COMPARATIVE STUDY OF MICROBIAL QUALITY AND HEAVY METALS CONTENT OF FRESH AND SMOKED DRY MULLET(*Mugil cephalus*) AND SILVER CATFISH (*Chrysichthys nigrodigitatus*)

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ABSTRACT
Seafood has continued to serve as a major source of protein and other useful nutrients, making it an important component of a healthy diet. The microbial and heavy metals content of fresh and smoked Mullet and Silver catfish (MSc) were evaluated. The skin, gills and liver were aseptically removed. Ten folds serial dilution was carried out on each sample, while bacterial and fungi were isolated and identified. Mineral analysis was carried out using standard method and data obtained were analysed using ANOVA while means were compared using Duncan Multiple Range Test. Total viable bacteria count for fresh MSc (CFU/ml); Skin($0.8 \times 10^6 - 9.7 \times 10^6$; $0.7 \times 10^6 - 7.6 \times 10^6$), Gills($0.3 \times 10^6$–$5.0 \times 10^6$; $0.1 \times 10^6 - 8.9 \times 10^6$), Liver($0.2 \times 10^6$–$4.5 \times 10^6$; 0.1×10^6 –5.3×10^6), while smoked MSc; Skin(1.0×10^6– 6.0×10^6; $1.8 \times 10^6 - 7.8 \times 10^6$), Gills($1.13 \times 10^6 - 8.0 \times 10^6$; 0.6×10^6 –3.2×10^6), Liver(1.2×10^6–6.2×10^6; 3.9×10^5-1.4×10^6). Probable organisms isolated include; *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus* spp, *Klebsiella pneumonia*, *Aspergillus* spp, *Rhizopus* sp, *Fusarium sp* and *Penicillium* spp. Metal concentrations (mg/kg) varied significantly ($p < 0.05$) within locations and in skin, gills and liver with ranged; Fe(297.00±1.41-32.50 ± 0.71) > Zn(3.65±0.07-112.80±3.11) > Mn(0.08±0.01-2.2±0.35) > Pb(0.00 ± 0.00-0.85 ± 0.01) > Cr(0.95 ± 0.01-0.20 ± 0.01) > Cd(0.00±0.00-0.40±0.00) > Cu(0.01±0.04-1.50±0.01) while Fe(59.95±0.21-222.0±2.82) > Zn(41.60±0.14-19.60±1.40) > Cu (7.01±0.01-2.40±0.01) > Mn(0.00±0.00-0.16±0.01) > Cr(0.34± 0.01-0.09±0.01) > Pb(0.67±0.01-0.02±0.00) > Cd(0.00±0.00-0.03±0.00) in Makoko and Epe samples respectively. In this study Zn, Mn and Fe concentrations in Skin, Gills of Mullet were higher than WHO/FAO permissible limits, while Cd was recorded below detection limits. The presence of pathogenic microorganisms in these fish samples calls for serious public concern. To ensure food safety, adequate processing is required before consumption.

Keywords: Bacterial, Fungal isolate, Heavy metals, Microbial quality, Silver Catfish.

INTRODUCTION
Seafood and seafood products are excellent sources of nutrients for human health. It is one of the most important components of a healthy diet due to its high protein value, vitamin D and E, iodine, and long-chain omega-3 polyunsaturated fatty acids content (Sioen *et al.,*2007). Seafood products contain many important biomolecules; peptides, fatty acids, minerals (iodine, zinc, potassium, and phosphorus), and many vitamins, especially the B group. These biomolecules play an important role in the nutritional value and sensory quality of seafood. Fish is a high-protein, low-fat food that provides a range of health benefits. Fish is a major source of protein and the most important protein food available in the tropics, representing about 14% of all animal protein on a global basis (Abolagba and Mella,2008). Fish harvesting, handling, processing and distribution provide a livelihood for millions of people.

