



Microbes from water and fish of Ose river in Ondo State, Nigeria

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Abstract

Keywords:

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This study shows some microbiological and physicochemical quality of Ose River, Ondo State. The water samples were plated on nutrient agar for bacterial enumeration using a pour plate technique. Similarly, cultural characteristics and characteristics appearance using lactophenol cotton blue stain for microscopic studies were adopted for identification of fungi using the compendium of fungi. Identified bacterial species include Staphylococcus aureus, Proteus spp., Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Salmonella paratyphi, Klebsiella spp., Escherichia coli, Serratia mercrescen and Shigella flexneri. The fungal isolates identified from sample sources were Mucor hiemalis, Aspergillus fumigatus, Aspergillus niger, Rhizopus stolonifer, Penicillium frequentans, Saccharomyces spp., Penicillium chrisogenium, Fusarium verticillodes and Chrysonilia sitophila.

The total bacteria count of fish ranged from 2-86 cfu/mL. The highest count was found in the intestine of Tilapia and the least in the gill and skin of Tilapia. The total bacteria count of water ranged from 3-198 cfu/mL. The highest count was found in shoreline and the least in no-activity zone. The average total fungi count of fish ranged from 0-32 sfu/ml, the average total fungi count was highest in the intestine of tilapia and the least count was observed in the gill of tilapia. The average total fungi count of water ranged from 62-221 sfu/ml, in which the average total fungi count was highest in open space and the least was observed in no-activity zone.

Some physicochemical parameters of the water sample show a pH value of 6.92, temperature 28^oC, conductivity, 161, and the turbidity as 4.0. These conditions are fair for fish optimum growth. The study thus suggests that the river water can further be protected from undue pollutants to enhance fishery practices and safeguard health of the community using the water from river.

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INTRODUCTION

Aquaculture has gained much attention as a fast growing sector of global food production as well as source of animal protein in the world today. Microorganisms contribute a significant fraction of importance in the aquatic ecosystem and they have been observed to be among the factors that can cause the emergence of infectious diseases in the practice of aquaculture (Noga, 2000; Ikpi and Offem, 2011). The prevalence of infectious diseases has been observed to depend on the interaction between fish pathogens and the aquatic environment (Noga, 2000). Hence, the need to quantify and monitor microbial population in this sector.

Some major rivers in Nigeria were dammed for different purposes, including electricity and portable

water supply. It is significant to maintain the quality of the water because the contamination of the water with pathogenic microorganisms may cause serious illness or even death among the cultured organisms in the water (Liguori *et al.*, 2010). This study, therefore helps in the determination of microbial population including the coliform counts in the water body and fish population. Similarly, the identification of bacterial and fungal species that populate the river body was intensified. This is complementary to the determination of some physicochemical properties of the water.

MATERIALS AND METHODS

Isolation, characterization and identification of microorganisms from water samples.

The water samples were plated on nutrient agar for bacterial enumeration using a pour plate technique. Bacterial isolates were identified using standard microbiological methods. Similarly, cultural characteristics and characteristics appearance using lactophenol cotton blue stain for microscopic studies were adopted for identification of fungi using the compendium of fungi. Generally, associated fungi were identified by their cultural and morphological features (Kelley *et al.*, 2003).

RESULT

This study shows the distribution of diversified forms of microorganisms in the water body. The total bacteria count of fish ranged from 2-86 cfu/mL. The highest count was found in the intestine of tilapia and the least in the gill and skin of tilapia. The total bacteria count of water ranged from 3-198 cfu/mL (Table 1). The highest count was found in shoreline and the least in no-activity zone. The average total fungi count of fish ranged from 0-32 sfu/ml, the average total fungi count was highest in the intestine of tilapia and the least count was observed in the gill of tilapia. The average total fungi count of water ranged from 62-221 sfu/ml, in which the average total fungi count was highest in open space and the least was observed in no-activity zone (Table 1).

The identified bacterial species during this study includes *Staphylococcus aureus*, *Proteus* spp., *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Klebsiella* spp., *Escherichia coli*, *Serratia mercersen* and *Shigella flexneri* (Table 2). The morphological characteristics of the nine fungal isolated from the fish and water samples were presented in table 3, which illustrate the characteristics of the distinct colonies in terms of growth rate, diameter of colony within 7 days on PDA, pigmentation during growth, hyphae form and conidium form. The fungal isolates identified from sample sources were *Mucor hiemalis*, *Aspergillus fumigatus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium frequentans*, *Saccharomyces* spp., *Penicillium chrisogenium*, *Fusarium verticilliodes* and *Chrysonilia sitophila* (Table 3).

Table 4 shows the distribution and percentage occurrence of bacterial isolates in fish and water samples. While the distribution and percentage occurrence of fungal isolates in fish and water samples were determined as shown in Table 5. In Table 6, some physicochemical parameters determined during the study shows respective values to be pH 6.92, temperature 28°C, conductivity 161 µS/cm, and the turbidity as 4.0. **These conditions are fair for fish optimum growth.** The study thus suggests that the river water can further be protected from undue pollutants to enhance fishery practices and safeguard health. The species of fish recovered

from this study site viz *Tilapia* Fish and Cat fish are shown in Plates 1 and 2.

