Journal of Advances in Microbiology
1(1): 1-10, 2016; Article no.JAMB.30285

SCIENCEDOMAIN *international*
www.sciencedomain.org

Effect of Habitat Locations on the Bacterial and Water Quality Changes in Freshwater Tilapia (*Oreochromis niloticus*) Using Small Scale Depuration

Anyiam C. Albert[1*], Ezeama C. Felix[2], Emeh C. Cynthia[1] and Ojokoh S. Eromosele[1]

[1]Department of Food Technology, Akanu Ibiam Federal Polytechnic, Unwana, P.M.B. 1007, Afikpo, Ebonyi State, Nigeria.
[2]Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267, Umuahia, Abia State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ACA designed the study, performed the statistical analysis and managed literature searches. Author ECF wrote the protocol, and wrote the first draft of the manuscript. Authors OSE and ECC managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2016/30285
Editor(s):
(1) Ana Claudia Correia Coelho, Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Portugal.
Reviewers:
(1) Grazyella Massako Yoshida, Universidade Estadual Júlio de Mesquita Filho, Brazil.
(2) Sudalayandi Kannappan, ICAR-Central Institute of Brackishwater Aquaculture, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/17517

Original Research Article

Received 30th October 2016
Accepted 30th November 2016
Published 13th January 2017

ABSTRACT

Aim: The aim of the study was to investigate the effect of habitat locations on the bacteriological and physicochemical assessment of aquaculture freshwater Nile Tilapia (*Oreochromis niloticus)* using a small scale depuration system.
Methodology: Nile Tilapia samples were harvested from two different locations Michael Okpara University of Agriculture (MOUAU) and Umugbalu fish farm. The Nile Tilapia sample was subjected to depuration for a period of 48 h. The total bacteria count (TBC) of the Tilapia samples were determined and isolates characterized before and after depuration. The total bacteria count (TBC)

**Corresponding author: E-mail: chuksbarto17@yahoo.com;*

and other selected pathogenic bacteria in water and different fish organs differed significantly ($p < 0.05$) with TBC being the highest (1.80×10^6 $cfuml^{-1}$) in water from Umugbalu habitat. The TBC and other pathogenic bacteria from different fish organs (gill, muscle and gut) differed, being 1.41×10^6, 1.10×10^6, 1.50×10^6 $cfug^{-1}$ (TBC); 8.3×10^5, 7.4×10^5, 9.5×10^5 $cfug^{-1}$ (*Coliform*); 1.4×10^5, $<10^1$, $<10^1$ $cfug^{-1}$ (*Listeria* spp); 2.7×10^5, 2.3×10^5, 3.9×10^6 $cfug^{-1}$ (*Salmonella* spp) respectively as typically observed in samples from MOUAU habitat. For pH, temperature, salinity and turbidity, water sample from Umugbalu location had the highest values of 6.80, 30.9℃, 6.50 ppt and 26.0NTU and 7.00 ml/g respectively.

Conclusion: Small scale depuration system was adequate for the assessment of bacterial quality of the water and freshwater Tilapia organs. Furthermore, habitats as investigated in this study revealed that the water sample was grossly contaminated with pathogenic bacteria and as such could affect fish cultivation and the consumers.

Keywords: Depuration; tilapia; coliforms; habitat.

1. INTRODUCTION

The significant growth in fish consumption has enhanced people's diets around the world through diversified and nutritious food [1]. Fish accounted for about 17 percent of the global population's intake of animal protein and 6.7 percent of all protein consumed in 2013. Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita consumption of animal protein. In addition to being a rich source of high quality proteins containing all essential amino acids, fish provides essential fats (e.g. long chain omega-3 fatty acids), vitamins (D, A and B) and minerals (including calcium, iodine, zinc, iron and selenium), particularly if eaten whole [1,2]. Fish are generally nutritious and beneficial but aquaculture products have sometimes been associated with certain food safety issues [3].

Several studies have demonstrated many bacteria encountered in different fish which are potentially pathogenic under certain conditions [4]. The affected fish produces fish diseases which cause economic losses not only from mortality but also treatment expenses to the final consumer [4]. Fish and shellfish not only transmit disease to man but are themselves subject to many diseases. They are capable of transmitting many of the established food borne microbial infection and intoxication. It has been observed that the speed with which a product spoils is also related to the initial microbial load on it; the higher the count, the sooner spoilage occurs [5]. That is why the initial microbiology of fish skin, gills and gastrointestinal tract has been subjected to many researches, as fishes take a large number of bacteria into their gut from water sediment and food [4]. The microbial safety of freshwater habitants is related to their feeding habits and the quality of their ecosystems as well as their handling during marketing and retail operations [6]. Fishes are reared in different controlled environment which includes; ponds (concrete or earthen) and vats (wood or fiber), but in most cases, concrete and earthen ponds are widely used [7].

