Isolation and Characterization of Fungal Strains from Tilapia (*Oreochromis niloticus*) and Machoiron (*Chrysichthys nigrodigitatus*) Fish in Taabo Lake, Kossou Lake, and Tagba Lagoon of Grand-Lahou, Côte d’Ivoire

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ABSTRACT

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| This study aimed to isolate and characterize fungal strains contaminating freshwater fish (Tilapia and Machoiron) in three water bodies in Côte d'Ivoire: Lake Taabo, Lake Kossou, and the Tagba Lagoon in Grand-Lahou. A total of 120 samples were collected during four seasonal campaigns (dry and rainy seasons) and analyzed in the laboratory. Molds were isolated on acidified Potato Dextrose Agar (PDA) medium (pH 3.5) at 30°C and identified through macroscopic (color, texture) and microscopic (spore morphology, hyphae) observations. Contamination frequencies were calculated for each fungal genus. Among the 156 isolated strains, five dominant genera were identified: *Aspergillus* (57.69%), *Rhizopus* (25%), *Fusarium* (11.54%), *Penicillium* (3.85%), and *Absidia* (1.92%). *Aspergillus* was ubiquitous, while *Rhizopus* and *Fusarium* were more abundant during the rainy seasons. The analyzed fish showed high contamination rates: 75% for *Aspergillus*, 32.5% for *Rhizopus*, and 15% for *Fusarium*. These results confirm the presence of potentially toxigenic molds in fish, with associated risks of mycotoxin production (aflatoxins, ochratoxins). The contamination reflects environmental conditions and local practices. The study recommends establishing regulatory standards to limit consumer exposure and improve food safety. |

*Keywords: Molds, freshwater fish, Côte d’Ivoire, seasonal variation, Tilapia, Machoiron.*

1. INTRODUCTION

Freshwater fish constitute an essential resource for many populations, notably those in Africa. In Côte d’Ivoire, these fishery resources play a crucial role in both the diet and the economy of riverside populations. In fact, freshwater fish represent 50% of total fish consumption,estimated at 650,000 tonnes in 2021, and provide 42% of animal protein intake while supporting more than 70,000 direct and 400,000 indirect jobs (Failler *et al*., 2014; FAO, 2022; Mason *et al*., 2022). However, freshwater fish may contain chemical, physical, and biological contaminants capable of causing health disorders in the fish, and potentially in consumers, particularly due to toxin production, which also degrades the fish’s market quality. In humid tropical zones, climatic conditions favor the proliferation of microscopic fungi, which frequently leads to the contamination of food products such as fish (Chelack *et al*., 1991). Molds can be found in natural environments, including freshwater ecosystems and fish, through feeding, runoff from mold-contaminated fields, and the degradation of organic matte. (Caruso *et al*., 2013; Yang *et al*., 2023). The contamination of freshwater fish by molds is thus a growing concern due to its potential impact on both fish health and human health. It is in this context, that the present study was undertaken. Its objective is to isolate and characterize the fungal strains which aims to isolate and characterize the fungal strains responsible for the contamination of freshwater fish, thereby contributing to the identification of bio-indicators of freshwater pollution in humid tropical environments.

2. material and methods

2.1 Material

2.1.1 Biological material

The study was conducted on two species of freshwater fish, Tilapia (*Oreochromis niloticus*) and Machoiron (*Chrysichthys nigrodigitatus*) (Fig. 1), collected from fishermen operating in Taabo Lake, Kossou Lake, and the Tagba Lagoon in Grand-Lahou.



**Fig. 1. Freshwater fish species used in this study. A: Machoiron fish (*Chrysichthys nigrodigitatus*); B: Tilapia fish (*Oreochromis niloticus*)**

2.1.2 Laboratory equipment

Several items of laboratory equipment and consumables were used throughout this study: Culture media (Sabouraud with chloramphenicol and PDA), an oven set at 25°C, stomacher bags, scalpel blades, pipettes, Petri dishes, slides and cover slips, methylene blue, and a light microscope.

2.2 Methods

2.2.1 Characterization of study areas

The freshwater fishwere collected from three (03) water bodies:  **Tagba Lagoon in Grand-Lahou**, which originates from the Bandama River, is located in the Grands-Ponts region of southern Côte d'Ivoire. The area is characterized by a Guinean equatorial climate, with alternating periods of two rainy and two dry seasons of varying duration. The average annual temperature is 27.5°C, and average annual rainfall reaches 1664 mm, primarily between June and July (Alexandre *et al*., 2019).

