



## Assessment of Physicochemical Properties and Fungal Diversity in Wastewater from an Industrial Glass Manufacturing Facility in Ughelli, Delta State, Nigeria

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### KEYWORDS

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### ABSTRACT

Fungi are known to inhabit diverse environments, including aquatic ecosystems. While some fungal species offer beneficial applications to humans, others are pathogenic and pose potential health risks. This study investigated the mycoflora present in wastewater discharged from an Industrial Glass Manufacturing Facility in Ughelli, Ughelli North Local Government Area, Delta State, Nigeria. Water samples were collected using sterile plastic containers during the dry season (January) and rainy season (July), and were analyzed for fungal content. In addition, physicochemical parameters and heavy metal concentrations were determined. The results were evaluated against the effluent discharge standards set by the National Environmental Standards and Regulations Enforcement Agency (NESREA) and the World Health Organization (WHO) to assess effluent quality and regulatory compliance. Physicochemical analysis revealed that most parameters, including dissolved oxygen (DO: 4.6–8.10 mg/L), biochemical oxygen demand (BOD: 5 mg/L), pH (6.5–6.9), chloride (Cl<sup>-</sup>: 350 mg/L), sulfates (3.5 mg/L), nitrates (5–50 mg/L), and phosphates (3.5–5 mg/L), were higher during the rainy season than the dry season. However, these values remained within the permissible limits set by NESREA and WHO. A total of five fungal species were isolated: *Fusarium* sp., *Rhizopus* sp., *Aspergillus flavus*, *Penicillium glabrum*, and *Cladosporium oxysporum*. Although the physicochemical properties suggest the wastewater is within acceptable discharge standards, the presence of potentially pathogenic fungi, particularly species known to cause opportunistic infections, indicates possible health and ecological risks. Continuous surveillance and treatment of industrial effluents are recommended to mitigate the risk of fungal contamination in receiving water bodies and ensure the protection of human and aquatic health.

### CITATION

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## INTRODUCTION

One of the major environmental challenges facing developing countries such as Nigeria is the improper disposal of waste and the resultant contamination of water resources. These wastes, which include harmful chemicals, decomposing organic matter, and untreated wastewater from households and industrial processes, are often discharged directly into the environment without adequate treatment. Such practices contribute significantly to environmental degradation and pose serious threats to public health and aquatic ecosystems (Abdel-Shafya & Mansour, 2018). Moreover, the accumulation of toxic pollutants in water bodies may lead to bioaccumulation in aquatic organisms, entering the human and animal food chain and potentially causing long-term health problems (Barakat, 2011).

Among the biological contaminants of concern in wastewater are fungi. Fungi are eukaryotic microorganisms that may exist as unicellular yeasts, multicellular molds, or as dimorphic forms capable of switching between both. While many fungi play beneficial ecological roles as decomposers, others are opportunistic pathogens capable of causing a range of diseases in humans, which could range from superficial infections to life-threatening systemic illnesses (Richa et al., 2012; Thambugala et al., 2024). Morphologically, fungi are characterized by features such as chitinous cell walls, the presence of ergosterol in their membranes, and reproduction through spores (Naranjo-Ortiz & Gabaldón, 2019). Their capacity to thrive and multiply in various environments, including aquatic systems, renders them a critical component of water quality evaluations (Cabral, 2010). In freshwater ecosystems, fungi, including members of the ascomycetes and basidiomycetes, can inhabit submerged or partially submerged organic substrates. These aquatic fungi may function as saprophytes, parasites, or symbionts, and their diversity is often influenced by the physical and chemical

characteristics of the water body, such as pH, temperature, salinity, dissolved oxygen, and nutrient concentrations (Jones et al., 2014; Baldy et al., 2002). As such, the composition and abundance of fungal communities can serve as bioindicators of water quality and ecological health (Graça et al., 2002; Rankovic, 2005). Industrial effluents, if not adequately treated, can introduce a complex mix of organic and inorganic pollutants, including heavy metals and microbial contaminants, into receiving water bodies. To provide valuable insights into the microbiological safety of industrial effluents and underscore the need for integrated water quality monitoring that includes microbial assessment, this study aims to assess the physicochemical parameters and fungal diversity in wastewater discharged from an Industrial Glass Manufacturing Facility in Ughelli, Delta State, Nigeria. By comparing measured values to national (NESREA) and international (WHO) standards, the study evaluates the quality of the effluent and explores the potential public health and environmental risks posed by pathogenic fungi in the wastewater.

