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Sanitary and Bacteriological Studies of Different Aquatic Environments in Ibadan, Nigeria

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Abstract The physico-chemical properties and microbial quality of water used for the culturing of fishes play important role in management of aquacultures. The physico-chemical attributes and bacteriological quality of different aquatic environments in Ibadan, Southwest, Nigeria were studied. The result revealed higher significant difference of total dissolved solid (>68.3%, $p<0.05$) in feral water. Lower significant difference of dissolved oxygen saturation (<0.15mg/L, $p<0.05$) was obtained in pond water used for culturing Nile tilapia, while ammonia was not detected in any of the aquatic environments studied. Others parameters were indifferent but could support bacteria proliferation particularly temperature ranged from 30.5-31.5℃. The heterotrophic bacteria count obtained from different aquatic environments ranged from $196.33\text{-}252.67\text{x}10^2$ ($\log_{10}$ cfu/cm^2 4.29 -4.40). The highest microbial load of $252.67\text{x}10^2$ cfu/ml was recorded in the feral water and this could be due to heavy environmental pollution. *Staphyloccous, Esherichia coli, Pseudomonas* sp*, Klebsiella* sp*, Streptococcus* sp*, Salmonella* sp and *Proteus* sp were all isolated from the water samples from different aquatic environments. The bacterial assemblage was of public health significance.

Keywords Aquatic environments, Bacteria, Physico-chemical parameters, Public health

1. Introduction

As the human populations continue to expand, its reliance on captured and farmed fish production as important source of protein will also increase. Fish like other aquatic animals largely depend on aquatic environments. Water quality is the main factor that determines the degree of production and quality of the fish products. The physico-chemical and biological properties of water play a significant role in the sanitary and bacteriological quality of water (Dorota, *et al*., 2002).

In Nigeria, the deposition of human, animal excreta and other environmental wastes into natural water and run-off containing fecal matter of various sources during rainy seasons ultimately emptied into water bodies are capable of elevating bacterial counts in water bodies and ponds (Doyle and Erickson, 2006). It has been known that the microorganisms associated with most fishery products indicate the microbial flora in their aquatic environments (Ashie *et al*., 1996; Gram and Huss, 1996).

Fishes which are farmed in poor and or polluted water are not only prone to diseases but can be of health hazard to handlers and consumers (Boyd, 1998). In previous studies on Lagos lagoon (Ekundayo , 1997), on Ele river (Ajiwe *et al*., 2000) and on Otamiri river (Ibe and Ozor 2000), different bacterial species with potential for causing high proportion of death and ill- health in population dependents on the water bodies for water related resources were identified. While majority of published works in Nigeria are on fish with few or none on physico-chemical properties and bacteriological quality of aquatic environments, the authors of this current research undertook this study to produce data on physcio-chemical properties and bacteria occurrence to determine the contamination level and sanitary state of different aquatic environments in Ibadan South west Nigeria.

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2. Materials and Methods

2.1. Study Location

Three study sites in Ibadan, South west, Nigeria were used for this study. A commercial farm fish ponds culturing *Clarias gariepinus* (African catfish) and a fishery institute ponds culturing *Oreochromis niloticus*) (Nile Tilapia), both situated in Ibadan South west Local Government Area, Oluyole Estate, Ibadan, and Eleyele river, a major source of different aquatic animals in Ibadan North West Local Government Area, Onireke Ibadan, Nigeria.

2.2. In Situ Measurement of Physico-chemical Parameters of Water Samples

At the site of the river, and cultured ponds, dissolved oxygen (DO), water temperature (℃), ammonia (NH_3), pH and total dissolved solids (TDS) were measured using a portable Hach meter (Hach Company, Loveland, USA), and Aquachek® (USA) water quality test strips. Mean readings were taken for Morning (8.00 a.m) and afternoon (4.00 p.m) for three (3) consecutive times at periodic interval of seven days. The probe of HACH meter was dipped inside the tested water, and stabilized readings of DO, pH, T℃, and Total Dissolved (TDS) displayed on the meter monitor immediately were recorded. Aquacheck® stripes were manually dipped inside the tested water. For pH reading, the stripe was removed immediately; for Ammonia, the stripe was vigorously moved up and down inside the tested water for 30 seconds. Colour change for pH and NH_3 were read against the standards colours in 15 seconds and 30 seconds respectively.

