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Physicochemical characteristic, molecular characterization of bacteria and fungi isolates found in the Nile Stream, Abuja Nigeria.

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Abstract

Increasing human population coupled with urbanization, industrialization and agricultural activities have led to significant pressure on freshwater resources globally. This study was carried out to characterize the bacteria and fungi from water samples collected from Nile stream, Abuja Nigeria. The water samples were collected from two sampling stations and were analysed using standard methods and procedures. The results of the physicochemical parameters showed temperature, resistance, turbidity, dissolved oxygen, biological oxygen demand, salinity and nitrate record highest in station A, while pH and sulphate record highest in sampling station B. There was no significant difference ($p>0.05$) between all the measured physicochemical parameters except resistance and nitrate which showed significant difference ($p<0.05$) between the sampling stations. Lowest mean colony count was recorded (1.54×10^{-3} CFU/mL) in station B and highest mean colony was recorded (1.60×10^{-3} CFU/mL) in sampling station A. A total of four bacteria (Enterobacter hormaechei, Enterobacter sp.,

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Salmonella sp. and Bacillus safensis) and three fungi (Aspergillus flavus, Aspergillus niger and Penicillium citrinum) were identified from the sampled stations of Nile Stream. Most of the observed physicochemical parameters in this study shows an impaired ecological system due to anthropogenic factors and, the presence of these microorganisms in the stream water can pose a health risk to humans.

Keywords: Bacteria: Fungi: Molecular characterization; Nile stream

INTRODUCTION

Water is an essential natural resource on earth since all living species rely on it for development and the preservation of all aspects of life. Freshwater is a scarce resource required for agriculture, industry, and even human survival. There are three basic metrics used to assess water pollution: physical, chemical, and biological, all of which are critical for biotic activities (Adamu *et al.,* 2022). Analyzing these factors provides insights into the interactions between the biotic and abiotic components of the aquatic ecosystem (Arimoro and Keke 2016). Pollution from industrial discharge, agricultural runoff, and urban expansion contaminates water bodies with chemicals, nutrients, and heavy metals, endangering aquatic life and compromising water quality. The construction of dams, channelization, and land reclamation disturb freshwater ecosystems, leading to habitat degradation and biodiversity loss (Blettler and Mitchell, 2021).

Biological parameters, such as bacteria, can predict the presence or absence of pathogens but not the extent of contamination. The presence of some bacterial species indicates pollution caused by human activities. Bacteria and fungi play an important role in the conversion of biological and non-biological materials by participating in several biogeochemical cycles. Microbial diversity and functionalities found in freshwater bodies are critical for the sustainable management of freshwater resources (Mohammed *et al.,* 2023a). The assessment of surface and groundwater quality remains a major public interest in the developed world. There is a high need for monitoring water quality (Mohammed *et al.,* 2021), hence determining the presence of pathogenic bacteria in water is a crucial problem for human and animal health protection. Microorganisms, including bacteria and fungi, are fundamental components of freshwater ecosystems, contributing to nutrient cycling, organic matter decomposition, and overall ecosystem functioning. These microscopic organisms play pivotal roles in shaping the ecological dynamics of aquatic environments. Despite their importance, microbial communities in freshwater systems, such as the Nile stream in Abuja, have received relatively limited attention, particularly in terms of comprehensive characterization at the molecular level (Grossart *et al.,* 2019).

Increasing human population, coupled with increase in urbanization and industrialization has led to a significant pressure on freshwater resources which is of a major concern. For the past few year Nile stream has been subjected to various anthropogenic activities such as channelling of drainage which serve as source of pollution to the water, in view of this understanding the microbial composition and diversity within the Nile stream is essential for elucidating its ecological dynamics, assessing potential risks to human and ecosystem health, and informing strategies for conservation and sustainable management. By characterizing the bacteria and fungi inhabiting the Nile stream, we can gain insights into the intricate relationships between microbial communities, environmental factors, and ecosystem processes.