Silver catfish and Mullet fishes are among the dominant fishes of commercial catches by fishermen in Nigeria (Soyinka, 2008), and are readily available all through the year in the Niger-Delta region of Nigeria (Akpan and Ubak, 2004), and Lagos state in particular. The availability and abundance of Mullet in Lagos State have been attributed to the presence of a lagoon (Lawson and Jimon, 2010). In the tropical and subtropical regions, Mullet and Silver catfish form two of the important fisheries due to their rich nutritional content and potential for aquaculture (Isangedighi *et al.,* 2009), and giving that both fishes can be found in seawater and freshwater (Moro *et al.,* 2019).

Fresh Mullet and Silver catfish are used directly for the preparation of meals or preserved for future usage. Fish is a highly perishable food material and deterioration usually sets in after harvest at high temperatures (Aberoumand, 2010). The preservation of fish and fish products includes using any of the following available methods; fermentation, salting, freezing, oven-drying, sun-drying and smoking (smoke-drying) (Asiedu and Sanni, 2002; Abu and Eli, 2018).

Seas and the entire water bodies in Nigeria have suffered from serious pollution in the last four decades owing to oil exploration activities in the Niger Delta region, indiscriminate disposal of municipal wastes and lack of treatment of most industrial wastes before disposal into the environment and water bodies at large. Lagos State, being a cosmopolitan city has suffered the worst pollution arising from the large tunes of municipal and industrial wastes generated daily and a majority of these industrial wastes are disposed into the water bodies either as partially treated or not treated at all. Several studies have reported the extent of pollution of Lagos lagoon arising from the indiscriminate disposal of water (Eruola *et al.,* 2011), the effect of pollution on water quality and aquatic animals (Nkwoji *et al.,* 2018; Nkwoji *et al.,* 2020)

Aquatic environments have been highly impacted by various pollution among which is heavy metal pollution (Tabari *et al.,* 2010) which has been recognised as a serious problem for seafood (Caçador *et al.,*2012) and a great concern to man (Ibrahim *et al.,*2018). The sources of heavy metals can be both and/or anthropogenic or natural. Naturally, heavy metal enters into the aquatic system through ore-bearing, rocks, wind-blown, dust, forest fires and vegetation in small amounts (Fernandoz and Herrero, 2010). Fishes possessed a great ability to accumulate heavy metals in their muscles and given that different metals are taken up through different organs of fish based on corresponding affinity, leading to variation in concentrations of heavy metals at different organs of the body (Rao and Padmaja, 2000). Metal concentrations in fish can also be influenced by the environments where ponds or lakes are located (Singh and Kalamdhad, 2011). Cadmium, Mercury, Chromium, Lead, Arsenic and Manganese are some of the heavy metals that can be toxic at very low concentrations, persistent and bioaccumulated in aquatic environments (Morgano *et al.,* 2014). Also, the smoking process of fish has been shown to affect both the chemical and nutritional composition of smoked-dried fish arising from heat generated during the drying process (Olaoye *et al.,* 2014; Adamu-Governor *et al.,* 2023).

Further, the ingestion of heavy metals by fish through food and water may affect both the productivity and reproductive capabilities of such fish (Fonge *et al.,* 2011). Fish is one of the major sources of protein and forms an essential part of the human diet, the intake rate of these heavy metals by humans through the consumption of fish causes serious health hazards (Luoma and Rainbow,2008; Ofudje *et al.,* 2014).

In general, fish are regarded as safe food given that the muscles of healthy fish are known to be sterile (Novoslavskij *et al.,* 2016). However, studies have revealed the presence of microorganisms on fish surfaces; gills and skin (Sheng and Wang, 2021), inside of the fish; digestive tract and internal organs; liver and kidney (Moro *et *al.,* 2019). Also, arrays of bacterial pathogens known to be responsible for human diseases have been identified in fish (Novotny *et al.,* 2004; Moro *et al.,* 2019). Bacterial pathogens in water gain access into the fish majorly through gills or skin and can remain on the surface of the fish's body (Douglas, 2007; Sheng and Wang, 2021), and consequently cause bacterial diseases in fish (Dong, 2018) In addition to human pathogens, bacteria are considered the primary cause of fish spoilage (Rippen and Skonberg, 2012). In fish and fish products, pathogenic and spoilage microorganisms can be introduced at any point throughout the production and supply chain. However, fish and fish products, especially raw or undercooked products, have been involved in outbreaks associated with bacterial pathogens, fungi, mycotoxins, viruses, and/or parasites (Galaviz-Silva *et al.,* 2009), and with corresponding acute gastroenteritis with low fever, abdominal cramps, vomiting, nausea, diarrhoea and headache (Odu and Imaku, 2013).

