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Mycological, Aflatoxin and Heavy Metals Composition of Dried Fish from Idi-ape Markets in Ilorin, Nigeria

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Abstract

Fish is a cherished and highly sort after food that is widely eaten among many communities in Nigeria and the world at large, with good nutritional values. This study is aimed at evaluating the mycological flora, aflatoxin, proximate composition and heavy metals content in different dried fish samples. A total of three samples were used in this study which are; Salvetinus fortinacis, Heterotis niloticus and Malapterurus electricus. Aflatoxin detection in samples were carried out using High Performance Liquid Chromatography. The proximate analysis was carried out to determine the percentage nutritional value in each of the samples. Heavy metals detection was carried out using Atomic Absorption Spectrometer Buck scientific model 230. The result of the mycological evaluation revealed that the mean fungal count of all samples ranged from 1.5 ± 0.71 to 22.5 ± 4.95 (x10^2 CFU/g). With H. niloticus having the highest while M. electricus had the least. The proximate analysis results revealed percentage moisture of samples ranged from 4.382 ± 0.02 to 6.869± 0.009 (%) with H. niloticus having the highest and S. fortinacis having least value. Ash ranged from 3.518 ±0.18 to 4.424 ± 0.06 (%) with H. niloticus having the highest and S. fortinacis having the least. Percentage carbohydrate ranged from 14.179 ± 0.26 to 15.728 ± 0.33 (%) with H. niloticuss having the highest and S. fortinacis having the least. Calorific value ranged 1270.115 ± 1.12 to 1407.66 ± 1.87 (KJ/100g) with H. niloticus having the highest and S. fortinacis having the lowest. Percentage lipid ranged from 10.549 ± 0.08 to 14.293 ± 0.10(%) with H. niloticus having the highest and S. fortinacis having the least. Percentage Crude fibre ranged from 22.389 ± 0.19 to 29.310± 0.03 (%) with S. fortinacis having the highest and H. niloticus having the least. Percentage Protein ranged from 35.529 ± 0.00 to 38.062 ± 0.00(%) with S. fortinacis having the highest and M. electricus having the least. The presence of mycotoxin producing fungi in the different dried fish samples led to the presence of different Aflatoxins (known carcinogenic toxin) in the samples which is of a threat to human health.

Keyword- Aflatoxins, Mycotoxin, Molecular Identification, Fish, Heavy Metals

INTRODUCTION

Fish is a key source of animal protein in Nigeria and the rest of the world, and it provides a living for the vast majority of people, especially those who live near rivers. According to research by John *et al*. (2020), fish exhibits a high amount of deterioration when it is left outside of water bodies. Complex enzymatic, microbiological, chemical, and physical changes lead to fish degeneration (Abba, 2012).Out of all the fish preservation procedures used in Nigeria, smoke drying is the most used method because it costs less (Akintola and Fakoya 2017). Fish drying does not entirely stop microbial growth, though.

The microbial presence lowers the quality of fish products. In most instances, fresh seawater fish are salted and dried in the sun in preference to professional food processing industry. Due to the difficulty in controlling the drying conditions, some fishermen choose to dry their catch on the streets or on open balconies of homes, a technique that is common in many Asian and African nations (Akwuobu *et al.,* 2019). Bacterial, fungal, insect, and rodent contamination, as well as unclean handling, poor quality salt and water used for fish processing, all damage the quality of dry fish processed outdoors by open sun drying (Deng *et al.,* 2021). According to several research, fungal growth has caused a reduction in the quality of dried fish (Sa'adatu *et al.,* 2019). Thus, microbial infection impacts meat quality by changing flavor, texture, and nutritional loss as well as creating issues with food safety (Singapurwa *et al.,* 2018), all of which can result in a huge economic loss to the industry.

Africa's fish farmers and consumers are seriously hampered by fungal infection. As the primary degrading agents or as a secondary contaminant as a result of mechanical damage, fungi may be the source of this infection (John *et al.*, 2020). Numerous fungi overgrowth in agricultural goods causes production and quality declines as well as huge monetary losses (Cinar and Onbaş, 2019).The low weight metabolites known as mycotoxins have a significant impact on public health because they injure humans, domestic animals, and livestock and are known as mycotoxicoses (Okungbowa and Kinge, 2021). *Aspergillus, Penicillium*, and *Fusarium* are the principal genera of mycotoxigenic fungus, but *Trichoderma, Trichothecium*, and *Alternaria* are also significant as food pollutants or plant diseases (Rashad *et al.*, 2023).