Materials and Methods

Study Area

Nile Stream is part of the Wupa River which runs in front of Nile University of Nigeria, Abuja. The Wupa River is part of the Jabi River watershed in Abuja. The study point A is located between latitude 9°1'8"N and longitude 7°24'17"E, study point B is located between latitude 9°0'46"N and longitude 7°23'53"E (Figure 1). The collection points are one kilometre (1 km) apart and ten metres (10 m) in width. Point A is surrounded by rocks by the bank and in the water whilst Point B has a sandy bank with plants. Point A is associated with intense to moderate human activity by bikers, nomad cattle rearers and surrounding communities. Point B is associated with farm activities by Nile University of Nigeria.

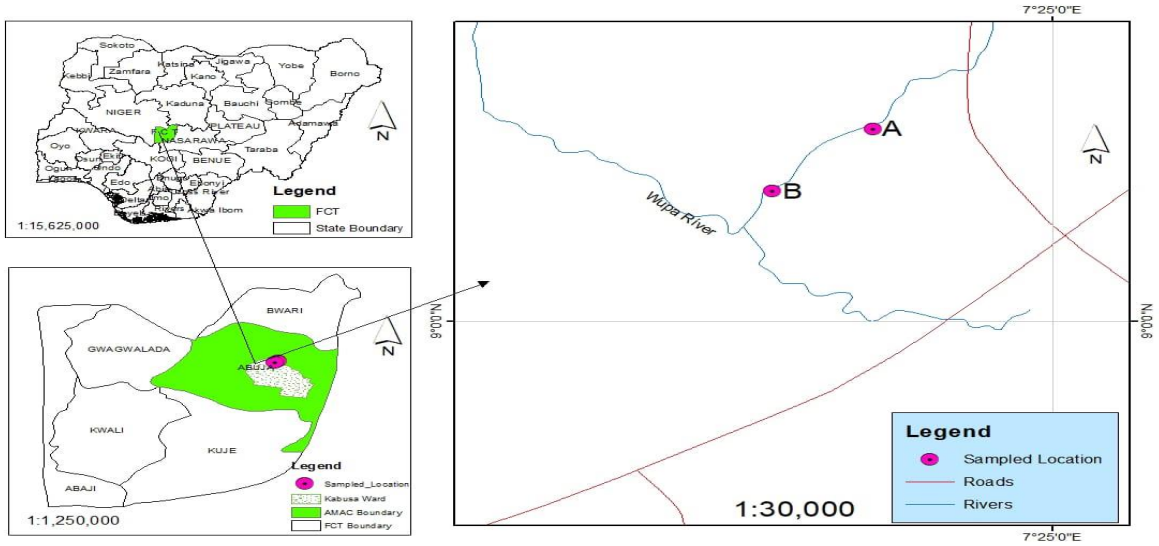

Figure 1: Map of the study area, showing Nile stream with the Sampling stations

Collection of samples

Water samples were collected in duplicates with one litre (1L) bottles which were rinsed with the sample water twice before collection at 10cm below the surface of the water and the bottles were labelled with the location, date and time. The samples were transferred to the school laboratory for physiochemical analysis, bacterial and fungal isolation and identification.

Determination of physicochemical parameters

All the physicochemical parameters were analysed using the standard methods and procedures of the American Public health association (APHA 2017). Water temperature, pH, turbidity, electrical conductivity, resistance, salinity, dissolved oxygen and total dissolved solid were determined *in-situ* using multifunction meter (Hanna HI991300/1) while biochemical oxygen demand, nitrate and phosphate were determined using titration method as described by APHA (2017).

Microbiological Analysis of river water samples

Serial dilution of the samples was carried out using the procedure described by (APHA, 2017) for the pour plate method was employed and 1ml of the river sample was aliquoted into a test tube containing 9ml of distilled water as a stock. Thereafter, 9ml of distilled water was measured into 6 other test tubes arranged serially and labelled and 1ml of the stock was then aliquoted into the next test tube and 1ml of that mixture was aliquoted into the next test tube;

this process was continued until the 6th test tube. 10^{-4} and 10^{-5} were chosen as the dilution factor. In each of the dilution 1ml was pipetted into peri dishes in duplicates.