The demand for fish consumption has increased in geometrical proportion owing to the prevalent high prices of meat (Olayele and Abegunde, 2015) and the health benefits associated with seafood. Similarly, fish is an indispensable portion of many Nigerian daily diets and given its rich source of polyunsaturated fatty acids, iodine, phosphorus, omega-3, Calcium, iron, trace element like copper and a fair proportion of vitamin B. Hence, this study was undertaken to determine the microbial quality and concentration of heavy metals and to isolate and identify bacteria and fungi associated with fresh and smoked Mullet and Silver catfish samples obtained from Makoko and Epe communities.

METHODOLOGY

Source of samples

Fresh fish samples; Mullet (*Mugil cephalus*) and Silver catfish (*Chrysichthys nigrodigitatus*) were collected directly from local fishermen while smoked fish samples were collected from local fish processors at Makoko (longitude 6.2940 and latitude 3.23103) and Epe (longitude 6.25332 and latitude 3.24208) fish markets. Fish samples were kept aseptically and separately in sterile polyethene zip-lock bags and transported in an ice-packed to College Central Research Laboratory, Yabatech, Lagos. Fish samples were dissected, organs (skin, liver and gills) removed, kept inside a sterile bottle containing normal saline and used immediately for microbial analysis.

Microbial analysis

Microbial analysis of liver, skin and gills from fresh and smoked Mullet and Silver catfish was done per the method described by Adamu-Governor *et al* (2023). For each sample, 25g was suspended in 225 ml of buffer peptone water (Oxoid Ltd, England), and homogenized for 2 min using a laboratory blender (Binatome, BL-1505 PRO, China). Serial decimal dilutions were made with buffer peptone water and inoculated in ten (10) different media: (i) Plate Count Agar (Bio-Rad) for total viable counts (incubated at 30°C / 3 days); (ii) Violet Red Bile Glucose (Bio-Rad) for *Enterobacteriaceae* (37°C / 24 ± 2 hr); (iii) TBX Agar (Bio-Rad) for *E. coli* (37°C / 21 ± 3 hr); (iv) TBX Agar (Bio-Rad) for *E. coli* (37°C / 21 ± 3 hr); (v) Mannitol Egg Yolk Polymyxin Mossel base (Biokar Diagnostics-Zac) with Egg Yolk (Biokar Diagnostics-Zac) for *Bacillus cereus* (30°C / 18–24 hr); (vi) Baird–Parker Agar (Bio-Rad) supplemented with Rabbit Plasma Fibrinogen (Bio-Rad) for *S. aureus,* (vii) *Pseudomonas* agar for *Pseudomonas* spp*,* (viii) Rapid' *Salmonella* agar enriched with buffered peptone water and active supplement (Bio-Rad) for *Salmonella* spp (37°C / 24 ± 2 hr). Each was confirmed with *Salmonella* Latex Kit (Bio-Rad) for agglutination test in compliance with the validated method BRD 07/11-12/05, (ix) Salmonella – Shigella agar for *Shigella* spp, and (x) PDA for fungi and molds (30 ± 2ºC / 72 hr).

Bacterial and fungi isolate Identification

Bacterial identification was done based on cultural, morphological, and series of biochemical tests; carbohydrate fermentation, catalase, citrate utility test, Sulphide, indole, methyl red, motility test, Kligler iron agar test using Bergey's Manual of Determinative Bacteriology. Before bacterial identification, distinct colonies were picked from the media and subcultured successively onto nutrient agar to obtain pure cultures.