Human health is seriously threatened by mycotoxins, especially ochratoxin A (OTA) and aflatoxins (AFTs). Aflatoxins are strong carcinogens that, along with the hepatitis B virus, cause many thousands of yearly fatalities in people, primarily in developing tropical nations (Niu *et al.*, 2021). AFTs are secondary metabolites called difuranocoumarins that *Aspergillus flavus* and *A. parasiticus* create. They are frequently detected in food and feeds and have been linked to a number of diseases in humans, domestic animals, and cattle around the world, including aflatoxicosis (Ashiq, 2015). Metal contamination of aquatic habitat has recently attracted attention on a global scale, particularly in emerging nations like Nigeria.

Due to their toxicity, lengthy persistence, bioaccumulation, and biomagnifications in the food chain, metals and metalloids from both natural and anthropogenic sources are constantly entering the aquatic environment and posing a severe threat to human and ecological health (Rahman *et al.*, 2013). The elevated levels of metals in bodies of water are a result of increased industrialization and vast agricultural operations (Paul *et al.*, 2021). Fish are particularly vulnerable to aquatic metal pollution since they are aquatic animals. Fish absorb food, assimilate particulate matter suspended in water, exchange ions with dissolved metals through lipophilic membranes like the gills, and adsorb metals to the surfaces of tissues and membranes. But in addition to the risk to fish, heavy metal pollution in fish has become a major global concern not only because of the threat to fish but also due to the public health risks associated with fish consumption (Islam *et al.*, 2015). This research work was aimed at evaluating mycological flora, Aflatoxin, proximate composition and heavy metal content in different dried fishes sold in Ilorin Market.

MATERIALS AND METHODS

Sample Location

Fish Samples were sourced from the popular fish market at Idi-ape market located at Ojo-oba axis of Ilorin West Local Government Area in Ilorin, Kwara State.

Sample collection

Three (3) samples of dried fishes of different species were purchased at the open fish market in Idi-ape, Ilorin west Local Government Area in Ilorin, Kwara State; *Salvelinus fortinacis* - (Ajede fish), *Heterotis niloticus* - (Bonga fish) and *Malapterurus electricus*- (Electric fish). The fish sample were bought and kept in a sterile polythene bags to avoid cross contamination and was taken aseptically to the microbiology lab for further studies.

Physical examination of dried fishes

The following characteristics were used in the selection of samples used in the study. They include odour, colour, texture, lack of infestation or rodents activities and attractiveness. These was maintained under sterile condition.

Mycological analysis

Ten grams of each sample of fish was homogenized and was immersed in 90ml sterile distilled water, a 10 folds serial dilution and 1ml of different dilution was dispensed into a sterile petri-dish, 2.5ml of streptomycin was added to the melted PDA before it was poured into the petri dishes, swirled gently to allow even distribution then allow it to solidify before it was incubated at 27°C for 120hrs.

Physical examination of all established colony, morphological examinations and microscopic examination took place as analysis progresses.

Inspection and isolation

The incubated plate were examined for fungal development, color, and colonial morphology. All observations were recorded. A single separate colony was subcultured on a freshly prepared PDA plate, incubated, and examined to obtain a pure culture utilizing microscopic examination and photomicrography method in order to obtain a pure culture after a visible growth was noticed (Zakariya *et al.*, 2022).

Characterization and Identification of Isolates

All isolates were identified based on colonial morphology, cellular morphology and molecular identity.

a) Fungi DNA Extraction

One hundred (100) mg of fungi mycellia was grinded with Dellaporta extraction buffer (100 mM Tris pH 8, 51 ml EDTA pH 8, 500 mM NaCl, 10 mM mcrcaptoethanol) and DNA extracted as described briefly. Each sample was grinded in 1000 µl of the buffer in a sterilized sample bags. Mix was collected in sterile eppendorf tube and 40 µl of 20% SDS was then added, this was followed by brief vortexing and incubated at 65

