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# Mycological and Physicochemical Assessment of Drinking Water from Pupils' Bottles

***[1]Emmanuel O. Olatunji, [2]Gift N. Amos, [2]Taiwo Stephen Okanlawon., [3]Olubi Olawale, [1]Abimbola Orukotan, [4]Ibrissa Kabore and [1]Tawakalit M. Momoh-Salami**

[1]Department of Biological Sciences, Lagos State University of Technology, Lagos State.
[2]Department of Biological Sciences, Glorious Vision University, Ogwa, Edo State
[3]Department of Surgery, College of Medicine, Lagos State University, Ikeja, Lagos State.
[4]Department of Animal Sciences, Universite Joseph Ki-Zerbo, Ouagadougou, Burkina Faso.

*Corresponding authors' email: omololuademola504@gmail.com

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**ABSTRACT**
This study investigated the microbial and physicochemical properties of drinking water samples collected from pupils' water bottles, with a focus on fungal contamination. Water samples were collected aseptically and analysed using standard microbiological and physicochemical methods. Fungi were isolated by serial dilution and cultured on Potato Dextrose Agar, followed by incubation. Morphological and cultural characteristics were examined, and microscopic identification was conducted using lactophenol cotton blue staining. Molecular identification was carried out via PCR amplification targeting the 18S rRNA gene, and amplified products were analysed by gel electrophoresis. The mean microbial load of fungal isolates ranged from $1.04\times10^4$ to $2.56\times10^4$ sfu/mL, exceeding the WHO permissible limit of $1.0\times10^2$ CFU/mL. Identified fungal species included *Aspergillus niger*, *Fusarium poae*, and *Penicillium chrysogenum*, with *A. niger* and *F. poae* each accounting for 40% occurrence, and *P. chrysogenum* 20%. Physicochemical analysis showed that most parameters were within WHO and FEPA limits, except for a slightly elevated pH (8.7) in one sample. Sequencing confirmed the identities of *A. niger* (573 bp) and *F. poae* (570 bp). The study highlights the presence of potentially harmful fungi in pupils' drinking water, emphasizing the need for routine water quality surveillance and public health education.

**Keywords:** Fungal Contamination, Drinking Water Quality, Physicochemical Parameters, Public Health Surveillance

## INTRODUCTION

In its purest form, water is a colourless, odourless and tasteless liquid that is mobile, although large volumes of water may appear blue (Abdulkareem *et al*., 2023). Its melting point is 0°C, while its boiling point is 100°C (Olatunji *et al*., 2019). The absence of water limits the survival of higher organisms to only a few days. Water is therefore a basic necessity for life (Amira & Yasir, 2011) and has multiple applications, including cooking, cleaning, food processing, swimming and drinking. However, because contaminated water can have direct adverse effects on human health, drinking water should be potable and free from harmful microorganisms, chemicals and other contaminants. Effective monitoring of water quality is therefore important for identifying contaminants and assessing the safety of water intended for human consumption. Recent developments in biosensor technology have provided opportunities for the rapid detection and monitoring of different water-quality parameters and contaminants (Adetunji *et al*., 2024).

Water contamination by fungal species is an emerging public-health concern, particularly in environments where water storage and hygiene practices are inadequate. Fungi such as *Aspergillus*, *Candida* and *Penicillium* can occur in water systems and moist environments because of their ability to survive under a range of environmental conditions (Biedunkiewicz *et al*., 2021). These fungi may originate from environmental sources, surrounding materials or human handling and may produce metabolites or contribute to allergic reactions and opportunistic infections, particularly among susceptible individuals (Paterson & Lima, 2017). The presence of fungi in water can also contribute to biofilm formation, which may complicate microbial control and promote persistence within water-containing systems (Pereira *et al*., 2020). Consequently, investigation of fungal contamination is relevant to the broader assessment of drinking-water quality.