However, fishes are sold most times without any sanitary control measures, thereby creating a public health risk [8] due to possible accumulation of pathogenic bacteria if the harvesting and selling areas are contaminated by contaminated water from land [9], or if they are handled without proper hygiene regulations [10]. Some of the most hazardous bacteria associated with the consumption of these fishes include *Salmonella* spp., *Escherichia coli*, *Listeria monocytogenes*, *Vibrio parahaemolyticus* and *V. cholera* [10,11]. The consumption of fresh African Tilapia (*Oreochromis niloticus*) is on the increase in both rural and urban centres in Nigeria [12], where most 'point and kill joints' (a restaurant where fish are kept alive, the customer chooses the desired size and it is killed and made into pepper soup as a delicacy) are present in our local restaurants and eatery houses, where they are sold as a delicacy. The improperly processing of these fishes posses a lot of public health hazards to the consumers.

Since depuration is a process by which fresh water species are held in tanks of clean seawater under conditions which maximize the natural filtering activity that results in expulsion of intestinal contents, which could enhance elimination of contaminants from the fish, and prevents their recontamination prior to sale [11]. These treatments have been of research interest to several workers [13] with emphasis on depuration of fishes harvested from marine

waters. In order to address these public health problems that are associated with Tilapia consumption, the aim of this research was to assess the bacteriological quality of freshwater Nile Tilapia before and after depuration. Furthermore, the physicochemical and bacteriological qualities of their habitat (pond) locations were evaluated and it effect on the depuration of freshwater Tilapia. The result of these assessments will invariably address the public health challenges associated with freshwater fish consumption.

2. MATERIALS AND METHODS

2.1 Study Site

The study was carried out in Ikwano local government in Abia state of Nigeria which covered two freshwater ecosystem or aquaculture medium (concrete ponds) where freshwater Tilapia (*Oreochromis niloticus*) were reared. The two sites were located at Michael Okpara University of Agriculture Umudike Fish Farm and Eze Chiaghanti Okeiyi Fish Pond at Umugbalu village, both in Ikwano LGA of Abia state. The minimum distance between one location and another was 2-3 km.

2.2 Description of the Depuration Tank

The tank consisted of connecting pipes and internal fittings which were constructed from materials under local regulations, which is permitted to have direct interaction with foods (SFIA, 1995). The tank was made with fibre, glass and PVC pipes were used for the connecting pipe work. The system was proposed to be rectangular in shape, having 550 mm depth, 800 mm wide and 1500 mm length; having a total volume of 660 litres of water approximately. Water flow to test tank was recorded at 46 $L.min^{-1}$. Mean daily water temperature of the water source was 29-30℃; the loading/stock density was 15 kg.

2.3 Sampling of Water

Samples of water (500 ml) were collected in sterile 1-litre conical flasks. The depth of water ranged from 0.8 m to 1.2 m. After on-site rinsing of the conical flasks, three 500 ml samples from each site or pond were collected from approximately 0.2 m above the bottom of the pond. Water samples were store in ice-packed cooler and transported to the laboratory within 1 h of collection for microbiological and physicochemical analyses.

2.4 Collection of Fish Sample

The fish species were identified at the Department of Fisheries and Aquatic Resource Management, Michael Okpara University of Agriculture Umudike, Abia state. Matured fish meant for human consumption were collected manually at each location. The fishes collected were kept inside plastic container of 50 litres capacity containing water from the location, method used was according to [14]. These were transported to Food Microbiology Laboratory in Department of Food Science and Technology, Michael Okpara University of Agriculture Umudike, within 30 min of collection for both microbiological analyses of the fresh water sample and depurated fish sample for 48 h.

2.5 Water Quality Characteristics of Water Samples

The various water quality parameters were analyzed using the standard method adopted by [15] and data were recorded during the study period. Water quality characteristics of water samples were carried in the Department of Environmental Management and Toxicology laboratory, Michael Okpara University of Agriculture Umudike, Abia State.

2.6 Depuration Period

The Fish sample (tilapia fish) was depurated separately for 48 h and readings taken at 12 h intervals (0, 12, 24, 36, 48 h each). Samples were collected and microbiological analysis was carried out as described by [14].