Table 1: Average total of bacterial and fungal count in water and fish samples in colony forming

Sample type	NA (cfu/ml)		PDA (sfu/ml)		EMB (cfu/ml)		MAC (cfu/ml)	
	10 ⁴	10 ⁶	10 ⁴	10 ⁶	10 ⁴	10 ⁶	10 ⁴	10 ⁶
Tilapia								
Intestine	86	51	32	32	6	4	42	34
Gill	34	26	3	0	3	2	28	24
Skin	43	21	11	8	4	2	48	36
Cat fish								
Intestine	68	60	3	2	32	31	16	9
Gills	14	6	7	4	17	14	11	4
Skin	12	9	12	6	4	4	6	4
Open space	126	41	221	180	4	0	18	7
No activity	107	72	128	62	3	0	0	0
Shore line	198	82	143	98	43	17	38	12

unit/ml (cfu/ml) and spore forming Unit per ml (sfu/ml).

LEGEND:

NA- Nutrient Agar
 PDA- Potato Dextrose Agar
 EMB- Eosin Methylene Blue Agar
 MAC- Mac Conley Agar

Table 2: Frequency of Bacterial Isolates identified.

S/NO	Probable organisms	Occurrence	Percentage (%) occurrence
1	<i>Escherichia coli</i>	1	5.88
2	<i>Bacillus</i> spp.	4	23.53
3	<i>Klebsiella</i> spp.	1	5.88
4	<i>Proteus</i> spp.	2	11.76
6	<i>Salmonella</i> spp.	2	11.76
7	<i>Shigella</i> spp.	1	5.88
8	<i>Staphylococcus</i> spp.	2	11.76
9	<i>Serratia</i> spp.	2	11.76
10	<i>Pseudomonas</i> spp.	2	11.76

TABLE 3: Morphological and Cultural Characteristics of Fungi Isolates from water and fish

S/N	Growth rate	Diameter of colony within 7days on PDA	Pigmentation during growth	Hyphae form	Conidium form	Probable Organisms
1	Fastidious	At 25°C on PDA 8CM 3days	White to yellow tater dark grey black sporangia	Upright phialide	Branched out from the phialide	<i>Mucor Hiemalis</i>
2	Moderate	7cm	Bluish green	Upright phialide	Branched out from the end of phialide	<i>Aspergillus Fumigatus</i>
3	Moderate	7cm	Brownish to black powdery	Upright phialide	Conidiophores arising from long broad thick – walled end of the phialide	<i>Aspergillus Niger</i>
4	Fastidious	8cm in 4days	White to brownish	Upright phialide	Branched out from the phialide	<i>Rhizopus Stolonifer</i>
5	Slow	3cm in 7days	Grey –green reverse intensely yellow	Upright phialide which forms basipetal chains of dry conidia	Penicillateconidiophres branched out as verticillatephialide	<i>Penicillium frequentans</i>
6	Moderate	5cm	Creamy	Pseudohypha formed	Ovid shape produced laterally mycelium	<i>Saccharomyces</i> spp.
7	Slow	3cm in 7days	Grey –green reverse intensely yellow	Upright phialide which forms basipetal chains of dry conidia	Penicillate conidiophres branched out as verticillatephialide	<i>Penicillium chrisogenium</i>
8	Moderate	7cm	White to pink	Short, broadly ending non-proliferating phialides	Conidiophores richly branched but not in spirodochia usually one celled macro-conidia	<i>Fusarium verticilliodes</i>
9	Fast growing	25mm in 24 hours	Colourless becoming pinkish to orange	Hyphae are more or less ascending, smooth-walled, septate with lateral branches	Conidia are one celled, produced in chain and connected by a narrow hyaline strand they are pinkish to orange in mass	<i>Chrysonilia sitophila</i>

TABLE 4: Distribution and percentage occurrence of bacterial isolates in fish and water samples

SAMPLE TYPE	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	<i>Salmonella paratyphi</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>	<i>Serratia mercescens</i>	<i>Pseudomonas Aeruginosa</i>
Tilapia										
Skin	-	-	-	+	-	-	-	+	-	-
Gill	+	+	+	-	-	+	-	+	-	-
Intestine	+	+	-	+	+	-	-	-	-	-
Cat fish										
Skin	-	+	-	-	-	-	-	+	+	-
Gill	-	-	+	-	-	-	-	-	+	+
Intestine	+	-	-	-	+	+	-	-	-	-
No activity	+	-	+	-	-	+	-	+	+	-
Open space	+	+	+	-	-	-	+	-	-	
Shore line	+	+	+	-	-	-	+	+	-	+
Percentage occurrence	17.1	14.2	14.2	5.7	5.7	8.5	5.7	14.2	8.5	5.7

Table 5: Distribution and percentage occurrence of fungal isolates in fish and water samples.

