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Research Article

Assessment of Heavy Metals and Mycological Flora in Various Dried Fish Samples from Idi-Ape Markets in Ilorin, Nigeria

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ABSTRACT

Fish is a highly nutritious food and a significant protein source widely consumed across Nigeria and globally. This study assessed the levels of heavy metals, aflatoxins, mycological flora, and proximate composition in three dried fish species *Clarias gariepinus*, *Gymnarchus niloticus*, and *Tilapia zilli* obtained from Idi-Ape Market, Ibadan. Heavy metals were analyzed using Atomic Absorption Spectroscopy (AAS), while aflatoxins were quantified via HighPerformance Liquid Chromatography (HPLC).Lead (Pb) concentrations ranged from 0.152 mg/kg in *T. zilli* to 0.214 mg/kg in *C. gariepinus*, exceeding the WHO permissible limit of 0.05 mg/kg in all samples. Cadmium (Cd) levels varied between 0.013 mg/kg and 0.028 mg/kg, also surpassing the FAO/WHO safe limit of 0.005 mg/kg. Aflatoxin B_1 levels ranged from 3.52 µg/kg to 7.84 µg/kg, exceeding the European Union threshold of 2 µg/kg in two of the samples. Fungal counts ranged from 1.0 ± 0.00a × 10^2 CFU/g in *T. zilli* to 8.5 ± 0.71c × 10^2 CFU/g in *G. niloticus*, with isolates including *Aspergillus flavus*, *A. niger*, and *Penicillium* spp., known producers of mycotoxins. Moisture content ranged from 4.136 ± 0.04(a)% to 5.778 ± 0.03(ab)%, ash content from 3.192 ± 0.05(a)% to 4.189 ± 0.12(b)%, and crude fibre from 24.254 ± 0.08(bc)% to 31.068 ± 0.20(a)%. Protein content was highest in *G. niloticus* (41.199 ± 0.00(c) %), while *T. zilli* had the highest carbohydrate content (18.138 ± 0.10(c) % and calorific value (1382.84 ± 2.78 (b) (KJ/100g). The detection of heavy metals and aflatoxins at levels exceeding safety limits, along with the presence of toxigenic fungi, indicates potential public health risks associated with the consumption of these dried fish samples.

Keywords: Afflatoxin; Health risk; Metal Composition; Mycological flora; Nutrition; Proximate

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INTRODUCTION

Fish and other seafood are unique dietary sources of cardioprotectivedocosahexaenoic (DHA) and eicosapentaenoic (EPA) fatty acids, which are linked to enhancing health and preventing diseases of old age (Ekanem and Udoma, 2021). In Nigeria, fish contributes approximately 40–50% of the total animal protein intake, with dried fish being a popular form due to its affordability, flavor, and long shelf life. The dried fish trade supports local economies and serves as a crucial protein source for low-income populations, especially in urban and peri-urban markets (Useh *et al.,* 2022).

However, dried fish products are often exposed to contamination during processing, transportation, and storage. One significant health concern is contamination by mycotoxins, which are poisonous secondary metabolites produced by certain fungi. When ingested by humans or animals, these toxins can lead to mycotoxicosis, a condition linked to cancers, liver damage, immune suppression, and neurological disorders such as alimentary toxic aleukia (ATA) (Alam *et al.,* 2022).

A variety of harmful health effects, including renal toxicity, immune system impairment, and carcinogenicity, can result from mycotoxins, including aflatoxins, which are extremely toxic compounds (Alshannaq and Yu, 2021; Al-jobory, 2025). The toxin is particularly dangerous in developing countries, where populations may already be dealing with nutritional and health issues and food safety laws and monitoring systems are usually insufficient (Wu *et al*., 2022).

The worldwide burden of mycotoxin contamination in food products has been extensively described in the literature; estimations indicate that the toxin may contaminate up to 25% of the world's food supply (Eskola *et al*., 2022).

In parallel, heavy metal contamination has become a global concern due to its persistence, toxicity, and bioaccumulation in aquatic ecosystems. While trace amounts of some metals such as zinc and selenium are essential for biological functions, manyincluding arsenic, lead, cadmium, mercury, and chromiumare toxic even at very low concentrations (Sankhla *et al.,* 2016). For instance, mercury can accumulate in fish tissue through feeding and gill absorption, posing risks to consumers (Ali *et al.,* 2021). These contaminants often accumulate in fish muscles and organs, which are commonly consumed by humans.

