: Physicochemical Characteristics of Pond C water (Abdullahi's Earthen Pond Dagiri)

The frequencies of isolates from both male and female fishes

The frequency of occurrence of isolated bacteria from fish scrapings was determined by taking the sum of all the numbers of male and female fishes per pond in which the isolation was carried out from and the percentage calculated.

Morphological characteristics of *P. aeruginosa* isolated from fish scrapings on different agar media was carried out base on the colony shape, size, elevation, edge, optical characteristics, consistency, surface, pigmentation and odour as shown in Table 1 below.

Table 2: Morphological characteristics of *P. aeruginosa* on different media

S/No.	Morphological feautures	Mac agar	NA agar	CLED agar
1.	Colony shape	Circular	circular	irregular
2.	Colony size	Large	medium	medium
3.	Colony Elevation	Raised	convex	raised
4.	Colony Edge	Entire	entire	undulate
5.	Optical characteristics	Opaque	opaque	opaque
6.	Consistency	Mucoid	mucoid	mucoid
7.	Colony surface	Smooth	smooth	rough
8.	Colony Pigmentation	Colourless	blue-green	blue-green
9.	Colony odour	Sweet grape	sweet grape	sweet grape

KEY: Mac agar = MacConkey agar, NA agar = Nutrient agar

Biochemical characteristics of *Pseudomonas aeruginosa*

The table 2 below shows the biochemical test results of *P. aeruginosa*. As an autochthonous member of an aquatic and soil habitat*, P. aeruginosa* is a motile, asporogenous, non-lactose fermentous bacteria which shows a positive reaction towards Catalase, Oxidase and Citrate test, with most of the biochemical test negative. Under microscope, the organisms appeared Gram negative rods with no particular arrangement.

Table 3: Biochemical characteristics of *Pseudomonas aeruginosa*

Biochemical Tests	Results
Gram staining	_
Motility test	+
Catalase	+
Oxidase	+
Citrate	+
Indole	_
Methyl red	_
Voges-proskauer	_

KEY:- + = Positive - = Negative

Table 4: The frequencies of isolates from both male and female fishes

Ponds	Male fishes [%]	Female fishes [%]	Total [%]
A	21[8.4]	19[7.6]	**40[16]**
B	23[9.2]	17[6.8]	**40[16]**
C	25[10]	15[6]	**40[16]**
Total	**69[83]**	**51[61]**	**120[144]**