**Lake Taabo**, also fed by the Bandama River, is located in the Agnéby-Tiassa region. It experiences an Attiéen climate, typical of transitional equatorial zones, with four distinct seasons: two rainy seasons (the main one from April to June and a shorter one from September to November) and two dry seasons (a long one from December to March and a shorter one from July to September) (Koffi *et al*., 2018; Kouassi *et al*., 2007).

**Lake Kossou**, located in the Yamoussoukro District of the Lacs region, also receives inflow from the Bandama River. He climate features two dry seasons (November–February and July–August) and two rainy seasons (March–June and September–October). Rainfall is heavier in the eastern part of the region, peaking in May, June, and September. Annual temperatures range between 19°C and 34°C (Groga *et al*., 2022).

2.2.2 Sampling

Four (04) sampling campaigns were conducted between December 2023 and November 2024 across the three study sites, covering the main seasonal periods: the long dry season (December 2023 to March 2024), the long rainy season (April to July 2024), the short dry season (July to September 2024), and the short rainy season (September to November 2024). During each campaign, ten fish were collected per site, totaling 120 freshwater fish samples (n = 120). The fish were obtained from fishermen between 8:00 and 10:00 GMT. During each sampling session, dissolved oxygen, water temperature, and pH were measured in situ using an HQ40d multi-parameter meter. Samples were transported under aseptic conditions in a cooler at -4°Cto the Food Microbiology Laboratory at National Polytechnic Institute Félix HOUPHOUET-BOIGNY (INP-HB) for analysis.

2.2.3 Isolation and Purification of Molds

Molds were isolated aseptically by culturing the skin, flesh, and internal organs of the fish on acidified PDA medium (pH 3.5). The pH was adjusted with 10% citric acid to inhibit bacterial growth and favor mold development. The PDA medium was prepared by dissolving 20 g of potato dextrose powder, 15 g of agar, and 15 g of glucose in 1L of distilled water, followed by autoclaving at 121°C for 15 minutes. After cooling, the citric acid solution was added, and the medium was poured into 90 mm Petri dishes (Emanfo *et al*., 2013). Once solidified, 10 g of tissue from each fish sample was inoculated onto agar surface. The cultures were incubated at 30°C for 3 to 5 days (Abdoullahi *et al*., 2019a). To obtain pure fungal strains, subcultures were made by transferring a fragment from an idolated colony to fresh media, avoiding contact with neighboring colonies (Guiraud, 1998).

2.2.4 Mold Identification

***2.1.1.1 Macroscopic Identification***

Macroscopic identification was performed by visually observing young cultures (<5 days old) grown on PDA medium. Observations were based on characteristics such as colony color, shape, texture, pigment production, spore density, and growth rate, following criteria byPitt & Hocking (1997, 2009); Samson *et al*. (2014). Colony surface morphology such as fluffy, velvety, powdery, granular, flat, or raised, was also noted (Compaore *et al*., 2016; Olga *et al*., 2015).

***2.1.1.1 Microscopic Identification***

Microscopic identification was carried out using fresh mounts stained with 1% methylene blue, prepared as described by Abdoullahi et al. (2019); Nguyen (2007). A sterile needle was used to collect a small amount of fungal material, which was spread onto a slide, stained, and examined under ×40 and ×100 magnification. Microscopic features examined included: mycelial morphology and branching, structure of conidiophores, presence and morphology of viesicles, arrangement of conidiogenous (uniseriate or biseriate), spore characteristics (size, shape, color, and surface features such as verrucose, smooth, or granular) (Abarca *et al*., 2004; Boudih, 2011; Compaore et al., 2016; J. I. Pitt & Hocking, 1997, 2009a; Schuster *et al*., 2002). Contamination prevalence and frequency were calculated using the formulas by Marasas *et al*. (1988).

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3. results and discussion

3.1 Results

3.1.1 Characterization of Isolated Fungal Strains

One hundred and fifty-six (52) fungal strains were initially isolated from fresh specimens of Tilapia and Machoiron collected from Lake Taabo, Kossou and the Tagba Lagoon in Grand-Lahou. Tables 1 and 2 summarize the cultural and microscopic features of representative fungal genera.

3.1.2 Identification of Isolated Fungal Strains

A total of 156 fungal strains were isolated from fresh fish samples (Machoiron and Tilapia) collected from Taabo Lake, Kossou Lake, and Tagba Lagoon across four distinct climatic seasons (Table 3). These isolates were distributed among five genera: *Aspergillus (*sections *Nigri* and *Flavi*), *Fusarium* (M4-B3), *Absidia* (M1-B3), *Penicillium* (H2O1), and *Rhizopus* (T-B1).