ºC for 10 minutes. At room temperature, 160 µl of 5 M potassium acetate was then added vortexed and centrifuged at 10000 g for 10 minutes. Supernatant where collected in another eppendorf tube and 400 µl of cold iso propanol was added mixed gently and kept at20° Cfor 60 minutes. Centrifugation was at 13000g for 10 minutes to precipitate the DNA after which supernatant was gently decanted and ensured that the pellet was not disturbed. DNA was then washed with 500 µl of 70 % ethanol by centrifuging at 10000g for 10 minutes. Ethanol was decanted and DNA air-dried at room temperature until no trace of ethanol was seen in the tube. Pellet was then re-suspended in 50 µl of Tris EDTA buffer to preserve and suspend the DNA (Odeyemi *et al.,* 2018).

a) Polymerase Chain Reaction Analysis

To use the ITS gene for characterization of fungi, ITS universal primer set which flank the ITS1, 5.8S and ITS2 region can be used;

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and - ITS 4: 5' TCC TCC GCT TAT TGA TAT GC 3'primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition include a cycle of initial denaturation at 940C for 5 min, followed by 35cycles of each cycle comprised of 30secs denaturation at 94ºC, 30secs annealing of primer at 55ºC, 1.5 min extension at 72ºC and a final extension for 7min at 72ºC (Odeyemi*et al.,* 2018; Gupta 2019).

b) Integrity

The integrity of the amplified gene fragment was checked on a 1% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100bp molecular weight ladder that was ran alongside experimental samples in the gel.

c) Purification of Amplified Product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then suspend with 20 µl of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel on a voltage of 110V for about 1hr , to confirm the presence of the purified product and quantified using a nanodrop of model 2000 from thermo scientific (Odeyemi *et al.,* 2018).

d) Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis (Odeyemi *et al.,* 2018).

Determination of Aflatoxins using High Performance Liquid Chromatography

i. Chemicals and materials

Methanol (lichrosolv) and acetonitrile (lichrosolv), AFT-Cil, AFT-G2, AFT-B1 and AFT-B2 (analytical grade) reference standard. 5.0g of powdered sample is soaked in 50ml of 70% methanol and was allowed to stand for overnight in a shaker. The extracted sample was decanted, centrifuged and filtered using a micron filter into a 5ml sample bottle.

ii. Procedure for analysis

Mobile phase is water/methanol
Acetonitrile (60: 20:20)
The wavelength is set at 365nm
Colour temperature is set to 35 degree centigrade
Run time is set at 7minutes

Sample volume of 40micro litres is injected.

The mobile phase is pumped to allow the sample to be carried into the column. The chromatogram is obtained from the display system after the run time, 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 more than 20 types of aflatoxin but the best known are B1, B2, G1, G2, M1 and M2.

Detection of Heavy metals

The triple acid digestion method of Sahrawat *et al.,* (2002) was employed. 2.0 g of dried sample was weighed into a conical flask to which 24cm^{3}of a mixture of concentrated nitric acid (HNO_3), sulphuric acid (H_2SO_4), and 60 % perchloric acid ($HClO_4$) (9: 2: 1 v/v) was added. The flask was put on a heating block/hot plate and digested for 10 minutes to a clear solution, cooled and the content was transferred into a 50cm^3 volumetric flask and made up to the mark with deionized water. The digest was analysed for minerals using Atomic Absorption Spectroscopy Buck scientific model 230.

Statistical Analysis

Data from the present study were analysed by Analysis of variance (2-way Anova) using statistical package for social science (SPSS) software (version 20.0) and Tukey range test was used to measure the differences between data means at 95% confidence level ($P < 0.05$).

RESULTS

Enumeration of Fungi

The mean fungal count of the different fish samples ranged from 1.5 ± 0.71 to 22.5 ± 4.95 (x10^2 CFU/g) with *H. niloticus* having the highest and *M. electricus* having the least count. This is presented in Table 1.

Morphological Characterization of Fungal Isolates

Morphological identification of fungi was carried out based on the physical appearances of isolates. This is shown in Table 2. The micrographs of *Aspergillus fumigatus, Aspergillus niger*.are presented in Plates 1 and 2.