For bacterial growth; 28.0g of nutrient agar was dissolved in 1000ml of distilled water in a corked conical flask and stirred until the agar had dissolved. For fungal growth; 39.0g of Sabouraud dextrose agar (SDA) was dissolved in 1000ml of distilled water in a corked conical flask and stirred till the agar had dissolved. The media were autoclaved 121°C for 15 minutes according to manufacturers instruction. The media were allowed to cool down in a sterilized chamber then poured into peri dishes containing the 1ml diluents in the presence of a flame. The bacterial culture dishes were incubated at 37°C for 24 hours whilst the fungi culture plates were incubated at 25°C for 6 days.

Biochemical characteristics of bacterial isolates

Bacterial colonies were enumerated using colony counting machine. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor, and a computation was performed for 1 mL of the original sample. An average count was used to produce the total count, and the results were reported as colony forming units per millilitre (CFU/ml) of sample. Bacterial samples were further sub-cultured to obtain pure cultures using nutrient agar. Furthermore, the pure culture of the bacteria isolates were subjected to various biochemical test such as catalase, indole, citrate utilization, coagulase, methyl red, Voges-Proskauer, urease and sugar fermentation to ascertain the phenotypic characteristics of the organisms (Ogodo *et al.*, 2021).

Fungal Identification

Potato dextrose agar was used to subculture the initial fungal colonies. The SDA was prepared according to manufacturer's instructions; As 500mg of mortar crushed ciprofloxacin was added into the agar after it had been autoclaved at 121°C for 15 minutes to inhibit bacterial growth. The agar was dispensed into sterile petri dishes in the presence of a flame. A sterile inoculating wire was used to collect fungal colonies of interest and placed in the middle of the agar plate. Fungal colonies were observed on SDA plates under normal room temperature. Each colony was observed with the following criteria: colour, texture of colony, elevation, form, border/margin. The fungal species were further characterised using morphological characteristic (Leber and Burnham, 2023).

Molecular characterization of Bacteria and Fungi

This involves DNA extraction, Polymerase Chain Reaction (PCR), gel electrophoresis, gel extraction and DNA sequencing using 16s for bacteria and ITS for fungi. DNA exteraction was carried out using the Zymo Research Corp Quick-DNA™ Miniprep kit (Irvine,California,USA), while PCR was done after extraction. PCR consists of three stages: pre-denaturing, denaturing (to separate the double-stranded template into two single strands), annealing (lowering the temperature to allow the DNA primers to attach to the template DNA), elongation (raising the temperature to allow the Taq Polymerase enzymes to make new strands of DNA), and final extension. According to BIOER GeneExplorer thermal cycler: Pre-denaturalization at 95°C for 5 minutes, denaturalization at 94°C for 1 minute, annealing at 52°C for 1 minute, elongation at 72°C for 1 minute, and final extension at 72°C for 7 minutes, cooling at 4°C. Gel Electrophoresis was carried out after PCR to view amplified DNA and the bands were viewed under and ultraviolet light, the base pair of the DNA were observed and read according to the ladder. Furthermore, Gel extraction was used to remove amplified genes for sequencing. Gel extraction was used to isolate desired fragments of intact

DNA from agarose gel electrophoresis. Lastly, DNA Sequencing was carried out using the Sanger technique.

Data Analysis

The mean and standard deviation were used to calculate the physicochemical parameters. Paleontological Statistical software (PAST software) version 4.05 was used to analyse the unprocessed data. Data were presented in Tables and Figures. DNA sequences were BLAST using the NCBI database. Phylogenetic trees were created using MEGA 11 software (v 11.0.13) 2024.