The emergence of fungi in water bottles raises concerns because apparently clean containers may become contaminated during filling, handling, storage or repeated use. Fungi, which are often less emphasized than bacterial indicators in routine water-quality investigations, can persist under suitable environmental conditions. The limited exploration of fungal diversity in water-bottle samples presents a challenge because identifying the species present is important for understanding their environmental significance and potential health implications. Factors such as prolonged storage, exposure to sunlight and fluctuating temperatures may influence fungal survival and proliferation in water bottles (Davies *et al*., 2021). In addition, the material of the bottle, cleaning practices, frequency of reuse and exposure to environmental contaminants may influence the microbiological quality of stored water.

A significant challenge in this field is the limited understanding of the variety of fungi that may occur in water-bottle samples. The absence of thorough investigations can limit the ability to identify specific fungal species, including potentially opportunistic organisms, that may be present in water consumed by pupils. The health implications of fungal contamination of water bottles also require attention because vulnerable populations, including children, elderly individuals and people with weakened immune systems, may be more susceptible to infections caused by opportunistic

fungi (Jain *et al*., 2010). Investigating fungal occurrence in water bottles used by schoolchildren is therefore important for improving understanding of potential environmental sources of contamination and promoting appropriate hygiene practices.

Existing water-quality standards predominantly focus on bacterial and physicochemical indicators, with comparatively limited attention to fungal indicators. The absence of universally established criteria for acceptable fungal levels in drinking-water bottles creates challenges for routine monitoring and interpretation of fungal contamination. Water-bottle samples may also be subjected to different storage conditions, including exposure to sunlight, fluctuating temperatures and extended storage durations. These variables may influence fungal proliferation and survival, yet comprehensive research on how different storage conditions contribute to fungal contamination in water bottles remains limited (Mohapatra *et al*., 2017). The application of appropriate analytical and monitoring approaches is therefore important for identifying changes in water quality and supporting effective management of drinking-water safety (Adetunji *et al*., 2024).

Microbiological contamination of drinking water remains an important public health concern in Nigeria, particularly where communities depend on borehole water and commercially packaged water for domestic consumption. The presence of potentially pathogenic microorganisms in these water sources raises concerns about their safety and suitability for human consumption. Recent investigations have identified multidrug-resistant bacteria in borehole and sachet water samples in Ilesha, Nigeria (Adebomi *et al*., 2026). Similarly, Alex and Kpormon (2023) documented the occurrence of bacterial and fungal contaminants in drinking-water sources in Port Harcourt, Nigeria, highlighting the need for comprehensive microbiological assessment. Although bacterial contamination has received considerable research attention, fungal contamination remains an important aspect of drinking-water quality that requires further investigation. Consequently, routine microbiological monitoring of drinking water is essential for identifying potential contaminants and safeguarding public health.

The impact of fungal contamination, particularly among vulnerable populations, further emphasizes the need for appropriate investigation and preventive measures. The application of modern water-quality monitoring approaches can also improve the detection and characterization of contaminants (Adetunji *et al*., 2024). Raising awareness about fungal contamination and appropriate cleaning practices is therefore important for promoting safer water-storage practices among pupils, parents, teachers and other members of the school community.

The present study therefore focuses on the fungal contamination and physicochemical characteristics of water bottles used by pupils in selected schools in Ogwa. Specifically, the objectives were to isolate and enumerate fungi present in water-bottle samples collected from pupils in Ogwa schools; to characterize and identify the fungal species isolated from these samples; to assess the physicochemical properties of the water in the pupils' bottles; and to educate the school community on effective methods for cleaning and maintaining pupils' water bottles. The study is expected to provide information on the occurrence, abundance and diversity of fungi associated with water bottles used by pupils in selected schools in Ogwa, as well as the physicochemical characteristics of the water contained in these bottles.

## MATERIALS AND METHODS

### Study Area

Ogwa is a town in Esan West Local Government Area in Edo State, Nigeria. It is among the constituent communities of Esan land. Having latitude 6° 30' 23" N and longitude 6° 12' 39" E in Edo state. The isolation identification and other assessment was carried out in the biological sciences laboratory of Glorious Vision University, Ogwa, Edo State, Nigeria. The samples were collected during the months of April and May, 2024.

