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# Culture-Based Characterization of Bacteria Associated with Fish Pond Wastewater Undergoing Treatment Using Plants

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Fish pond wastewater management is one of the problems having the greatest impact on the environment. This untreated fish pond waste water contains organic materials, pathogenic microorganisms, nutrients and toxic compounds, which when discharged into the environment and it runs off into the water bodies in excess could cause algal bloom (eutrophication) of the receiving waters. Port Harcourt is a riverine area, many of the fish farmers discharge their wastewater direct into the water bodies or into drainages that flows into the water bodies and this act is harmful to humans and the environment as a whole.

The determination of the microbiological and physicochemical characteristics and remediation of fish wastewater using *Eichhornia crassipes* and *Pistia stratiotes* were investigated. The physicochemical and microbiological parameters of fish pond wastewater were monitored at intervals from 1-70days. Fish wastewater samples were collected from twelve fish ponds (6 plastic tanks and 6 concrete tanks) using standard procedures. Identification of bacteria was carried out using colonial morphological and biochemical characteristics of the isolates. A total of 194 bacterial isolates belonging to eleven genera were identified from the twelve fish ponds with 6 concrete tanks having total heterotrophic bacteria, coliform counts, *Salmonella Shigella* counts, feacal coliform count, *Vibro* count, and Pseudomonad count that ranged from 4.78 ± $0.5\times10^4$ to 5.74 ±

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$0.39\times10^5$, 4.06 ± 0.06 $\times10^4$, to 5.8 ± 0.43 $\times10^5$, 4.3 ± 0.24$\times10^4$ to 4.99 ±0.42 $\times10^4$, 4.18 ± 0.39 $\times10^4$ to 5.08 ± 0.43 $\times10^5$ , 4.08±0.35 x $10^5$ to 5.24±0.46 x $10^5$ and 4.1 ± 0.3× $10^4$ to 5.15 ± 0.44 $\times10^4$ cfu/ml, respectively and 6 plastic tanks having total heterotrophic bacteria, coliform counts, *Salmonella Shigella* counts, feacal coliform count, *Vibro* count, and pseudomonad count that ranged from 4.55 ± 0.46$\times10^4$ to 5.74 ± 0.4$\times10^5$, 4.43 ± 0.23 $\times10^4$, to 5.78 ± 0.36 $\times10^5$, 4.00 ± 0.5$\times10^4$ to 5.00 ±0.47 $\times10^5$, 4.18 ± 0.39 $\times10^4$ to 5.17 ± 0.45 $\times10^5$ , 3.78±0.35x$10^3$ to 5.24 ± 0.46x$10^5$ and 3.81 ± 0.26× $10^3$ to 5.15 ± 0.44 $\times10^5$ cfu/ml, respectively. The bacteria isolates were *Staphylococcus, Micrococcus, Bacillus, Enterococcus, Proteus, Pseudomonas, E. coli, Salmonella, Klebsiella, Vibrio and Shigella* sp. The presence of these organisms is an indication of lack of qualitative pond management which could become harmful to both fishes andhumans in the food web systems. Therefore, there is the need to protect our water sources for aquaculture purposes and sustainable development through the detection of aquatic infectious substances and possible control of these microbes.

*Keywords: Fish ponds; bacterial isolates; heterotrophic bacteria; microbes; concrete tanks; plastic tanks.*

## 1. INTRODUCTION

The temperature of water supplied to a fish pond ranges from 25°C to 35°C as this supports the growth of the microorganisms and fishes found in the pond. There are various sources of water, including well water, borehole water, stream water, river water, etc., that can be supplied to the fish pond. There are several microorganisms found in ponds including bacteria, fungi, algae, protozoa, nematodes and viruses [1]. Bacteria has a unique characteristics, they are ubiquitous in every habitation on earth, growing in soil, acidic hot springs, radioactive wastes, water and the live bodies of plants and animals [1]. Thus, bacteria are important microorganisms in ponds, whereby, some are beneficial, others are not [2].

Beneficial pond bacteria are natural and safe for fish, pets and people [3]. Beneficial bacteria are microorganisms that occur naturally in water gardens, streams, ponds, etc. They are responsible formaintaining crystal clear healthy water, breaking down organicwaste, breaking down ammonia from fish waste, reducing nitriteand nitrate, reducing nutrient load in ponds and balancing the ecosystem (3). Aquatic bacteria, through the process of decompositionand as sources of food, play an important role in pond ecosystemsand also in fish production [2]. Non-beneficial bacteria cause offensive odour to ponds and also diseases in fishes [3].