Despite growing awareness of these risks, few studies in Nigeria have investigated the simultaneous occurrence of heavy metals, fungal contaminants, and aflatoxins in dried fish samples from local open-air markets, particularly in Ilorin. This represents a critical gap in food safety research, especially considering the high consumption rate of dried fish in urban households.

This investigation was therefore motivated by the need to assess the microbial and chemical safety of dried fish sold in a typical Nigerian market setting.

This study aimed to determine the levels of selected heavy metals, identify fungal contaminants, and assess the presence of aflatoxins in dried fish samples obtained from Idi-Ape Market, Ilorin, Nigeria.

MATERIALS AND METHODS

Sample Collection and Study Location

Dried fish samples were collected from the Idi-Ape Market, located along the Oja-Oba axis in Ilorin West Local Government Area of Kwara State, Nigeria. This market was selected due to its high consumer patronage and the frequent sale of traditionally processed fish, which are often exposed to poor handling and storage conditions, increasing their susceptibility to contamination. A total of nine (9) dried fish samples, comprising three different species, *Gymnarchus niloticus* (knife fish), *Clarias gariepinus* (catfish), and *Tilapia zilli* (tilapia) were randomly selected from different vendors. For each species, three replicate samples were obtained to ensure data reliability and representation. All samples were immediately labeled, placed in sterile polythene bags to avoid external contamination, and transported in cooled containers to the microbiology laboratory.

Upon arrival at the laboratory, the samples were stored at 4°C until further processing and analysis to maintain sample integrity and prevent microbial growth or chemical degradation

Examining dried fish physically

The following criteria were used to select the samples used in the study. Colour, texture, odour, lack of rodent activity or infestation, and attractiveness were the criteria, and they were then stored in a sterile setting (Ajimati *et al.*, 2023).

Preparation and Mycological analysis of samples

The preparation of the media (potato dextrose agar, or PDA) followed the manufacturer's instructions. After homogenizing 10 g of each fish sample, it was submerged in 90 ml of sterile distilled water, a 10-fold serial dilution, and 1 ml of a different dilution. The melted PDA was then mixed with 2.5 ml of streptomycin, swirled gently to ensure even distribution, allowed to solidify, and then incubated at 27°C for three to five days to count the number of fungi (Useh *et al.,* 2022). Every analysis was done three times. As the analysis goes on, morphological, microscopic, and physical inspections of the culture plates based on their developed colony are conducted (Ajimati *et al.*, 2023).

Inspection and Isolation

The incubated plate's fungal development, colonial morphology, and colour were assessed. Every observation was noted. A single unique colony was subcultured on a freshly prepared Potato Dextrose Agar (PDA) plate, incubated, and examined using the microscopic examination and photomicrography method to achieve a pure culture once noticeable growth was seen (Etim *et al.*, 2024). The observed colony development was counted using a colony counter and the counts were expressed as colony-forming unit per gram (cfu/g) of the samples (Useh *et al.*, 2022)

Characterization and Identification of Isolates

All isolates were characterized according to colony morphology, cellular morphology, and molecular identification (Odeyemi *et al.*, 2018).

Determination of Aflatoxins using High Performance Liquid Chromatography

This was achieved using HPLC as adopted by Ajimati *et al.* (2023).

Procedure for analysis

Mobile phase is water/methanol

Acetonitrile (60: 20:20)

The wavelength is set at 365nm

Colour temperature is set to 35 degrees centigrade

Run time is set at 7 minutes

A sample volume of 40 micro litres is injected.

The mobile phase was pumped to allow the sample to be carried into the column. The chromatogram is obtained from the display system after the run time, the standard is prepared using reagents listed as reference above. The retention time of the standard is compared with that of chromatogram obtained from the sample to determine the Aflatoxin content/concentration in the sample.

There are over 20 types of Aflatoxin, but these are best known B1, B2, G1, G2, M1 and M2. Aflatoxin and Aflatoxin Q1. Some of these forms are derivatives or metabolites of animal metabolism for example Aflatoxin M1 and Aflatoxin M2 are the metabolites of Aflatoxin B1 and Aflatoxin B2 which are found in the lactating mammals feed of Aflatoxin contaminated feed (Zhang and Banerjee, 2020).