## MATERIALS AND METHODS

### Collection of Wastewater Samples

Wastewater samples were aseptically collected from three distinct sampling points (designated as Points I, II, and III), located at different discharge sources around the from an Industrial Glass Manufacturing Facility in Ughelli. This is depicted in Plate 1. Sampling was conducted during both the dry season (January 2024) and rainy season (July 2024) to capture seasonal variations. Samples, which contained visible particulates, were collected using sterile plastic sampling containers. All collected samples were immediately transported in insulated coolers to the Department of Botany, Delta State University, for physicochemical and microbiological analyses.



Spd 1



Spd 2



Spd3

Plate 1: Waste water collection points

Key: SP1= sample collection Point 1, SP2 = sample collection point 2 and SP3 sample collection point 3

### Determination of Physicochemical Parameters

The collected wastewater samples were analyzed for various physicochemical parameters following standard analytical procedures. The parameters measured included pH, biochemical oxygen demand (BOD), dissolved oxygen (DO), calcium ( $\text{Ca}^{2+}$ ), sulphates ( $\text{SO}_4^{2-}$ ), nitrates ( $\text{NO}_3^-$ ), phosphates ( $\text{PO}_4^{3-}$ ), chlorides ( $\text{Cl}^-$ ), and magnesium ( $\text{Mg}^{2+}$ ). Each analysis was conducted in triplicate, and the results were compared with permissible limits set by the National Environmental Standards and Regulations Enforcement Agency (NESREA) and the World Health Organization (WHO).

### Fungal Load Analysis (Serial Dilution and Culturing)

To determine fungal load, serial dilution of the wastewater samples was performed according to the method described by Odeyemi et al. (2013). One milliliter (1 mL) of each sample was transferred into a test tube containing 9 mL of sterile distilled water to obtain a 10-fold serial dilution. From the  $10^3$  and  $10^5$  dilutions, 0.1 mL aliquots were aseptically dispensed into sterile Petri dishes in triplicate. Subsequently, 20 mL of cooled, molten Potato Dextrose Agar (PDA) was poured into each dish, gently swirled to mix, and allowed to solidify. Plates were incubated at ambient room temperature (25–28 °C) for 5 to 7 days.

After the incubation period, fungal colonies were counted. Plates with colony counts between 30 and 300 were

selected for enumeration, and the results were expressed as colony forming units per milliliter (CFU/mL), following the protocol of Odeyemi et al. (2013).

### Isolation and Identification of Fungal Isolates

Distinct fungal colonies were sub-cultured onto fresh PDA plates to obtain pure isolates. The identification of fungal species was carried out based on colony morphology, microscopic features, and staining characteristics using Lactophenol Cotton Blue (LCB) staining, in accordance with the method described by Leslie and Summerell (2006).

### Lactophenol Cotton Blue Microscopy

A drop of 70% ethanol was first placed on a clean microscope slide to help spread the fungal material. A small portion of mycelium from a pure culture was gently removed using a sterile inoculating needle and placed on the slide. One drop of Lactophenol Cotton Blue stain was added, and a coverslip was carefully placed over the preparation. The slide was examined under a compound light microscope using 10x and 40x objective lenses to observe hyphal structures and reproductive features for species-level identification.

### Ethical consideration

The ethical approval for this research was granted by the Ethical Committee of the Department of Botany, Delta State University, Abraka, Delta State, Nigeria.

## RESULTS AND DISCUSSION

**Table 1: Physicochemical parameters of the various wastewater samples (mg/L)**

Parameters	Dry Season			Rainy season			NESREA		WHO	
	Spd1	Spd2	Spd3	Spp1	Spp2	Spp3	DU	AQ	DU	AQ
pH	9.75	9.45	9.65	10.3	9.66	9.35	6-9	6-8.5	6.5-9.2	6.5-8.5
DO	9.00	12.00	12.00	11.00	6.50	6.00	NS	8-10	5.0	4-6
BOD	5.00	7.00	10.50	8.50	3.00	3.50	NS	6-30	NS	5.0
$\text{Mg}^{2+}$	4.62	4.13	3.90	4.30	4.40	4.40	NS	NS	NS	NS
$\text{Ca}^{2+}$	7.62	6.81	6.33	7.13	6.81	6.23	NS	NS	NS	100
$\text{Cl}^-$	1.12	1.03	0.68	1.16	1.55	0.11	NS	350-600	NS	100-250
Sulphate	5.27	3.19	2.10	3.42	2.19	2.01	NS	100	500	500
Nitrate	0.37	0.12	0.12	0.17	0.19	1.01	20	10-20	5	10-50
Phosphate	1.46	0.25	1.20	0.65	1.33	1.35	NS	3.5	5	3-5