2.7 Sample Preparation for Microbial Count Using Serial Dilution Technique

The fresh fishes were cleaned with cotton wool and sterile distilled water, after which they were beheaded. Their gill, gut and muscle (meat) were carefully removed by fine dissection using sterile scalpel. Twenty five grams (25 g) of gills, guts and muscles were separately crushed using a sterile mortar and pestle. Two hundred and twenty five milliliter (225 ml) of sterilized peptone water (oxoid) were added to the crushed samples and homogenized to obtain a dilution of 1:10. For the fish species and for the entire experiment 1 ml of appropriate dilution was used for inoculation on the Tryptone soy agar and the selective media for the isolation of *E. coli, Listeria* and *Salmonella*. This experiment was carried out

on the water sample (from different habitat locations), depuration medium and on the fish before (0 h) and after depuration at 12 h intervals (12, 24, 36, 48 h). Microbial analysis was carried out on the water and fish sample according to the method described by [14]. For the fish species and for the entire experiment 0.1 ml of appropriate dilution was used for inoculation on the molten Tryptone soy agar and the selective media for the enumeration of Coliforms, *Listeria* and *Salmonella* spp*.* The microbial load for each water and fish samples were recorded in $cfuml^{-1}$ for water samples and $cfug^{-1}$ for fish samples.

2.7.1 Enumeration of coliforms

Sorbitol MacConkey agar (CM0813) was prepared according to manufacturer's instruction (Thermo Fisher Scientific Inc, Oxoid Limited). Cefixime Tellurite selective supplement (SR0172E) was used for the isolation of *Coliform*. Pink colonies which were observed and counted with colony counter indicated the presence of *Coliform*.

2.7.2 Enumeration of *Listeria* spp

Brilliance *Listeria* agar base (CM1080) was prepared according to manufacturer's instruction (Thermo Fisher Scientific Inc, Oxoid Limited). One vial of Brilliance *Listeria* selective supplement (SR0227E) and Brilliance *Listeria* differential supplement (SR0228E) were added for the isolation of *Listeria* spp. Green colonies which indicated the presence of *Listeria* spp were observed and counted with colony counter.

2.7.3 Enumeration of *Salmonella* spp

Brilliance *Salmonella* agar base (CM1092) was prepared according to the manufacturer's instruction (Thermo Fisher Scientific Inc, Oxoid Limited). One vial of Brilliance *Salmonella* selective supplement (SR0194E) was added for the isolation of *Salmonella spp*. Purple and blue colonies were observed and counted with colony counter, indicated the presence of *Salmonella* spp.

2.8 Statistical Analysis

Data from laboratory analysis are expressed using tables. Results are expressed as mean ± standard deviation of triplicates. Data obtained were analyzed by one-way analysis of variance (ANOVA) and correlation analysis. Least significance difference (LSD) test was used for mean separation for statistical significance at 95% ($P<0.05$) confidence level, using the statistical software SPSS 17.0 for Windows (SPSS Inc., Chicago, III., USA).

3. RESULTS AND DISCUSSION

3.1 Results

Table 1 shows the bacterial load of water sample (habitat) from MOUAU and Umugbalu locations. Water sample from Umugbalu recorded the highest TBC of 1.80×10^6 $cfuml^{-1}$. Among other pathogenic bacteria, Coliform proves to be the most predominating bacteria with the highest value of 1.10×10^6 $cfuml^{-1}$ from Umugbalu water sample. The next prevalent pathogenic bacterium was *Salmonella* spp*,* which had a population of 7.0×10^5 $cfuml^{-1}$ from Umugbalu water sample. *Listeria* spp was not isolated from all the water samples.

Table 1. Bacterial count ($cfuml^{-1}$) of water samples (habitats) for tilapia from different locations

Isolates (Bacterial species)	Locations	
	MOUAU	Umugbalu
TBC	$1.60^c \times 10^6$	$1.80^c \times 10^6$
Coliforms spp	$1.05^c \times 10^6$	$1.10^c \times 10^6$
Listeria spp	$<10^{1a}$	$<10^{1a}$
Salmonella spp	$5.0^b \times 10^5$	$7.0^b \times 10^5$

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different ($p<0.05$)
MOUAU= Michael Okpara University of Agriculture Umudike

Table 2 shows the physiochemical properties of water samples from different locations/habitats where the fish species were harvested. The parameters include: pH, temperature, salinity and turbidity. The water sample from Umugbalu location had the highest values of 6.80, 30.9℃, 6.50 PSU and 26.0NTU and 7.00 ml/g for pH, temperature, salinity and turbidity respectively; while water sample from MOUAU had the lowest value of 6.75, 30.1℃, 5.20 ppt and 22.0NTU which was recorded for pH, temperature, salinity and turbidity respectively. MOUAU sample had a higher dissolved oxygen value of 7.20 mg/l than Umugbalu sample with a value of 7.00 mg/l.

