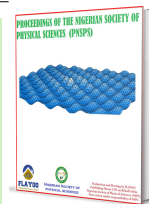


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Assessment of aquaculture wastewater impact on Osun river, Ede, Nigeria

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ABSTRACT

Water samples were collected from four different points around a fish pond in Ede, Osun State, and subjected to physicochemical and metal content analyses to determine the heavy metal levels. The physicochemical tests included pH, temperature, colour, electrical conductivity, chloride, dissolved oxygen, nitrate, phosphate, and turbidity. Biological tests such as fecal coliform, heterotrophic plate count, and adenosine triphosphate tests were also performed. The results showed that the samples had acceptable pH, temperature, and electrical conductivity levels. The colours of the samples ranged from light greenish, greenish, and light brown to no colour. The samples had an average chloride content ranging from 99.4 ± 0.2 mg/L to 149.9 ± 0.2 mg/L, dissolved oxygen from 1.94 ± 0.01 mg/L to 2.31 ± 0.02 mg/L, nitrate content from 2.99 ± 0.01 mg/L to 3.12 ± 0.01 mg/L, and turbidity from 2.44 ± 0.01 mg/L to 2.97 ± 0 mg/L. All of these values were within the regulatory limits. The metal content analyses showed that zinc, lead, mercury, and chromium were within acceptable limits. The bacterial counts ranged from 1.5×10^2 CFU/mL to 4.5×10^2 CFU/mL, leading to four genera of gram-negative and gram-positive bacteria, respectively. *Aspergillus* spp., *Mucor*, and *Penicillium* were the fungal isolates obtained from the samples. Therefore, to prevent harmful bacteria from the fish pond, the microbial load should be reduced by regulating and monitoring the water supplied and discharged from the pond ensuring it is free from harmful bacteria.

Keywords: Aquaculture, wastewater, heavy metals.

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1. INTRODUCTION

Aquaculture is an essential part of global food production and contributes greatly to meeting the growing demand for fishery products. It has played a crucial role in the economic development of many nations, providing valuable employment opportunities and stabilizing the economic situation of vulnerable communities [1]. However, most rural communities rely on streams,

dams, springs or shallow dug wells for their water supply, which often becomes contaminated due to human activities, making it unsafe for consumption [2].

In Nigeria, the government recently announced the implementation of import quotas and a 25% reduction in fish imports each year. This move aims to promote the development of aquaculture and boost domestic fish production, which will contribute to the growth of the economy and support sustainable livelihoods for many communities [3]. However, this increase in aquaculture has led to a corresponding rise in aquaculture waste output,

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which is causing concern among the public. This waste can be classified into two types - solid and dissolved waste. Solid waste is mainly made up of fish feed and faeces, which can create eutrophic conditions characterized by high phytoplankton blooms, high ammonia buildup, high nitrite, low dissolved oxygen and high turbidity. Dissolved waste, on the other hand, comes from the metabolites excreted by the fish through the gills and urine [4]. The accumulation of these wastes can negatively affect the water quality of the system, leading to diseases in fish [5]. To dispose of this waste, it is discarded with effluent water into nearby rivers, streams or lakes, which can cause eutrophication and harm the benthic ecosystem. The discharged organic wastes with high levels of nitrogen (N) and phosphorus (P) can contaminate the waters and sediments, leading to increasing residues of chemicals and drugs used for disease control, loss of biodiversity and other impacts [6, 7].

Aquatic pollution caused by heavy metals is also a serious concern, as they can be introduced into water bodies by leachates of agrochemicals and fertilizers containing heavy metals from farm settlements, as well as during transportation and harvesting of fish [8]. The accumulation of metals in the organs of aquatic fauna and their host environment poses a significant risk to their health. Exposure to heavy metals has been linked to developmental retardation, various cancers, kidney damage, and even death [9, 10]. Therefore, it is essential to conduct a scientific study to evaluate the concentrations of heavy metals in the riverine ecosystem.

