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AN INVESTIGATION OF THE MICROBIOLOGICAL AND PHYSICOCHEMICAL PROFILE OF SOME FISH POND WATER WITHIN THE NIGER DELTA REGION OF NIGERIA.

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ABSTRACT: *The microbial profile of some concrete and earthen fish ponds within the Niger delta region were carried out. The result of physico-chemical properties of the water samples showed that alkalinity was significantly higher in concrete ponds (99.7±47.1 to 150±69.7) than (18±6.9 to 24±14.5 mg/L) in earthen ponds. Electrical conductivity varied significantly between the ponds with (200±84.1 to 290±74.9 μ s/m) in concrete ponds and (18±6.9 to 24±14.5 μ s/m) in earthen ponds. Sulphate was higher in concrete ponds (0.25±0.36 to 1.53±14.9ng/L) than (0.25±0.36 to 0.4±0.77mg/L) in earthen ponds. The mean total heterotrophic bacteria count was higher in concrete ponds ($6.5x10^5$to $7.4x10^5$ cfu/ml) than ($6.3x10^5$ to $6.5x10^5$ cfu/ml) in earthen pond. The mean fungal count ranged between ($2.11x10^5$ to$2.25x10^5$ cfu/ml) in concrete pond and ($1.8x10^5$ to $2.4x10^5$cfu/ml) in earthen ponds. The bacteria genera isolated from the ponds were Escherichia coli, Staphylococcus sp., Aeromonas sp., Streptococcus sp., Salmonella sp., Vibrio sp, Serratia sp., Shigella sp., Proteus sp., Pseudomonas sp., Klebsiella sp. and Enterobacter species. While the fungal isolates were Aspergillus sp., Penicillum sp., Cladosporium sp., Fusarium sp., and. Mucor sp. The study revealed that the ponds were grossly contaminated with pathogenic microorganisms which poses a risk to human health, thus of significant public health concern.*

KEYWORDS: Heterotrophic bacteria, fish ponds, fungal isolates, pathogens, physico-chemical properties.

INTRODUCTION

Most of the world's population (56 percent) derives at least 20 percent of its animal protein intake from fish (FAO, 2002). Fish is the preferred source of much desired animal protein compared to poultry, beef, mutton or pork. It is comparatively cheaper and highly acceptable with little or no religious bias which gives it advantage over other proteins (Philips, 2004).

Fishes are reared in different culture media or controlled environment which could be ponds (concrete or earthen), vats (wooden or fiber glass) and plastics (Osawe, 2004). Among these culture systems, concrete and earthen ponds are widely used (Ezenwa, 2006). Earthen pond culture system has been the conventional method of fish culture in Nigeria, until recently, concrete tanks culture system is gaining grounds as land become costly, scarce and readily unavailable (Onome and Ebinimi, 2010). Research has shown that a higher number of fish farmers use concrete ponds (73%) compared to 27% using earthen ponds (Ugwumba, 2010). Fishes cultivated in these controlled environments has been found to be contaminated by microorganisms (pathogenic and opportunistic organisms) (Fafioye, 2011, Nguyen *et al.*, 2007). This contamination has been attributed to questionable water quality and high stocking densities (Okpokwasili and Ogbulie, 1998). The feed used for the fish in these ponds contain

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organic materials and introduces a wide variety of microorganisms into the ponds (Okpokwasili and Ogbulie, 1999).

As a result of high cost of feeding, animal manure has been one alternative that has been used to supplement or completely replace conventional feed in country like China, India, and also in Nigeria .However, organic manuring also leads to the release of high concentration of opportunistic and pathogenic microorganisms into the ponds which are of public health concerns. Their presence in fish intended for human consumption may constitute a potential danger not only in causing disease (Omojowo and Omojosola, 2013). Fish is in direct contact with micro flora in the environment and the opportunistic pathogens already present in the water invade the host under stress. It is therefore, important to understand the micro flora associated with fish culture environment, since the microbial flora of a cultivated fish is a reflection of its aqueous environment (Erondu and Ayanwu, 2005). It is therefore significant to evaluate and compare the microbial quality of the different fish ponds within the Niger delta region of Nigeria.

