

Physicochemical and Microbial Quality of the Asa River, Ilorin, Southwestern Nigeria

Ogidi, T. J.^{1*}, Sanni, B. M.², Bewaji, S.³, Olabode, J. A.⁴

¹*Department of Science Laboratory Technology, Newland Polytechnics, Ilorin, Nigeria*

²*Department of Microbiology, Kwara State University, Malete, Nigeria*

³*Department of Geological Sciences, Achievers University, Owo, Nigeria*

⁴*School of Nursing Sciences, Achievers University, Akure, Nigeria*

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This study assessed the physicochemical and microbiological quality of water samples from a segment of the Asa River in Ilorin, Nigeria, over an eight-week period. Water samples were analysed using the multiple tube fermentation technique and spread plate method, with microbial identification performed via standard microbiological procedures. Physicochemical parameters included pH (7.47–8.95), temperature (22.9–28.2°C), biological oxygen demand (BOD) (6.40–9.10 mg/L), total suspended solids, total dissolved solids, dissolved oxygen, and total solids. Microbial analysis revealed total heterotrophic counts ranging from $2.70\text{--}7.25 \times 10^4$ cfu/mL, total coliform counts from $1.45\text{--}6.05 \times 10^4$ cfu/mL, and faecal coliform counts from $1.45\text{--}3.40 \times 10^4$ cfu/mL. Identified bacterial species included *Streptococcus* sp., *Escherichia coli*, *Shigella* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Vibrio cholerae*. Antibiotic susceptibility tests against eight common antibiotics showed multiple resistance, particularly in *Klebsiella pneumoniae*. Fungal isolates included *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus fumigatus*, and *Penicillium* sp. The presence of antibiotic-resistant microorganisms indicates significant contamination of the Asa River, posing a public health risk if used for domestic purposes.

Keywords: Asa River, Microbiological quality, Total coliform count, Faecal coliform count, Isolates

Introduction

Water is the most abundant and vital substance provided by nature (Foppen, 2018). Despite its abundance, access to potable water remains a critical challenge, with over one billion people lacking safe drinking water (Onuorah and Odibo, 2019; WHO, 2022). Ensuring access to safe and sufficient water for families in Ilorin, Kwara State, Nigeria, could significantly reduce mortality rates. Safe and reliable water is essential for agricultural, domestic, and industrial activities in the region (Igbinsola and Aisien, 2020; Oyediran et al., 2018). The lack of potable water, coupled with inadequate sanitation and poor hygiene, contributes to 9.1% of global infections and 6.3% of all deaths worldwide (WHO, 2022; Igbinsola, 2020). Microbial pollution affects various water sources, including surface water and piped water supplies (Alotaibi, 2009; Bekuretsion et al., 2018). Annually, 37.7 million people are affected by waterborne diseases, with 1.5 million children succumbing to diarrhea (WHO, 2022). Factors such as urbanisation, economic challenges, and climatic changes exacerbate the inaccessibility of safe drinking water (Hussain et al., 2013; Protor and Hammes, 2015).

Microbial pathogens from human and animal wastes contaminate various water bodies (Habtu and Zewdu, 2019; Hassan et

al., 2018). These pathogens include bacteria, viruses, and parasites (Caggiano et al., 2020). Common waterborne infections include diarrhea caused by *Escherichia coli*, salmonellosis caused by *Salmonella*, and shigellosis caused by *Shigella* (Luyt et al., 2012).

The Asa River, located in the heart of Ilorin Metropolis, is a critical resource of economic, agricultural, and environmental significance. However, it is heavily impacted by pollutants such as lead, cadmium, and chromium, alongside human activities that degrade its quality (Oyediran et al., 2018). Industrial effluents, agricultural waste, and runoff containing pollutants are discharged directly into the river, making them primary sources of pollution (Adekunle and Eniola, 2008). Additionally, high levels of eutrophication, driven by organic matter from pig faeces and soap residues from laundry activities, further compromise the river's quality (Eletta et al., 2005). This study aims to characterise the microbial contaminants and evaluate the water quality of the Asa River.