This research aims to evaluate the influence of aquaculture wastewater on the Osun River ecosystem. The Osun River is one of the longest rivers in Nigeria, measuring approximately 267 km in length [10]. However, for this study, the catchment area of the Osun River is limited to the Odo-Eja region in Ede, where fish farming is prevalent and the river water is used for domestic, agricultural, and irrigation purposes. The assessment will involve a comprehensive analysis of various physicochemical parameters such as pH, temperature, nitrogen compounds, phosphates, dissolved oxygen, and heavy metal concentrations. Additionally, the study will also include biological assessments by examining the microbial load, fecal coliform counts, and the presence of specific pathogens. By integrating these analyses, we can gain a complete understanding of the ecological health of Osun River and assess the potential risks associated with aquaculture effluent discharge.

2. EXPERIMENTAL METHODS

2.1. SAMPLE SITE

Ede is a town located in Osun State, which is situated in south-western Nigeria. It is located along the Osun River and is also a stop on the railway from Lagos, which is about 180 kilometres southwest of the town. Additionally, Ede is at the crossroads of roads from Osogbo, Ogbomosho, and Ile-Ife. The town is known for its agricultural production, thriving trade and commercial activities, which benefit from its central location and proximity to major cities such as Osogbo, Iwo, Ife, and Ejigbo. The study was carried out at Alhaji Aagberi Fish Farm, Odo-Eja area (7°73'56.40"N, 4°42'24.59"E), Ede North Local Government Area, Osun State, Nigeria, where the major species cultivated is the catfish (*Clarias spp.*).

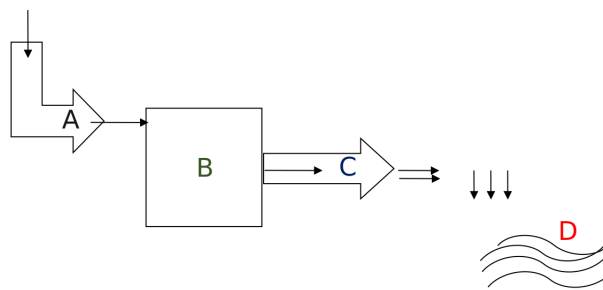


Figure 1. Schematic representation of sample site.

2.2. SAMPLE COLLECTION

The water samples were collected at four different points labelled A (source of fresh water into the pond), B (pond water itself), C (pond water outlet where wastewater is released into the flowing river) and D (receiving river which is 50 m away from the pond) as represented in Figure 1.

The samples were collected in sterile plastic sample bottles and labelled accordingly, subsequently transported to the laboratory immediately for analysis using recommended procedures.

2.3. PHYSICOCHEMICAL ANALYSIS

The pH of the water samples was measured using a Jenway pH meter (model 3310) that was calibrated beforehand using standard buffer solutions of pH 4.0, 7.0 and 10.0. Temperature was measured using a calibrated mercury thermometer (Jenway model 3015) by suspending the thermometer around 10 cm below the water surface at the sampling sites for at least 2 minutes before taking the reading. The turbidity of the water samples was measured using a turbidimeter, while electrical conductivity and total dissolved solids were measured using a HACH conductivity meter (model 4510). The dissolved oxygen content of the samples was measured using a digital dissolved oxygen meter. Nitrate, nitrite and sulphate contents were analyzed using a calibrated V2000 multi-analyte photometer, while the concentrations of chloride, total hardness and alkalinity were determined using standard titrimetric methods [11].

To analyze the presence of heavy metals in the water samples, 5 mL of HNO₃/HCl (3:2) was added to 100 mL of the sample. The mixture was then digested at 130°C on a hot plate, and after cooling, the digested sample was filtered using a 0.45 mm membrane filter. The filtrate was then made up to 100 mL with de-ionized water and stored in clean polyethylene bottles [10]. The heavy metals that were analyzed using the Shimadzu Atomic Absorption Spectrophotometer Model AA-6300 were Zinc (Zn), Lead (Pb), Mercury (Hg), and Chromium (Cr).