Mineral and heavy metal analysis

Mineral and heavy metal content (Fe, Cu, Zn, Mn, As, Pb, Cd and Cr) of liver, skin and gills from fresh and smoked Mullet and Silver catfish were determined using Atomic Absorption Spectrophotometer (Agilent Technologies, 200 series AA, Australia) as described by Adamu-Governor *et al.* (2023).

Statistical analysis

Data obtained were subjected to statistical analyses using a one-way Analysis of Variance test (ANOVA) with Duncan's Multiple Range post hoc tests to identify significant differences in means where it exists ($p < 0.05$) with the aid of SPSS (IBM SPSS Inc. 26. Chicago, IL, USA).

RESULTS

Microbial analysis

Microbiological analysis of different organs; skin, gills and liver of fresh and smoked Mullet (*Mugil cephalus*) and Silver catfish (*Chrysichthys nigrodigitatus*) collected from Makoko and Epe fish markets are shown in Figure 1-5. Total bacterial aerobic count as shown in figure 1.0, smoked dried Liver of Mullet and fresh gills of Silver catfish from Makoko had the highest mean microbial load respectively. Generally, microbial load on the dried liver were higher compared to the liver from fresh fish samples except Mullet from Epe. Similarly, mean values of microbial load in fresh gills were higher than in smoked dried gills in all samples examined in this study. The mean values of microbial load on the skin vary, with fresh skin of Mullet and Silver catfish from Epe and Makoko being higher than smoked dried skin. On the contrary, microbial load mean scores for smoked dried skin were higher than fresh skin of Mullet and Silver's catfish obtained from Makoko and Epe fish markets respectively.

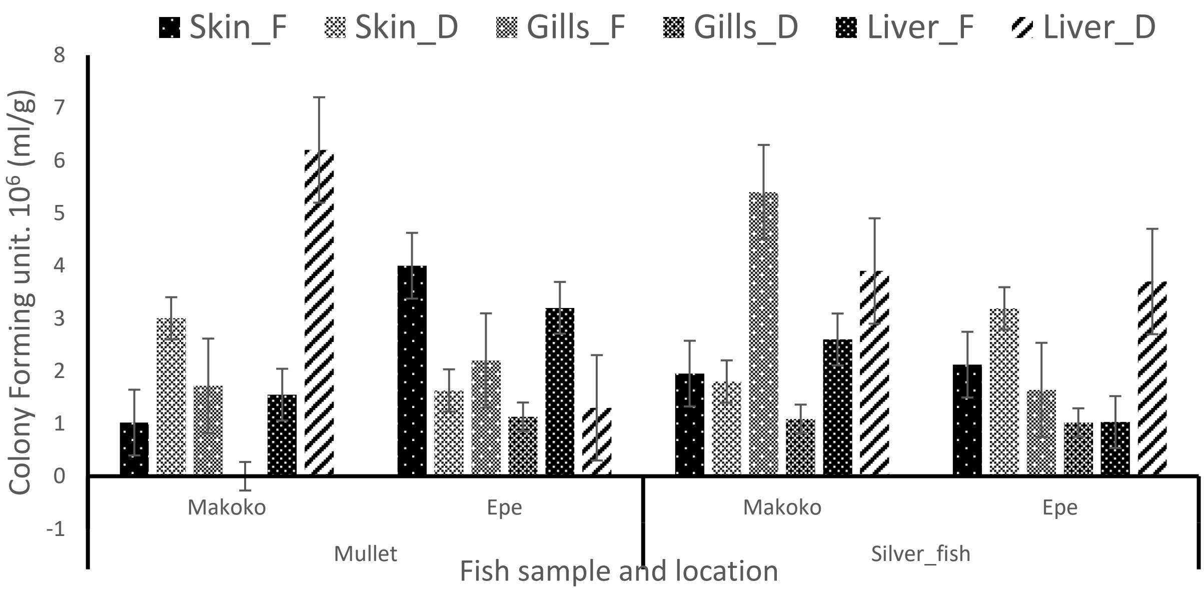

Figure 1.0: **Total aerobic bacterial count**

Expectedly and as shown in figure 2, the mean scores of coliform bacterial counts varied within fish samples, locations and across the different organs of fish examined. Smoked dried gills from Mullet had the highest mean value for microbial load, followed by smoked dried skin of Silver catfish obtained from Makoko. Generally, the mean values for microbial load for smoked dried gills were higher than fresh gills except for Mullet from Epe Market. A similar trend was observed in the mean values of smoked dried skin and liver except in Mullet from Makoko and Epe markets.