Detection of Heavy Metals

A conical flask was filled with the homogenized fish samples, which weighed 2.0g. To minimize volatile metal losses, 1 millilitre of pure HNO_3 was added to the samples prior to ashing, and they were left to predigest for the whole night. After being charred on electric hot plates, the samples were ash-treated for 24 hours at 55°C in a muffle furnace. Five millilitres of 1:1 HCL and a solution prepared in a 50 millilitre standard flask were used to dissolve the ash. In accordance with the procedure outlined by Ibanga *et al.* (2019), the metal concentrations of the materials were measured using an atomic absorption spectrometer, Buck Scientific Model 230 against suitable blank standard solutions of Cd, Zn, Ni, Co, and Pb.

Data Analysis

Data obtained from the assay in this study were analysed statistically using one-way analysis of variance to determine whether there were any significant differences. Differences of the mean were determined by the least significant differences, and a 95% confidence level was utilized as an indication of statistical significance ($P < 0.05$).

RESULTS

Enumeration of Fungi

The mean fungal count of the different fish samples ranged from 1.0 ± 0.00 to 8.5 ± 0.7x102 CFU/g, with *G. niloticus* having the highest (value) and *Tilapia zilli* having the least count (value). This is presented in Table 1.

Morphological Characterization of Fungal Isolates

The physical characteristics of the isolates were used to perform morphological identification of the fungi. Table 2 illustrates this, while plates 1 and 2 display the micrographs of *Trichoderma viride* and *Fusarium oxysporum*.

Molecular Identification

Isolate K was identified as *Trichoderma viride* by molecular identification of the two sample fungal isolates, whereas Isolate T was identified as *Fusarium oxysporum*. Table 3 displays this.

Determination of Mycotoxins

Aflatoxin B1 was found for *C. gariepinus* at RT 0.507 min with an area of 434.600 and a concentration of 0.2944, and Aflatoxin B2 was also found at RT 1.215 min with an area of 147195.203 and a concentration of 2.7056, according to the High-Performance Liquid Chromatography (HPLC) study performed on the samples. In *Tilapia zill*i, aflatoxin B1 was found at an RT of 0.448 minutes, an area of 761.800, and a concentration of 0.1100. The *G. niloticus* sample's aflatoxin was not detectable.

Proximate Analysis

The highest percentage mean moisture content, 5.778± 0.03ab, was found in *G. niloticus* (knife fish) and the lowest, 4.136± 0.04a, in *C. gariepinus* (catfish), according to the proximate analysis performed on all fish samples. The tilapia fish, *T. zilli*, had the highest mean percentage ash content at 4.189 ± 0.12b, while the catfish, *C. gariepinus*, had the lowest at 3.192 ± 0.05a. The greatest percentage mean carbohydrate value was 18.138 ± 0.10 for tilapia fish (*T.zilli*) and the lowest was 11.668 ± 0.11 for knife fish (*G.niloticus*). The tilapia fish, *T.zilli*, had the lowest % mean calorific value

at 1217.859 ± 4.48a, while the knife fish, *G. niloticus*, had the greatest at 1382.84± 2.78b.*G. niloticus* (knife fish) had the highest percentage mean lipid content (13.261± 0.12b), whereas *C. gariepinus* (catfish) had the lowest value (9.004 ± 0.02a). With a percentage mean value of 31.068 ± 0.20, *C. gariepinus* (catfish) had the highest crude fiber content, while *G. niloticus* (knife fish) had the lowest, at 24.254 ± 0.08 bc. *G. niloticus* (knife fish) had the highest percentage mean value of crude protein at 41.199 ± 0.00c, while *T. zilli* (tilapia fish) had the lowest at 34.528 ± 0.00b. Table 4 displays these.

Detection of heavy metals in samples

According to the results of the heavy metal analysis, the concentrations of Zn, Pb, Cr, and Fe in catfish were 2.04, 0.214, 0.1255, and 2.1055 (mg/L), respectively. 1.2 mg/L of zinc, 0.119 mg/L of lead, 0.073 mg/L of Cr, and 2.1515 mg/L of iron were found in tilapia. Zn, Pb, Cr, and Fe concentrations in knife fish were 0.00445 mg/L, 0.051 mg/L, and 0.1175 mg/L, respectively. Table 5 displays these.