RESULTS

Physicochemical parameter of water samples collected from Nile Stream Abuja

The mean physicochemical parameters of water samples collected from sampled station of Nile Stream Abuja is presented in table 1. Temperature, resistance, turbidity, dissolved oxygen, biological oxygen demand, salinity and nitrate record highest in station A while pH and sulphate record highest in sampling station B. There was no significant different ($p>0.05$) between all the measured physicochemical parameters except Resistance and Nitrate which showed significant different ($p<0.05$) between the sampling stations.

Bacteria isolated from water samples collected from Nile Stream Abuja

The mean bacterial colony count of water samples collected from Nile Stream Abuja is presented in table 2. Lowest mean colony count was recorded (1.54×10^{-3} CFU/ml) in station B and highest mean colony was recorded in (1.60×10^{-3}) in sampling station A. The biochemical characteristics of bacteria isolated from water sampled collected from Nile Stream is shown in table 3. A total of three bacterial isolates were identified from the sampled station of Nile Stream. The bacteria comprised of three Gram-negative bacteria which are *Enterobacter hormaechei, Enterobacter* sp. and *Salmonella* sp. while *Bacillus safensis.* is the only Gram-positive bacteria isolated in this study

Table 1: Mean physicochemical parameter of water samples collected from Nile Stream Abuja

Parameters	Station A	Station B	WHO permissible limit
Temperature (°C)	32.33 ± 1.89	32.38 ± 1.66	40
pH	7.37 ± 0.05	7.41 ± 0.02	6.5-7.5
Electrical conductivity (µS/Cm)	0.24 ± 0.02	0.24 ± 0.02	125
Resistance	4106 ±359	2696 ± 2115*	
Total dissolved solid (mg/L)	0.12 ± 0.01	0.12 ± 0.01	500
Salinity (mg/L)	0.11 ± 0.01	0.11 ± 0.00	0.00001
Turbidity (NTU)	4.05 ±0.78	3.91 ± 1.78	<40
Dissolved oxygen (mg/L)	2.71 ± 2.97	2.57 ± 3.03	5
Biological oxygen demand (mg/L)	3.17 ± 2.79	2.93 ± 2.77	5
Nitrate (mg/L)	0.43 ± 0.23	0.09 ± 0.01*	50
Sulphate (mg/L)	247 ± 4.82	277.2 ± 29.09	250

Table 2: The mean bacterial colony count of water samples collected from Nile Stream Abuja

Station	Dilution factor	Mean colony	Colony form per mil (CFU/ml)
A	10^4	160	1.60×10^{-3}
	10^5	156	1.56×10^{-4}
B	10^4	TNTC	TNTC
	10^5	154	1.54×10^{-3}

TNTC= too numerous to count

Table 3: Microscopic and biochemical characteristics of bacteria isolated from water samples collected from Nile Stream Abuja

Station	Gram reaction	Shape	Coagulase	Catalase	Urease	Voges [illegible]	Methyl red	Indole	Citrate	Glucose	Fructose	Lactose	Suspected organism
A	-	Rod	+	+	+	+	+	-	+	+	+	+	*Enterobacter hormaechei*
	+	Rod	+	+	-	-	+	-	-	+	+	+	*Bacillus safensis*
B	-	Rod	+	+	-	-	+	-	+	+	-	-	*Salmonella sp*
	-	Rod	+	+	+	+	+	-	+	+	+	+	*Enterobacter sp*

Note; + =positive - = negative

Fungi isolated from water samples collected from Nile Stream Abuja

The fungi isolated and identified from water samples collected from Nile Stream Abuja are presented in Table 4. *Aspergillus* species were found in both sampling stations, while *Penicillium* sp. was found only in sampling station B.

Table 4 Fungi isolated from water samples collected from Nile Stream Abuja

Sampling point	**Macroscopic observation**	**Microscopic observation**	**Possible fungi**
A	Green with a powdery surface, circular form, flat on agar, curled margin, reverse has a yellow to white pigmention with green border	septate hyphae, sporangia, branched sporangiospore, mycelium	*Aspergillus* sp.
B	Dark green with a wrinkled surface, water droplets on growth, flat on agar, circular form, lobate margin, reverse has yellow pigmentation with white border	septate hyphae, branched condiospores, ellipsodial conidia, high sporulation	*Penicillium* sp.
	Black with grainy surface, circular form, flat on agar, curled margin, reverse is wrinkled and white to yellow pigmentation	septate hyphae, long and branched sporangiospore, round spore-filled sporangia	*Aspergillus* sp.

