

Assessment of mycological profile and heavy metals concentration of Orogodo River in Delta State, Nigeria

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ABSTRACT

Fungal and heavy metals pollution of rivers are of public health concerns. The mycological and heavy metal characteristics of surface water from Orogodo River in Delta State, Nigeria, were assessed during a single sampling period (February, 2025), across three sampling stations namely; Mbiri, Owa Nta and Agbor, using standard analytical methods including culture-based fungal enumeration, morphological identification and atomic absorption spectrophotometry (AAS) for the determination of metals. Fungal colony forming units (CFUs) were recorded as $10.44 \pm 2.14 \times 10^3$ CFU/mL, $10.44 \pm 2.26 \times 10^3$ CFU/mL, and $9.88 \pm 1.99 \times 10^3$ CFU/mL for Site I (Mbiri), Site II (Owa Nta), and Site III (Agbor), with identified aquatic fungi; *Saccharomyces* sp., *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Rhizopus* sp. Species distribution analysis revealed *Saccharomyces* sp. as the most prevalent isolate, present in the respective stations surveyed while *Penicillium chrysogenum*, *Rhizopus* sp., and *Fusarium oxysporum* were among the least common. Heavy metal analysis of the river's surface water showed elevated concentrations of iron (0.76 mg/L) and cadmium (0.05 mg/L), both of which exceeded the World Health Organization and Nigeria Standards for Drinking Water Quality permissible limits (0.3 mg/L and 0.003 mg/L). These results suggest localized fungal and metal contamination and warrant broader seasonal monitoring.

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INTRODUCTION

The Orogodo River originates near Mbiri village at approximately 150 m above sea level, draining a 465 km² catchment within the sand-silt complex of the Benin Formation (Issa *et al.*, 2011). Flowing southwest for about 75 km, it eventually merges with the Ethiope basin at Urhuoka in northern Delta State. As a third-order tropical stream, its width ranges from 6–8 m in the upland areas to nearly 20 m across the Agbor floodplain. Peak flows, typically occurring between April and September during convective storms, can elevate the river's stage by over 1.2 m within six hours (Iwegbue *et al.*, 2012). Rivers are dynamic ecosystems that host diverse microbial communities, including fungi, which are key agents in nutrient cycling and organic matter decomposition. The study of aquatic fungi, or riverine mycology, has gained attention due to their ecological significance and responsiveness to environmental changes (Gulis & Suberkropp, 2009; Shearer *et al.*, 2007). Saprophytic and pathogenic fungi contribute to the breakdown of leaf litter and organic debris, enhancing nutrient turnover and energy flow within aquatic food webs (Bärlocher & Boddy, 2016; Gulis & Suberkropp, 2003). Aquatic fungi are generally grouped into aquatic hyphomycetes, chytrids, and yeasts, with hyphomycetes being particularly prevalent in freshwater systems (Suberkropp, 1992). These organisms colonize plant detritus and biofilms, producing extracellular enzymes that degrade complex compounds like cellulose and lignin, thereby accelerating decomposition and influencing carbon cycling (Findlay, 2010; Kaushik & Hynes, 1971). Fungi also interact with other microbes, such as bacteria and algae, forming

complex biofilms that stabilize sediments and modulate nutrient dynamics (Hofmann & Wurzbacher, 2019; Grossart *et al.*, 2019).

Fungal diversity and abundance in river systems are shaped by factors such as temperature, pH, organic matter availability, and pollutant load (Shearer *et al.*, 2007; Gulis & Suberkropp, 2014). Among pollutants, heavy metals are particularly impactful, often altering fungal community structures by promoting metal-tolerant species and suppressing sensitive ones (Singh *et al.*, 2016; Qian *et al.*, 2020). In rivers affected by mining or industrial discharge, reduced fungal diversity but increased presence of resistant taxa has been observed (Srivastava & Thakur, 2014; Mohan *et al.*, 2016).