There are various factors affecting the distribution of bacteria in fish pond which includes predatory protozoa present in water.This has significant impact in decreasing the number of bacteria.Protozoa require living or dead bacteria for food and easily engulf large number of these organisms, provided the water contains sufficient dissolved oxygen [4].

Also the feed used for the fish in these ponds contain organic materials and introduces a wide variety of microorganisms into the ponds [4].

In this study, we determined various types of bacteria and their occurrence including coliforms that are commonly associated with fish pond wastewater.

### 1.1 Water Hyacinth (*Eichhornia crassipes*)

Water hyacinth is a noxious floating weed that has received great attention due to its ability to spread fast, and it is a valuable resource with many unique properties [5], it belongs to the kingdom plantae, the division magnoliophyta, class magnoliopsida, order commelinales, the famly of the pontederiaceae and the genus Eichhornia. Water hyacinth seems to be one of the most promising aquatic plants for wastewater treatment hence the need to carry out this study, to check its ability to remediate fish wastewater. Water hyacinth is a vigorous grower that is known to double its size in two weeks and it is highly tolerant and has a high capacity to naturally absorb pollutants due to the type of roots system.

### 1.2 Water Lettuce (*Pistia stratiotes*)

Water lettuce is also known as 'Jal Kumbhi', water cabbage, Nile cabbage or shellflower. It is a free floating aquatic plant that is found in streams, lakes and ponds. Water Lettuce

belongs to the kingdom plantae, the division magnoliophyta, class magnoliopsida, order Alismatales, the famly of the Araceae and the genus Pistia.Water lettuce forms dense mats on surface of water bodies thereby distrusting the aquatic flora and fauna that are underneath and thus highly affects the water ecosystem. Water lettuce grows very fast, in a matter of days. Water lettuce has the ability to remove nutrients and heavy metals from the sewage sludge drainage ditches; the physicochemical parameters will reduce progressively as it continues to remain in the wastewater and so it is the most suitable plant for waste phytoremediation in tropical areas [6].

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area

Two study locations were chosen for this research which includes: a private large scale fish farm in Sangana Street, Diobu, and Rivers State University (RSU) Nkpolu, Oroworukwu, areas of the Port Harcourt metropolis. The study locations were designated Station 1 and Station 2 respectively. Station 1 which is a private farm in Sangana Street has its GPS coordinates as longitude 4.4735ºN and latitude 6.5954ºE, 20m elevation. Station 2 which is RSU campus fish farm has its GPS coordinates as longitude 4.4754ºN and latitude 6.5846ºE, 10m elevation. Station 1 had six plastic fish ponds while station 2 had six concrete ponds. Rivers State is one of the coastal States in Nigeria with one of the highest rainfalls and the climate is that of tropical wet climate with long and heavy rainy seasons and very short dry season according to Gobo et al. [7].

### 2.2 Experimental Setup

A total of twelve tanks, consisting of six plastic fish ponds (labeled 1-6) as Station 1 and six concrete fish ponds (labeled 1-6) as Station 2 were used for the experiment. After the