MATERIALS AND METHODS

Study area

The study area is within the Niger delta region of Nigeria lies between latitude 4-6^{o}N and longitude 5-8^{o}E with high biodiversity, characteristic swamp, water ways, vast plains and mangrove forest. The farms combine fish production and livestock production, thus they operate integrated fish production having both concrete and earthen fish ponds stocked with Tilapia and Catfish.

Sample Collection

Water samples were aseptically collected from the ponds biweekly using sterile screw capped bottles. Composites samples were obtained by collecting at different sampling points and depths of 30cm below the water surface. The water samples were transported to the laboratory in an ice-packed container for microbiological and physiochemical analysis.

Isolation of total heterotrophic bacteria and fungi

Ten-fold serial dilution of each pond water sample was prepared aseptically in sterile physiological saline up to 10^{-5} and 0.1ml aliquot of each dilution was inoculated on dried nutrient agar and sabouraud dextrose agar plates, in triplicate using the spread plate technique. The nutrient agar plates were incubated at 35^{o}C for 24h under aerobic condition, while the sabouraud plates were incubated at room temperature for five days. Plates containing 30 -300 colonies were selected and counted. The number of colony forming units per ml (cfu/ml) was calculated by multiplying the number of colonies per dilution factor.

Enumeration of total Coliform

The most probable number (MPN) was adopted in determining the total coliform bacteria using MacConkey broth and five tubes techniques .All positive tube from the MPN tubes were sub-cultured on an EMB agar plates in duplicate and incubated at 35^{o}C for 24h.

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Isolation of *Salmonella* and *Shigella*.

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

Isolation of *Vibrio* species

The thiosulphate citrate bile salt agar (TCBS) was prepared and poured on to sterilized Petri dishes. On solidification, 0.1ml of each pond water sample previously enriched in alkaline peptone water was transferred unto the dried agar plate in duplicate using a 1ml pipette and spread evenly with a sterile hockey stick. It was incubated at 35ºC for 24 - 48h. After incubation, yellow and green colonies were counted and identified using biochemical reactions.

Identification of isolates

The cultural, morphological and biochemical characteristic of the respective isolates were compared with the criteria in Bergey's manual of Determinative Bacteriology (1994). The biochemical test used in the identification and characterization of the isolates include: gram staining, motility, indole production, methyl red –Voges proskauer. Citrate utilization, oxidase, catalase, coagulase and sugar fermentation test.

Physico-chemical analysis of water sample

The water samples from the different ponds were examined for physiochemical characteristics using standard procedures of the American Public Health Association (APHA). pH was measured in-situ using a Phillips model of PW 9418 Ph meter after it has been calibrated with standard buffer 7 . Temperature and conductivity were measured using Hach conductivity meter Model (CO150) The winkler's iodometric titration method was adopted for dissolved oxygen determination, biochemical oxygen demand was determined using the 5days approach, while chemical oxygen demand was measured using Walkley and Black (1934) dichromate reflux condensor. Alkalinity was done by titrating 100ml of samples with 0.02ml of HCL solution using methyl orange as indicators, sulphate was determined using the Barium chloride (Turbidimetric) method, while the spectrophotometric method at 555 nm UV visible light PC UNICO 2102 ,USA was used in the determination of nitrite. Ammonia and phosphate were measured using Nessler reaction and Ascorbic acid method respectively.

Statistical analysis

Results collected were subjected to analysis of variance and the least significant difference (LSD) test was used to separate differences between means at 5% probability level.