In Nigeria and other tropical African regions, studies on riverine fungi remain limited. However, emerging research has reported the presence of genera such as *Cladosporium*, *Aspergillus*, and *Penicillium* in river sediments, water, and submerged plant materials, often showing tolerance to heavy metals and organic pollutants (Okhuoya *et al.*, 2000; Ekwu & Iwuoha, 2021; Ekwu *et al.*, 2018). The Orogodo River which is subject to urban runoff and abattoir waste, is a conducive environment for such fungi, some of which may possess bioremediation potential (Ogbe & Arimoro, 2019; Asibor, 2017). Beyond ecological roles, fungi in polluted waters also present public health concerns, as certain species can act as opportunistic pathogens. Waterborne fungi such as *Candida*, *Aspergillus*, and *Fusarium* have been detected in contaminated rivers, raising concerns for communities that rely on these waters for domestic use (Khan & Ahmad, 2018; Chowdhury *et al.*, 2019). Thus, mycological assessments are

vital for both environmental monitoring and health risk evaluation (Gulis & Suberkropp, 2009; Wang *et al.*, 2021). Fungal communities in rivers also exhibit seasonal variability. Studies have reported fluctuations in spore counts and species richness between wet and dry seasons, corresponding with changes in water flow, temperature, and organic matter input (Harrison, 2005; Shearer *et al.*, 2007). Such temporal dynamics highlight the need for long-term monitoring to understand fungal ecology in fluvial systems (Suberkropp *et al.*, 2016).

Rivers often act as conduits for heavy metals introduced through industrial effluents, mining, agriculture, and domestic waste (Kumar & Singh, 2017; Olawale *et al.*, 2020). In countries like Nigeria, rapid urbanization and inadequate waste management have intensified the contamination of rivers with metals such as lead (Pb), cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), and zinc (Zn) (Eze *et al.*, 2021; Okoye & Ezema, 2020). Even at low concentrations, these metals are toxic due to their non-biodegradable nature and their ability to interfere with biological processes (Sarkar *et al.*, 2016; Zhang *et al.*, 2018). Their distribution in rivers is influenced by factors such as geology, hydrology, and seasonal weather patterns. Metals can bind to suspended particles or settle on the sediments, affecting their bioavailability and toxicity (Wu *et al.*, 2017; Li *et al.*, 2019). During dry seasons, reduced water flow and low water volume can lead to higher concentrations due to limited dilution (Zhang *et al.*, 2018; Olawale *et al.*, 2020). The Orogon River, located in an industrially active part of Delta State, is especially vulnerable to heavy metals and other contaminants capable of causing pollutions (Ogbe & Arimoro, 2019; Asibor, 2017). Activities such as automobile repair, waste dumping, and livestock slaughtering introduce contaminants through runoff and direct discharge. This poses

significant ecological and public health risks (Gimba, 2026), affecting both aquatic organisms and nearby communities. Heavy metals represent some of the most persistent and hazardous pollutants in aquatic environments. Their accumulation threatens biodiversity, degrades water quality, and impairs ecological services like nutrient cycling and water purification (Alloway, 2013; Tchounwou *et al.*, 2012). Unlike biological contaminants, heavy metals do not degrade biologically. Instead, they accumulate in sediments and organisms, leading to biomagnification across trophic levels (Zhou *et al.*, 2016; Singh & Kumar, 2017). Heavy metals adversely impact aquatic life, including fungi, algae, invertebrates, and fish, by disrupting physiological and reproductive functions (Sarkar *et al.*, 2016; Li *et al.*, 2019). Yet, some fungi demonstrate metal tolerance and bioaccumulation abilities, suggesting potential for bioremediation (Mohan *et al.*, 2016; Singh *et al.*, 2016). Given the river's exposure to various pollutants, and lack of mycological data on the River Orogon this study aimed to assess the mycological profile and heavy metals concentrations in River Orogon in Delta State.