SAMPLE TYPE	<i>Mucor hiemalis</i>	<i>Aspergillus fumigatus</i>	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium frequentans</i>	<i>Saccharomyces</i> spp.	<i>Penicillium chrisogonium</i>	<i>Fusarium verticilliodes</i>	<i>Chrysonilia sitophila</i>
Tilapia									
Muscle	-	+	+	+	-	-	-	+	-
Gill	+	+	+	-	-	-	+	+	-
Intestine	+	+	-	+	+	-	-	-	-
Cat fish									
Muscle	+	+	-	-	-	-	-	+	+
Gill	+	+	+	-	-	-	-	-	+
Intestine	+	-	-	-	+	-	-	-	-
No activity	+	-	+	-	-	+	-	+	-
Open space	+	+	+	-	-	-	+	-	+
Shore line	+	+	+	-	-	-	+	+	-
Percentage occurrence	21.8	18.9	16.2	5.4	5.4	2.7	8.1	13.5	8.1

Table 6: PHYSICOCHEMICAL PARAMETERS OF WATER SAMPLES

pH	Temp (°C)	Conductivity (µS/cm)	Turbidity (FNU)
6.92	28	161	4.0

LEGEND

µS/cm – Micro-siemens per centimeter
FNU – Formazin Nephelometric Unit

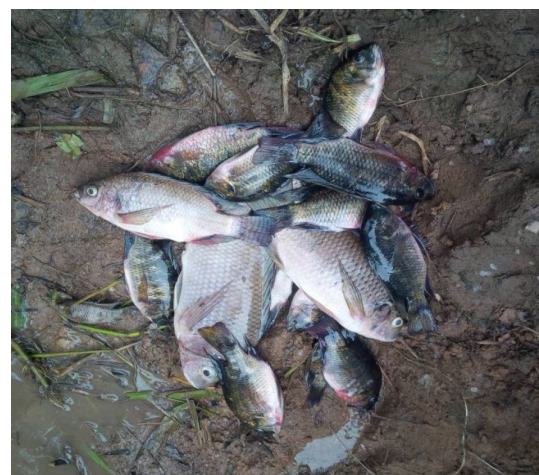


Plate 1: *Tilapia* Fish



Plate 2: Cat fish

DISCUSSION

This study shows the microbiological and some physicochemical qualities of water and fish samples that populate the Ose river. Four replicate samples from Ose-Owo river were tested quantitatively and qualitatively. The microbial analyses of the water collected from the Ose dam sources shows high microbial load. The finding is in agreement with the report of Oni *et al.* (2013) on associated microbial load of artificially cultured *Clarias gariepinus* fingerlings, in which high microbial load with low mortality of the fingerlings was recorded. This may be an indication that *Clarias gariepinus* is adequately suited to withstand high microbial load. Bacteria constitute the most economically significant group of pathogenic agents to fish (Muniruzzaman and Chowdhury, 2004) because of their ubiquity. Bacterial diseases are responsible for heavy mortalities in both culture and wild fishes worldwide (Muniruzzaman and Chowdhury, 2004) and most causative microorganisms are naturally occurring opportunistic pathogens which invade the tissue of a fish host and rendered it susceptible to infection.

The study revealed the presence of *Escherichia coli*, *Klebsiella* spp., *Bacillus* spp., *Salmonella paratyphi*, *Staphylococcus aureus* and *Shigella* spp in the fish water and this may pose a threat to the health of the fishes and consumers. Gram negative bacteria were the most predominant bacteria isolated and this is in agreement with findings reported by Molokwu and Okpokwasili, (2002) and Akinwale *et al.*, (2007) in their study on the diversity of micro-flora of fish pond water. Also aquatic fungi and water mold from fresh water sources have been reported to be responsible for the infections of aquatic species (Huchzermeyer and vander Waal, 2012). The presence of toxic aquatic fungi in water tends to infect aquatic animals, including those that are consumed by humans. *Aspergillus* spp, *Rhizopus* spp was also isolated from Ose River and the fish constituting this aquatic environment. These are harmful to human and are in agreement with findings reported by Gottlich *et al.*, (2002), who recovered similar species of organisms from groundwater-derived drinking water.

Variety of bacterial species populates Ose river sources as shown in this study. This includes some *Cyanobacteria* which are very commonly found microorganism in water. The bluish-green colour in water or ditches is attributed to some of these organisms. For example, *Cyanobacteria*, *Pseudomonas* species, *Escherichia coli*, *Streptococcus* species, *Staphylococcus* species, *Klebsiella* species and so on (FAO, 2014).

It has been observed that infectious disease is one of the most important constraints to efficient and sustainable aquaculture production, impacting on food security, socio-economic development, profitability and trade (Walker, 2004). The

persistence of pathogens in the water environment also is considered as one of the crucial factors for infection transmission. The population today is very high and will continue to grow. Hence, the management of water resources, including transport of water to the areas where it is needed, separation and depuration of wastewater and production of freshwater by desalination, have become increasingly important (Iaccarino, 2019).

Since water is ubiquitous and essential to human life, it is therefore recommended in this study that, our water bodies should be properly monitored by appropriate agencies. Similarly, fish is an important component of human food and animal feeds, it is one of the main food components of humans for many centuries. Fishermen and inhabitants of riverine areas should also be enlightened on sources of pollutants and protection of our water bodies.

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