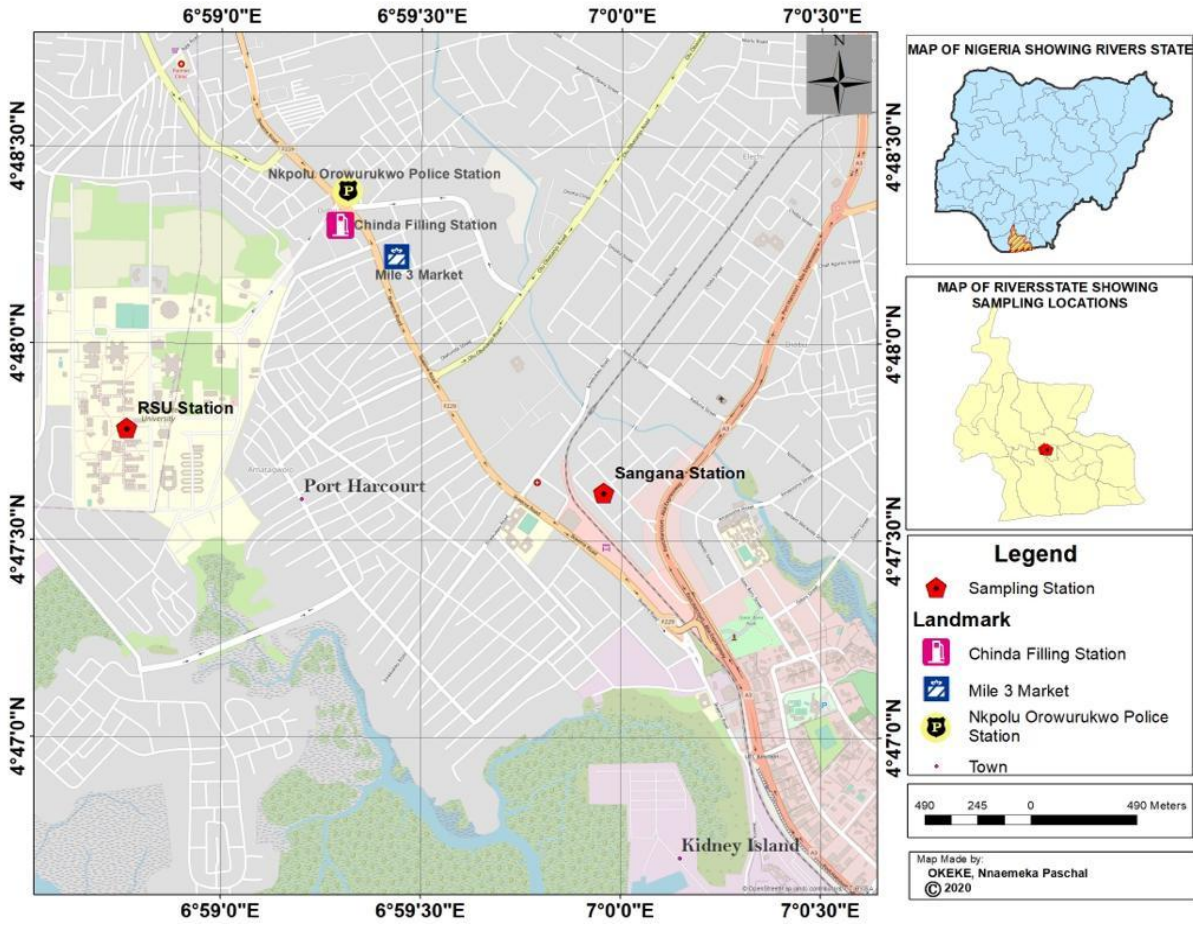


**Fig. 1. Map of study area**

fertilization period and the tanks washed, two tanks each in Station 1 and Station 2 were stocked with post fingerlings catfish and left within the tank for a period of 1 month without changing the water. During this one month the fishes were fed, microbiological analysis and physicochemical parametersof the fish pond wastewater were done, at the end of the one month the fishes were removed and kept in another tank and few stalks of *Eichhornia crassipes* was introduced into the fish pond wastewater and left for one month for phytoremediation to take place, during this period microbiological analysis and physicochemical parameters of the fish pond wastewater were done. At the end of the one month of the phytoremediation, the fishes were reintroduced into the remediated fish pond wastewater containing *Eichhornia crassipes* to stay with the treatment plant till the end of the experiment, microbiological and physicochemical parameters of the wastewater were taken.

A similar setup was employed for another set of two tanks each in Stations 1 and 2, to which *Pistia stratiotes* was added for remediation. The last set of two tanks each in Stations 1 and 2 were treated in a similar way but no plant was added for remediation. These tanks served as the control.

## 2.3 Sample Collection

Wastewater samples were collected from the tanks in Station 1 and Station 2 that have been remediated with water hyacinth and water Lettuce respectively. These were collected with sterilized plastic bottles. Each sample bottle was rinsed three times with the appropriate sample before it was finally collected according to the Standard Methods [8]. When collecting the water samples, the base of each sterilized sample bottle was held with one hand, and plunged about 30cm below the water surface with the mouth of the sample bottle positioned in an opposite direction to water flow [8]. After the samples were collected, they were labeled and immediately carried in a cooler packed with ice blocks for analysis, this ensures rapid cooling so as not to have the microbes killed during transit which will bring about an erroneous result. The sampling was done for a period of two and a half months which covered the period of the bioremediation.(at the beginning of the experiment and at the end of the experiment) Samples were collected and analyzed at the intervals of Day 1, Day 14, Day 28, Day 42, Day 56 and Day 70.