Table 1: Fungal Counts of the analysed fish samples

Samples	Mean ± SD Fungal count ($x10^2$ CFU/g)
C. gariepinus	2.0 ± 1.41^a
G. niloticus	8.5 ± 0.71^c
T. zilli	1.0 ± 0.00^a

NB: Counts are replica of 3 samples.

Table 2: Morphological features of Fungal isolates

Isolate	Morphological characteristics
K	Greenish powdery growth
T	Whitish growth with reddish background

KEY: K= *Gymnarchus niloticus,* T= *Tilapia zilli*

Table 3: Molecular identification of fungal isolates

SAMPLE ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
K	*Trichodermaviride*	993	993	100%	0	100.00%	OQ918657
T	*Fusariumoxysporum*	847	847	100%	0	100.00%	OQ918658

0

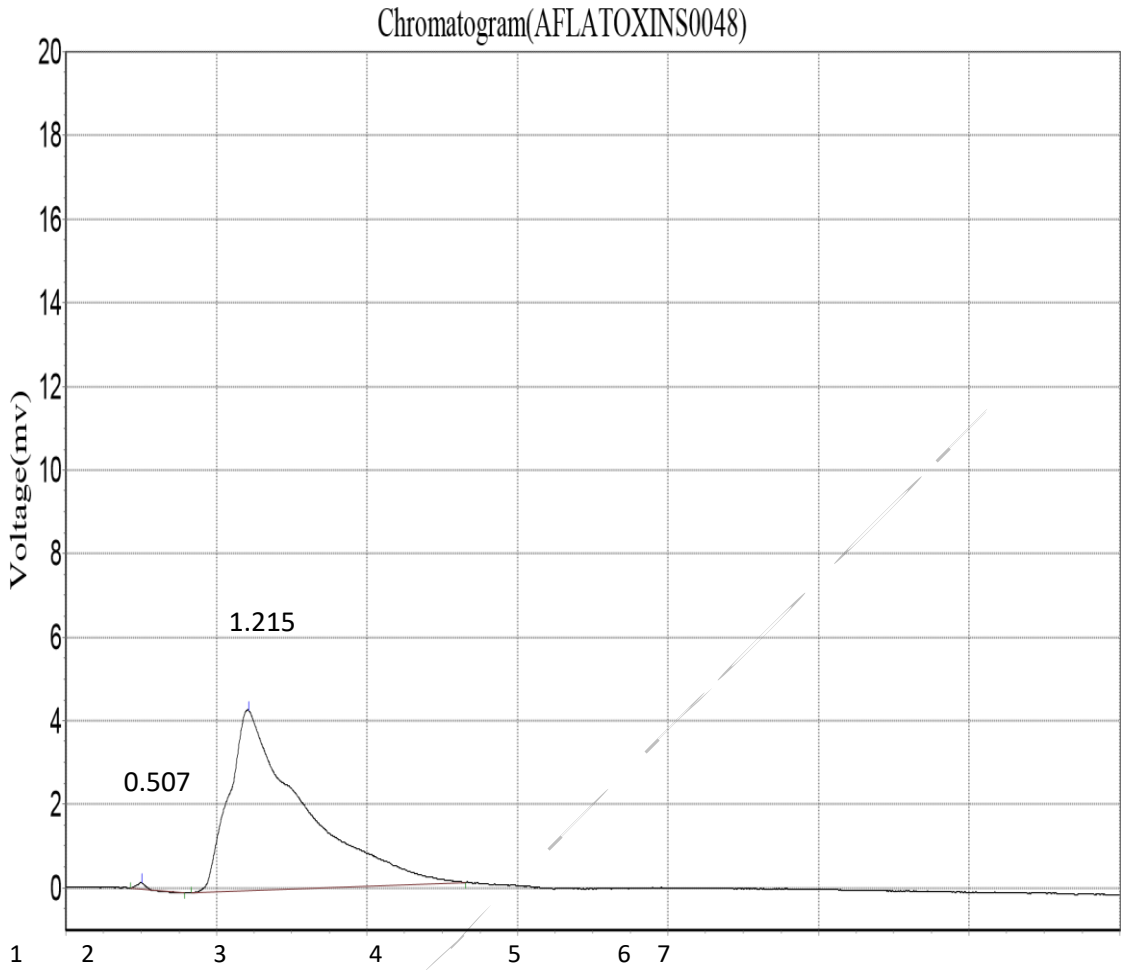

Figure 1: Aflatoxin detection in *C. gariepinus*

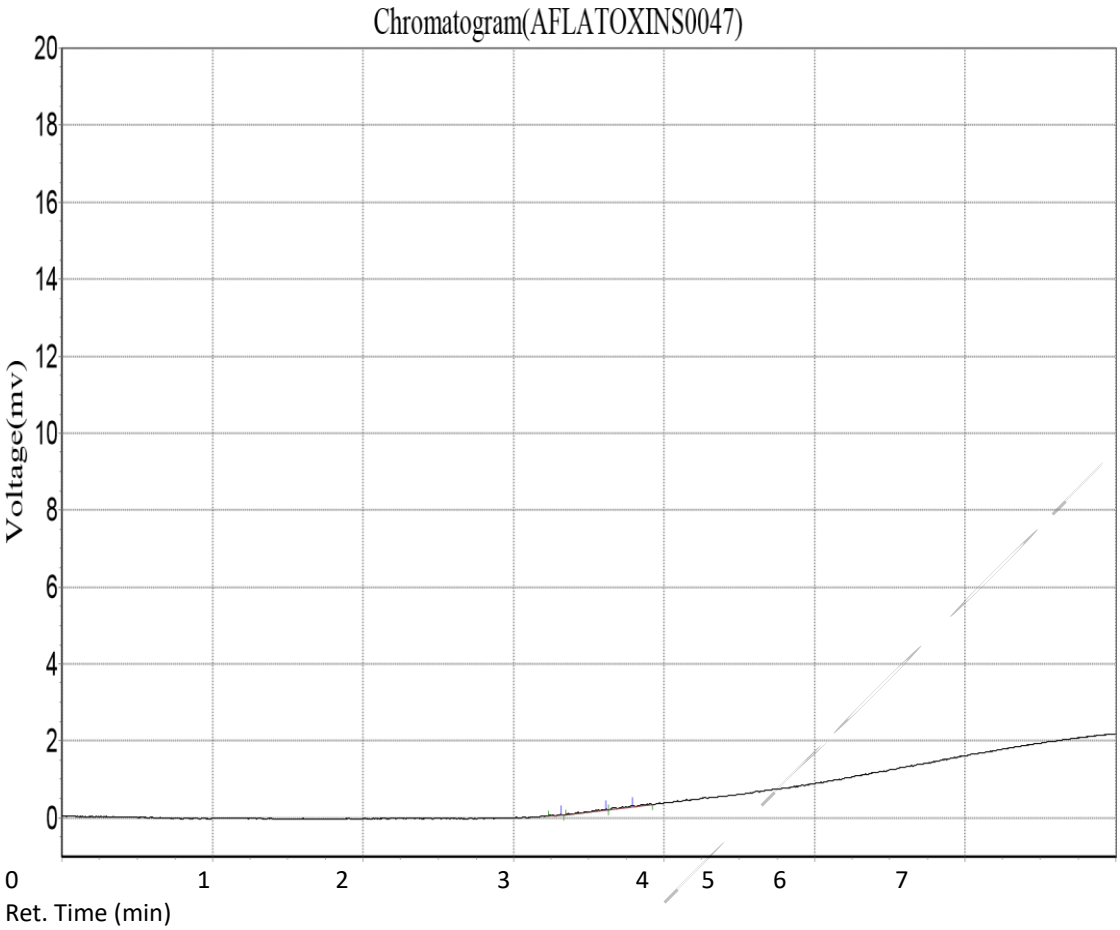

Figure 2: Aflatoxin detection in *G. niloticus*

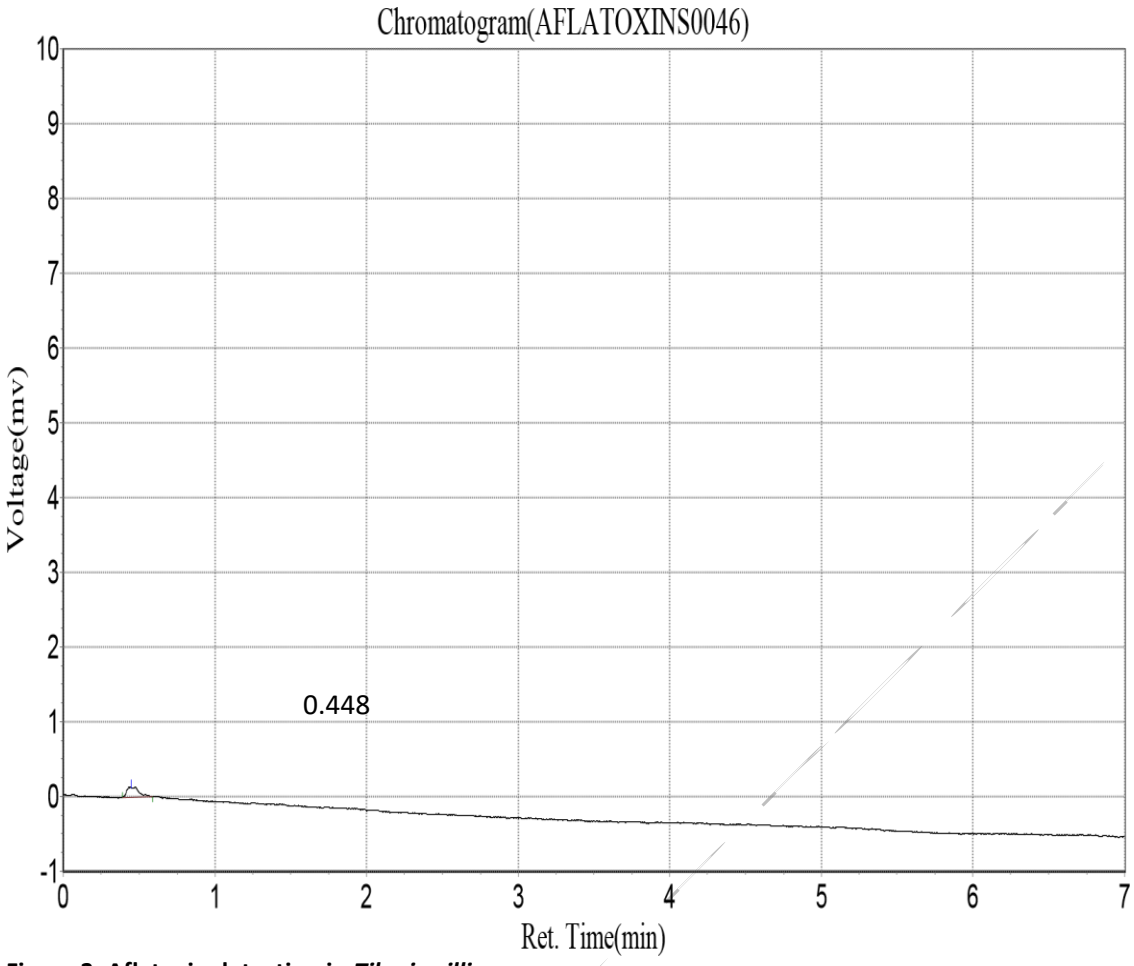

Figure 3: Aflatoxin detection in *Tilapia zilli*

Table 4: Proximate analysis of the fish samples

Sample (Scientific Name)	% Moisture	% Ash	%CHO	Calorific Value Kj/100g	% Lipid	% Crude Fiber	% Protein
Gymnarchus niloticus	5.778± 0.03^{ab}	3.839 ± 0.12^{a}	11.668 ±0.11^{b}	1382.84 ± 2.78^{b}	13.261 ± 0.12^{b}	24.254 ± 0.08^{bc}	41.199 ± 0.00^{c}
Tilapia zilli	5.289± 0.03^{ab}	4.189 ±0.12^{b}	18.138 ±0.10^{c}	1320.164 ±4.22^{b}	11.688 ± 0.07^{ab}	26.166 ± 0.01^{c}	34.528 ± 0.00^{b}
Clarias gariepinus	4.136 ± 0.04^{a}	3.192 ± 0.05^{a}	13.123 ±0.31^{b}	1217.859 ± 4.48^{a}	9.004 ± 0.02^{a}	31.068 ± 0.20^{a}	39.478 ± 0.01^{a}