MATERIALS AND METHODS

The Orogon River is located between latitudes 5°10' and 6°20' N and longitudes 6°10' and 6°21' E (see Fig. 1). Its primary source is groundwater seepage from an aquifer beneath the dense rainforest of Mbiri, with additional inputs from rainfall, municipal discharge, and surface runoff from surrounding riparian communities. The river flows through Agbor, a major town in southern Nigeria. The riverbed is composed predominantly of fine sand mixed with mud, with occasional deposits of coarse sand and pebbles. Decomposing macrophytes and organic debris also contribute to the composition of the substratum.

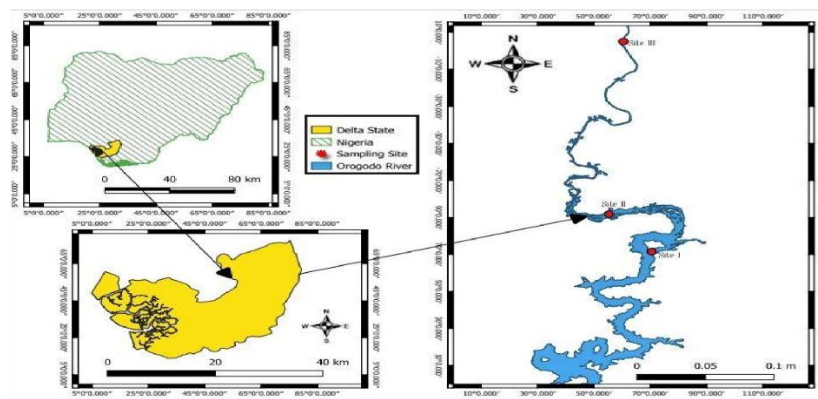


Figure 1: Sampling site locations and corresponding coordinates

Water sampling was carried out in February 2025, just prior to the onset of the rainy season. The grab sampling method was used in accordance with standard procedures outlined by the World Health Organization (WHO) and the American Public Health Association (APHA) for surface water analysis. At each sampling point, clean, sterile 1-liter plastic containers were used to collect water samples. Samples from each sampling stations were collected in duplicates. To preserve sample integrity, the containers were immediately stored in ice-filled coolers and transported to the laboratory within 4 to 6 hours. The selected sampling sites were:

- i. Site I (Upstream) – Mbiri
- ii. Site II (Midstream) – Owa Nta
- iii. Site III (Downstream) – Agbor

These locations were chosen based on their potential exposure to varying degrees of anthropogenic activities.

Fungi Enumeration

Fungal isolation was conducted following the standard protocol described by Musa *et al.* (2025). All samples were microbiologically analyzed on the day of collection to minimize the risk of microbial degradation. Upon arrival at the laboratory, the samples were allowed to stand at ambient temperature (28 °C) on a sterile workbench prior to processing. 9.0 mL was aseptically dispensed in duplicate into sterile centrifuge tubes. The samples were centrifuged using a centrifuge 800D (G. Bosch, Germany) at 250 rpm for 10 minutes to concentrate the microbial content. After centrifugation, the supernatant was carefully decanted, and 0.1 mL of the resulting residue was aseptically spread onto two types of culture media: Potato Dextrose Agar (PDA) and Rose Bengal Chloramphenicol Agar. Each plating was performed in duplicate using a sterile bent glass rod. To

inhibit bacterial growth, 50 µg/L of chloramphenicol was added to the PDA plates. The inoculated plates were incubated at ambient temperature (28 °C) for 5 days to allow for fungal growth.

Determination of Heavy Metals (Pb, Fe, Cr, Cd and Ni)

The analysis of lead (Pb), iron (Fe), chromium (Cr), cadmium (Cd), and nickel (Ni) concentrations in the samples was performed using the APHA 3111B standard protocol (APHA, 2017). A 100 mL aliquot of each sample was measured using a graduated cylinder and transferred into a clean conical flask. To each sample, 10 mL of a concentrated 1:1 hydrochloric acid/nitric acid (HCl/HNO₃) mixture was added, along with 23 g of anti-bumping granules. The mixture was heated on an electrothermal heater until the volume was reduced to approximately 30–50 mL. After digestion, the sample was allowed to cool to room temperature and then filtered through Whatman No. 42 filter paper. The filtrate was quantitatively transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. Single-element calibration standards were prepared by serial dilution of 1000 mg/L stock solutions for each metal (Pb, Fe, Cr, Cd, and Ni). A minimum of three working standard solutions ranging from 0.1 mg/L to 10 mg/L was prepared for each element. External calibration