2.4. BIOLOGICAL TESTS

The microbiological tests included the determination of the total viable bacterial count, as well as the presence of total coliform, fecal *E. coli*, and *Pseudomonas aeruginosa* using standard methods of examination. For identifying other pathogenic enteric bacteria, water samples were diluted and spread on various types of agar medium, including nutrient agar, Macconkey agar, blood agar, eosin-methylene blue agar (EMB), and thiosulfate citrate bile sucrose agar (TCBS). The plates were then incubated

for a day at 37°C, and the cultures were examined for distinct colonies. These colonies were streaked on nutrient agar slant and incubated for 24 hours at 30°C to be kept as stock cultures. Conventional bacteriological methods and API20E were used for the identification of each isolate [11].

3. RESULTS AND DISCUSSION

3.1. PHYSICOCHEMICAL ANALYSIS

The results of the physicochemical parameters analyzed showing the pH, temperature, electrical conductivity, colour, chloride, dissolved oxygen, nitrate, phosphates and turbidity are as presented in Table 1. The pH of the water samples ranged between 6.5 and 8.0. The pH values are consistent with those measured in other countries ranging from 6.5 to 8.5 for irrigation water and aquatic fauna [12]. The pH of water can be affected by various factors such as photosynthesis, respiration, air temperature, carbon dioxide concentration in the atmosphere, and the accumulation and decomposition of organic matter in the water, which leads to the production of weak carbonic acids that have an impact on pH levels [13]. However, low pH can cause more metals to dissolve in the river leading to an increase or decrease in the toxicity of poisons in water [14].

The temperature readings obtained from the samples ranged between 26.0±1°C-30±1°C, as shown in Table 1. Sample B had the lowest temperature value, while sample A had the highest. These results indicate that the temperature is within the acceptable limits set by regulatory standards [2, 15]. However, it is important to note that extreme temperatures can adversely affect the metabolism, physiology, and overall growth of the fish. Therefore, it is crucial to maintain a suitable temperature range for optimal fish culture and promotion.

Freshwater typically has a low conductivity range because it has little to no salt present. However, the result of the water samples showed a high conductivity range of 2700±0 μS/cm to 3000±100 μS/cm, higher than the WHO optimal requirement limit for aquaculture (100 – 2000 μS/cm). This indicates that the water has been contaminated, likely due to flowing through clay and limestone soils that contain materials which ionize and increase electrical conductivity as well as use of manure for pond fertilization and fish feeds in ponds. This high conductivity range is not ideal for drinking water supplies as it suggests increased salinity and water pollution. Additionally, it can lead to the smothering of the stream bottom, especially if the stream is deep [16].

Water colour is an important quality parameter that affects its acceptability. The colour of water is determined by the abundance of phytoplankton and zooplankton in the aquatic body. For example, green, bluish-green, and brownish-green colours indicate the presence of good plankton populations, which are essential for fish health. However, the samples collected gave different colours ranging from light greenish to greenish, light brown, and no colour, as presented in Table 1. Sample A was observed to have a light greenish colouration, while sample B showed a greenish colouration, suggesting that the pond has an abundance of microscopic algae, which forms the base of the pond's food chain and supports healthy oxygen levels for fish and other aquatic life. Sample C shows a light brown colouration, indicating excess nutrients in the pond, as well as warm temper-

atures that create ideal conditions for the rapid, dense growth of algae, known as algae bloom. Sample D has a clear colour as it has already mixed with the flowing river.

Sample A which is fresh water inlet has a mean chloride content of 106.5±0.5 mg/L, with sample B being the water obtained from the pond itself giving 149.1±0.2 mg/L. Samples C and D gave 99.4±0.1 mg/L and 106.5±0 mg/L, respectively. The samples were found to be within the acceptable limits of 250 mg/L value of chloride according to WHO. Chloride values above this standard will cause many environmental impacts that will threaten the biological integrity of aquatic ecosystems [17, 18].