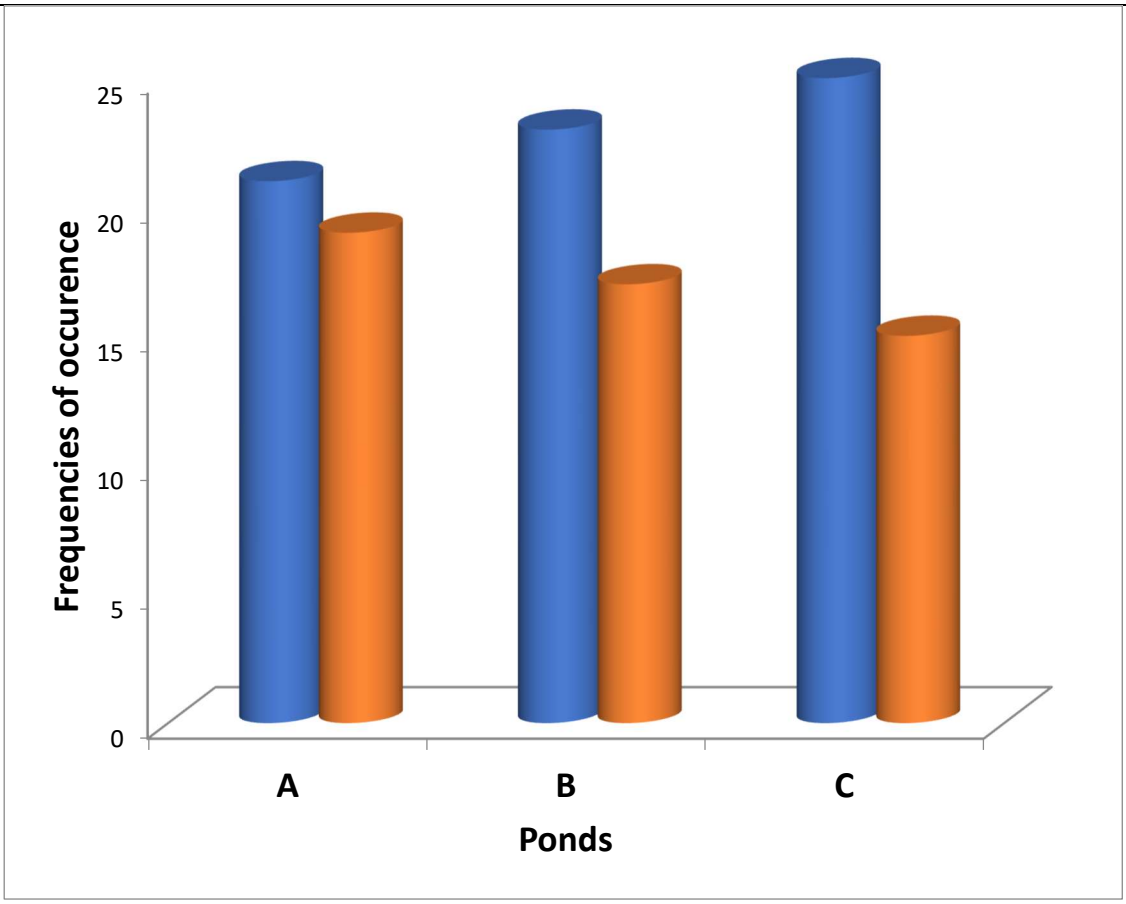

Plate 6: Frequencies of occurrence of both male and female fishes from the three ponds

Keys:
Pond A = (Andrew Azazi Barack Earthen Pond Dukpa)
Pond B = (Muhammad Salihu's Concrete pond phase 3) and
Pond C = (Abdullahi's Earthen Pond Dagiri).

Discussion

About One hundred milliliters (100 ml) of water samples (from three different portions) and a total of (120) one hundred and twenty swab sticks were collected from three different ponds each such as; Pond A, (Andrew Azazi Barack Earthen Pond) Pond B, (Muhammad Salihu's Concrete pond phase 3) and Pond C, (Abdullahi's Earthen Pond Dagiri). Physicochemical characterization was carried out using with the water samples collected. Some parameters where examined such as; pH, Temperature, Ammonia, Alkalinity, Total hardness, Dissolve oxygen (DO) and Biological oxygen demand (BOD).

The pH is a major factor that determines the growth of microorganisms. The pH value helps to ascertain if the water is a proper environment for fishes, plants and algae. The pH obtained from all the ponds in this study were within the range of pH 6.25 to 6.73, with the highest pH of 6.73 from Andrew Azazi Barack pond. It is almost within the range required for aquaculture as reported by Njoku *et al*. (2015). Ehiagbonare and Ogunrinde (2010), and Kamal *et al*. (2007). The temperature obtained from this study ranges from 25.3^{o}C – 25.8^{o}C which is still within the limit that supports fish productivity. It has been reported by Ntegwu and Edema (2008) that the optimum temperature for increased fish productivity ranges from 20^{o}C - 30^{o}C. This finding corroborates with the report of Fafioye (2011) who observed a temperature range of 27^{o}C – 28^{o}C in the preliminary studies and water characteristic of fish ponds. The ammonia value ranges from 0.26-0.55, the alkalinity value is within 46.8-53.6, the hardness ranges from 19.6-25.7, the dissolved oxygen ranges from 2.8-5.5, the B.O.D ranges from 2.9-4.4, while the turbidity is within the range of 34.1-52.2.

The morphological features of *P. aeruginosa* on different media such as; MacConkey agar, Cetrimide agar, and Cled agar are very similar, even though *P. aeruginosa* produces circular, Mucoid, smooth colonies with emits of sweet grape odour in all the agar, *P. aeruginosa* grew well on MacConkey agar, but did not ferment lactose sugar. The characteristics colonies were similar with finding of (Wahba and Darrell, 1965). Some subcultured isolate of *P.* aeruginosa did not produce any characteristics pigment neither on nutrient agar, nor on MacConkey agar which was observed to be due to minimal in temperature level (i.e. temperature below 37^{o}c) and some atypical strain of *P. aeruginosa* may not produce pigment on agar media has been reported by some investigators (Wahba and Darrell, 1965). Many strains of *P. aeruginosa*produce various types of pyocins and this pyocin producing strain of *P. aeruginosa* give pigment on agar media
In Gram's staining, the morphology of *P. aeruginosa* showed gram-negative, pink coloured, medium rodshaped appearance. While the biochemical test revealed that *P. aeruginosa* shows a positive test towards; Catalase, Oxidase, and citrate. With indole, methyl red, sugar fermentation test, and Voges-Proskauer negative. This supports the observation of (Quinn*et al.,* 2002 and Cheesbrough, 1985).

5. Conclusion

The findings presented showed that the physicochemical properties were within range for ideal cat fish production in the region and indicated a fair hygienic practice among the commercial fish farmers. It was concluded that *P.aeruginosa*. remains a constant contaminant of fresh pond water in Gwagwalada Area Council, Abuja, Nigeria.

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