was performed using deionized water and the respective standard solutions. Calibration curves were generated for each metal by plotting absorbance against concentration. Both the digested samples and reagent blanks were analyzed using Atomic Absorption Spectrophotometry (Spectraa 55B, USA). The concentration of each metal in the samples was determined by interpolating the absorbance values on the corresponding calibration curves.

RESULTS AND DISCUSSION

The heterotrophic fungal counts from the Orogo River sampling points are summarized in Table 1. Colony-forming unit (CFU) counts per milliliter of water were recorded as $10.44 \times 10^3 \pm 2.14$ CFU/mL at Mbiri (Site I), $10.44 \times 10^3 \pm 2.26$ CFU/mL at Owa Nta (Site II), and $9.88 \times 10^3 \pm 1.99$ CFU/mL at Agbor Town Center (Site III). The cultural and morphological characteristics of fungal isolates recovered from the various sampling sites along Orogo River are detailed in Table 2. The distribution pattern of fungal species across the sampling locations is presented in Table 3, where *Saccharomyces* species were the most frequently isolated, while *Penicillium chrysogenum*, *Rhizopus* sp., and *Fusarium oxysporum* were among the least common. Heavy metal concentrations at each sampling point are reported in Table 4.

Table 1: Total Heterotrophic Fungal Count of the Orogo River

Sampling Site	Name of Location	Fungi (CFU) Mean $\pm$ SD
I	Mbiri	$10.44 \times 10^3 \pm 2.14$
II	Owa Nta	$10.44 \times 10^3 \pm 2.26$
III	Agbor	$9.88 \times 10^3 \pm 1.99$

Table 2: Cultural and Morphological Characteristics of the Fungal Isolates from the Orogo River

Cultural Characteristics	Morphological Characteristics		
Nature of Colony	Nature of Hyphae	Spore Type	Organism
Cream colonies with pale reverse side	Pseudohyphae	Chlamydospore	<i>Saccharomyces</i> sp.
Fluffy colonies with black spores and a pale reverse side	Septate	Conidiospore	<i>Aspergillus niger</i>
White fluffy colonies with cream reverse side	Septate	Chlamydospore	<i>Fusarium oxysporum</i>
Green dome shaped colonies with dirty white colouration	Septate	Conidiospore	<i>Penicillium chrysogenum</i>
Fluffy grey colonies with black spores at the air interface	Non-septate	Sporangiospore	<i>Rhizopus</i> sp.

Table 3: Distribution Patterns of the Fungal Isolates across Orogo River

Sites	<i>Saccharomyces</i> sp.	<i>Rhizopus</i> sp.	<i>Aspergillus niger</i>	<i>Fusarium oxysporum</i>	<i>Penicillium chrysogenum</i>
Ia*	+				
Ib		+			
Ic	+				
IIa**			+		+
IIb			+		
IIc	+				
IIIa***	+				
IIIb	+			+	
IIIc	+				

*- Different points within site I (Mbiri) **- Different points within site II (Owa Nta)***- Different points within site III (Agbor)

Table 4: The Concentration of Heavy Metals from the Various Points along Orogo River

Heavy metal	Site I	Site II	Site III	WHO (mg/L)
Iron, Fe (mg/L)	0.76	<LoD	<LoD	0.3
Lead, Pb (mg/L)	<LoD	<LoD	<LoD	0.01
Cadmium, Cd (mg/L)	<LoD	0.05	<LoD	0.003
Chromium, Cr (mg/L)	<LoD	<LoD	<LoD	0.05

Heavy metal	Site I	Site II	Site III	WHO (mg/L)
Nickel, Ni (mg/L)	<LoD	<LoD	<LoD	0.01

Key: <LoD = Below Limit of Detection (Fe (0.005-0.01mg/L); Pb (0.01-0.015 mg/L); Cd (0.0015-0.002 mg/L); Cr (0.003-0.004 mg/L); Ni (0.005-0.007)

As shown in the table above, iron (Fe) and cadmium (Cd) were detected in water samples collected from Sites I and II. The concentrations of these metals exceeded the World Health Organization (WHO) permissible limits for safe drinking water. In contrast, none of the analyzed heavy metals were detected in samples from Site III.