Molecular Identification

The molecular identification carried out on the three fungal isolates revealed isolate A to be *Aspergillus fumigatus*, Isolate B *Aspergillus niger* and Isolate E *Aspergillus niger*,. This is presented in Table 3

Determination of Aflatoxin

From the High-Performance Liquid Chromatography (HPLC) analysis carried out on the samples, Aflatoxin B1 was detected at retention times (RT) 0.290 and 5.182 (min), with areas 1709.800 and 238.473 and concentrations 2.3484 and 0.9749 while Aflatoxin M2 was detected at RT 4.707 min with area of 1709.800 and concentration 0.7067 in *M. electricus*. Aflatoxin B2 was also detected at RT 1.215 min, area of 147195.203 and concentration 2.7056. For *S. fortinails*, Aflatoxin B1 was detected at RT 0.207 min, area 1714.800 and concentration 1.1492, Aflatoxin M1 was also detected at RT 5.732, area of 33441.232 and concentration 0.0867, Aflatoxin M2 were also detected at RTs 4.065 and 5.732 (min) with areas of 129.325 and 31441.232, and concentrations 0.0867 and 1.0715. For *H. niloticus*, Aflatoxin B1 was detected at RT of 0.440 with an area of 500.000 and concentration 0.1629, Aflatoxin B2 was detected at RT of 1.140 with area of 306443.406 and concentration 3.8371.

Proximate analysis

From the proximate analysis carried out on all fish samples, *H. niloticus*(bonga fish) had the highest percentage mean moisture content of 6.869 ± 0.09 while *S. fortinacis* (Ajede Fish) had the least value of 4.382 ± 0.02. For mean percentage ash content, *H. niloticus* (bonga fish) had the highest value of 4.424 ± 0.06 while *S. fortinacis* (Ajede Fish) had the lowest value of 3.518 ± 0.18. *H. niloticus*(bonga fish) had the highest percentage mean carbohydrate value of 15.728 ± 0.33 while *S. fortinacis* (Ajede Fish) had the lowest 14.179 ± 0.26. *H. niloticus* (bonga fish) had the highest percentage mean calorific value of 1407.66 ± 1.87 while *S. fortinacis* (Ajede Fish) had the lowest value of 1270.115 ± 1.12. Percentage mean lipid content was highest in *H. nioticus* (bonga fish) with 14.293 ± 0.10 while *S. fortinacis* (Ajede Fish) had least value of 10.549 ± 0.08. Crude fiber was highest in *S. fortinacis* (Ajede Fish) with a percentage mean value of 29.310 ± 0.03 while *H. niloticus* (bonga fish) had the lowest value of 22.389 ± 0.19. Crude protein was highest in *S. fortinacis* (Ajede Fish) with percentage mean value of 38.062 ± 0.00 while lowest in *M. electricus* with 35.529 ± 0.00. These are presented in Table 4.

Detection of heavy Metals in samples

The result from the heavy metal analysis revealed that Electric fish (*M. Electricus)* contained Zinc (Zn), Lead (Pb), Chromium (Cr) and Iron (Fe) at concentrations 0.7085, 0.1135, 0.0445 and 1.624 (mg/L) respectively.. Ajede fish (*S. fortinacis)* contained 0.5245 mg/L of Zn, 0.0785mg/L of Pb, 0.0795 mg/L of Cr and 1.289 of Fe. Bonga fish (*H. Niloticus)* contained 0.2805 mg/L of Zn, 0.144 mg/L of Pb, 0.155 mg/L of Cr and 2.2015 mg/L of Fe. These are shown in Table 5.

Table 1: Fungal Counts in different fish samples

Samples	Mean ± SD Fungal count (x10^2 CFU/g)
S. fortinacis	2.5± 0.71^a
H. niloticus	22.5 ± 4.95^b
M. electricus	1.5 ± 0.71^a

Key: a= The result show that there is no significant different between Mean b= The results show that there is a significant different between Mean NB: Counts are replica of 3 samples.

Table 2: Morphological Characterization of Fung al isolates

Fish Samples	Morphological characteristics of isolate
A	Greenish with yellowish background, they have a green spiked conidia i.e the surface has small spikes covering its surface.
B	They have a cottony appearance; initially white to yellow and then turning black. Made up of felt-like conidiophores. The reverse is white to yellow. In microscopy, the conidial heads are radiate with conidiogenous cells biseriate. Conidia brown.
E	They have a cottony appearance; initially white to yellow and then turning black. Made up of felt-like conidiophores. The reverse is white to yellow. In microscopy, the conidial heads are radiate with conidiogenous cells biseriate. Conidia black.