## 2.4 Enumeration and Isolation of the Bacteria

Ten-fold Serial dilutions of all the samples were made according to the methods described by Oliveira et al*.* (26). One millimeter (1ml) of the wastewater was aseptically introduced into 9ml of sterile distilled water giving an initial dilution of 1:10ml. Then aliquots (0.1 ml) of $10^{-2}$ and $10^{-3}$ were inoculated in duplicate onto sterile solidified Nutrient agar (NA), Salmonella-Shigella agar, Eosin methylene blue agar (EMB), Thiosulphate citrate bile salt agar (TCBS), MacConkey agar (MA) and Cetrimide agar (CA) and evenly spread out with a sterile flamed glass spreader. These were incubated at 37$^{o}$C for 24 hours and observed for growth and that of EMB were incubated at 44$^{o}$C for 24 hours. The colonies on the plates were counted and recorded using colony forming unit of the sample [9*,*10].

## 2.5 Isolation of *Salmonella* and *Shigella*

The *Salmonella/Shigella* agar (SSA) was prepared according to the manufactures instructionand 0.1ml aliquot of each water sample was transferred onto the surface of dried sterilized SSAplate. The plates were inoculated in triplicate and incubated at 37$^{o}$C for 24 - 48h.Thereafter,pure cultures were obtained by sub-culturing onto freshly prepared SSA plates and purecolonies were identified using biochemical reactions.

## 2.6 Isolation of *Vibrio* species

The thiosulphate citrate bile salt agar (TCBS) was prepared and poured on to sterilized Petridishes. On solidification, 0.1ml of the diluents $10^{-2}$ – $10^{-3}$ water sample previously enriched in alkaline peptone water was transferred unto the dried agar plate in duplicate using a 1 ml pipette andspread evenly with a sterile hockey stick. It was incubated at 35$^{0}$c for 24 - 48h. After incubation, yellow and green colonies were counted and identified using biochemical reactions.

## 2.7 Identification of Bacterial Isolates

The bacterial isolates were identified based on the method of [11] and Bergey's Manual of Determinative Bacteriology [12]. The isolates

were characterized based on their appearance on the culture media and these appearances include: shape, colour, wetness, dryness, elevation, opacity, margin, size and texture. Then the microscopy was done under light microscope to check for the Grams reaction, shape and arrangement. Biochemical tests including: Catalase, Indole test, VogesProskaurer, Motility, Coagulase, Methyl Red Test, Citrate utilization, Sugar fermentation (sucrose, glucose, fructose, galactose, lactose, and maltose were used to characterized isolates.

## 3. RESULTS

The results of the mean total heterotrophic bacterial counts, *Salmonella shigella* counts, fecal coliform, vibriod and Pseudomonad counts from the various fish ponds are shown in Tables 1 and 2.

### 3.1 Bacterial Types Isolated

Bacteriological analysis of the wastewater samples showed eleven different genera (Table 3) they include *Staphylococcus* spp, *Micrococcus* spp, *Bacillus* spp, *Enterococcus* spp, *Proteus* spp, *Pseudomonas* spp, *E. coli*, *Salmonella spp*, *Klebsiella* spp, *Vibrio* spp and *Shigella* spp. *Staphylococcus* spp was the highest occurred bacteria in both plastic fish pond and concrete fish ponds with a range of 25% and 26% in Stations 1 and 2 (plastic fish ponds and concrete fish ponds) respectively. While *Shigella* spp occurred at a range of 6% in both types of tanks. *Enterococcus* spp and *Klebsiella* spp being the lowest occurred at a range of 4% in both types of tanks. The bacterial types isolated and their occurrence pattern are shown in Table 5.