RESULT

The physico-chemical parameters of the water samples of the four ponds are presented in Figures 1-4. The parameters did not vary significantly within the ponds except for alkalinity, **(**99.7$\pm$47.1 to 150$\pm$69.7) which was higher in concrete ponds than in earthen ponds (18$\pm$6.9 to 24$\pm$14.5 mg/L**)** conductivity was higher in concrete ponds (200$\pm$84.1 to 290$\pm$74.9 μ s/m) than

18±6.9 to 24±14.5 µ s/m in earthen ponds, sulphate was found to be higher in concrete ponds (0.25±0.36 to 1.53±14.9mg/L) than (0.25±0.36 to 0.4±0.77mg/L) in earthen ponds. All the parameters were within the range that supports fish production except ammonia which was above the limit specified by the FAO for aquaculture. The microbial count did not very significantly between the ponds (Table 1). Total heterotrophic bacteria count was highest in concrete pond 1 (9.5x10^5cfu/ml) and lowest in earthen pond 2 (3.6 X10^5cfu/ml)). Fungal count was also highest in concrete pond 1(5.5x10^5cfu/ml) and lowest in earthen pond1 (1.2x10^5). *Salmonella /Shigella* count was highest in earthen pond 1(8.5x10^5) and lowest in concrete pond1 (1.0x10^5cfu/ml). *Vibrio* count was higher in concrete pond 2(8.3x10^4 cfu/ml) and lowest concrete pond1 (1.0x10^5 cfu/ml), while total coliforms was highest in concrete pond 1 94mg/100ml and lowest in earthen pond 2 (2mgl100ml). Bacteriological analysis of the water samples showed twelve different genera (Table 2) which are *Escherichia coli*, *Staphylococcus* sp., *Salmonella* sp., *Shigella* sp., *Aeromonas* sp., *Vibrio* sp., *Pseudomonas* sp., *Proteus* sp., *Enterobacter* sp., *Klebsiella* sp., *Serattia* sp. and *Streptococcus* sp. *Enterobacter* sp. occurred only in concrete pond 1 and 2, *Serattia* sp. occurred only in earthen pond 1, *Proteus* sp. and *Streptococcus* sp. occurred in all the ponds except earthen pond2 while others occurred mainly in all the ponds. The fungal genera isolated are presented in table 3 and they include *Aspergillus* sp., *Penicillum* sp., *Cladosporium* sp., *Mucor* sp. and *Fusarium* sp.