KEY: A= *Salvelinus fortinacis(Aspergillus fumigatus)*, B= *Heterotis niloticus(Aspergillus niger)*, E=*Malapterurus electricus (Aspergillus niger)*

Plate 1: Photomicrograph of *Aspergillus fumigatus*

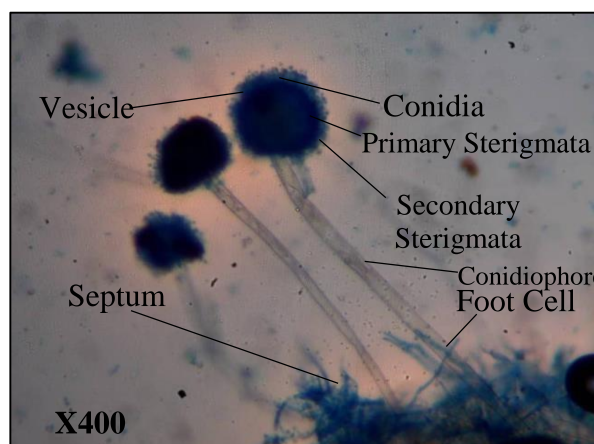

Plate 2: Photo-micro-graph of *Aspergillus niger*

Table 3: Molecular identification of Fungal Isolates

SAMPLE	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
A	*Aspergillus fumigatus*	978	978	99%	0	100.00%	OQ918654
B	*Aspergillus niger*	982	982	100%	0	100.00%	OQ918655
E	*Aspergillus niger*	982	982	100%	0	100.00%	OQ918656

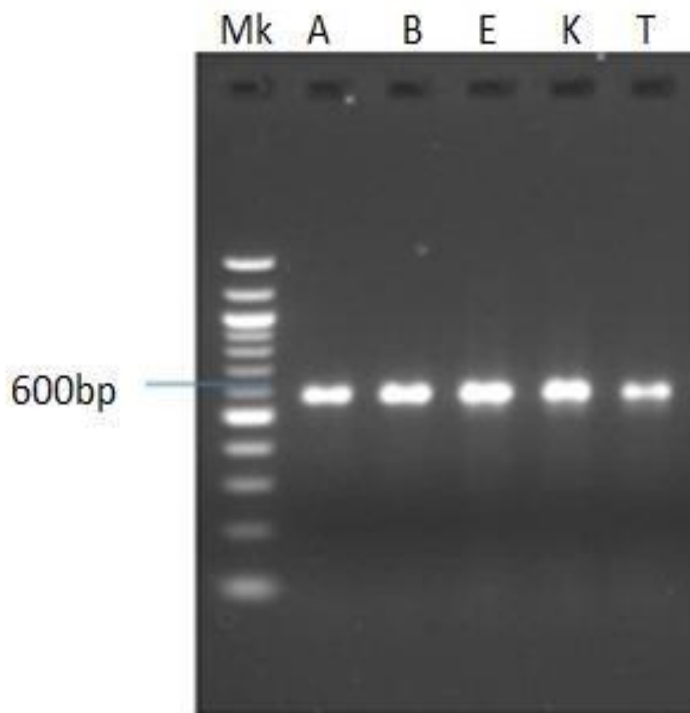

Figure 1: Gel electrophoresis of fish sample
KEY: A= *Salvelinus fortinacis (Aspergillus fumigatus)*, B= *Heterotis niloticus (Aspergillus niger)*, E=*Malapterurus electricus (Aspergillus niger)*,