The *Aspergillus* genus was the most prevalent, with 90 strains representing 57.69% of the total isolates. *Rhizopus* accounted for 39 isolates (25%), followed by *Fusarium* with 18 isolates (11.54%). Penicillium and Absidia were less frequently isolated, with 6 (3.85%) and 3 (1.92%) strains respectively.

Within the *Aspergillu*s genus, 76.67% (69/90) belonged to section *Nigri*, 16.67% to section *Flavi*, and 6.66% to *section Fumigati*. Figure 2 illustrates the relative prevalence of the isolated fungal genera.

**Table 1. Macroscopic characteristics of fungal strains isolated from fresh fish**

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| **Codes** | **PDA Culture Description** | **Macroscopic Images** | | **Reference Images** |
| **Obverse** | **Reverse** |
| Moi 21 | Rapid growth with rounded margins, compact thalli initially yellow, becoming olive-green at maturity with fluffy texture; red pigment production on colony reverse. |  |  | A. flavus isolated from aural debris  **(Pitt & Hocking, 1997)** |
| T-B1 | Highly invasive, filamentous strain displaying white to gray coloration with black sporangia at margins; culture reverse appears colorless. |  |  | **(BEUGRE, 2024)** |
| H201 | Rapid growth with velvety appearance, white thallus, and dark green conidia; red pigments visible on colony reverse. |  |  | **(BEUGRE, 2024)** |
| Moi 09 | Very rapid growth with rounded margins, extremely compact black thalli displaying granular texture; no diffusible pigments observed on colony reverse. |  |  | **(Pitt & Hocking, 1997)** |
| M4-B3 | Rapid growth with aerial mycelia and crescent-shaped, septate conidia of cream-white coloration; pink to red pigments visible on colony reverse. |  |  | **(Chabasse et al., 2002)** |
| M1-B3 | Rapid growth covering the Petri dish, forming low white colonies with granular texture; beige toyellow pigments visible on colony reverse. |  |  | **(Chabasse et al., 2002)** |

**Table 2. Microscopic characteristics of fungal strains isolated from fresh fish**

|  |  |  |  |
| --- | --- | --- | --- |
| **Codes** | **Microscopic Description** | **Microscopic Images** | **Reference Images** |
| Moi 21 | Septate and branched hyphae with rough-walled conidiophores. Vesicles bearing biseriate phialides and metulae are arranged radially, forming conidial heads containing oval, green-colored spores. |  | **(Pitt & Hocking, 2009b)** |
| T-B1 | Nonseptate (coenocytic) and branched hyphae bearing terminal vesicles. Endogenous conidia occur in clusters, displaying oval morphology and gray coloration. |  | **(Pitt & Hocking, 2009b)** |
| H201 | Penicillium-like branched conidiophores with terminal ends containing sparse, oval-shaped spores having smooth walls. Hyphae are intertwined and septate. |  | **(Pitt & Hocking, 2009b)** |
| Moi 09 | Presence of erect conidiophores terminating in circular aspergillate heads with biseriate arrangement (phialides and metulae), producing round, verrucose, black-colored spores**.** |  | **(Pitt & Hocking, 2009b)** |
| M4-B3 | Hyaline, septate hyphae with branched and clustered conidiophores. Conidia are ovoid-elongate, bean-shaped. |  | **(Pitt & Hocking, 2009b)** |
| M1-B3 | Broad, aseptate hyphae. Aerial mycelia form globose, flattened sporangia. |  | Voir les détails de l’image associée. ATLAS MICOLOGIA: ABSIDIA CORYMBIFERA / Lichtheimia corymbifera  **(Pitt & Hocking, 2009b)** |

**Fig. 2. Prevalence of different fungal genera isolated from fish**

The genus *Aspergillus*, contaminating 75% of the analyzed fish, was detected across all sampling sites (Lake Taabo, Tagba Lagoon, and Lake Kossou) and throughout all climatic seasons. The genus *Rhizopus* was identified in 32.5% of the samples, predominantly during the rainy season. *Fusarium* was found in 15% of fish, mostly during the transition between dry and rainy seasons. *Penicillium* (5%) and *Absidia* (2.5%) were detected mainly during periods of rainfall.