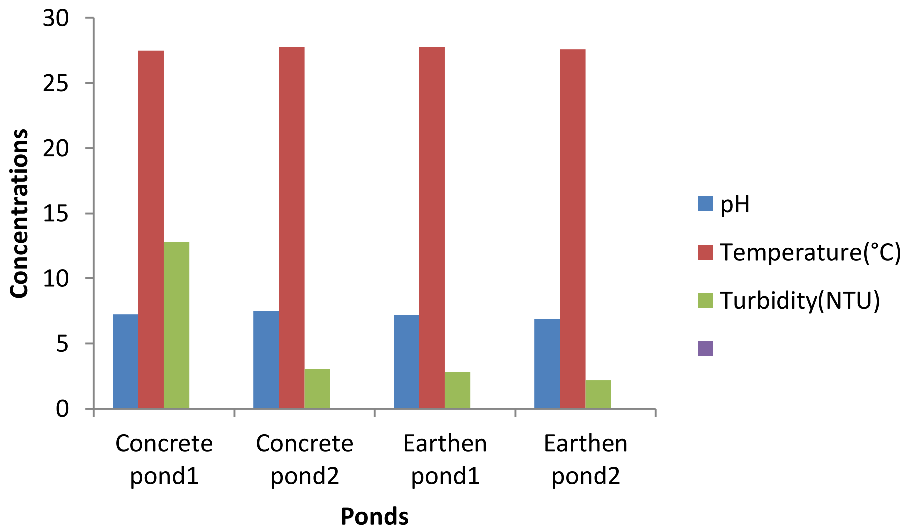

Figure 1: Concentration of pH, temperature and turbidity within the ponds

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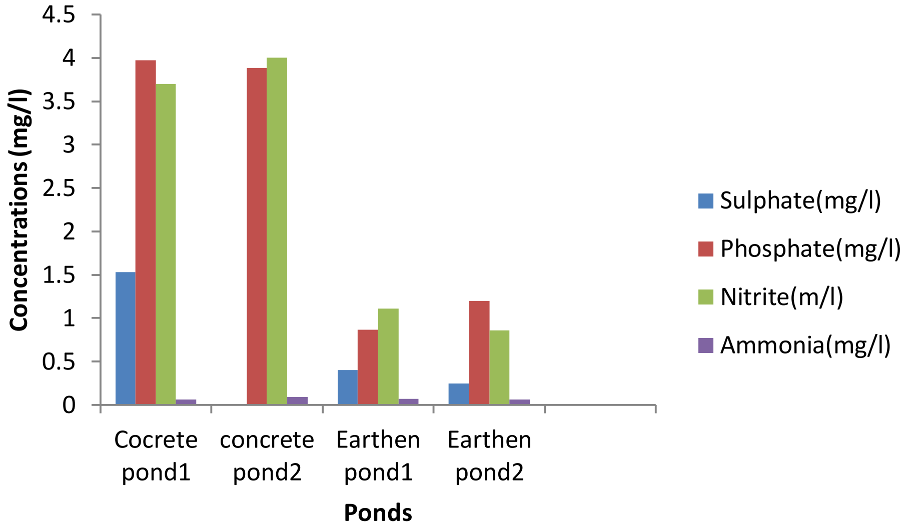

Figure 2: Concentration of sulphate, phosphate, nitrite and ammonia.

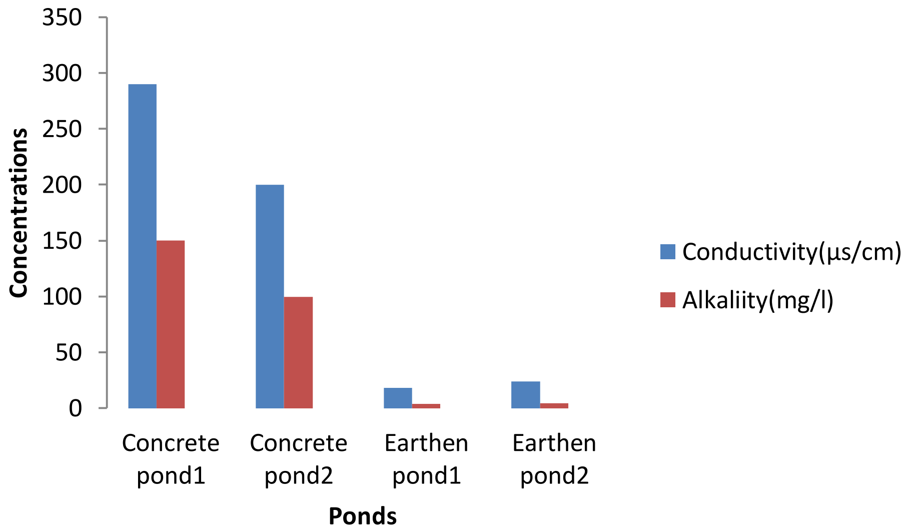

Figure 3: Concentration of conductivity and alkalinity

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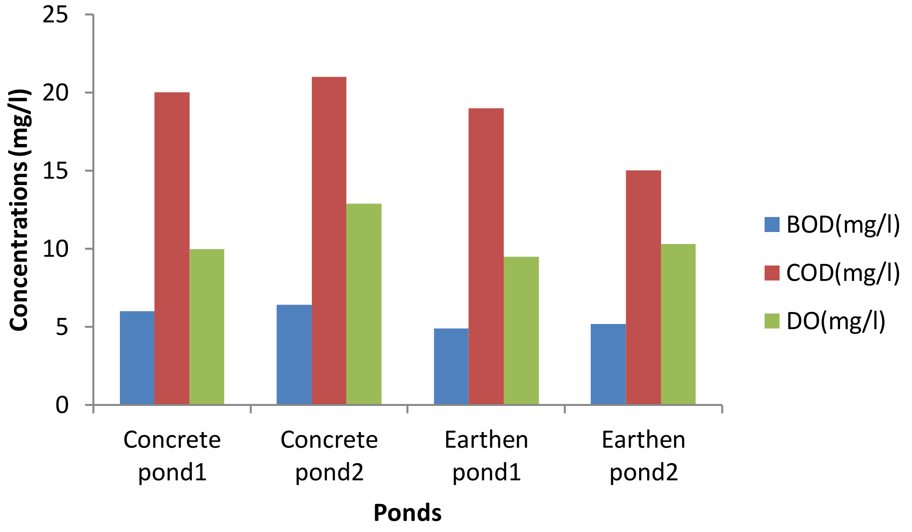

Figure 4: Concentration of BOD, COD and DO

TABLE 1: Mean total heterotrophic Bacteria count, fungal count, *Salmonella/Shigella* count, *Vibrio* count and Coliforum count of the pond water.