## 4. DISCUSSION

A total of one hundred and ninety four bacterial species were isolated during the study. Ninety nine bacteria out of the number of bacteria isolated occurred in station 1 tanks while ninety five bacteria occurred in station 2 tanks. Stations 1and 2 recorded progressive increase in the total heterotrophic bacterial counts. In stations 1 and 2, there was considerable coliform counts throughout the duration of the study with Station 2 having higher counts than Station 1. This higher coliform counts recorded in Station 2 could be attributed to the number of fishes that were introduced into the tanks, as the number of fishes introduced into Station 2 was higher than the number introduced into Station 1 thereby increasing the fish droppings and the feed that remains after feeding. The feacal coliform count in Station 1 and Station 2 was low. Station 1and Station 2 recorded high counts of total Salmonella shigella counts.

**Table 1. Mean Bacterial counts of fish wastewater from station 1 in cfu/ml**

| | ***Eichhornia crassipes*** | ***Pistia stratiotes*** | **Control** |
|---|---|---|---|
| THB | $5.27\pm0.50a \times 10^5$ | $5.35\pm0.46a \times 10^5$ | $5.27\pm0.48a \times 10^5$ |
| CC | $5.78\pm0.46a \times 10^5$ | $4.77\pm0.44a \times 10^5$ | $4.64\pm0.46a \times 10^5$ |
| SSC | $4.55\pm0.43a \times 10^4$ | $4.50\pm0.43a \times 10^4$ | $4.29\pm0.59a \times 10^4$ |
| FCC | $4.58\pm0.52a \times 10^4$ | $4.66\pm0.45a \times 10^4$ | $4.49\pm0.41a \times 10^4$ |
| VBC | $4.63\pm0.51a \times 10^4$ | $4.45\pm0.59a \times 10^4$ | $4.41\pm0.53a \times 10^4$ |
| PC | $4.63\pm0.59a \times 10^4$ | $4.45\pm0.54a \times 10^4$ | $4.56\pm0.49a \times 10^4$ |

*Key: THB: Station 1: plastic fish ponds, total heterotrophic bacteria, CC: coliform count, SSC: salmonella shigella count, FCC: feacal coliform count, VBC: vibriod count, PC: pseudomonad count*

**Table 2. Mean Bacterial counts of fish wastewater from station 2 in cfu/ml**

| | ***Eichhornia crassipes*** | ***Pistia stratiotes*** | **Control** |
|---|---|---|---|
| THB | $5.13\pm0.49a \times 10^5$ | $5.12\pm0.55a \times 10^5$ | $5.06\pm0.55a \times 10^5$ |
| CC | $4.62\pm0.47a \times 10^5$ | $4.72\pm0.55a \times 10^5$ | $4.63\pm0.50a \times 10^5$ |
| SSC | $4.51\pm0.43a \times 10^4$ | $4.51\pm0.43a \times 10^4$ | $4.38\pm0.44a \times 10^4$ |
| FCC | $4.64\pm0.55a \times 10^4$ | $4.56\pm0.48a \times 10^4$ | $4.59\pm0.45a \times 10^4$ |
| VBC | $4.60\pm0.48a \times 10^4$ | $4.51\pm0.50 \times 10^4$ | $4.62\pm0.52a \times 10^4$ |
| PC | $4.49\pm0.48a \times 10^4$ | $4.50\pm0.51a \times 10^4$ | $4.55\pm0.48a \times 10^4$ |

*Key: Station 2: Concrete fish ponds, THB: Total heterotrophic bacteria, CC: coliform count, SSC: salmonella shigella count, FCC: feacal coliform count, VBC: vibriod count, PC: pseudomonad count*

**Table 3. Biochemical and morphological features of the isolated gram positive bacteria**

| Isolate code | Colony/cell characteristics | Gram | Stain | Coagulase | Catalase | Oxidase | $H_2S$ | Spore | MR | VP | Indole | Citrate | Glucose | Sucrose | Maltose | Lactose | Galacatos | Fructose | Motility | Starch | Probable | Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B1 | Cream, small, circular, raised, opaque, cocci | + | + | + | + | - | + | - | - | - | - | + | + | + | + | - | + | + | - | - | | *Staphylococcus* sp. |
| B2 | Orange, large, circular, raised and opaque; cocci | + | + | + | + | - | + | - | - | - | - | + | - | + | + | - | + | + | - | - | | *Micrococcus* sp. |
| B3 | Cream, dry round raised and opaque; rods | + | - | + | + | - | + | - | + | - | + | + | + | + | + | - | - | + | + | + | | *Bacillus* sp. |
| B4 | Yellow, dry and opaque | + | - | + | - | + | - | - | - | - | + | + | + | + | - | + | + | - | - | | | *Staphylococcus* sp. |
| B5 | White cream large, smooth surface, flat, opaque cocci | + | - | - | - | - | - | - | + | - | - | + | + | + | + | + | + | - | + | | | *Enterococci* sp |