2.3. Collection of Water Sample

Water samples were drawn in sterile 500ml bottles from the three different sites of each pond and river in each occasion of sampling. The water samples were brought to the laboratory in ice box at temperature below 4^{o}C within 2 hrs of sampling. Samples were drawn between 8.00 and 10.00am for morning and 3.00-4.00pm for afternoon.

2.4. Processing of Water Samples

Each water sample collected from river and cultured ponds was analyzed and total bacterial count (TBC) and *Enterobacteriaceae* count (EC) were determined. In brief, for the enumeration of TBC and EC in water, 1ml sample water was mixed with 9 ml of sterile normal saline (0.85% NaCl solution) to prepare 10^{-1} dilution. Then 0.1ml of diluted water samples was inoculated on Nutrient Agar (NA) and MacConkey Agar (MAC) for enumeration of TBC and EC respectively. The plates were then incubated at 37^{o}C for 18-24 hrs. The distinct colonies were counted. The distinct colonies were further sub-cultured on freshly prepared NA and MAC for colonial purification. The isolates were equally identified using Gram-staining method, physiological, biochemical reactions and fermentation of sugar according to standard taxonomic schemes (Buchanan and Gibbons, 1974).

2.5. Statistical Analysis

The bacterial density data were transformed into natural log before statistical analysis. The means of physico-chemical parameters and bacterial load were compared by using analysis of variance (ANOVA) and significant means separated using Duncan multiple range test (DMRT) as outlined by Steels and Torrie (1980). The values of $p<0.05$ were considered significant.

3. Results and Discussion

The physico-chemical attributes of water samples of different aquatic environments are as presented in Table 1. The temperature of the water ranged from 30.5-31.5℃ with higher values obtained in the noon. The pH values in the range of 7.33-7.50 were obtained in study. The pH is interdependent with other water quality parameters such as carbon dioxide, alkalinity and hardness. pH can be toxic in itself at a certain level and also known to influence the toxicity as well of hydrogen sulphide, cyanides, heavy metals and ammonia (Klont,1993). A pH between 6.5-9.0 is ideal for freshwater animal (Boyd, 1998). Below pH 6.5, some species expresses slow growth and some organism's ability to maintain salts balance is affected (Lloyd, 1992).

The values of dissolved oxygen obtained in this study ranged from 0.15-5.42mg/L with significantly lower level of dissolved oxygen recorded in water using for culturing of Nile tilapia. Nile tilapia can survive low dissolved oxygen (Thomas and Michael, 1999), this could put undue stress on the fish, leading to low survival rate of the tilapia (Boyd, 1990). A concentration of at least 5 mg/l (Lawson, 1995; ANZECC, 2000) is recommended as ideal for fresh water fish aquaculture. Dissolved oxygen (DO) is considered as one of the most important aspect of aquaculture. It is needed by aquatic animals to respire and perform metabolic activities; low levels of DO are often linked to fish kill incidence. Ammonia is the initial product of the decomposition of nitrogenous organic wastes and respiration.

The high concentration of ammonia cause an increase in pH and ammonia concentration in the blood of fish can damage the gills, the red blood cells, affects osmoregulation, reduces oxygen carrying capacity of blood and increases the oxygen demand of the tissue (Lawson, 1995). However in this study, there was no indication of the presence of ammonia in the water samples.

Feral water showed a significantly higher total solids (>68.30%) as obtained in this .study and this value is above the recommended level of less than 40% (ANZECC, 2000). This could be as a result of mass pollution of the environments, hence higher bacteria load observed in the feral water studied. All the water quality variables studied were suitable for bacterial proliferation particularly temperature ranging from 30.50-31.50℃ (Shankar *et al*., 2009). A low level of organic substance in the water, low temperature, good oxygenation and the appropriate pH assure that the bacteriological status of the water is good and abundance of heterotrophic bacteria remaining low (Dorota, *et al*., 2002).