NESREA - National Environmental Standards and Regulations Enforcement Agency, WHO - World Health Organisation, DU - domestic use, AQ - aquaculture, NS - not stated (Amoo et al., 2017; Akharam et al., 2017; Egboduku and Olorunfemi, 2016)

Table 1 presents the physicochemical properties of wastewater for both the dry and wet seasons, in comparison with NESREA and WHO standards. The physicochemical analysis revealed variations in water quality parameters across the different sampling points and seasons. The pH values of the wastewater samples ranged from 9.45 to 10.30, exceeding the NESREA and WHO recommended range of 6.5–8.5, indicating an

alkaline nature of the effluent. Such elevated pH levels may pose risks to both aquatic organisms and the structural integrity of ecosystems.

Dissolved oxygen (DO) values ranged from 6.0 to 12.0 mg/L, which falls within acceptable limits and supports the survival of most aquatic organisms. Biochemical oxygen demand (BOD) values ranged from 3.00 to 10.50 mg/L, suggesting the presence of organic matter at

concentrations that may not immediately threaten aquatic life but still require monitoring for long-term ecological balance.

Other parameters, including Magnesium ( $Mg^{2+}$ ): 3.90–4.62 mg/L, Calcium ( $Ca^{2+}$ ): 6.23–7.62 mg/L, Chloride ( $Cl^-$ ): 0.11–1.16 mg/L, Sulphates ( $SO_4^{2-}$ ): 2.10–5.27 mg/L,

Nitrates ( $NO_3^-$ ): 0.12–0.37 mg/L, Phosphates ( $PO_4^{3-}$ ): 0.25–1.46 mg/L. All fell within the recommended permissible limits for effluent discharge, as adapted from NESREA, WHO, and corroborated by earlier studies (Amoo et al., 2017; Akharam et al., 2017; Egboduku & Olorunfemi, 2016).

**Table 2: Fungal count for the various water samples**

Sample	Fungal count (CFU mg/ml)	
1. SPP 1 (Raining season)	$1.7 \times 10^4$	17000
2. SPP 2 (Raining season)	$1.1 \times 10^4$	11000
3. SPP 3 (Raining season)	$2.7 \times 10^4$	27000
4. SPD1 (Dry season)	$9 \times 10^4$	90000
5. SPD 2 (Dry season)	$7 \times 10^5$	700000
6. SPD 3 (Dry season)	$3 \times 10^5$	300000

SPP – species in rainy season; SPD – species in dry season

**Table 3: Fungi isolated from water samples**

Samples	Fungi isolated
1. SPP 1 (Raining season)	<i>Aspergillus flavus</i> , <i>Fusarium</i> spp
2. SPP 2 (Raining season)	<i>Aspergillus flavus</i> , <i>Penicillium glabrum</i> , <i>Cladosporium oxysporum</i>
3. SPP 3 (Raining season)	<i>Rhizopus</i>
4. SPD1 (Dry season)	<i>Fusarium</i> spp, <i>Penicillium glabrum</i> ,
5. SPD 2 (Dry season)	<i>Penicillium glabrum</i> ,
6. SPD 3 (Dry season)	<i>Penicillium glabrum</i> , <i>Rhizopus</i> spp

**Table 4: Percentage of occurrence of fungi isolated from water samples collected Ofuanfo River**

Isolates	Water samples collected from Ofuanfo River							Total	% of occurrence
	SPP1 RS	SPP2 RS	SPP3 RS	SPD1 DS	SPD2 DS	SPD3 DS			
<i>Fusarium</i> spp	+	+	-	-	-	-	2	20	
<i>Rhizopus</i> spp	-	+	-	-	-	+	2	20	
<i>Aspergillus flavus</i>	+	+	-	-	-	+	2	20	
<i>Penicillium glabrum</i>	-	-	-	+	+	+	3	30	
<i>Cladosporium oxysporum</i>	-	+	-	-	-	-	1	10	
							10	100	

RS – rainy season, DS - dry season

The percentage of fungi isolated from the samples, as presented in Table 4 shows that *Fusarium* sp. was present in the samples collected during the rainy season but absent in those collected during the dry season. *Rhizopus* sp. and *Aspergillus flavus* were present in samples from both the rainy and dry seasons. *Penicillium glabrum* was

found only in the samples collected during the dry season and absent in the rainy season samples. *Cladosporium oxysporum* was present exclusively in the rainy season samples. The predominant fungal species was *Penicillium glabrum*, with a 30% occurrence, while the least predominant species was *Cladosporium oxysporum*, with 10% occurrence.