Tables 3, 4 and 5 shows the effect of habitat on depuration of tilapia organs (gill, muscles and gut). The total bacteria count (TBC) for the gills,

muscle and gut ranged from 2.0 x 10^5 - 1.44 x 10^6 cfug^{-1}, 1.6 x 10^5 – 1.19 x 10^6 cfug^{-1} and 3.8 x 10^5 – 1.54 x 10^6 cfug^{-1} at 48 h and 0h of depuration respectively from both habitat. At 48h of depuration there was no bacterial isolates reported from both habitats *Coliform* values ranged from 1.0 x 10^5 – 9.0 x 10^5 cfug-^{1}, 2.7 x 10^5 – 8.7 x 10^5 cfug^{-1} and 2.8 x 10^5 – 9.7 x 10^5 cfug^{-1} (at 36 h and 0 h) from MOUAU and Umugbalu locations respectively, there were no isolation at 48 h of depuration from both locations. Similarly, *Salmonella* spp recorded values of 1.0 x 10^5 - 2.9 x 10^5 cfug^{-1}, 1.2 x 10^5 – 4.6 x 10^5 and 1.2 x 10^5 – 4.0 x 10^5 cfug^{-1} (at 24 h and 0 h). Finally, *Listeria* spp values ranged from 1.3 x 10^5 cfug^{-1}– 1.4 x 10^5 at 0 h of depuration but there was no isolation observed from 12 h – 48 h in the gills. It was also observed during the study that there was no isolation of *Listeria* spp before and after depuration at the muscles and gut organs of the fish sample.

3.1.1 For gills

Tilapia from Umugbalu was reported to have the highest TBC value (1.44 x 10^6) while thelowest value was from MOUAU (<10^1) at 48 h. *Listeria* was isolated only from MOUAU habitat at 0h.

3.1.2 For muscle

There was significant difference between the TBC (muscles) obtained from both habitats at 0h – 24 h and 36 h of depuration. Similarly, there was significant difference between the *Coliform* (gills) from both habitats at 0 h-36 h of depuration. Furthermore, at 0h – 36 h of depuration the *Salmonella* spp load were significantly different from both habitats.

Table 2. Physico-chemical characteristics of water (habitat) from which tilapia (*Oreochromis niloticus*) were harvested

Characteristics	Location	
	MOUAU	Umugbalu
pH	6.75^{c}±0.05	6.80^{c}±0.05
Temperature (℃)	30.1 ^{c}±0.65	30.9^{a}±0.00
Salinity (PSU)	5.20^{c}±0.20	6.50^{c}±0.20
Turbidity (NTU)	22.0^{b}±2.00	26.0^{b}±2.00
Dissolve oxygen (ml/g)	7.20^{c}±0.10	7.00^{c}±0.10

Each value represents the mean ±SD of triplicate determinations.
Means on the same row with different superscript are significantly different ($p < 0.05$).
MOUAU= Michael Okpara University of Agriculture Umudike
NTU: Nephelometric Turbidity Units
PSU: Practical Salinity Unit

3.1.3 For gut

The Least square difference (LSD) of 0.067 shows that there was no significant difference between the TBC (guts) from both habitats. Furthermore, there was significant difference between the Coliform load from both habitats at 0 h – 36 h hours of depuration. No significant difference was observed at 48 h of depuration between both habitats. The Least square difference (LSD) of 0.382 shows that there was no significant difference between the *Salmonella* spp load (guts) from both habitats.

Table 3. Effect of habitat on bacterial load (cfu/g) of depurated tilapia gills

		Isolates (Bacterial Spp)			
Depuration period (h)	Habitat	TBC	*Coliform*	*Listeria*	*Salmonella*
0	MOUAU	1.41^{a} x 10^6	8.3^{b} x 10^5	1.4^{a} x 10^5	2.7^{ab} x 10^5
	UMUGBALU	1.44^{a} x 10^6	9.0^{a} x 0^5	1.3^{b} x 10^5	2.9^{a} x 10^5
12	MOUAU	1.10^{c} x 10^6	7.2^{c} x 10^5	<10^{1c}	1.6^{c} x 10^5
	UMUGBALU	1.19^{b} x 10^6	8.4^{b} x 10^5	<10^{1c}	2.4^{d} x 10^5
24	MOUAU	6.8^{e} x 10^5	4.0^{e} x 10^5	<10^{1c}	1.0^{d} x 10^5
	UMUGBALU	7.5^{d} x 10^5	4.6^{f} x 10^5	<10^{1c}	1.8^{c} x 10^5
36	MOUAU	3.3^{f} x 10^5	1.0^{f} x 10^5	<10^{1c}	<10^{1e}
	UMUGBALU	3.6^{f} x 10^5	1.3^{f} x 10^5	<10^{1c}	<10^{1e}
48	MOUAU	2.0^{g} x 10^5	<10^{1g}	<10^{1c}	<10^{1e}
	UMUGBALU	<10^{1h}	<10^{1g}	<10^{1c}	<10^{1e}
LSD		0.361	0.272	0.937	0.189

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different ($p<0.05$)
MOUAU = Michael Okpara University of Agriculture Umudike
TBC = Total Bacteria Count

Table 4. Effect of habitat on bacterial load (cfu/g) of depurated tilapia muscles