	TOTAL HETEROPHIC COUNT (cfu/ml)	FUNGAL COUNT (cfu/ml)	*SALMONELLA/SHIGELLA* (cfu/ml)	VIBRO COUNT (cfu/ml)	TOTAL COLIFORM COUNT (cfu/100ml)
Concrete Pond 1	6.5×10^5	2.11×10^5	3.2×10^5	4.9×10^4	59.6
Concrete Pond 2	7.4×10^5	2.5×10^5	1.9×10^5	5.9×10^4	21.0
Earthen Pond 1	6.4×10^5	1.8×10^5	3.1×10^5	5.1×10^4	23.0
Earthen Pond 2	6.3×10^5	2.4×10^5	1.7×10^5	4.9×10^4	70.3

Table 2 Distribution of isolates from the different ponds

Isolates	concrete pond1	concretepond2	earthen pond1	earthen pond2	frequency	%frequency
E.coli	+	+	+	+	43	20.7
Staphylococcus	+	+	+	+	28	13.5
Salmonella	+	+	+	+	10	4.8
Aeromonas	+	+	+	+	33	15.7
Shigella	+	+	+	+	21	10.1
Pseudomonas	+	+	+	+	5	2.4
Proteus	+	+	+	_	8	3.8
Enterobacter	+	+	_	_	4	1.9
Vibrio	+	+	+	+	25	12
Serattia	-	-	+	_	2	0.96
Streptococcus	+	+	+	_	12	5.7
Klebsiella	+	+	+	+	17	8.2

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Table 3 Occurrence of fungal isolates within the ponds

Ponds	*Aspergillus*	*Penicillum*	*Cladosporium*	*Fusarium*	*Mucor*
Concrete pond1	+	+	+	_	_
Concrete pond2	+	+	+	_	_
Earthen pond1	+	+	+	+	+
Earthen pond2	+	+	+	+	+
Frequency	28	21	10	2	2
% frequency	44.4	33.3	15.9	3.2	3.2

DISCUSSION

The result of the microbiological characteristic showed that Gram negative bacteria were dominant in the bacteria isolated from the ponds. The microorganisms isolated were *E-coli, Salmonella* sp. *Shigella* sp. *Pseudomonas* sp. *Proteus* sp. *Klebsiella* sp. *Vibrio* sp. *Enterobacter* sp., *Serratia* sp., *Aeromonas* sp., *Staphylococcus* sp., and *Streptococcus* sp. The coliforms isolated were an indication of the contamination of the pond water with fecal materials which may result to the presence of pathogenic organisms. The fecal material may be as a result of fertilization of the ponds with animal manure which is discharged directly into the fish ponds, or excreted by the fish into the ponds or through runoff (Kay *et al.*, 2008). The diverse groups of bacteria isolated from these ponds are in line with the report of Okpokwasili and Ogbulie (1999) who worked on pond water suggesting that allochthonous bacteria from feed added to the ponds are the principle source of bacteria of health importance and Dabbor (2008) who reported similar organisms in the microbiological study of El-quanter fish pond. 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 (Piet, 2009) on consumption of improperly cooked fish cultivated in these ponds. *E. coli* was the most dominant organism occurring in both concrete and earthen ponds. The presence of *E.coli* in water or food indicates the possible presence of causative agents of many gastro intestinal diseases (Ampofo and Clerk, 2010). *Pseudomonas*, *Proteus*, *Staphylococcus* species have been implicated in food poisoning (Oni *et al.*, 2013). *Aeromonas* species were also predominantly present in both ponds. This organism is one of the most opportunistic pathogen for fresh water fish and he main etiological agents in disease outbreak were several mortalities were recorded (Das and Mukheyce,1999).