Materials and Methods

Study Site

Ilorin, the capital city of Kwara State in North Central Nigeria, has a population of approximately 1,099,959. The Asa

River, a significant water body in Ilorin, is located at coordinates 8.4875°N and 4.5588°E. The river supports fishing and irrigation for vegetable farming. Approximately 4.5% of the population relies on piped water, while the majority depend on wells, boreholes, and streams. Water samples were collected from the Station Segment of the Asa River, specifically at points with heavy commercial activities, which contribute significantly to the river's pollution.

Sample Collection

Water samples for microbiological analysis were collected in sterilised glass bottles autoclaved prior to use. Each bottle was filled, leaving a 20 mm headspace to allow for effective shaking. Samples were transported in an icebox and analysed within 4 hours of collection. Sampling occurred twice weekly over an eight-week period.

Microbiological Analysis of Water Samples

Most Probable Number Technique

The presence of total and faecal coliforms was assessed using the multiple-tube fermentation technique, comprising presumptive, confirmed, and completed tests. For the presumptive test, 0.1 ml of each water sample was inoculated into five replicate test tubes, each containing 10 ml of sterile single-strength MacConkey broth and an inverted Durham tube. The tubes were incubated at 37°C for 24 hours. Gas production in the Durham tubes and a colour change to yellow indicated the presumptive presence of coliform bacteria. One ml from presumptive positive tubes was plated onto Eosin Methylene Blue Agar and incubated at 37°C for 24 hours. Dark blue colonies with a green metallic sheen suggested the presence of *Escherichia coli*. These colonies underwent biochemical tests, including oxidase, catalase, urease, motility, indole, and sugar fermentation tests (Hemraj et al., 2013).

Microbial Isolation

Bacteria were isolated using the spread plate method. Water samples underwent serial dilution, and 0.1 ml of 10⁻³ and 10⁻⁵ dilutions were uniformly spread onto nutrient agar using an L-rod. Plates were incubated at 37°C for 24 hours. Distinct isolates were subcultured and subjected to biochemical tests, including oxidase, catalase, urease, motility, indole, coagulase, methyl red, Voges-Proskauer, and sugar fermentation tests (Hemraj et al., 2013).

Isolation of *Salmonella*, *Shigella* sp., and *Vibrio cholerae*

For *Salmonella* and *Shigella*, 20 ml of water sample was enriched with 10 ml of sterile F broth and incubated at 37°C for 24 hours to enhance the growth of these low-concentration organisms. The enriched culture was streaked onto Salmonella-Shigella agar and incubated at 37°C for 24 hours. *Salmonella* colonies appeared colourless with a black center, while *Shigella* colonies were colourless without a black center.

For *Vibrio cholerae*, 20 ml of water sample was enriched with 10 ml of peptone water and incubated at 37°C for 24 hours. The enriched culture was streaked onto thiosulphate-citrate-bile-salt

agar and incubated at 37°C for 24 hours. *Vibrio cholerae* colonies were 2–3 mm in diameter and yellow. Salmonella-Shigella agar and thiosulphate-citrate-bile-salt agar were used for selective growth of the target organisms.

Isolation and Characterisation of Fungi

Fungi were isolated using the spread plate method following serial dilution of the water samples. Aliquots of 0.1 ml from 10⁻³ and 10⁻⁵ dilutions were inoculated onto sterile potato dextrose agar supplemented with 1% streptomycin. Plates were incubated at 25°C for 48–72 hours. Pure cultures were obtained through subculturing and stored in agar slants. Fungal isolates were characterised using macroscopic, microscopic, and biochemical tests, with morphology determined according to Omomowo et al. (2015).