Molecular Characterization of bacteria and fungi isolated from Nile stream

Gel electrophoresis results of bacteria isolates showed all bands were with a 750-1000bp range using a 2000bp DNA ladder and the bands of fungal isolates were between 500-750bp using a

2000bp DNA ladder. The results of the Molecular Characterization of bacteria isolated from Nile stream is presented in table 5. Bacteria sample S01 has 77.28% similarity with *Enterobacter hormaechei* with accession number of CP043853.1. Bacteria sample S02 has 70.33% similarity with *Salmonella enterica* with accession number CP009088.1. Bacteria sample S03 has 72.61% similarity with *Bacillus safensis* with accession number OR394189.1. Bacteria sample S04 has 98.94% similarity with *Enterobacter* specie with accession number HQ441562.1. The phylogenetic trees of the identified species are shown in figure 2. The fungi sample MO1 has 90.59% similarity with *Aspergillus flavus* with accession number KU687322.1. Fungi sample MO2 has 92.86% similarity with *Aspergillus niger* with accession number MK156708.1. Fungi sample MO3 has a 97.62% similarity with *Penicillium citrinum* with accession number MN861067.1. The phylogenetic trees of the identified fungal species are shown in figure 3.

Table 5 Molecular Characterization of bacteria isolated from Nile stream

Sample code	Accession number	Similarity (%)	Organism
S01	CP043853.1	77.28%	*Enterobacter hormaechei*
S02	CP009088.1	70.33%	*Salmonella enterica*
S03	OR394189.1	72.61%	*Bacillus safensis*
S04	HQ441562.1	98.94%	*Enterobacter* sp.

Table 6 Molecular Characterization of fungi isolated from Nile stream

Sample code	Accession number	Similarity (%)	Organism
MO1	KU687322.1	90.59%	*Aspergillus flavus*
MO2	MK156708.1	92.86%	*Aspergillus niger*
MO3	MN861067.1	97.62%	*Penicillium citrinum*