Isolates (Bacterial Spp)					
Depuration period (h)	**Habitat**	**TBC**	***Coliform***	***Listeria***	***Salmonella***
0	MOUAU	$1.10^{a} \times 10^{6}$	$7.4^{b} \times 10^{5}$	$<10^{1a}$	$2.3^{c} \times 10^{5}$
	UMUGBALU	$1.19^{a} \times 10^{6}$	$8.7^{a} \times 10^{5}$	$<10^{1a}$	$4.6^{a} \times 10^{5}$
12	MOUAU	$7.0^{c} \times 10^{5}$	$3.0^{d} \times 10^{5}$	$<10^{1a}$	$1.7^{d} \times 10^{5}$
	UMUGBALU	$9.0^{b} \times 10^{5}$	$4.4^{c} \times 10^{5}$	$<10^{1a}$	$3.9^{b} \times 10^{5}$
24	MOUAU	$3.0^{e} \times 10^{5}$	$1.5^{e} \times 10^{5}$	$<10^{1a}$	$<10^{1e}$
	UMUGBALU	$4.6^{f} \times 10^{5}$	$2.7^{d} \times 10^{5}$	$<10^{1a}$	$1.2^{d} \times 10^{5}$
36	MOUAU	$<10^{1f}$	$<10^{1f}$	$<10^{1a}$	$<10^{1e}$
	UMUGBALU	$1.6^{f} \times 10^{1}$	$<10^{1f}$	$<10^{1a}$	$<10^{1e}$
48	MOUAU	$<10^{1f}$	$<10^{1f}$	$<10^{1a}$	$<10^{1e}$
	UMUGBALU	$<10^{1f}$	$<10^{1f}$	$<10^{1a}$	$<10^{1e}$
LSD		0.128	0.102	0.522	0.186

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different (p<0.05)
MOUAU = Michael Okpara University of Agriculture Umudike
TBC = Total Bacteria Count

Table 5. Effect of habitat on bacterial load (cfu/g) of depurated tilapia guts

Isolates (Bacterial Spp)					
Depuration period (h)	**Habitat**	**TBC**	***Coliform***	***Listeria***	***Salmonella***
0	MOUAU	$1.50^{a} \times 10^{6}$	$9.5^{a} \times 10^{5}$	$<10^{1a}$	$3.9^{b} \times 10^{5}$
	UMUGBALU	$1.54^{a} \times 10^{6}$	$9.7^{a} \times 0^{6}$	$<10^{1a}$	$4.0^{a} \times 10^{5}$
12	MOUAU	$1.20^{b} \times 10^{6}$	$8.0^{c} \times 10^{5}$	$<10^{1a}$	$3.1^{d} \times 10^{5}$
	UMUGBALU	$1.21^{b} \times 10^{6}$	$8.7^{b} \times 10^{5}$	$<10^{1a}$	$3.4^{c} \times 10^{5}$
24	MOUAU	$7.2^{d} \times 10^{5}$	$2.8^{d} \times 10^{5}$	$<10^{1a}$	$1.2^{f} \times 10^{5}$
	UMUGBALU	$7.7^{c} \times 10^{5}$	$3.0^{d} \times 10^{5}$	$<10^{1a}$	$2.6^{e} \times 10^{5}$
36	MOUAU	$1.2^{f} \times 10^{5}$	$<10^{1e}$	$<10^{1a}$	$<10^{1g}$
	UMUGBALU	$3.8e \times 10^{5}$	$<10^{1e}$	$<10^{1a}$	$<10^{1g}$
48	MOUAU	$<10^{1g}$	$<10^{1e}$	$<10^{1a}$	$<10^{1g}$
	UMUGBALU	$<10^{1g}$	$<10^{1e}$	$<10^{1a}$	$<10^{1g}$
LSD		0.663	0.408	0.522	0.116

Each value represents the mean of triplicate determination
Means in the same column with different superscript are significantly different (p<0.05)
MOUAU = Michael Okpara University of Agriculture Umudike
TBC = Total Bacteria Count

3.2 Discussion

3.2.1 Bacterial load of water sample (habitat)

The results of the bacteriological characteristics showed that the selected pathogenic bacteria which were isolated from the water habitat (pond), are Gram-negative bacteria. However, the occurrence of these Gram–negative bacteria in all of the locations indicated the prevalence of these bacteria in aquatic environment [16,17]. Additionally, the bacterial variation in different habitat, clearly demonstrates the influence of food ecosystem on the bacterial profile of fish [18]. The microbes enumerated were Coliforms and *Salmonella* spp. The Coliforms isolates would be an indication of the contamination of the pond with fecal matter which resulted to the presence of pathogenic organism in fish. The fecal matter may be as a result of fertilization of the pond with animal manure which was discharged directly into the fish ponds, or excreted by the fish into the ponds [19].