**Table 5: Identification of Fungi Isolated**

Samples	Macroscopic	Microscopic
1. <i>Aspergillus flavus</i>	The colonies on the upper surface were olive green with a white edge. The reverse side exhibited a green coloration. The colonies were ovoid in shape, measuring 2.7 cm in diameter after 5 days of incubation at room temperature on Sabouraud Dextrose agar.	It contained thick-walled conidiophores that were hyaline, slightly roughened, erect, long, and aseptate, with a vesicle at the top bearing phialides and short conidial chains. The length of the conidiophore was 95 µm.
2. <i>Penicillium glabrum</i>	The colonies had a green, cottony upper surface. They also had a white border and a brown coloration on the reverse side when grown on Sabouraud Dextrose Agar. The colonies were round in shape, varied in size, and measured up to 1.2 cm within 5 days at room temperature.	The conidiophore was long, thick-walled, and hyaline, measuring up to 87 µm in length. The conidial head was bluish near the apices with an irregular shape. The conidia measured approximately 5 µm in diameter, with an average size of 4 µm.
3. <i>Fusarium</i> spp	The colony was pink with white patches on the surface, and the reverse side was brown in coloration on Potato Dextrose Agar. The colony was round in shape and measured 4 cm in diameter after 5 days at room temperature.	The macroconidia were canoe-shaped, multiseptate, containing 3–6 septations, and slightly pointed at the ends. They measured 32 µm in length. The microconidia were oblong, borne singly, non-septate, and single-celled.
4. <i>Cladosporium oxysporum</i>	The color was greyish-green, while the reverse side was dark brown to black. The colonies were slow-growing. They had vegetative hyphae, and the conidiophores and conidia were pigmented.	It contained septate brown hyphae, erect pigmented conidiophores, and conidia that occurred in branching chains which readily disarticulate.
5. <i>Rhizopus</i>	Non-septate or sparsely septate broad hyphae (6–15 µm in diameter), sporangiophores, rhizoids (root-like hyphae), and sporangia.	The texture was cotton-candy-like. From the front, the color of the colony was initially white and then turned grey. The reverse was white to pale.

## Discussion

This study assessed the physicochemical characteristics and fungal diversity of wastewater discharged from the Industrial Glass Manufacturing Facility in Ughelli, Delta State, Nigeria., Delta State, Nigeria. The findings revealed that while most physicochemical parameters of the wastewater fell within the permissible limits set by NESREA and WHO, the elevated pH and presence of potentially pathogenic fungi raise important environmental and public health concerns.

The pH values recorded in this study (9.45–10.30) exceeded the recommended standards of 6.5–8.5 (Table 1). Elevated pH levels can alter aquatic ecosystems by reducing biodiversity and influencing the solubility and toxicity of other contaminants (Barakat, 2011). High alkalinity may result from industrial discharges containing soda ash or other glass-manufacturing by-products. Similar findings were reported by Akharam et al. (2017), who observed increased pH in wastewater from glass industries. The dissolved oxygen (DO) values (6.0–12.0 mg/L) indicate that the wastewater has the potential to support aerobic microbial and aquatic life, especially during early discharge. However, DO levels above 10 mg/L

in industrial effluents may sometimes reflect chemical oxidation processes rather than biological health, suggesting the need for further investigation into the effluent composition. The BOD values (3.00–10.50 mg/L) also suggest the presence of moderate organic pollution. According to Ani et al. (2016), high BOD levels may result in oxygen depletion in receiving water bodies, which can adversely affect aquatic organisms. The levels of other ions, including magnesium, calcium, sulphates, nitrates, chlorides, and phosphates, remained within permissible limits. These findings are in agreement with earlier studies (Amoo et al., 2017; Egboduku & Olorunfemi, 2016), indicating that industrial wastewater, when moderately treated or diluted, may not pose immediate chemical threats to surface water systems. However, continuous accumulation of even trace elements can lead to long-term ecological impacts.