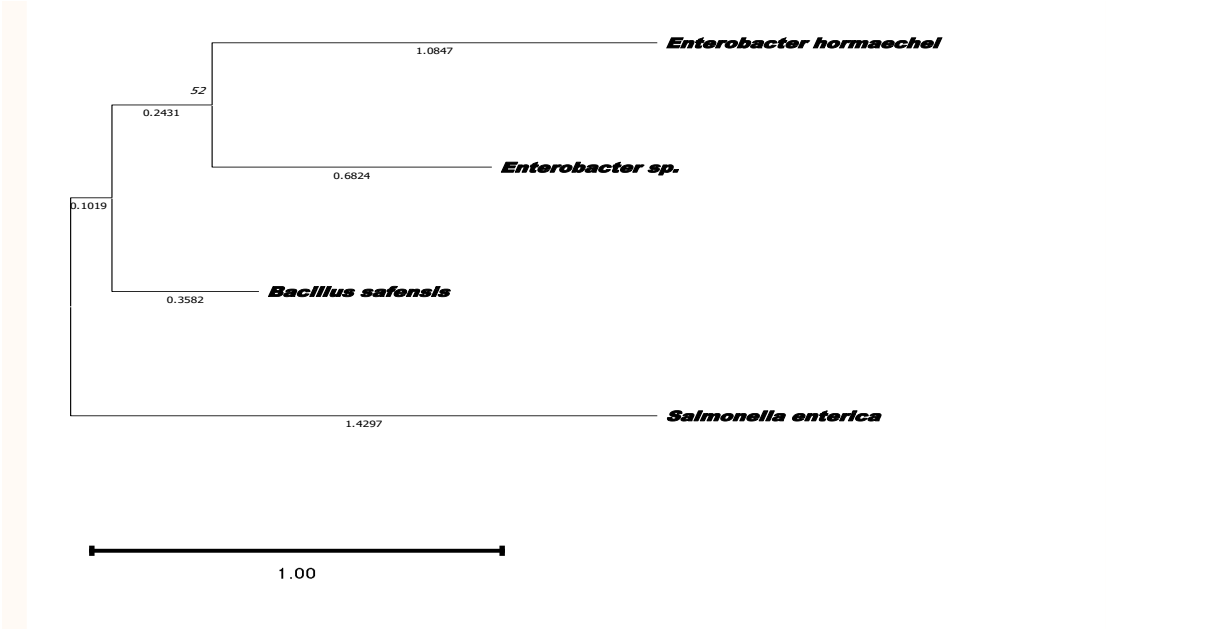

Figure 2: Phylogenetic tree of the bacteria species identified from the Nile stream

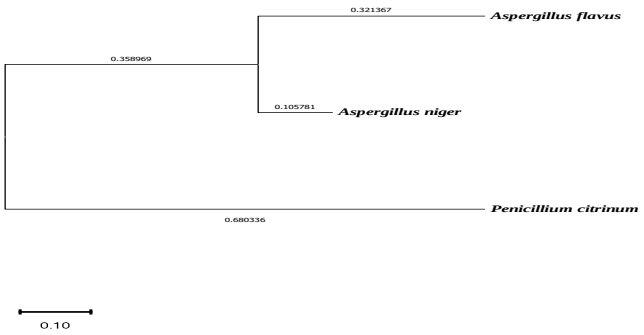

Figure 3: Phylogenetic tree of fungi species identified from the Nile stream

DISCUSSION

Water quality is critical to the health and sustainability of aquatic ecosystems and hydrology. Renewable freshwater supports humanity by providing water for drinking, irrigation, industrial applications, fish production, recreation, transportation, and trash disposal (Hassan and Babatunde, 2015; Adama *et al.,* 2023). Water also plays a significant role in the cycling of materials and can be a vector if it becomes a source of dangerous compounds and diseases (Mohammed and Adamu, 2019; Maishanu *et al.,* 2022). River water's quality and stability are determined by elements such as catchment lithology, climatic conditions, atmospheric and anthropogenic inputs (Mohammed *et al.,* 2023b).

In this study the water temperature ranged between 32.33 ± 1.89 to 32.38 ± 1.66 °C. The high temperature value observed in this study could be as a result of time of sampling and expose vegetation cover of the stream which has been disrupted by human activities. Temperature is one of the basic parameters that influence aquatic biotas as all physiological activities of organisms are temperature dependant (Mohammed *et al.,* 2021). The water pH ranged between 7.37 ± 0.05 and 7.41 ± 0.02. The observed pH value in this study is within the permissible limit of 6.5 - 8.5 according to the World Health Organization (WHO 2021).

The concentration of oxygen gas in a body of water is referred to as dissolved oxygen (DO). All life forms, including those responsible for self-purification mechanisms in aquatic habitats, require oxygen (Ostroumov, 2017). The observed DO in this study ranged from 2.57 ± 3.03 to 2.71 ± 2.97 mg/L. the observed low dissolved oxygen in this study could be as result of increase in human activities as a result of urbanization, industrialization and construction activities close to the water bodies. High dissolved oxygen as bee used as benchmark for unperturbed or less perturbed water (Arimoro and Keke 2016; Adamu *et al.,* 2022). In this study the BOD_5 ranged from 2.93 ± 2.77 to 3.17 ± 2.79 mg/L which is an indication of moderate pollution. Bilewu *et al.,* (2022) also reported that water bodies with BOD_5 range of 2 to 8 mg/L are moderately polluted as BOD_5 has been used as benchmark to determine the pollution status of water.