**Table 4. Biochemical and morphological features of the isolated gram negative bacteria**

| Isolate Code | Colony/cell characteristics | Gram | Stain | Coagulase | Catalase | Oxidase | $H_2S$ | Spore | MR | VP | Indole | Citrate | Glucose | Sucrose | Maltose | Lactose | Galacatos | Fructose | Motility | Starch | Probable Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B6 | Bluish, moist, opaque, raised, round rods | - | - | - | + | - | - | - | - | - | - | + | - | - | - | - | - | - | + | - | *Pseudomonas* sp |
| B7 | Greyish, moist, opaque, raised, round rods | - | - | - | + | - | - | - | + | - | + | - | + | + | - | + | + | - | + | - | *Escherichia* sp |
| B8 | Cream large, circular mucoid rods | - | - | - | + | - | - | + | - | + | - | + | + | + | + | + | + | + | + | * | * | *Klebsiella* sp |
| B9 | Swarming, cream, opaque rods | - | - | - | + | - | + | - | + | - | - | + | + | - | - | - | - | + | + | - | *Proteus* sp |
| B10 | Cream circular mucoid transparent rods | - | - | - | + | - | + | - | + | - | - | - | - | - | + | - | - | + | - | - | *Salmonella* sp |
| B11 | Bluish circular, moist translucent rods | - | - | - | + | + | - | - | + | - | + | + | + | + | + | + | + | + | + | + | *Vibrio* sp |
| B12 | Greyish white circular, convex, smooth translucent rods | - | - | - | + | - | + | - | + | - | + | - | + | - | - | - | - | + | + | - | - | *Shigella* sp |

**Table 5. Bacterial isolates and their percentage occurrence in stations 1 and 2**

| Bacterial Isolates | Station 1 | Station 2 |
|---|---|---|
| *Staphylococcus* spp | 25 (26.59) | 26 (26.26) |
| *Micrococcus* spp | 14 (14.89) | 15 (15.15) |
| *Bacillus* spp | 14 (14.89) | 15 (15.15) |
| *Enterococcus* spp | 4 (4.25) | 4 (4.04) |
| *Proteus* spp | 6 (6.38) | 7 (7.07) |
| *Pseudomonas* spp | 5 (5.31) | 6 (6.06) |
| *E.coli* spp | 6 (6.38) | 6 (6.06) |
| *Salmonella* spp | 5 (5.31) | 5 (5.05) |
| *Klebsiella* spp | 5 (5.31) | 4 (4.04) |
| *Vibrio* spp | 5 (5.31) | 5 (5.05) |
| *Shigella* spp | 6 (6.38) | 6 (6.06) |

*Key: Percentage occurrence in parenthesis*

Station 1and Station 2 had high vibriod counts and Pseudomonad counts.

The presence of *E. coli*, *Klebsiella* sp, *Staphylococcus* sp, *Shigella* sp, *Salmonella* sp and *Vibrio* sp observed in this study could pose a threat to the health of fishes and consumers and can lead to the transmission of water borne diseases such as typhoid fever, cholera, food poisoning and gastroenteritis [13]. The predominant bacteria isolated were the Gram negative bacteria. This was also reported by other researchers [14,15,16,2]. The European food safety authority lists pathogens such as *Salmonella* and *E. coli* as responsible for most of the food borne infections worldwide [17]. Also, *Staphylococcus* sp and *Pseudomonas* sp have also been involved in food poisoning. In addition *Salmonella* and *Shigella* were listed amongst the organisms isolated from catfish and named Bacillus also as an organism isolated from fish which is responsible primarily for toxin mediated disease as opposed to infections [18].

The presence of human pathogenic bacteria like vibrio could be attributed to the fact that vibrio spp are regarded as indigenous bacteria in aquatic environment and present in fish up to $10^2$ – $10^3$ cfu/g and can multiply under favourable temperature conditions of above $15^0$C [2]. Total aerobic heterotrophic bacteria count, *Salmonella/Shigella* count, *Vibrio* count and *Coliform* count were high and varied within the ponds. These values was due to the water temperature which was within optimum for bacterial growth and also due to the organic matter load found within pond water resulting from the diet used in feeding the fish.