Five fungal species, *Aspergillus flavus*, *Fusarium* sp., *Penicillium glabrum*, *Cladosporium oxysporum*, and *Rhizopus* sp.—were isolated from the wastewater samples. These genera are commonly associated with industrial and polluted environments due to their adaptive metabolic strategies and resistance to environmental

stressors (Leslie & Summerell, 2006). *Aspergillus flavus* is particularly notable for its ability to produce aflatoxins, secondary metabolites that are carcinogenic and have been implicated in both acute and chronic health disorders (Richa et al., 2012). The presence of *Fusarium* sp. also raises concern, as some species can produce mycotoxins such as fumonisins and trichothecenes, which are harmful to both humans and animals.

The detection of these fungi in wastewater suggests that the effluent may serve as a reservoir for opportunistic pathogens, especially if discharged into natural water bodies used for domestic or agricultural purposes. This aligns with findings by Medeiros et al. (2009), who emphasized that wastewater fungi could act as emerging contaminants due to their ability to persist in aquatic environments and influence both ecological and human health. Moreover, the presence of fungal spores in effluents may contribute to biofilm formation in aquatic systems, potentially affecting water quality, infrastructure, and treatment processes. The findings underscore the need to integrate microbiological monitoring, particularly of fungal species, into routine water quality assessments. Although this study sampled during both dry and rainy seasons, seasonal comparisons of the data indicate that rainfall may dilute some chemical parameters, while runoff could introduce additional contaminants into the system. This suggests that effluent monitoring should be conducted year-round to capture temporal variations and potential peak pollution periods, as emphasized by Rankovic (2005) and Roache et al. (2006).

Given the limitations of this study, which include the absence of toxicological assessments and molecular characterization of the fungal isolates, there is a need for further research to deepen understanding of the potential risks posed by industrial wastewater. Future studies should investigate the toxic effects of elevated pH and fungal metabolites (e.g., aflatoxins) on aquatic organisms and local populations reliant on these water resources. Employing molecular tools would allow for more precise identification of fungal species and provide insights into their pathogenicity and resistance profiles. Long-term, seasonal monitoring is also essential to capture temporal variations in both physicochemical and microbial parameters, thereby offering a more comprehensive risk profile. Additionally, exploring the bioremediation potential of native fungi or other microorganisms from the wastewater could offer sustainable treatment solutions. Finally, epidemiological studies are recommended to evaluate the health risks faced by workers and nearby communities exposed to untreated or inadequately treated effluents. Nonetheless, this study highlights the importance of integrating microbiological assessment into routine wastewater monitoring. While the wastewater from the Beta Glass Company may not be overtly toxic based on physicochemical criteria, the detection of pathogenic

fungi suggests the potential for long-term environmental and public health implications. Continuous surveillance and treatment of industrial effluents are recommended to mitigate the risk of fungal contamination in receiving water bodies and ensure the protection of human and aquatic health.

## CONCLUSION

This study demonstrated that the wastewater discharged from the Industrial Glass Manufacturing Facility in Ughelli, Delta State, Nigeria., Ughelli, generally complies with NESREA and WHO standards for most physicochemical parameters, except for pH, which was consistently above the recommended range, indicating an alkaline effluent. The presence of five fungal species, *Aspergillus flavus*, *Fusarium* sp., *Penicillium glabrum*, *Cladosporium oxysporum*, and *Rhizopus* sp. raises concerns about potential pathogenic risks to both aquatic ecosystems and human health. While the wastewater may not be overtly toxic chemically, the elevated pH and microbial profile suggest that the effluent could pose ecological disturbances and public health challenges if discharged untreated or inadequately treated into the environment. Continuous monitoring and improved wastewater management are essential to mitigate these risks. Based on the findings, it is recommended that Glass Manufacturing Facilities and allied industries should implement or upgrade their existing wastewater treatment technologies to ensure that pH levels are regulated within acceptable standards before discharge. In addition, routine microbial monitoring, particularly of fungal biodiversity, should be integrated into wastewater quality control programs to identify and mitigate potential pathogenic threats. Environmental authorities should also conduct comprehensive environmental impact assessments (EIAs) to evaluate the effects of alkaline wastewater and fungal contaminants on surrounding aquatic ecosystems. Furthermore, there is a need to increase public and industry awareness about the importance of effluent quality compliance, while regulatory agencies such as NESREA must enforce stricter adherence to environmental standards to protect public health and the environment.

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