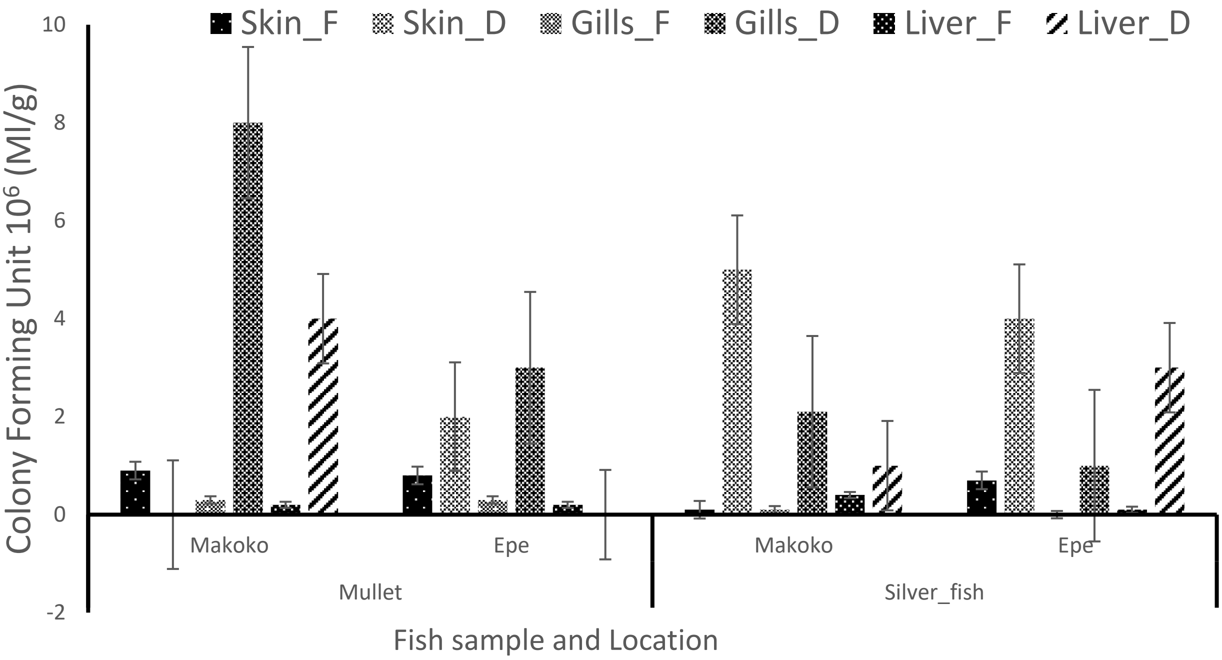

Figure 2.0: **Coliform bacterial count**

Presented in figures 3 and 4 are *Staphylococcus* and *Pseudomonas* species counts recorded in skin, gills and liver of fresh and smoked dried Mullet and Silver catfish obtained from Makoko and Epe fish markets respectively. For Staphylococcus species count, smoked dried skin and gills had the highest mean values, while dried gills and liver had the highest mean values in microbial load in Mullet for Makoko and Epe fish markets respectively. A similar trend was observed in the microbial load of the dried liver in Makoko while fresh skin had the highest in the Epe fish market for silver catfish. Pseudomonas species count, fresh skin, gills and liver had the highest mean values and correspondingly followed each other; with fresh skin recording the highest while fresh liver had the least mean scores for Mullet in Makoko. A similar trend was also