### Sample Collection

Samples were collected from Glorious Vision Nursery and primary school; Kings delight primary school and Eguare primary school using sterile capped sample bottle and swab stick. Samples collected in the sample bottle was done by pouring the water from the pupil water bottle directly into the sample bottle. The swab stick was used to swab inside the water bottle. Samples were collected around 9am-10am. The samples were immediately transported to the laboratory where analysis was initiated within 1hr of collection.

### Microbiological Analysis

#### *Isolation of fungi Isolates*

One milliliter was measured and transferred into a test tube containing sterile distilled water for serial dilution. Fifteen ml (15 ml) of the prepared molten potato dextrose agar (PDA) which was left to cool was poured aseptically into the dishes and gently swirled in clockwise and anticlockwise direction to allow for even distribution of the colonies on the surface of the agar. The agar was allowed to set (Okafor *et al*., 2023). PDA plates were incubated without inverting at 25°C for 2-5 days. Identification and Characterization fungi isolates were characterized based on their colonial morphology, cellular morphology and biochemical characteristics using lactophenol cotton blue staining. The fungi isolates were identified with reference to Compendium of Soil Fungi and Pictorial Atlas of Fungi (Okanlawon *et al*., 2023).

Macroscopic analysis was conducted daily to observe colony growth rate, surface consistency, pigment presence, margin distinctness, and folding patterns, examining both the upper and reverse sides of the colonies (Olutiola *et al*., 1991). For microscopic analysis, triangular portions from 5–7-day-old fungi colonies were prepared using 70% alcohol and lactophenol stain, then examined under low and oil immersion lenses to identify structures such as hyphae, conidia, and conidiophores.

#### *Total Fungi Count*

After the incubation period, different colonies were observed and plates within 30-300 colonies were counted and recorded, this was taken as the total viable count. fungi Isolates were counted using SFU/g.

### Molecular Identification of the Isolates

#### *Fungal DNA Extraction*

Genomic DNA of fungal isolates was extracted following the protocol of Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research Group, California, USA) as described by the manufacturer, with slight modification. A sterilized surgical blade was used to scrape off the fungal mycelium. This was homogenized in a sterilized mortar using 750µl of Bashing Bead Buffer and liquid nitrogen. DNA quality was determined by gel electrophoresis using 1% agarose gel

#### *PCR Amplification*

Fungal universal primer pair ITS4, forward: (5´-TCCTCCGCTTATTGATATGS-3´) and ITS5, reverse: (5´-GGAAGTAAAAGTCGTAACAAGG- 3´), were used to amplify the ITS1-2 region of the isolates. The PCR cocktail consisted of 3µL of genomic DNA (10ng/µL), 0.1µL of Taq polymerase, 2.5µL of 10X PCR buffer, 1.0 µL of DMSO, 1.0µL of 2.5mM DNTPs, 1.0µL of 25mM MgCl2(Promega), 1.0µL of each primer (concentration of 5µM) and 13.4µL of Nuclease-free water, making a total volume of 25µL. Amplifications were performed in a thermal cycler (GeneAmp® 9700 PCR System, Applied Biosystems, California, USA) using an initial denaturation step of 94°C for 5 minutes, followed by 36 cycles of denaturation at 94°C for 30 seconds, annealing at 54°C for 30 seconds and elongation at 72°C for 45 seconds with a final extension at 72°C for 7 minutes. The amplicons were stored at 10°C. On a 1.5% agarose gel, the amplification product was separated, and electrophoresis was then done at 80 V for 1 h and 30 min. DNA bands were visible after electrophoresis, thanks to ethidium bromide staining. As a benchmark for DNA molecular weight, a 100 bp DNA ladder was employed (Gibert *et al*., 2022)

## RESULTS AND DISCUSSION

The mean microbial load for fungal isolates is presented in Table 1 and ranges from $1.04x10^4$sfu/ml to $2.56.0x10^5$ sfu/ml.