Table 5: Heavy metals determination in different fish samples

Sample Name	Zn (mg/L)	Pb (mg/L)	Cr (mg/L)	Fe (mg/L)
Clarias gariepinus	2.04 ± 0.00^{b}	0.214 ± 0.00^{a}	0.1255 ± 0.00^{b}	2.1055 ± 0.02^{b}
Tilapia zilli	1.2 ± 0.02^{ab}	0.119 ± 0.03^{a}	0.073 ± 0.00^{a}	2.1515 ± 0.01^{b}
Gymnarchus niloticu	0.0045 ± 0.00^{c}	0.051 ± 0.01^{b}	0.1175 ± 0.00^{a}	2.3135 ± 0.08^{b}
WHO standard	5.0	0.05	0.05	0.1

KEY: Cat=*Clarias gariepinus,* Tilapia=*Tilapia zilli,* Knife=*Gymnarchus niloticus*

DISCUSSION

The mycological assessment of the dried fish samples revealed mean fungal counts ranging from 1.0 ± 0.00 to 2.0 ± 1.41 CFU/g, indicating relatively low levels of fungal contamination. Two fungal species, *Trichoderma viride* and *Fusarium oxysporum*, were consistently

isolated across all three fish species (*Gymnarchus niloticus*, *Clarias gariepinus*, and *Tilapia zilli*). The presence of *Fusarium oxysporum*, a known mycotoxin producer, raises concerns over potential toxin accumulation. This fungal profile contrasts with that reported by Ajimati *et al.,* (2023), suggesting that environmental conditions, drying methods, and storage practices may influence fungal diversity in dried fish products.

The moisture content ranged from 4.136 ± 0.04% to 5.778 ± 0.03%, consistent with values reported by Ajimati *et al.* (2023) and Akinwumi *et al.* (2022). These low moisture levels are favorable for shelf stability, as microbial growth, including that of toxin-producing fungi—is inhibited under dry conditions. This supports the microbial safety of the dried fish under current storage practices.

The ash content (3.192 ± 0.05% to 4.189 ± 0.12%) aligns with that reported by Ogunbambo (2020), while carbohydrate content (11.668 ± 0.11% to 18.138 ± 0.10%) corresponds with prior findings by Ajimati *et al.,* (2023) but is higher than the levels recorded by Akinwumi *et al.,* (2022). These differences may reflect variations in fish species or preparation methods. Elevated crude protein levels further reaffirm the nutritional richness of dried fish, echoing findings by Neranjala *et al*. (2022). Lipid content, while generally consistent with Ajimati *et al.,* (2023), deviates from Jim *et al.,* (2017), who reported lower fat concentrationspossibly due to species differences or lipid degradation during processing.

The heavy metal analysis revealed varying degrees of contamination among the fish samples. Zinc (Zn) concentrations ranged from 0.0045 to 2.04 mg/L, which remained within the WHO permissible limit of 5.0 mg/L. However, lead (Pb) levels were notably concerning, ranging from 0.051 to 0.214 mg/L, and thereby exceeding the WHO safety threshold of 0.05 mg/L in all samples. This is particularly alarming, as the highest Pb concentration exceeding the limit by over 300%. Chromium (Cr) was detected at concentrations between 0.073 and 0.1175 mg/L, while iron (Fe) levels ranged from 2.1055 to 2.3135 mg/L. Although the concentrations of Cr and Fe did not surpass established regulatory limits, their presence still warrants attention due to their potential for bioaccumulation in the food chain (Hussaini *et al.*, 2022).

These findings reinforce the dual burden of contamination in dried fish: while fungal growth appears controlled under current storage conditions, toxic heavy metals persist at unsafe levelsmost critically Pb. The co-occurrence of fungi and metals highlights the need for integrated food safety surveillance in informal markets**.**

CONCLUSION

This study identified the presence of *Trichoderma viride* and *Fusarium oxysporum*, low moisture and microbial loads, but concerning levels of lead (Pb) in dried fish sold at the Idi-Ape market, Ilorin. While the nutritional value of dried fish remains high, the detection of Pb above WHO safety limits underscores a significant public health risk.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data set used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare that they have no competing interest.

Conflict of Interest

The authors declare no conflict of interest. All the contributor were captured in either in the authorship or acknowledgment base on their contribution.

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Authors' contribution

A.S and A.F.M were responsible for the conceptualization and overall project administration. The methodology was developed collaboratively by A.S., A.F.M and B.S.I. the original draft of the manuscript was written by A.S., A.F.M and B.S.I. while B.S.I., A.A.E., O.A.A., A.A.A., O.S.A., A.K.O., A.K.A and K.H.M. contributed o the review and editing process.

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