Thus, the pond water becomes an ideal culture medium for the proliferation of bacterial pathogens causing bacterial infection in fish and an important cause of food poisoning [19].

The same diverse groups of bacteria were isolated from these ponds in the two stations which is in line with the report of Okpokwasili and Ogbulie [4] who worked on pond waste water and this suggests that no matter the type of fish pond, there are certain types of bacteria that will be seen. Probably some of the bacteria from feed added to the ponds are the principal source of bacteria of health importance. Daboor [20] also reported similar organisms in the microbiological study of El-quanter fish pond. *E. coli* was found in both Stations 1 and 2 fish ponds. The presence of *E. coli* in water or food indicates the possible presence of causative agents of many gastro-intestine diseases [21]. *Pseudomonas, Proteus and Staphylococcus* species have been implicated in food poisoning [22]. The presence of pathogenic microorganisms especially *E. coli, Salmonella, Shigella* and *Vibrio* can lead to the transmission of water borne diseases such as, Typhoid fever, Cholera, food poisoning and gastroenteritis [13] on consumption of improperly cooked fish cultivated in these ponds.

These results of the total heterotrophic bacterial counts could be as a result of the feed used for the fish in these ponds which contain organic materials and introduces a wide variety of microorganisms into the ponds. This observation is in line with Okpokwasli and Ogbulie, [4] who in their investigation of fish pond waste water suggested that bacteria from feed added to the ponds are the major sources of bacterial pathogens of clinical importance. Another influencing factor linked to the bacterial load in the fish pond waste water was poor management [23], as the water in the fish ponds were not

changed throughout the study period. The high microbial counts obtained throughout this study is in consonance with studies by Njoku et al*.,* [2] and Sule et al*.,* [24] who carried out microbiological analysis of fish pond water in Port Harcourt and Ilorin respectively. However, results of microbial counts in this study were higher than the 1.2 x $10^4$ cfu/ml reported by Danba et al*,* [1] in Kano, Nigeria. This study revealed high range of microbial load which was also reported by Torimiro et al., [14] with a range of 1.50 x $10^4$cfu/ml – 1.13 x $10^6$ cfu/ml.

These high counts may be as a result of the organic substance/material used in fertilizing the ponds as well as contamination resulting from air droplets and living things such as insects entering the ponds, waste excreted by the fish into the ponds or through water runoff [25]. Some of the microorganisms found in this study can be used in the secondary treatment of waste water to remove dissolved organic matter [26]. Similar organisms isolated in this study were also identified in other studies. Apart from solid matter reduction, these microorganisms are also involved in nutrient recycling such as phosphate, nitrogen and heavy metal [26].

The coliforms isolated from the fish pond water indicated contamination of the pond water with fecal materials as also reported by Njoku et al, [2] and excrete by the fishes into the pond [25]. This could be as a result of the presence of pathogenic organisms in fish when their concentration is above ($10^4$ – $10^6$) in the skin and ($10^4$ – $10^7$cfu/g) [2] which poses a health risk to the cultured fish in these ponds and it is a possible problem in the management of fish pond waste water [19].

There was always the presence of microbes throughout the study period for the tanks in the two stations that had plants and the control as microbes in waste water play a vital role for the releasing of nutrient to the waste water by utilizing the organic compounds for their growth and development. The plants have well developed root systems which facilitated the microbes to colonise well to form a satisfactory habitat for their growth and development.

## 5. CONCLUSION

This study identified the high microbial load throughout the study period. The total heterotrophic bacteria counts, coliform counts, *Salmonella/Shigella* counts, feacal coliform counts, *Vibrio* counts and Pseudomonad counts varied in the various tanks throughout the study period. *E. coli*, *Klebsiella* sp, *Staphylococcus* sp, *Shigella* sp, *Salmonella* sp and *Vibrio* sp were identified from both the plastic and the concrete tanks, which revealed that both ponds were grossly contaminated with identical pathogenic bacteria that could affect fish cultivation. These could pose a serious threat to the fishes and the consumers by lowering fish yield, cause diseases and economic loss and endanger the consumers.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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