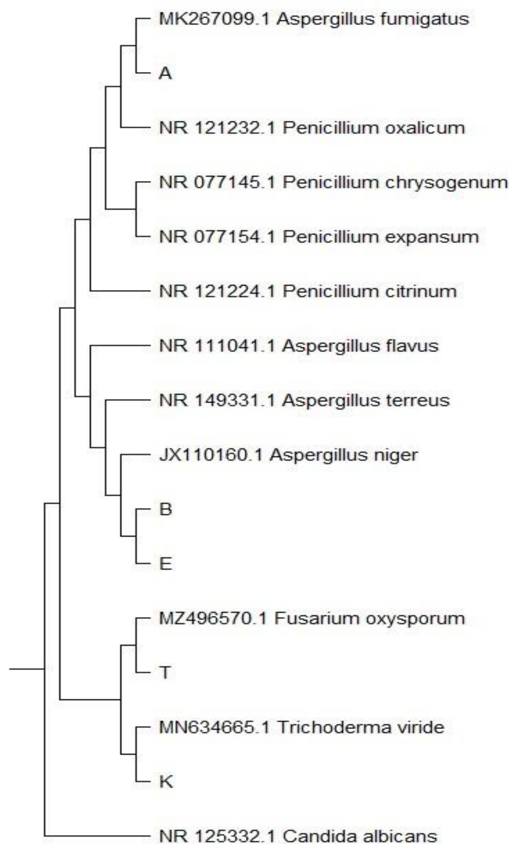

Figure 2: Phylogenetic tree of fish samples X400

Table 4: Proximate composition of different fish samples

Sample (Scientific Name)	% Moisture	% Ash	%CHO	Calorific Value KJ/100g	% Lipid	% Crude Fiber	% Protein
Salvelinus fortinacis	4.382 ± 0.02[a]	3.518 ± 0.18[a]	14.179 ± 0.26[a]	1270.115 ± 1.12[a]	10.549 ± 0.08[a]	29.310 ± 0.03[a]	38.062 ± 0.00[a]
Heterotis niloticus	6.869 ± 0.09[b]	4.424 ± 0.06[b]	15.728 ± 0.33[a]	1407.66 ± 1.87[b]	14.293 ± 0.10[b]	22.389 ± 0.19[b]	36.296 ± 0.00[b]
Malapterurus electricus	4.547 ± 0.03[a]	3.691 ± 0.18[a]	15.481 ± 0.27[a]	1309.47 ± 1.39[b]	12.138 ± 0.08ab	28.614 ± 0.02[a]	35.529 ± 0.00[b]

a= The result show that there is no significant different between Mean b= The results show that there is a significant different between Mean

Table 5: Heavy metals content in different fish samples

Sample Name	Zn (mg/L)	Pb (mg/L)	Cr (mg/L)	Fe (mg/L)
Malapterurus electricus	0.7085 ± 0.00[a]	0.1135 ± 0.00[a]	0.0445 ± 0.00[a]	1.624 ± 0.02[a]
Salvelinus fortinacis	0.5245 ± 0.03[a]	0.0785 ± 0.01[b]	0.0795 ± 0.00[a]	1.289 ± 0.02[a]
Heterotis niloticus	0.2805 ± 0.01[a]	0.144 ± 0.02[a]	0.155 ± 0.00[b]	2.2015 ± 0.03[b]
WHO standard	5.0	0.05	0.05	0.1

KEY: Electric=*Malapterurus electricus* Ajede = *Salvelinus fortinacis,* Bonga= *Heterotis niloticus*.

DISCUSSION

The fungal enumeration carried out on different dried fish samples revealed mean fungal load ranging from 1.5 to 22.5 (x10^2 CFU/g). The fungal species isolated from the samples were *Aspergillus niger* and *Aspergillus fumigatus*. Some of the fungi isolated from this study have previously been reported to be present in dried fish samples (Ibanga *et al*., 2019; Deng *et al*., 2021). The growth of these fungi in fish is due to favorable conditions that support their growth. The presence of *Aspergillus sp*. in the sample may be due to the fact that *Aspergillus* is a common soil fungus and can colonize these fish in dirty environments. Penicillium is also a soil-borne fungus that can get into fish due to poor hygiene practices, leading to spoiled fish.

Other spoilage microorganisms present in fish can also cause fish to spoil and negatively impact consumers. All these fungi in fish show that fish is a good substrate for them to function and grow well.