The study showed that the dissolved oxygen (DO) values obtained ranged from 1.94±0.01 mg/L to 2.31±0.02 mg/L. Sample A had 1.94±0.01 mg/L, sample B had 2.31±0.02 mg/L, sample C had 1.98±0.01 mg/L, and sample D had 1.95±0.02 mg/L. These values are below the permitted limits of water, which is between 4-6 mg/L [15]. Sample B had the highest value among all samples, which could be attributed to the presence of algae on the surface of the water. However, the results showed a healthy population of phytoplankton or microalgae in the pond.

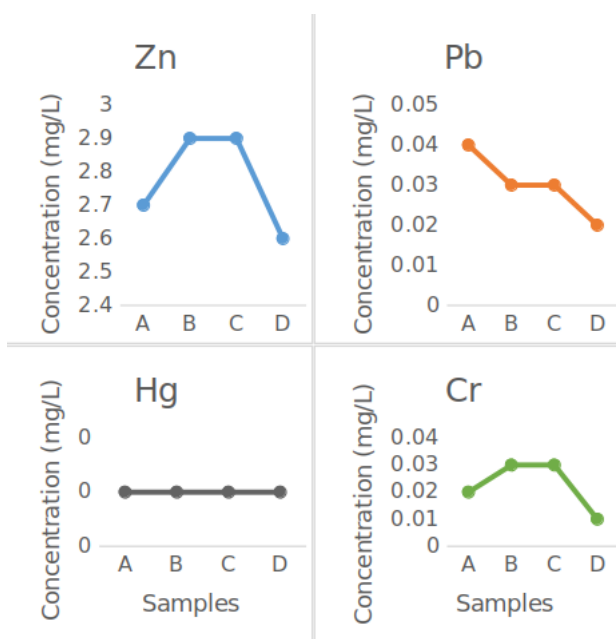
Samples A, B, C, and D were analyzed for their nitrate content. Sample A had a mean nitrate value of 2.99±0.01 mg/L, while samples B, C, and D had 3.12±0.01 mg/L, 3.04±0.01 mg/L, and 3.02±0.02 mg/L, respectively. All of these values were found to be within the permissible nitrate content limit set by the World Health Organization (50 mg/L) and were also within the optimal range for aquaculture, which is 0.1 to 4.5 mg/L. This means that the water samples contained low levels of oxidized organic matter and were relatively non-toxic to fish and other aquatic animals, except at exceedingly high levels above the permissible limit. If nitrate levels exceed the permissible limit, they can result in eutrophication, which leads to the loss of diversity in the aquatic biota and overall ecosystem degradation through algal blooms, excessive plant growth, oxygen depletion, and reduced sunlight penetration [19]. Excess levels of nitrate above 10 mg/L can cause methemoglobinemia, a condition that reduces the blood's ability to carry sufficient oxygen, which can lead to blue baby syndrome. Although nitrate levels that affect infants do not pose a direct threat to older children and adults, they do indicate the possible presence of other more serious residential or agricultural contaminants such as bacteria or pesticides [20, 21].

The mean phosphate level of sample A was found to be 1.21±0.01 mg/L. Samples B, C, and D had mean phosphate levels of 1.49±0.01 mg/L, 1.23±0.01 mg/L, and 1.21±0.01 mg/L, respectively. These values are within the permissible limit of 5.00 mg/L and are not harmful to fish [15]. High levels of phosphate can be dangerous for fish as they create an imbalance and produce harmful toxins that can destroy them. Phosphate concentrations in fish ponds are influenced by the use of fertilizers and phosphorous-rich fish feeds. At low concentrations, phosphate is not harmful, but it can become harmful at higher levels. Higher doses of phosphate can also interfere with digestion in both humans and animals [22].

The mean turbidity value of sample A is 2.44±0.01 NTU. On the other hand, samples B, C, and D have turbidity values of 2.97±0 NTU, 2.47±0.02 NTU, and 2.44±0.02 NTU, respectively. The higher turbidity value of sample B could be due to the presence of suspended materials in the water. The turbidity value

Table 1. Physicochemical parameters analysis of water samples.