**Table 3**. frequency of collected fish by detected genus across different locations.

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| **Fungal genera** | **Localities** | | | | | | | | | | | | **Number of contaminated fish** | **Frequency of genus contamination of samples** |
| **Lake Taabo** | | | | **Tagba Lagoon in Grand-Lahou** | | | | **Lake Kossou** | | | |
| LDS | LRS | SRS | SDS | LDS | LRS | SRS | SDS | LDS | LRS | SRS | SDS |
| *Aspergillus* | + | + | + | + | + | + | + | + | + | + | + | + | 90 (120) | 75 % |
| *Rhizopus* | - | + | - | + | - | + | + | + | + | + | - | + | 39 (120) | 32.5 % |
| *Fusarium* | + | + | - | + | - | - | + | + | + | + | - | + | 18 (120) | 15 % |
| *Penicillium* | - | + | - | - | - | + | - | - | - | + | - | + | 6 (120) | 5 % |
| *Absidia* | - | + | - | - | - | + | - | - | - | + | - | - | 3 (120) | 2.5 % |

*\*The numbers in parentheses represent the total sample count; +: Mold presence; -: Mold absence; LDS: Long Dry Season; LRS: Long Rainy Season; SRS: Short Rainy Season; SDS: Short Dry Season.*

3.2 Discussion

This study highlights the presence of toxigenic molds in freshwater fish from Lake Taabo and the Tagba Lagoon in Grand-Lahou. The 156 fungal strains identified belong to five genera: *Aspergillus*, *Rhizopus*, *Fusarium*, *Absidia*, and *Penicillium*. These findings are consistent with those ofBashorun *et al*. (2023), who reported similar fungal species in aquaculture fish tissues and feed in Doha, Qatar. Comparable studies from São Paulo and Dourados (Brazil), Giza (Egypt), and Iran have also reported the presence of these fungal genera in fish or their feed (Alinezhad et al., 2011; Anees et al., 2023; Gomes et al., 2022; Mohamed et al., 2017).

The distribution of genera observed here, *Aspergillus* (75%), *Rhizopus* (32.5%), *Fusarium* (15%), *Penicillium* (5%), and *Absidia* (2.5%), aligns closely with previous studies, particularly those by Alinezhad et al. (2011) and Mohamed et al. (2017), who also found *Aspergillus* to be predominant (57% and 64%, respectively). In contrast, Gomes et al. (2022) reported *Penicillium* as the second most common genus after *Aspergillus*.

The predominance of *Aspergillus* can be attributed to its ubiquity; its spores are commonly found in air, soil, and water. According to Pitt and Hocking (2009), *Aspergillus* species from the Ascomycota subphylum can reproduce sexually and readily colonize food products under favorable environmental conditions.

Luan et al. (2023) noted that fungal contamination in freshwater fish depends on ecological factors such as geographic location, temperature, humidity, water quality, and fish feeding habits. Liu et al. (2016), for instance, found distinct microbial communities in the gut content of carnivorous versus herbivorous fish species in the same freshwater environment.

In addition, studies by Bashorun et al. (2023) and Gomes et al. (2022) demonstrated a strong link between fungal contamination of fish feed and the subsequent contamination of fish tissues. The situation is worsened by environmental pollution due to industrial, agricultural, and domestic waste, which favors fungal proliferation (Vieira et al., 2023). Certain species, such as *Penicillium implicatum*, which produces citrinin, contribute to the degradation of organic matter in ponds, thereby facilitating fungal contamination (Damasceno et al., 2019). Chronic exposure to water contaminated by toxigenic molds can lead to pathological conditions in fish and increase health risks for human consumers.

In Côte d'Ivoire’s humid tropical climate, these results suggest widespread fungal contamination of freshwater fish and raise concerns about potential mycotoxin production, such as aflatoxins, ochratoxins, and fumonisins, in fish tissues.

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4. Conclusion

This study provides clear evidence of fungal contamination in freshwater fish from Lake Taabo and the Tagba Lagoon (Grand-Lahou). The fungal isolates exhibited considerable taxonomic diversity, with *Aspergillus* emerging as the most dominant genus, followed by *Rhizopus*, *Fusarium*, *Absidia*, and *Penicillium*. Several of these genera are known producers of potent mycotoxins. The presence of these fungi raises serious concerns regarding fish health and food safety. Mycotoxins not only contribute to fish diseases and mortality, causing significant economic losses, but also pose severe risks to human health, even at low exposure levels. These findings underscore the urgent need for systematic monitoring of fungal contaminants in freshwater fish in Côte d'Ivoire and the implementation of strategies to mitigate their presence along the fish value chain.

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**Disclaimer (Artificial intelligence)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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