The presence of these fungi in fish may be due to unhygienic display of these fish in markets or poor storage in unventilated environments where the pest acts as a carrier of the disease. The amount of fungus present is small, which may be less harmful but can be harmful in immunocompromised individuals, where the fungus can act as an opportunistic pathogen. Because they cannot thrive in a sterile environment but are very vital, these fungi are not only hazardous to immunocompromised individuals but also have the capacity to produce extremely deadly toxins when aggregated in the body, to reduce the contamination of fish. Some researchers have reported the ability of some of these fungi to produce mycotoxins. *Aspergillus flavus* and *Penicillum species* (Camardo *et al*., 2019). The presence of various fungi does not necessarily mean that aflatoxin is present in the food, but the presence of toxin-producing fungi such as *Aspergillius flavus* may be an indicator of the presence of aflatoxin species. different. Factors that contribute to the presence of large amounts of aflatoxins in fish samples include, for example, fish being susceptible to fungal colonization during storage and packaging. These fungi have found a favorable environment for fish to grow and develop abundantly to produce these metabolites (Kumar *et al*. 2021). Deng *et al*. (2021). Mycotoxins (Aflatoxin B1, B2, M2) found in various dried fish samples were also reported. The presence of aflatoxin in samples poses a significant health threat to consumers, as aflatoxin is known to cause health problems, including liver damage, when consumed in high concentrations. . Many of these aflatoxins are reported to be deposited in samples via microorganisms such as Fusarium sp. and Aspergillus sp. The average percent moisture of different dried fish samples ranged from 4.382 ± 0.02 to 6.869 ± 0.09.

Reported by Akinwumi *et al.* (2022) obtained low moisture contents of 6.62 ± 0.01 and 4.20 ± 0.00 from charcoal and oven-dried fish, respectively. The presence of low humidity can limit the extent of microbial reversion because microorganisms grow well in humid environments. Low humidity also improves the shelf life and quality of dried fish and other food samples. However, the ash content reported by Akinwumi *et al.* (2022) differs from the results obtained from this study ranging from 3.192 ± 0.05 to 4.424 ± 0.06. Ash content represents the amount of minerals present in a particular food sample, indicating that the mineral content present in that sample is low. The higher carbohydrate content obtained in this study, ranging from 14.179 ± 0.26 to
15.728 ± 0.33, is in contrast to that reported by Akinwumi *et al.* (2022) reported lower levels in their different samples. The high crude protein ratio reported in this study has been supported by various researchers, including Neranjala *et al.* (2022), the high protein value further confirms the protein nature of fish. The lipid content reported in this study contrasts with the results of Jim *et al.* (2017) reported low lipid percentage (1.74%), while Ogbonnaya and Shaba (2009) reported relatively high values (21.20% and 29.60%) for different types of dried fish .
The results of heavy metal analysis showed that Zn concentration in all samples ranged from
0.2805 to 0.7085 mg/L, Pb concentration ranged from 0.0785 to 0.144 mg/L, Cr ranged from 0.0445 to 0.155 mg/L and Fe Concentration ranged from 1.289. since February 2015. Zn concentrations obtained and reported by Daniel *et al.* (2013) challenged the results obtained from this study with Zn concentrations ranging from 5.49 to 14.05. The obtained results of Pb concentration are consistent with the results of this study ranging from 0.01 to 0.29 mg/L. A study conducted by Oyekunle *et al.* (2020) reported higher Zn concentrations in all samples, ranging from 1.50 to 3.15. The reported values for Pb are similar to those obtained in this study. The Zn levels obtained in this study were within the WHO-recommended concentration (5.0 mg/L). Excessive consumption of Zn can lead to digestive disorders, accompanied by some symptoms such as vomiting, nausea, cramps, diarrhea and epigastric pain (Hussain et al., 2022). All Pb values obtained from this study are higher than the WHO recommended concentration (0.05 mg/L), so may be harmful if consumed. Exposure to or inhalation of Cr may increase the risk of lung, nose and sinus cancer. Severe dermatitis and generally painless skin ulcers can be caused by exposure to Cr compounds (Burnase *et al.*, 2022). The iron (Fe) content obtained in this study was higher than the WHO recommended limit of 0.1 mg/L. Fe accumulation in the body can cause serious damage leading to organ failure. It can also lead to chronic diseases, such as cirrhosis, diabetes and heart failure. Many people have genetic changes that cause hemochromatosis (Yiannikourides and Latunde-Dada, 2019).

CONCLUSION

Aflatoxin-producing fungus were found in all the three dried fish samples, which resulted in the presence of this carcinogenic toxin. The samples are unsuitable for human consumption due to the high concentrations of heavy metals that exceed the recommended limits set by the World Health Organization. The soil-borne Two *Aspergillus niger* and *Aspergillus fumigatus* were the three fungal species that were isolated. *M. electricus* has a high metal content that is deemed dangerous for ingestion by humans. This content includes zinc, lead, chromium, and iron. Because *H. niloticus* has the maximum moisture content, spoiling organisms may be able to affect it.

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