Results

The physicochemical properties of water samples from the Station Segment of the Asa River are presented in Table 1. Parameters include temperature, pH, total dissolved solids (TDS), dissolved oxygen (DO), biological oxygen demand (BOD), total solids (TS), and total suspended solids (TSS). The pH ranged from 7.47 to 8.95, temperature from 22.9 to 28.2°C, TDS from 630.0 to 793.5 mg/L, DO from 20.10 to 22.20 mg/L, BOD from 6.40 to 9.10 mg/L, TS from 486.2 to 816.1 mg/L, and TSS from 14.10 to 32.10 mg/L. Peak values were observed as follows: temperature in week 1 (26.95°C), pH in weeks 1 and 6 (8.95), TDS in weeks 1 and 6 (793.5 mg/L), DO in weeks 1 and 7 (22.20 mg/L), BOD in week 3 (9.10 mg/L), TS in week 7 (816.1 mg/L), and TSS in weeks 3 and 6 (32.10 mg/L).

Microbial loads, including total heterotrophic count (THC), total coliform count (TCC), total faecal count (TFC), and most probable number (MPN) values, are shown in Table 2. The THC was highest in week 3 (7.25×10^4 cfu/mL) and lowest in week 5 (2.70×10^4 cfu/mL). The TCC peaked in week 3 (6.05×10^4 cfu/mL) and was lowest in weeks 5 and 7 (1.85×10^4 cfu/mL). The TFC was highest in week 3 (3.40×10^4 cfu/mL) and lowest in week 5 (1.45×10^4 cfu/mL). MPN values, expressed per 100 mL, were highest in weeks 3 and 7 (0.94×10^4) and lowest in week 5 (0.30×10^4).

Bacterial isolates showed varying frequencies of occurrence (Figure 1). *Escherichia coli* was the most prevalent at 36.59%, followed by *Vibrio cholerae* (14.15%). *Salmonella* sp., *Streptococcus* sp., and *Staphylococcus aureus* each had a frequency of 11.71%, *Klebsiella pneumoniae* at 9.27%, and *Shigella* sp. was the least frequent at 4.87%.

Fungal isolates included *Rhizopus stolonifer* with the highest frequency of occurrence at 33.33%, followed by *Aspergillus fumigatus* at 26.67%. *Penicillium* sp. and *Aspergillus niger* each had the lowest frequency at 20% (Figure 2).

Antibiotic sensitivity tests (Figure 3) revealed that *Klebsiella pneumoniae* and *Staphylococcus aureus* exhibited multiple resistance, each resistant to at least three antibiotics (Ofloxacin, Nitrofurantoin, Gentamicin, Augmentin, Amoxicillin, Tetracycline, Ciprofloxacin, and Chloramphenicol). Ofloxacin was the

Table 1 Physicochemical parameters of water samples from Station Segment of Asa River

Week	T (°C)	pH	TDS (mg/L)	DO (mg/L)	BOD (mg/L)	TS (mg/L)	TSS (mg/L)
1	26.95	8.95	630.0	22.20	8.05	648.7	18.80
2	24.50	7.77	681.5	21.40	6.40	696.3	15.40
3	24.50	8.26	780.0	20.70	9.10	814.1	32.10
4	28.20	8.24	768.5	21.30	7.60	798.6	31.10
5	22.90	8.09	698.5	20.20	8.10	777.2	26.30
6	26.40	8.95	793.5	21.30	8.65	731.0	32.10
7	26.10	7.47	793.5	22.20	8.95	816.1	23.40
8	26.00	8.81	674.5	20.10	7.60	486.2	14.10

Keys: T (°C) – Temperature, TDS – Total Dissolved Solids, DO – Dissolved Oxygen, BOD – Biological Oxygen Demand, TS – Total Solids, TSS – Total Suspended Solids.