**Table 1: Mean Microbial Load of Fungal Isolates**

| Isolation | Mean Microbial Load (sfu/ml) |
|---|---|
| First Isolation | $2.56x10^4$ |
| Second Isolation | $1.04x10^4$ |
| Third Isolation | $1.25x10^4$ |

Acceptable viable count by WHO: 1.0×102 CFU/ml
Mean microbial load of fungi isolates sfu/ml= Spore forming unit per milliliter.
The morphological and cultural characteristics of fungal isolates are presented in Table 2. Some of the isolates had white hyphae, green spores, scanty in occurrence, creamy on the reverse side of the agar and did not produce pigment.

**Table 2: Colony Morphology of Fungal Isolates**

| Isolate Code | Colour of Spores | Reverse Side of Agar | Nature of Aerial Hyphae | Pigmentation | Type of Aerial Hyphae |
|---|---|---|---|---|---|
| F1 | Creamy | Creamy | Embedded | None | None |
| F2 | Creamy | Creamy | Embedded | None | None |
| F3 | White | Creamy | Embedded | Brown | Fluffy |
| E1 | White | Creamy | Embedded | Black | Fluffy |
| E2 | White | Creamy | Embedded | Brown | Fluffy |

The microscopic and macroscopic characteristics of fungal isolates are shown in Table 3. Some of the fungal isolates had white hyphae, green spores, scanty in occurrence, creamy on the reverse side of the agar, powdery in patches and did not produce pigment. The percentage occurrence for fungal isolates is shown in Table 3. *Aspergillus candidus* had the highest occurrence (40%) as well as *Fusarium poae (40%) and penicillium chrysogenum* had the lowest occurrence (20%) altogether.

**Table 3: Microscopic and Macroscopic Characterization of Fungal Isolates**

| Isolate Code | Characterization | Probable Identity |
|---|---|---|
| F1 | Characterised by thin, spreading colonies velvety and grass-green to bluish, green with pals to bright yellow sometimes vinaceous reverse and yellow exudate conidiophores two to three stage branched conidia smooth walled odour often fruity conidia are tri-nucleated | *Penicillium chrysogenum* |
| F2 | They are typically powdery black, conidiophores arising from long, broad thick-walled, sometimes branched for cells, it has a tall conidiophore. Conidia are large with radiating heads, mostly globose and irregularly roughed | *Aspergillus niger* |
| F3 | They are fast growing aerial mycelium, loosely cottony, white/pink at the agar surface, odour characteristically fruity, conidiospore richly branched with short and broadly ellipsoidal, spores not produced but thickened, swollen hyphal portions may function as chlamydospores | *Fusarium poae* |
| E.1 | They are typically powdery black. Conidiophores arising from long, broad thick-walled, sometimes branched for cells, it has tall conidiophores. Conidia are large with radiating heads, mostly globose and irregularly roughed. | *Aspergillus niger* |
| E.2 | They have a fast-growing aerial mycelium. Loosely cottony, white/pink at the agar surface, odour characteristically fruity. Conidiospore richly branched with short and broadly | *Fusarium poae* |

| Isolate Code | Characterization | Probable Identity |
|---|---|---|
| | ellipsoidal, spores not produced but thickened, swollen hyphal portions may function as chlamidiospores. | |

Table 4 summarizes the physicochemical properties of water samples from pupils' bottles, showing that most parameters fall within WHO and FEPA limits. However, Sample 1 recorded a slightly high pH (8.7), exceeding the WHO recommended range.

**Table 4: Physiochemical Properties of Water Samples**

| Parameters | Sample1 | Sample2 | Sample3 | WHO Limit | FEPA Limit |
|---|---|---|---|---|---|
| PH | 8.7 | 7.0 | 7.9 | 6.5-8.5 | 6.0-9.0 |
| Temperature | 13 | 10 | 12.5 | 25 | 30 |
| Turbidity | 1.1 | 0.3 | 0.32 | 5-25 | 5 |
| EC | 58 | 54 | 57 | 300 | NL |
| BOD | 0.15 | 0.19 | 0.18 | 5 | 5 |
| Dissolved | 7.2 | 6.8 | 7.1 | 14 | NL |
| Oxygen TDS | 28 | 39 | 45 | 500 | NL |

Legends: EC= Electrical conductivity, BOD= Biological oxygen demand, TDS= Total dissolved solids, NI= Not indicated. All values in mg/l, except pH, EC. Drinking water quality as recommended by FEPA and WHO

Table 5 displays the frequency of fungal isolates, with *Aspergillus niger* and *Fusarium poae* each occurring in 40% of samples, while *Penicillium chrysogenum* appeared in 20%, indicating notable fungal presence.