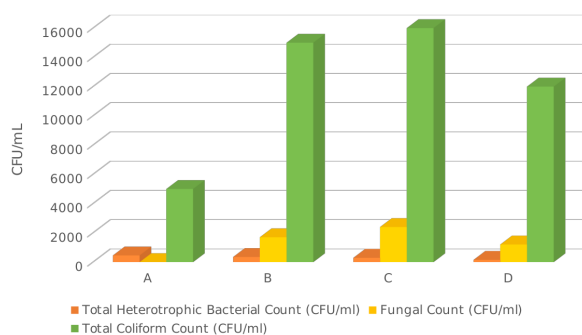
Parameters	Samples			
	A	B	C	D
pH	6.5±0.1	8.0±0.2	7.2±0.1	7.0±0.2
Temperature (°C)	30±1	26±1	28±0	27±1
Conductivity (µs/cm)	2700±0	2900±100	3000±100	2900±0
Colour	Light greenish	Greenish	Light brown	Clear
Chloride (mg/L)	106.5±0.5	149.1±0.2	99.4±0.1	106.5±0
Dissolved oxygen (mg/L)	1.94±0.01	2.31±0.02	1.98±0.01	1.95±0.02
Nitrate (mg/L)	2.99±0.01	3.12±0.01	3.04±0.01	3.02±0.02
Phosphate (mg/L)	1.21±0.01	1.49±0.01	1.23±0	1.21±0.01
Turbidity (NTU)	2.44±0.01	2.97±0	2.47±0.02	2.44±0.02

**Figure 2. Concentrations of heavy metals in the water samples.**

of sample D, on the other hand, could be a result of the natural filtration process that occurs as the water flows through the soil in the river. These turbidity values are acceptable and within the limit of not more than 5.00 NTU. High turbidity levels may lead to an increased risk of gastrointestinal diseases such as diarrhoea, vomiting, and abdominal cramps due to contaminants such as viruses or bacteria that attach to the suspended solid particles. In addition, highly turbid water has an altered taste and odour and its visual properties are negatively impacted. When necessary, treating highly turbid water significantly increases water treatment costs due to the amount of flocculants needed to clarify the water [23].

The presence of heavy metals in water can lead to kidney complications in humans and cause ionic imbalance in pond water. Therefore, tests were conducted on water samples to identify the presence of heavy metals such as Zn, Pb, Hg, and Cr, with the results shown in Figure 2.

Sample A had 2.7 mg/L of Zn, while samples B, C, and D had 2.97, 2.47, and 2.6 mg/L, respectively. However, zinc is considered an essential element for the human body, with the recom-

**Figure 3. Bacteria, fungi and coliform count.**

mended level between 5-15 mg/L by WHO [24]. The amount of lead in the samples ranged from 0.02-0.04, with sample A having the highest amount and sample D having the least. These values fall within the acceptable limit for drinking water set by WHO, which is 0.05mg/L. The chromium (Cr) concentrations in samples A, B, C, and D were 0.02 mg/L, 0.03 mg/L, 0.03 mg/L, and 0.01 mg/L, respectively. These values were below the recommended values of 0.05 mg/L for pond water and agricultural water due to minimal agrochemical usage around the study area [25]. All samples had the same amount of mercury (Hg), which was 0.002 mg/L when tested.

3.2. BIOLOGICAL TESTS

Figure 3 shows that the bacterial count ranged between 1.5×10^2 CFU/mL to 4.5×10^2 CFU/mL. These coliform counts exceed the recommended limit of 1000 CFU/100 mL of total coliforms for aquaculture. While microbes can provide a source of food for fish, some nutrients can also be obtained from sediment. Therefore, a high microbial load can be harmful to fish health.