Table 2 Microbial load of water samples from Station Segment of Asa River

Week	THC (cfu/mL)	TCC (cfu/mL)	TFC (cfu/mL)	MPN/100mL
1	2.55×10^4	2.45×10^4	1.85×10^4	0.36×10^4
2	4.05×10^4	2.55×10^4	2.25×10^4	0.72×10^4
3	7.25×10^4	6.05×10^4	3.40×10^4	0.94×10^4
4	4.35×10^4	2.30×10^4	1.65×10^4	0.61×10^4
5	2.70×10^4	1.85×10^4	1.45×10^4	0.30×10^4
6	3.60×10^4	2.45×10^4	2.35×10^4	0.74×10^4
7	3.50×10^4	1.85×10^4	1.80×10^4	0.94×10^4
8	3.50×10^4	2.50×10^4	2.40×10^4	0.72×10^4

Keys: THC – Total Heterotrophic Count, TCC – Total Coliform Count, TFC – Total Faecal Count, MPN – Most Probable Number.

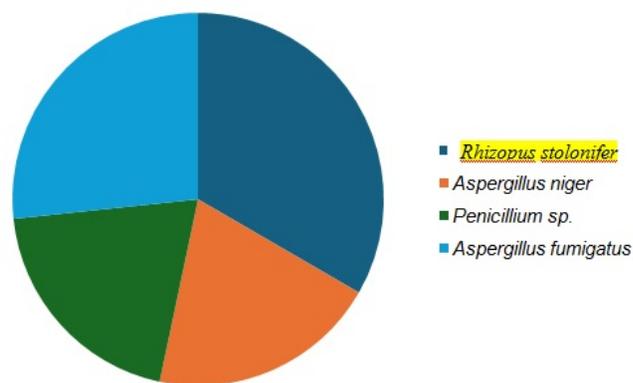


Figure 2 Fungal frequency of distribution from water samples obtained from Station Segment of Asa River

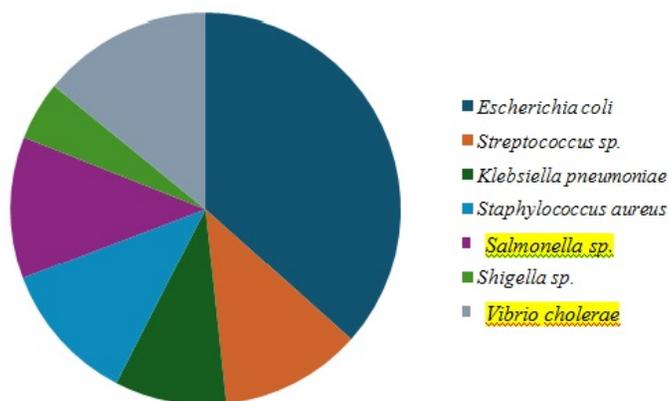


Figure 1 Bacterial frequency of distribution from water samples obtained from Station Segment of Asa River

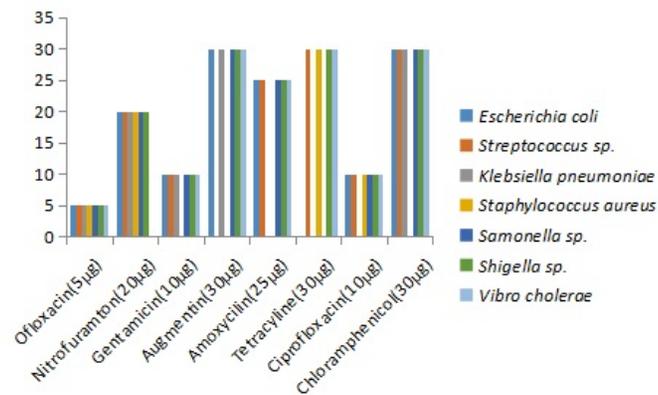


Figure 3 Antibiotic sensitivity of bacterial isolates obtained from Asa River water sample

most effective antibiotic, while Amoxicillin was the least effective. Variations in BOD and microbial counts across weeks were notable but not statistically analysed.