Discussion

Fungal presence in freshwater ecosystems, particularly rivers intersecting urban and peri-urban areas, is typically linked to organic pollution, sewage discharge, and decaying vegetation (Adeniyi et al., 2020). In this study, total heterotrophic fungal counts in the Orogo River exceeded WHO (2022) and Nigeria Standards for Drinking Water Quality (NSDWQ) (2015) guidelines for potable water, which recommend a limit of 1.00 CFU/mL. While no specific standards exist for untreated surface water, the elevated values ($9.88 - 10.44 \times 10^3$ CFU/mL) raise public health concerns, especially in communities relying on such water for domestic use. These findings are consistent with earlier studies (Eze et al. 2019; Oyetibo et al., 2021). Eze et al., 2019 reported fungal counts ranging from $4.2 - 7.8 \times 10^3$ CFU/mL in relatively unpolluted rivers, suggesting higher organic or anthropogenic loads in the Orogo River. The uniform fungal counts across Sites I, II, and III indicate widespread contamination rather than isolated hotspots, likely due to continuous non-point source pollution such as agricultural runoff, sewage, and domestic waste (Oyetibo et al., 2021). Species distribution analysis revealed *Saccharomyces* sp. as the most prevalent isolate, present in six subsites. Its dominance indicates sugar-rich organic inputs. *Aspergillus niger* found at multiple downstream locations, reflects its ecological adaptability and pathogenic potential. Less frequent isolates, such as *Penicillium chrysogenum*, *Rhizopus* sp., and *Fusarium oxysporum*, suggest localized sources from decaying plant matter, or agricultural runoff. Sites II and III exhibited greater species diversity, likely due to their location downstream of dense human settlements and market activities. This aligns with findings by Chukwura and Okpokwasili (2020), who noted higher microbial diversity in river segments near peri-urban or commercial zones. These fungi, some of which are opportunistic pathogens, pose risks of skin, respiratory, and gastrointestinal infections, especially in immunocompromised individuals. Spores from *Aspergillus* and *Penicillium* are particularly concerning due to their potential for aerosolization during domestic use. Heavy metal analysis revealed iron (Fe) and cadmium (Cd) contamination at Sites I and II, respectively. Iron was detected at 0.76 mg/L—more than double the WHO guideline of 0.3 mg/L. While essential in trace amounts, excess iron can contribute to biofilm formation, turbidity, and aesthetic degradation. Its presence at Site I may stem from lateritic soil weathering, rusting infrastructure, or runoff from construction debris. Cadmium, detected at 0.05 mg/L at Site II, significantly exceeded the WHO and NESREA limit of 0.003 mg/L. Unlike iron, cadmium is non-essential and highly toxic, associated with kidney damage, skeletal deformities, and carcinogenic effects. Its occurrence suggests point source pollution, possibly from battery disposal, electronic waste, or agrochemical runoff. The absence of cadmium in Sites I and III indicates localized contamination rather than widespread distribution. Lead (Pb), chromium (Cr), and nickel (Ni) were

not detected in any of the samples. While encouraging, this does not rule out their presence in sediments or bioavailable forms. Metals can be adsorbed onto particles and later re-released under changing environmental conditions such as pH shifts or hydrological disturbance (Jaboro et al., 2013). Hence, sediment monitoring is recommended for a more comprehensive risk assessment.

CONCLUSION

In conclusion, the Orogo River shows clear signs of fungal diversity and heavy metals such as Fe and Cd contamination. The detected Cd concentration and elevated Fe warrant concern, while the cultured fungi indicated notable fungal presence in the sampled river water.

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