Virtually all the water samples studied were heavily contaminated. The mean total bacteria and *enterobacteria* count of water samples obtained from the ponds and the river are as shown in Table 2. The highest bacteria count 252.67 ± 24.53 10^2 cfu/ml ($\log_{10}$ cfu/cm^3=4.40) and *enterobacteria*

count 138 ± 22.69 10^2 cfu/ml($\log_{10}$ cfu/cm^3=4.14) were obtained from feral water while the least total bacteria count 196.33 ± 42.31($\log_{10}$ cfu/cm^3=4.29) was obtained from cultured tilapia water and least count of *enterobacteria* 71.00 ± 27.50 10^2 cfu/ml($\log_{10}$ cfu/cm^3=3.85) was obtained in the cultured catfish water (CCW).

In each case, the *enterobacteria* count was lower than the total bacteria count of the samples. This could be due to rapid die off of *enterobacteria* in the aquatic environments (Edward and Pullin, 1990). The *enterobacteria* count obtained from cultured catfish water (CCW) was significantly lower than the *enterobacteria* counts obtained from feral and cultured tilapia water (CTW).

This could be possibly due to heavy pollution of the feral water studied and the same may not be said of cultured tilapia water. A study is underway to study what could be responsible for lower *enterobacteria* count obtained from cultured catfish water (CCW). In this regard, we may be able to establish what is responsible for different degree of level of proliferation of heterotrophic bacteria in different aquatic environments. Comparing the microbial loads of different aquatic environments studied, there was no significant difference in the microbial loads. However, highest microbial load of 252.67 x 10^2 cfu/ml ($\log_{10}$ cfu/cm^3=4.40) was recorded in the feral water. This could be due to heavy environmental pollution which was evidenced by the high total dissolved solid (>68.30%) recorded in the water. Many industries that lack effluent treatments plants discharged their untreated wastes into this river.

Chukwura and Okpokwasli(1997) and Odiete (1999) reported that many industries deposited their wastes into nearby natural water bodies and this environmental discharge has a contributory effect on the upsurge in the microbial load (Lateef, 2004). Lateef (2004) obtained a bacteria count of 2.15 x 10^5 cfu/ml ($\log_{10}$ cfu/cm^3=5.50) from a pharmaceutical effluent studied. Some workers observed the bacterial counts in order of 10^5($\log_{10}$ cfu/cm^3=5.00) in some polluted rivers studied that were exposed to human, agricultural and industrial wastes in Nigeria (Chukwura and Okpokwasli, 1997; Bakare, *et al*., 2003; Adewoye and Lateef, 2003).

The toxic substances discharged into water bodies can accumulate through the food chain (Odiete, 1999) and may also either limit the number of species or produce dense population of micro-organisms (Okafor, 1985), some of which could be pathogenic and be human health risk to population dependent on water resources.

The heavy bacterial load could be a source of stress to aquatic vertebrates, fishes inclusive. Stress and consequently immune suppression was probably the commonest underlying cause of disease in fish. Usually pathogens such as *Aeromonas*, *Pseudomonas*, and *Flavobacteria* are environmental contaminants, and are usually secondary invaders of otherwise stressed fish. Several bacteria isolated in the course of this study are species in the genera: *Staphylococcus*; *Escherichia*, *Pseudomonas*, *Klebsiella*, *Streptococcus*, *Salmonella* and *Proteus*. Some bacterial species such as *Proteus* sp and *Streptococcus* sp have been linked with certain diseases condition in fish (Sagua, 1986) which resulted in huge loss of stock. *Salmonella* sp, *E. coli*, *Streptococcus* and *Staphylococcus* were also implicated in fish borne (Babu, 2000) and shrimp borne (Raghavan, 2003) diseases of human. Staphylococci are gram positive facultative anaerobic bacteria. They are widespread among mammalian where they belong to the healthy microbial flora of skin and mucosa.