Discussion

The World Health Organization (2022) standards for potable water specify a pH of 6.8–8.5, dissolved oxygen of 6–8 mg/L, biological oxygen demand (BOD) of 6 mg/L, total suspended

solids (TSS) of 20 mg/L, and total dissolved solids (TDS) of 500 mg/L (WHO, 2022). The Asa River water samples exhibited values exceeding these standards. TDS (630.0–793.5 mg/L) and BOD (6.40–9.10 mg/L) consistently surpassed 500 mg/L and 6 mg/L, respectively. Total solids (486.2–816.1 mg/L) and TSS (14.10–32.10 mg/L) also indicated contamination. These findings align with Okonko et al. (2008) and Shittu et al. (2008), who reported elevated physicochemical parameters in contaminated water, exceeding WHO (2022) standards. The physicochemical parameters of the Asa River suggest significant contaminant presence.

The microbial load and diversity in the Station Segment of the Asa River were substantial. High microbial counts, comparable to those reported by Ogidi and Oyetayo (2013) (up to 10^5 cfu/mL), reflect river characteristics and pollution sources (Igbinosa and Aisien, 2020). Isolated species, including *Escherichia coli*, *Vibrio cholerae*, *Salmonella* sp., *Shigella* sp., *Klebsiella pneumoniae*, *Streptococcus* sp., and *Staphylococcus aureus*, are consistent with findings by Amoo et al. (2018) and Akinpele et al. (2014). These organisms indicate microbial contamination, posing public health risks if the water is used domestically (Caggiano et al., 2020; Akinbile, 2004).

Total coliform counts ($1.85\text{--}6.05 \times 10^4$ cfu/mL) in all samples exceeded the WHO (2022) maximum permissible limit of 0/100 mL for drinking water. Faecal coliform counts ($1.45\text{--}3.40 \times 10^4$ cfu/mL) further confirm contamination. While total coliform presence does not always indicate faecal contamination or pathogens (Adekunle and Eniola, 2008), these results align with Anthony and Renuga (2012), who detected coliforms in drinking water samples.

From eight samples, 41 isolates representing eight genera were identified, consistent with Anthony and Renuga (2012), who isolated similar species. This microbial diversity likely results from multiple environmental contamination sources, such as refuse dumping and industrial effluents (Hussain et al., 2013; Grifith et al., 2003; Arshad and Shakoore, 2017). *E. coli* was the most prevalent (36.59%), followed by *Vibrio cholerae* (14.15%), corroborating Asfaw et al. (2016). These bacteria thrive in water bodies impacted by refuse and industrial discharges (Abera et al., 2011; Amira and Yassir, 2011). *Klebsiella* sp., a slow-growing environmental bacterium, was also detected, posing risks to vulnerable populations (e.g., elderly, children, immunocompromised) if consumed (Chissaque et al., 2018; Antony and Renuga, 2012).

Fungal isolates included *Rhizopus stolonifer* (33.33%), *Aspergillus fumigatus* (26.67%), *Penicillium* sp. (20%), and *Aspergillus niger* (20%), consistent with Monika et al. (2017), who isolated similar fungi from drinking water. Fungal cell wall components and loads may cause allergies or opportunistic infections, particularly in immunocompromised individuals. *Aspergillus* sp. can lead to aspergillosis, and fungi are increasingly recognised as causative agents of respiratory, mucosal, and systemic infections (Moat et al., 2016).

Antibiotic susceptibility tests showed *Klebsiella pneumoniae* and *Staphylococcus aureus* exhibited multiple resistance to at least three antibiotics (Ofloxacin, Nitrofurantoin, Gentamicin, Augmentin, Amoxicillin, Tetracycline, Ciprofloxacin, Chloramphenicol). Ofloxacin was the most effective, while Amoxicillin and Ciprofloxacin were the least effective, aligning with Onifade and

Ilori (2008). Multiple antibiotic resistance, also reported by Akinpele et al. (2014) in the Obere River, underscores public health risks from consuming water containing resistant organisms.

Conclusion

It can be concluded from the study of the Station segment of the Asa River that the water is heavily contaminated with faecal-originated microorganisms. The Station segment of the Asa River is unsatisfactory for drinking. Also, the presence of antibiotic-resistant microorganisms suggests that the Asa River is significantly contaminated and poses a public health risk, particularly if the water is used for domestic purposes.

Limitations of the Study

Variations were not assessed, potentially affecting microbial load.

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