**Table 5: Percentage Occurrence of Fungal Isolates**

| Organisms | Frequency | Occurrence (%) |
|---|---|---|
| Aspergillus niger | 2 | 40 % |
| Penicillium chrysogenum | 1 | 20 % |
| Fusarium poae | 2 | 40% |
| Total: 5 | 100 | |

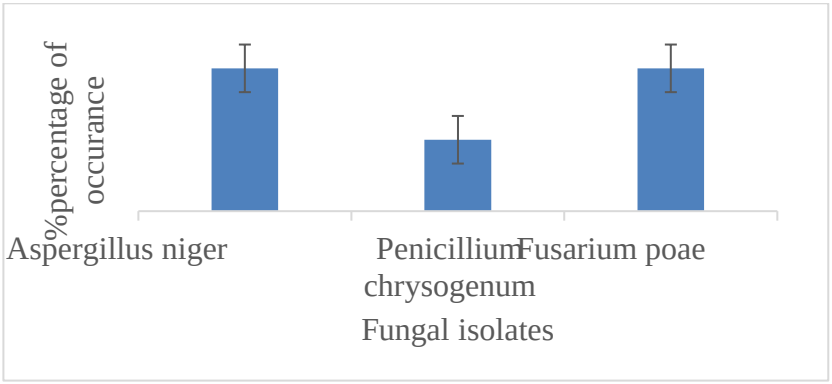



Figure 1: The Percentage Occurrence of Fungal Isolates

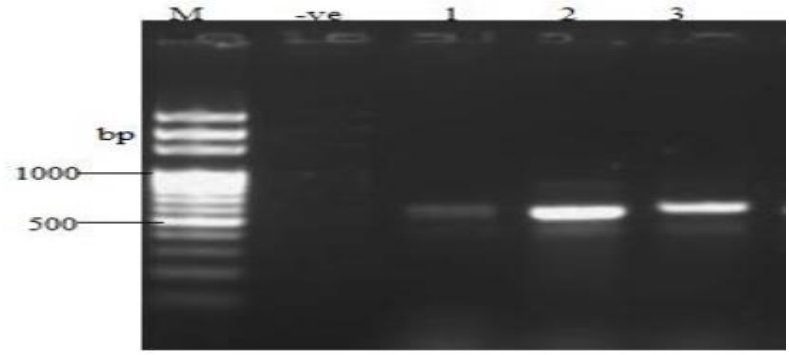



Figure 2: PCR Amplification Image of 18srrna Gene Bands of the Fungi Isolates

Lane 1: DNA marker, Lane 2-3 are the 18SrRNA of the isolates

The two sequenced fungi isolate include;

1. Sample OS… *Aspergillus niger* Band size: 573
2. Sample OS4…. *Fusarium poae* Band size: 570

**Discussion**

Contamination of water bottles can be attributed to factors such as handling, particularly when bottles are not properly cleaned and dried, which can encourage fungal growth and contaminate the water. Other contributing factors include leaching from plastic materials, cross-contamination, human error, environmental factors, storage conditions, ageing infrastructure, poor maintenance, natural disasters and intentional contamination (Figueras and Borrego, 2010). These factors may influence the microbiological quality of stored water and increase the opportunity for microorganisms to enter and persist within water containers.

In this study, the total viable count ranged from $1.04 \times 10^4$ to $2.50 \times 10^4$ CFU/mL, indicating a relatively high microbial load in the analyzed water samples. Earlier work by Hussain *et al*. (2024) also reported considerable microbial contamination in water samples. Similarly, Okanlawon *et al*. (2024) reported variations in microbial quality among water reservoirs in Ogwa and Ebelle communities, Edo State, while Avoseh *et al*. (2024) demonstrated the importance of bacteriological assessment in evaluating the quality of River Obazagbon water. The presence of a high microbial load in water may indicate contamination arising from the source, handling, storage or environmental exposure. Such contamination may become more significant where storage temperature, pH and other environmental conditions favor microbial survival and multiplication.