Fungal infection is an important economic and limiting factor in intensive fish production. The fungi genera isolated from the ponds were *Aspergillus* sp., *Penicillum* sp., *Cladosporium* sp., *Mucor* sp., and *Fusarium* sp. *Aspergillus* and *Penicillum* species formed the dominant group of fungi in this study. The observation is consistent with the work of Obire and Anyanwu, (2009) who noted that *Aspergillus* and *Penicillum* species are believed to penetrate into the environment through dead plants materials and remains for long period of time. Similarly, Eze and Ogbaran, (2010) cited *Penicillum* sp. as the most abundant fungi during his study on the microbiological and physiochemical of fish pond water in Ugheli Delta state Nigeria. In contrast to the present result, Fafioye, (2011) reported *Cladosporium* sp. as the dominant fungi specie. The occurrence of *Fusarium* sp. and *Mucor* sp. in earthen ponds could be attributed to the fact that the earthen ponds was a more conducive environment for their growth and proliferation due to the presence of soil and plants in the earthen ponds.

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Total aerobic heterotrophic bacteria count, fungal counts, *Salmonella/Shigella* count, *Vibrio* count and *Coliform* count (Table 1) were high and varied within the ponds. The values was due to 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 (Eze and Ogbaran, 2010).

Among water and food borne pathogens in coastal ecosystems, *Vibrios* contribute the major part. *Vibrio* sp. has been found to play a vital role in fish and shrimp culture system (Amand *et al.*,2010). As they damage water quality causing disease and mortality to the fish as secondary and primary pathogens. The presence of specific pathogenic human species of *Vibrio* can serve as an indicator of public health risk of water and food destined for human consumption (Ganesh *et al*.,2009) .

The pH recorded in all the ponds were within range required for aquaculture (6.5-9.5). pH measurement helps to determine if the water is a proper environment for fish although most fish can tolerate pH as low as 5.0. The pH obtained in this study was similar to that of Ehiagbonare and Ogunrinde (2010) who studied the physiochemical analysis of fish pond water in Okada in Edo State. Ntengwe and Mojisola, (2008) observed that the appropriate pH for increased fish production is 6-9. While Mohamed, (2005) reported that rapid changes can cause extreme stress in the fish similar to shock in humans.

Temperature is a factor of great importance for aquatic ecosystem, as it affects the organisms as well as the chemical and physiochemical parameters of water. The optimum condition for increased fish productivity were found to be at 20 -30^{o}C (Ntegwu and Mojisola , 2008). The temperature obtained from this study ranged from 26-29^{o}C and was within the limit that supports fish productivity. This corroborates with the report of Fafioye, (2011) who observed a temperature of 27-28^{0}C in the preliminary studies and water characteristic and bacterial population in Kojalo fish pond.

The dissolved oxygen, obtained in this study was below the 5mg/L required for fish production. Generally concentration below 5mg/L may adversely affect the functions and survival of biological organisms while below 3mg/L leads to death of most fish (Swan, 2006). The low dissolved oxygen recorded in ponds could be attributed to elevated temperature ,increased microbial and organic load and resultant increase in metabolic activity may also account to low dissolved oxygen concentration (FAO, 2005). Similar result were also reported by Okpokwasili and Ubah, (1991) who worked on water quality and bacteria disease in fish ponds but different from the reports of Onome and Ebinimi, (2010) who recorded higher DO of 4.34 – 6.33mg/l as a result industrial input from the surrounding industries near the fish farm.

Alkalinity of water is the amount of dissolved calcium, magnesium and other compounds in water. The optimum alkalinity for increased fish production is 20-300mg/l. The alkalinity in this study was higher in the concrete ponds than the earthen ponds. This could be attributed to the leaching outs of these substances (lime) from the walls of the concrete ponds into the water (UNESCO/WHO/UNED, 1996). The values obtained in the earthen ponds were below 20mg/l and therefore should be adjusted for maximum productivity by liming.