Table 1. Physico-chemical properties of the different aquatic environments

Systems	Temp (°C)		DO (mg/L)		pH		NH_3 (ppm)		TDS (%)	
	AM	PM	AM	PM	AM	PM	AM	PM	AM	PM
CCW	30.5± 0.27^a	31.33± 0.33^a	5.40± 0.03^a	5.42± 0.07^a	7.50± 0.03^a	7.33± 0.09^a	0.00	0.00	8.83± 0.08^b	8.83± 0.18^b
CTW	30.6± 0.04^a	31.40± 0.25^a	0.15± 0.01^b	0.15± 0.01^b	7.50± 0.03^a	7.50± 0.03^a	0.00	0.00	23.47± 11.69^b	23.47± 11.69^b
FW	30.67±0.07^a	31.5± 0.20^a	4.91 ± 0.92^a	4.61±0.0^a	7.33±0.09	7.50±0.03^a	0.00	0.00	68.3±012.56^a	64.50±10.95^a

CCW, Cultured Catfish Water (A) CTW, Cultured Tilapia Water (B) FW, Feral (Natural) Water (C); *Means in the same column with the same letters are not significantly different using Duncan Multiple Range Test at p<0.0

Table 2. Mean total bacteria load and *Enterobacteria* count (x 10^2) of different aquatic environments studied

	SAMPLES	Total Bacteria count Mean± SEM n=9	Enterobacteria count Mean± SEM n=9
A	Cultured Catfish Water (CCW)	224.27± 41.79^a (4.35)*	71.00± 27.50^b (3.85)*
B	Cultured Tilapia Water (CTW)	196.33± 42.31^a (4.29)*	111.33± 18.44^a (4.05)*
C	Feral Water (FW)	252.67± 24.53^a (4.40)*	138± 22.69^a (4.14)*

*Means in the same column with the same letters are not significantly different according to Duncan Multiple Range Test at P<0.05; *, values in parenthesis are $\log_{10}$ CFU/ml.

However, *Staphylococci* are also common human- animal pathogen. The coagulase- positive species *Staphyloccous aureus* are the species with the broadest pathogenic potential. In contrast to *S. aureus,* members of the heterogeneous group of coagulase negative staphylococci (CNS) are regarded as less pathogenic bacteria. However, in the recent decades, CNS has emerged as nosocomial pathogens in immunocompromised individuals. Specifically, *Staphylococcus epidermidis* is a common cause of line associated septiceamia and other polymer related infections.

Some strains of *E. coli* are capable of causing food borne diseases, ranging from mild enteritis to serious illness and death. There is good evidence for the occurrence of water borne infection caused by *E. coli* 0157: H7. As the occurrence of this strain in cattle is well established and its infections dose is low, it poses a potential risk to public health where bovine manure is used as pond fertilizer (WHO, 1997). *Salmonella* sp are among the most important causes of human gastrointestinal disease world wide and previous studies established that aquatic birds spread these organisms and other pathogens in the environment (Felon, 1983).

Most bacteria species isolated in this study have been implicated as bacteria of public health importance in the previous studies by some researchers (Babu 2000; Raghavan, 2003; Lateef, 2004; Sowunmi, 2008).

The implication of this is that the aquatic environments studied portray danger being potential sources of biological health hazards to population dependent on water bodies for water related resources. In Nigeria, the physico-chemical and biological parameters are not adequately monitored and indiscriminate deposition of wastes into water bodies all contributed to proliferation of dense microorganisms in the aquatic environments. Study of bacteria in the context of their environment and their hosts physiology, led to the conclusion that bacterial diseases of fish and other aquatic animals are almost invariably stress related (Inglis, 1993).

Therefore precaution should be taken to maintain good water quality for aquaculture purpose in order to prevent build up of microbial populations in the aquatic environments. The indiscriminate discharge of environmental wastes into water bodies should be discouraged as this will go a long way to safe guard the life of public from biological health hazards associated with aquatic environments. The safety of public can also be ensured by improving sanitation within metropolis by provision of public toilets, enactment of effective policy for the collection and disposal (management) of municipal solid wastes. All these would drastically reduce the pollution of running Water Rivers with industrial and domestic wastes.

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