In this study TDS values were observed to be high probably because of the increase in anthropogenic activities along the stream. Bilewu *et al.,* (2022) also reported that TDS in bodies of water can come from a variety of sources, including natural sources, wastewater, urban and agricultural runoff, and industrial effluent. The amount of dissolved solids in water controls light transparency, which is required for both the flora and fauna activities for normal

development and production (Hassan and Babatunde, 2015). Nitrate recorded 0.09 ± 0.01 to 0.43 ± 0.23 mg/L. The high nitrate value observed in this study could be as a result of surface run off from agricultural activities close to the stream. Nitrate in aquatic environment have been mostly linked with surface run off from agricultural activities (Adamu *et al.,* 2022; Mohammed *et al.,* 2023b).

Microbial contamination can pose a significant threat to human health, as exposure to contaminated water can lead to a range of illnesses, including gastrointestinal infections, skin rashes, and respiratory problems. In developing countries, microbial contamination of freshwater is a major cause of waterborne diseases such as cholera, typhoid fever, and dysentery. *Enterobacter hormaechei, Salmonella enterica, Bacillus safensis* and *Enterobacter* sp. are common bacteria found in surface water, including streams. These bacteria are known to pose potential risks to human health and the environment (Olaoye and Onilude, 2020; Mohammed *et al.,* 2023a). The presence of gram negative bacteria in surface water or any source of water is of public health concern as gram-negative bacteria have been reported to cause infections in humans, particularly those with compromised immune systems (Olaoye and Onilude 2020). *Salmonella enterica* is another pathogenic bacterium that can be found in surface water. *Salmonella enterica* is known to cause gastrointestinal illnesses in humans and animals (Adamu *et al.,* 2019; Mohammed 2023a). *Bacillus safensis* is a species of bacteria that has been isolated from various environments, including soil and water. While *Bacillus safensis* is generally considered non-pathogenic, some strains have been shown to exhibit antibiotic resistance (Satomi *et al.,* 2006). *Enterobacter* sp. is a genus of bacteria that includes various species, some of which are opportunistic pathogens. *Enterobacter* sp. has been detected in surface water samples collected from rivers and streams. The presence of this group of bacteria in surface water may indicate faecal contamination or other sources of pollution (Fuhrimann *et al.,* 2015). *Aspergillus flavus, Penicillium citrinum,* and *Aspergillus niger* are common fungi found in various environments, including surface water sources such as streams. Some studies have reported the presence of *Aspergillus flavus, Penicillium citrinum*, *Aspergillus niger* and other fungal species in some freshwater bodies of Nigeria (Adamu *et al.,* 2018; Adamu *et al.,* 2022; Maishanu *et al.,* 2022). These fungi are known to produce mycotoxins, which can be harmful to human health and the environment.

Conclusion and recommendation

Freshwater pollution by human activities is becoming a matter of urgent concern as it threatened environmental productivity and its sustainability. Most of the observed physicochemical parameters in this study shows an impaired ecological system due to anthropogenic factors. Also, the presence of *Enterobacter hormaechei*, *Enterobacter* sp.,*Salmonella enterica* and *Bacillus safensis* in the stream water can pose a health risk to humans and wildlife if the water is consumed or in contact with open wounds. The presence of *Aspergillus flavus*, *Aspergillus niger*, and *Penicillium citrinum* in freshwater offers serious health dangers because they produce dangerous mycotoxins such as aflatoxins and citrinin. These fungus can pollute drinking water, causing major health concerns such as liver damage, cancer, respiratory disorders, and allergic responses. They also have a harmful influence on plants, resulting in crop rotting and poor quality.

Water-borne infections are clearly caused by inappropriate waste disposal, sewage pollution of water, and surface runoff; consequently, required efforts must be made to mitigate the impact of anthropogenic activity on water bodies. Monitoring and testing of stream water for bacteria contamination is important for ensuring the safety of recreational activities such as swimming, fishing, and drinking water sources. Implementing proper water treatment and

sanitation practices can help reduce the risk of exposure to these harmful bacteria in stream water.

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