The physicochemical parameters of the water samples were measured both in situ and in the laboratory. The parameters measured included air and water temperature, pH, biological oxygen demand (BOD), dissolved oxygen (DO), electrical conductivity (EC) and total dissolved solids (TDS). The range values of the measured parameters were compared with relevant World Health Organization (WHO) and Federal Environmental Protection Agency (FEPA) standards. The findings showed that some of the physicochemical parameters were slightly outside the recommended range. The pH of the three water samples was 8.7, 7.0 and 7.9, respectively, indicating neutral to slightly alkaline conditions. The values of 7.0 and 7.9 were within the WHO guideline range of 6.5–8.5, while the value of 8.7 was slightly above this range but remained within the broader FEPA range of 6.0–9.0. Similar variations in physicochemical characteristics have been reported in Nigerian water studies. Okanlawon *et al*. (2024) reported variations in pH, temperature, dissolved oxygen, total dissolved solids and biological oxygen demand in water reservoirs in Edo State. Likewise, Avoseh *et al*. (2024) assessed River Obazagbon using physicochemical and bacteriological parameters, demonstrating the importance of combining physicochemical and microbiological measurements when evaluating water quality. The temperature values recorded in the present study were 13.0, 10.0 and 12.2°C, respectively, which were within the tolerable values reported by Kelechi *et al*. (2021).

Also, the colony morphology of the fungal isolates in this study was noted because of its relevance to isolate identification. Some of the fungal isolates were fluffy, embedded, black and pigment-producing. These morphological characteristics are useful in the preliminary identification and differentiation of fungal isolates because colony colour, texture, growth pattern and pigmentation provide important taxonomic characteristics. Okanlawon *et al*. (2023) similarly demonstrated the relevance of microbial isolation and identification in their study of microorganisms associated with Jollof rice, in which several fungal organisms were recovered and characterised. The morphology of microorganisms may also influence their observed growth characteristics and interaction with environmental conditions (Nkere *et al*., 2011).

The microorganisms recovered from the water samples analysed in this study included five fungal isolates. The fungi isolated from the water and their occurrence were *Penicillium chrysogenum*, *Aspergillus niger* and *Fusarium poae*. These organisms are environmentally distributed fungi and their presence in water may indicate contamination from environmental sources, containers, handling practices or airborne spores. *Aspergillus niger* had the highest occurrence (40%), as did *Fusarium poae* (40%), while *Penicillium chrysogenum* had the lowest occurrence (20%). The recovery of *Aspergillus* species is consistent with previous findings by Okanlawon *et al*. (2023), who reported several fungal organisms, including *Aspergillus* species, among microorganisms isolated from Jollof rice. The occurrence of these fungi in the present study indicates that fungal contamination may occur in water as well as in other exposed food and environmental materials.

In addition, the varieties of microorganisms obtained from the water samples in this study may be attributed to several factors, including primary contamination and handling of the water. The bottle used in collecting the water and the sanitary condition of the processing environment, particularly exposure to air and airborne dust, may have contributed to increased levels of contaminants. The pH, environmental conditions, temperature and prolonged storage may also influence the survival and multiplication of microorganisms and potentially increase their numbers in stored water (Yamaguchi *et al*., 2007). These observations are consistent with Okanlawon *et al*. (2024) reported variations in microbial and physicochemical characteristics among water reservoirs, and Avoseh *et al*. (2024) demonstrated the usefulness of combined physicochemical and bacteriological assessment in determining water quality. Furthermore, Oladeji *et al*. (2024) reported the isolation of *Pseudomonas aeruginosa* from an oxidation pond at Obafemi Awolowo University, further demonstrating the persistence of microorganisms within water-associated environments.