Mohammed [20] who has reported that the contamination has been attributed to questionable water quality and high stocking densities. The feeds used for fish in these ponds may contain organic materials and introduces a wide variety of microbes into the ponds [20]. However, organic manure also leads to the release of high level of opportunistic and pathogenic microbes in the ponds which are of public health concern.

According to [21], their presence in fish intended for human consumption may constitute a potential danger not only in causing disease but could act as a reservoirs of antibiotic resistance organism leading to treatment failure and high cost of treatment when improperly cooked fish is consumed.

The presence of pathogenic microbes especially *Coliforms* and *Salmonella* spp can lead to the transmission of water-borne disease such as typhoid fever, gastroenteritis and food poisoning [22] on consumption of improperly cooked fish cultivated in these ponds or through contact with the contaminated fish and water. The bacteria isolated from these ponds are in agreement with the report of [20] who worked on pond (ecosystem) water suggesting that allochthonous bacteria from feed added to the ponds are principal source of bacteria of health importance and [23] who also reported similar organism in the microbiological study of Elquanter fish pond. Coliforms were the most predominant microbes occurring in the water sample (pond) from different locations. Its presence in water or food indicates the possible presence of causative agents for many gastrointestinal diseases [15]. Total bacteria count for Coliforms and *Salmonella* spp (see Table 1) were high and varied within the water samples (pond) from different locations. The observed value may be due to water temperature (Table 2) which fell within the optimum condition for bacterial growth and also due to the organic matter load found in pond water resulting from the diet used in feeding the fish. Thus, the pond water may be an ideal culture medium for the proliferation of pathogens capable of causing bacterial infection in fish and an important cause of food poisoning [24].

3.2.2 Water quality parameters of water sample (habitat)

The Water quality parameters of fresh tilapia species (*Oreochromis niloticus*), play a major role in their distribution and microbial profile. Apparently, the temperature obtained (Table 2) is highly favourable for the growth of mesophilic bacteria which explains the high number of *Coliform* and *Salmonella spp* isolated from the habitat or ecosystem. Furthermore temperature is a factor of great importance for aquaculture ecosystem, as it affects the organism as well as water quality parameters. The optimum condition for increasing fish productivity was found to be at 20 – 30℃ [25]. The temperatures observed from this study ranged from 30.0 – 31.5℃ and was at close range that supports fish production.

Similarly, the pH values recorded in all the ponds were within the range of 5.45 – 7.45, thus suitable for fish production. The pH values obtained in this study was similar to that of [15], who studied the physicochemical parameters of fish pond water in Okada in Edo state. [34] Accordingly observed that the appropriate pH for increased fish production is 6 – 9, while [20] reported that rapid changes in pH can cause extreme stress in that similar to shock in humans. Also, the pH value of the habitat must have enhanced the growth of the microbes in both fish species. A pH range of 7.5 to 8.7 has been reported to enhance the survival of fresh fish species [26].

Slightly high salinity level of 4.10 - 6.50 PSU in all locations may be due to lack of changing of the water in aquaculture pond at appropriate time which invariably must have brought the water salinity to a moderate level [27]. Salinity is also a major driving factor that affects the density, physiology and growth of aquatic organisms' population like freshwater fish [28].

Dissolved oxygen is one of the important water quality parameters that determine the dynamics of the biota in natural waters because it is a regulator of metabolic processes [29]. Similarly, dissolve oxygen obtained in this study was above 5 mg/l required for fish production. Generally, concentration below 5 mg/l may adversely affect the functions and survival of biological organism and below 3 mg/l can lead to death of most fish [30]. Low dissolved oxygen observed in a fish pond (water habitat) could be attributed to elevated temperature, increased microbial and organic load and the resultant increase in metabolic activity may also account to low dissolved oxygen concentration [31].

3.2.3 Effect of habitat (pond) on depuration of tilapia

The results (Tables 3, 4, 5) above showed that fish living in natural/aquaculture environment are known to harbor pathogenic *Enterobacteriaceae* [32]. Furthermore, the presence of *Coliforms* in fish demonstrates the level of pollution of their environment because *Coliforms* are not the normal bacterial flora in fish [33]. *Coliform* were higher than other bacteria (*Listeria* spp and *Salmonella* spp) and there is a significant difference ($p < 0.05$) in bacteria count among the

bacteria species. Generally the gut had a higher TBC and other selected pathogenic bacteria load than gills and muscles. The significantly higher bacterial loads observed in the intestine regardless of the species of freshwater fish, demonstrates that the intestines are better microhabitats due to the presence of more nutrient (being reservoirs of food).This corroborates that retention and multiplication of microbes are tissue related [34,35]. Furthermore, the higher TBC found in the intestine as compared with that of the gill and muscle was not surprising as this had confirmed that some portions of sea foods and more susceptible than others [35]. Also the microbiological diversity of fresh water fish muscle depends on the fishing grounds and environmental factors around it [36].