Ammonia occurs in fish ponds as a result of accumulation of left over rich protein feed, fish waste, and microbial decomposition of organic matter (Dourborow *et al*., 1997). An ammonia

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concentration in all the ponds was above the permissible level of 0.2mg/l recommended for aquaculture (Table 1). Normally, the concentration of ammonia in pond water should be zero. Studies have shown that ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and longtime gill damage (Durborow *et al*., 2000). Similarly, higher ammonia concentration was also obtained by Onome and Ebinimi, (2010) who attributed the high ammonia concentration in new Calabar river to industrial nitrogenous waste.

Nitrite is termed the invisible fish killer as it is deadly to the smallest fish at a concentration as low as 0.25ppm. Nitrite concentration obtained in this study exceeded the maximum permissible limit of 0.1mg/l (Figure 2). It has been observed that nitrite is a skin irritant and will cause the fish to display symptoms of irritability such as rubbing themselves, jumping and skimming across the surface of the pond (Eze and Ogbaran ,2010). Nitrite prevents the blood cells from absorbing oxygen from water. This process turns their blood to a dull brown color and hence the popular name of nitrite poisoning “brown blood disease” (Dourborow *et al*., 1997)

Phosphate level in concrete ponds was above the 3mg/l. This limit should be controlled to avoid eutrophication of the ponds (Eze and Okpokwasili, 2010). Phosphate may be introduced into the pond through fish feed or through surface run off, and could also be from the building materials used in the construction of the ponds. These fishes can also store phosphate in their organs and when they die, they release the previously absorbed into the water which triggers the growth of new algae (Durborow *et al*., 1997).

Sulphate concentration in the ponds varied from 0.25-4.0 (mg/l) with the concrete ponds significantly higher than the earthen ponds. These values are similar to that of Ehiagbonare and Oguunrinde (2010) (0.66-1.09m/l) and different from Utang andAkpan (2012) who reported 42.46-57.36mg/l. He suggested the use of detergent and soaps by residents which got into the water body may be responsible for the high value of sulphate.

The electrical conductivities of the water samples generally varied significantly ($P < 0.05$) and ranged from 9 µs/cm to 400µs/cm for both ponds throughout the period of study. Higher conductivities were observed in concrete ponds than in earthen ponds. Electrical conductivity is a useful indicator of mineralization and salinity or total salt in a water sample. The FAO acceptable limit for conductivity in aquaculture is 20-1500 µs/cm (DWAF, 1996). This limit was not exceeded in these ponds. Thus, the parameter is suitable for fish production.

Turbidity values obtained from the concrete ponds and earthen ponds were within the range that supports aquatic life. Turbidity is the result of several factors including suspended soil particles, planktonic organisms and humic substances produced through decomposition of organic matter (FAO, 2002). It hinders the penetration of sunlight in the pond making it difficult for aquatic habitat to receive the positive effect of light (Ali *et al*., 2004).

The BOD and COD were low and within the permissible range of (<10mg/l and <20mg/l) respectively. The result is different from that obtained by Odewumi and Zakari, (2010) who recorded a higher value of COD and BOD in the study of cat fish ponds in Jos suggesting that the fish ponds were grossly polluted. Similarly, Ehiagbonare and Ogurinde (2010) reported BOD of 2.2mg/l, 2.36mg/l in concrete ponds in Oluko and Igusa and1.6mg/l in earthen pond at Afugle during the study of physiochemical analysis of pond water in Okada and its environs.

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In conclusion, water quality parameters and microbial quality of the two ponds was not significantly different, but there were slight differences in the concentration of nutrients which could be attributed to leaching of these substances into the soil of the earthen ponds. The study also revealed that both ponds were grossly contaminated with pathogenic bacteria that could affect fish cultivated since the microbial quality of any fish pond water is a reflection of the microbial flora of the fish itself. These organisms could lower fish yield, causes diseases and economic loss and equally endanger the ultimate consumers (humans) particularly if the fish harvested from the ponds are under processed.

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