*Aspergillus niger* isolated from the water samples is a filamentous fungus commonly associated with soil, plants, food materials and other environmental substrates. *Aspergillus* species may occur in water where organic materials and environmental contamination provide suitable conditions for their persistence. The presence of *A. niger* in the present study may therefore be associated with environmental exposure, airborne spores, contaminated containers or handling practices. Okanlawon *et al*. (2026a), in their review of enzyme-producing and antimicrobial microorganisms from local environments, discussed the importance of environmental microorganisms in biotechnology and public health. This provides a broader context for investigating microorganisms recovered from environmental samples, although it does not establish the specific source of *A. niger* in the present study. The detection of this fungus in water should not automatically be interpreted as evidence that the water will cause fungal pneumonia or other severe diseases, as the health significance of environmental *Aspergillus* depends on factors such as species

identity, concentration, route of exposure and the susceptibility of the exposed individual (Ravikiran *et al*., 2022).

*Fusarium poae* is an environmental fungus associated mainly with soil and plant materials. Members of the genus *Fusarium* may occur as saprophytes, plant-associated organisms and, in some circumstances, opportunistic human pathogens. Some species have been associated with superficial infections such as keratitis and onychomycosis, as well as more invasive infections in susceptible individuals (Dinolfo *et al*., 2014). The detection of *F. poae* in the present study contributes to the overall assessment of fungal contamination in pupils' drinking-water bottles, although its isolation alone does not establish that the water caused disease. Okanlawon *et al*. (2026a) highlighted the broader relevance of microorganisms obtained from local environments, while Okanlawon *et al*. (2026b), in their review of microbial bioprospecting organisms like fungi and natural products against multidrug-resistant pathogens.

*Penicillium chrysogenum* occurs in a variety of habitats, including soil, vegetation and food products. Exposure to *Penicillium* spores may be associated with allergic reactions and hypersensitivity conditions, while some species may occasionally cause opportunistic infections in susceptible individuals (Suhaib & Bandh, 2011). Its recovery from the present water samples may reflect environmental contamination through airborne spores, surrounding materials, storage conditions or handling practices. The importance of examining microbial contamination in everyday environments is also reflected in Okanlawon *et al*. (2026c), who investigated bacterial contamination and antimicrobial resistance among street-vended foods in Ogwa and Ebelle, Edo State, Nigeria. Although their research concerned bacteria in food rather than fungi in drinking water, its local setting provides contextual evidence of the relevance of microbiological investigations to public health in the study area.

Environmental pollution and microbial contamination can also occur alongside other water-quality concerns. Enerijiofi *et al*. (2026) investigated the bioadsorption of heavy metals by bacterial isolates obtained from solid-waste dumpsites which could be cloer top water sources in the environment. Although their study focused on heavy-metal removal rather than fungal contamination or drinking-water safety, it illustrates another dimension of environmental microbiology and pollution management.

Finally, Todd *et al*. (2007) highlighted the importance of appropriate hygiene and handling practices in preventing the introduction and spread of microorganisms in materials intended for consumption. This principle is relevant to pupils' drinking-water bottles, where contamination may occur during filling, transportation, storage or repeated handling. The present findings therefore emphasise the importance of maintaining appropriate hygiene throughout water collection, handling, transportation and storage. However, the specific sources of the fungal isolates cannot be established without further investigation of the water supply, bottle-cleaning practices, storage conditions and handling procedures.

## CONCLUSION

This study investigated the microbiological quality of pupils' water bottles in selected primary schools in Ogwa, Edo State, with particular emphasis on fungal contamination. The findings indicate that some water bottles contained one or more fungal microorganisms, suggesting possible contamination associated with inadequate cleaning, storage and handling practices. Such contamination may present potential health concerns for schoolchildren. Daily cleaning of water bottles with hot soapy water, followed by thorough drying, should be encouraged to reduce microbial growth. Schools should incorporate water-bottle hygiene education into health lessons, discourage bottle sharing, and encourage parents to replace damaged or degraded bottles regularly. These measures can promote safer water-storage practices among pupils.

## CONFLICT OF INTEREST

Authors declare that there is no conflict of interest

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