Furthermore, [37] discovered that fish in direct contact with microflora in the environment; the opportunistic pathogens already present in the water may invade the host under stress and undesirable water quality conditions. Since some of the selected microflora associated with fish culture environment has been reported, it is important to note that the microflora of a cultivated fish is a reflection of it aqueous environment [38]. Hence, there is need for good fish culture and water management to reduce the occurrence of disease in fish in order to achieve the financial and nutritional benefits of the fish production venture. This can be inferred, that the reason for higher population of bacteria in the water and tilapia sample from Umugbalu location when compared to the MOUAU location was a function of pollution they were expose to. There was significant difference ($p<0.05$) between tilapia samples from MOUAU and Umugbalu, difference in their bacteria population was obvious. The reason was simply because the fish species from MOUAU location were better catered for and the level of bacterial pollution was controlled through regular change of water. They were also not prone to farming activities that causes domestic discharge unlike the Umugbalu location.

The study finally showed that bacterial populations accumulated in freshwater fish (Tilapia) generally reduced to acceptable limit of less than 10^5 cfu/gm [39] after 48 h of depuration.

4. CONCLUSION

In conclusion, small scale depuration system was adequate for the assessment of bacterial quality of the water and the organs of freshwater fish (*Oreochromis niloticus*). Furthermore, the study also reveals that the water sample (habitat/pond) was grossly contaminated with pathogenic bacteria and these could affect fish cultivation. These organisms could lower fish yield, cause disease and economic loss and equally endanger the ultimate consumers (humans), particularly if the fish harvested from the water (habitat/pond) are under processed. Finally, the presence of pathogenic bacteria, in the fresh water fish as well as the pond water investigated, is likely to pose high health risk to humans who use the fresh water fish as source of protein. If it becomes necessary to use fresh water fish as source of protein, then it should be depurated, since the TBC and other pathogenic bacteria observed initially were not within the acceptable limit of but following depuration for 48h, the fish organs bacterial load were brought down to a safe level of $<10^5$ cfu/gm.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. The State of World Fisheries and Aquaculture 2016. Rome. 2014;223. Available:http://www.fao.org/3/a-i5555e.pdf
2. Hernandez MD, López MB, Alvarez A, Ferrandini E, Garcia B, Garrido MD. Sensory, physical, chemical and microbiological changes in aquacultured meager (*Argyrosomus regius*) fillets during ice storage. J. Food Chem. 2009; 114:237-245.
3. Thampuran N, Surendraraj A, Surendran PK. Prevalence and characterization of typical and atypical 73. Nemetz, TG and EB Shotts, Jr. 1993. Zoonotic diseases. In: Fish Medicine. MK Stoskopf, ed. W.B. Saunders Company, Philadelphia. 2005; 214-220.
4. Douglas D. Identifying freshwater aquarium fish disease; 2007. Available:http://fishsuite101.com/article.cfm/identifyingfishdiseases.04-03-2015.pdf
5. Adebayo-Tayo BC, Odu NN, Okonko IO. Microbiological and physicochemical level of fresh cat fish (*Arius hendelotic*) from different markets in Akwa Ibom State, Nigeria. New York Sci. J. 2010;5(4):46-52.
6. Miceal W, Suen F, Carina K, Tor M. Journal of Clinical Microbiology. Published

by the American Society for Microbiology. 2007;45:1-7.