The water at the inlet of the pond did not show any signs of fungal growth, but there were some levels of fungal growth in the middle of the pond. This may be due to the pH level and the temperature in the pond. The bacterial and fungal growth at 50 meters away from the pond is less than at the outlet, which could be explained by the water movement over sand and soil that may have trapped some of these organisms along the way. Also, the environment is different from the pond, which may not support the survival of some of these organisms. The coliform counts per unit sample sources indicate some levels of contamination, which could be alarming as high levels of contamination

Table 2. Biochemical identification of microbes.

Isolates	Cell shape	Gram reaction	Starch hydrolysis	Indole	Catalase	Coagulase	Glucose	Sucrose	Lactose	Fructose	Mannitol	Hydrogen sulphide	Motility	Ornithine	Methyl red	Voges proskauer	Oxidase	Identification
A1	Rod	+	+	-	+	-	-	-	ND	-	-	+	+	+	-	+	+	<i>Bacillus</i> spp.
A2	Short rod in cluster	+	+	-	-	-	A	A	ND	A	AG	+	-	+	-	-	-	<i>Lactobacillus</i> spp.
A3	Short rod	-	+	+	+	+	-	+	NLF	-	-	-	+	+	+	-	-	<i>Escherichia coli</i>
A4	Short rod in cluster	-	+	-	+	-	AG	-G	ND	AG	AG	-	+	-	-	+	+	<i>Pseudomonas</i> spp.
A5	Short rod	-	-	-	+	-	AG	AG	-G	AG	AG	-	-	+	-	-	+	<i>Flavobacterium</i> spp.
A6	Cocci	+	+	-	+	-	AG	-G	ND	AG	AG	+	-	+	-	+	-	<i>Staphylococcus</i> spp.
A7	Rod in chain	-	+	-	+	-	AG	AG	ND	AG	AG	-	-	-	+	+	-	<i>Klebsiella</i> spp.
A8	Cocci in chain	+	+	-	-	-	-	-	ND	A	-	+	-	+	+	-	-	<i>Streptococcus</i> spp.

Key: + (positive), - (negative), ND (not determined), NLF (non-lactose fermenters)

with the presence of these indicator organisms could be associated with neglecting good fishpond management practices. It could also be due to an increase in the rate of microbial infiltration as a result of fecal contamination either of animal or human origin. Therefore, it is crucial to maintain good water quality for fish pond management practices to enhance the quality of fish yields, reduce the outbreak of diseases, decrease the mortality rate of fish, and also prevent infections in humans [26].

Table 2 presents the results of the biochemical analysis of the microbes found in the water samples. The study identified four genera of gram-negative bacteria: *Escherichia coli*, *Pseudomonas* spp., *Klebsiella* spp. and *Flavobacterium* spp. Similarly, four genera of gram-positive bacteria were found during the study: *Bacillus* spp., *Lactobacillus* spp., *Staphylococcus aureus* and *Streptococcus* spp. Conducting a bacteriological examination of fish pond water is crucial to detect the presence of microorganisms that may pose a health risk to fish and cause fatalities. It is equally important to examine the water at the inlet to the fish pond to determine the type of bacteria being transferred. The presence of certain bacteria species, such as *Pseudomonas* and *Streptococci*, indicates contamination that could negatively affect the growth and survival of the aquaculture system. Therefore, it is necessary to reduce the microbial load and ensure that the water supplied to the pond is free from harmful bacteria to prevent them from entering and harming the fish in the pond.

4. CONCLUSION

The impact of aquaculture wastewater on the Osun river from Al-haji Aagberi Fish Farm, Ede, Nigeria was conducted in this study. The physicochemical properties, metal contents, and biological tests were performed. The results showed that the water samples had acceptable pH, temperature, and electrical conductivity. The average chloride, dissolved oxygen, nitrate, turbidity and heavy metals were within regulatory limits. The bacterial count and microbial load were slightly above acceptable limits. To prevent harmful bacteria, the microbial load of the ponds could be reduced by ensuring the water supplied and discharged is free from harmful bacteria.

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