7. Ezenwa BIO. Aquaculture research and fish farm development potential in the Niger Delta. Paper presented at a workshop on Niger Delta fisheries potentials 10th May 2006. Port Harcourt, Nigeria. 2006;1-12.
8. Oliveira J, Cunha A, Castilho F, Romalde JL, Pereira MJ. Microbial contamination and purification of bivalve shellfish: Crucial aspects in monitoring and future perspectives - A mini-review. Food Control J. 2011;22(6):805-816.
9. Nester EW, Anderson DG, Roberts JCE, Pearsall NN, Nester MT. Microbiology - A human perspective. 4th Ed., McGraw-Hill Higher Education, New York. 2004;786-796.
10. Pouch F, Ito K. Compendium of methods for the microbiological examination of foods. Washington, USA: American Public Health Association; 2001.
11. Lee R. Lovatelli A, Ababouch L. Bivalve depuration: Fundamental and practical aspects. FAO Fisheries Technical Paper. No. 511. Rome, FAO. 2008;139.
12. FDF. Federal Department of Fisheries. Fisheries Statistics of Nigeria, Fourth edition. Abuja, Nigeria; 2007.
13. Andrew LS, Grodner RM, Moody MW. Microbial safety of *Rangia cuneata.* Louisiana estuarine clam at harvest and following relaying. Food Microbiol. 1990;7: 107-111.
14. Ezeama CF. Food microbiology. Fundamentals and Applications. Natural Prints Ltd Lagos, Nigeria. 2007;64-72.
15. Ampofo JA, Clerk G. Diversity of bacterial contaminants in tissues of fish culture in organic waste fertilized ponds. Health publications. The Open Fish Sci J. 2010;3: 142–146.
16. Mattle GR, Matte MH, Satto MIZ, Sanchez PS, Rivera IG, Martins MT. Potentially pathogenic vibrios associated with mussels from tropical region on the Atlantic Cost of Brazil. J. Applied Bacteriol. 1994;77:281-287.
17. Efiuvwevwere BJO, Ajiboye MO. Control of microbiological quality and shelf life of catfish (*Clarias gariepinus*) by chemical preservatives and smoking. J. Applied Biotechnol. 1996;80:465-470.
18. Fleet GH. Microorganisms in food ecosystems. Inter. J. Food Microbiol. 1999;50:107-117.
19. Kay D, Crowther J, Stalpleton CM, Wyer MD, Ewtrell LF, Edward A, Francis CA, McDonald AT, Watkinson J. Fecal indicator organism concentrations in sewage and treated effluents. J. Water Res. 2008;42: 442–445.
20. Mohammed HA. Physicochemical characteristics of Abuzabaal pond, Egypt. Egypt Journal of Aquatic Research. 2005; 316:1–13.
21. Omojowo FS, Omojasola PF. Microbiological quality of fresh catfish raised in ponds fertilized with raw and sterilized poultry manures. American J. Res Com. 2013;2325–4046.
22. Piet K. Waste disposal technology. Mpumalaugh, South Africa. 2009;24–27.
23. Dabbor SM. Microbiological profile of El. Quanater EL- Khairia Fish Farm. Global Veterinarian. 2008;2:51-55.
24. Eze VC, Ogbaran IO. Microbiological and physicochemical characteristic of fish pond water in Ughelli, Delta State, Nigeria. Inter. J. Current Res. 2010;8:082-087.
25. Ntengwe FN, Edema MO. Physicochemical and microbiological characteristic of water for fish production using small ponds. J. Physics and Chem of the Earth. 2008;33:701-707.
26. Olofintoye KK. Population and infection dynamics of *Bulinus globosus* and *Biomphalaria pfeirfferi* in Elemi River, Ekiti State, Nigeria. Nigeria Journal of Science and Engineering. 1999;6:1884-1891.
27. Manaham SE. Water pollution. In: Environmental Chemistry. 6th edn. Lewis Publishers, London; 1994.
28. Jamabo NA. Ecology of *Tympanotonus fuscatus* (Linnaeus, 1758) in the mangrove swamps of the upper Bonny River, Niger Delta, Nigeria. Ph.D. Thesis, Rivers State University of Science and Technology, Port Harcourt, Nigeria. 2008;231.
29. Beadle LC. The inland waters of the Tropical Africa. An Introduction to Tropical Limnology. Longman Series Ltd, London. 1981;274.
30. Swan L. A fish farmer guide to understanding water quality; 2006. Available:http/aquanic.org/publicat/il-in/as – 530htlm
(09-19-2015)
31. FAO. The state of world fisheries and aquaculture. FAO. United Nation Rome. Food Control. 2005;18:1391-1396.

32. Pillay TVR. Fish and public health and disease. In: Aquaculture, Principle and Practices. Pillay, T.V.R. eds. Fishing New Book. Farrihan. New York. 1990;174-215.
33. Claucas IJ. Fish handling, processing and preservation in the tropics. Report of the Tropical product Institute, London. 1982; G144(Part 2):145.
34. Kueh CSW, Chan K. Bacteria in bivalve shell fish with special reference to the oyster. Journal of Applied Bacteriology. 1985;59:41-47.
35. Efiuvwevwere BJO, Iweanoge HA. Microbiological and physicochemical quality of various tissue types of fresh and potassium sorbate treated and untreated smoked mullet (*Mugi cephalus).* World Journal of Microbiology and Biotechnology. 1991;7:562-566.
36. Cahill MM. Bacteria flora of fishes. Springer, New York. Journal of Microbial Ecology. 1990;19(1):21-41. ISSN-0095-3628 (print) 1432-184x (on-line)
37. Erondu ES, Anyanwu PE. Potential hazards and risk associated with the aquaculture industry. African J. Biotechnol. 2005;4(13):1622–1627.
38. Ajayi AO. Bacteriological study of catfish, *Claria gariepinus,* from fish pond sources in Akungba-Akoko community, Nigeria. British Microbiol Res J. 2012;2(1):1-9.
39. ICMSF. (International Commission on Microbiological Specifications for Foods). Microorganisms in foods VI*:* Microbial ecology of food commodities; 2005. Available:http://www.spring.com/us/book/9780306486753. 18-08-2015

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17517