observed in mean values of microbial load in Silver catfish from the Makoko fish market. Fungi count as shown in figure 5, smoked dried gills had the highest microbial load compared to the fresh gills for Millet in Makoko. Similarly, fresh skin and liver had a higher microbial load compared to smoked dried skin and liver in Mullet from Makoko while a microbial load of smoked dried skin and liver were higher in fresh skin and gills for Mullet for Epe fish market. In Silver catfish, smoked dried skin had the highest mean value of microbial load compared to fresh skin. Contrarily, fresh gills and liver had the highest mean value compared to dried gills and liver in Makoko while Silver catfish from Epe market; fresh skin, gills and liver microbial load mean values were marginally higher than the mean scores of smoked dried skin, gills and liver

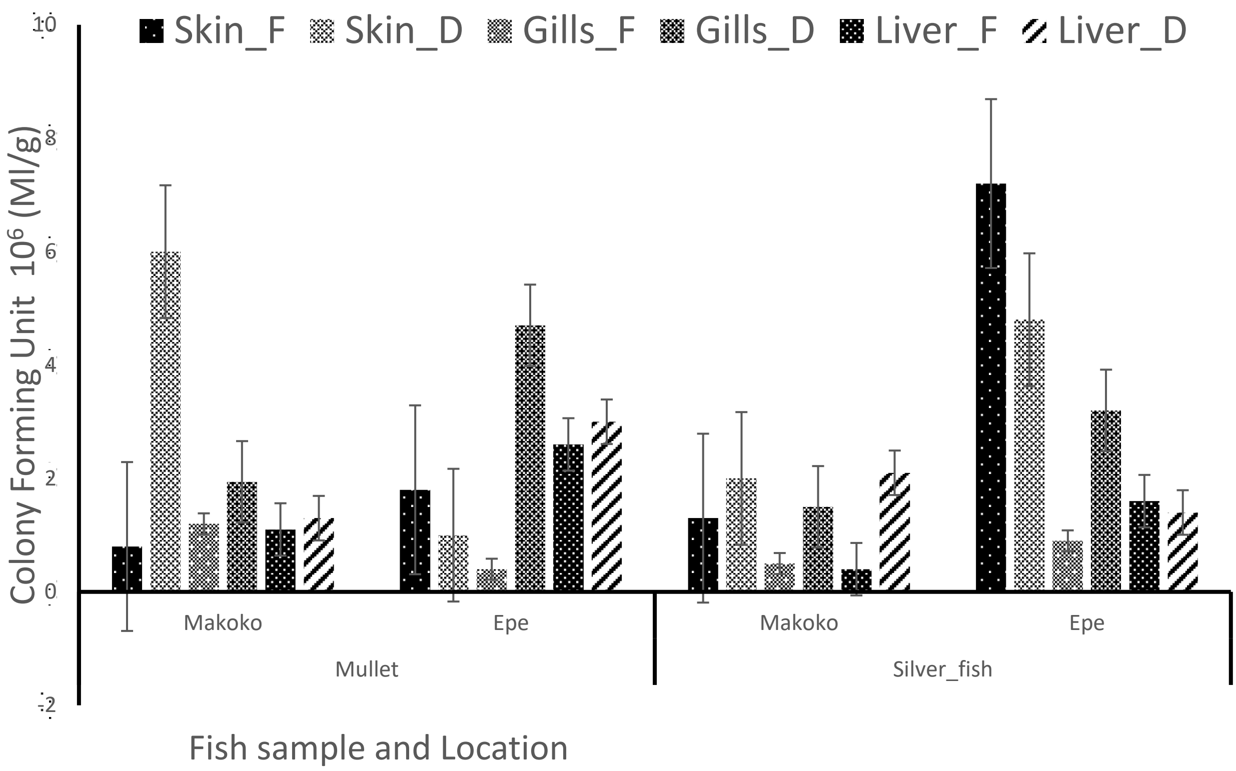

Figure 3.0: **Staphylococcus species count**

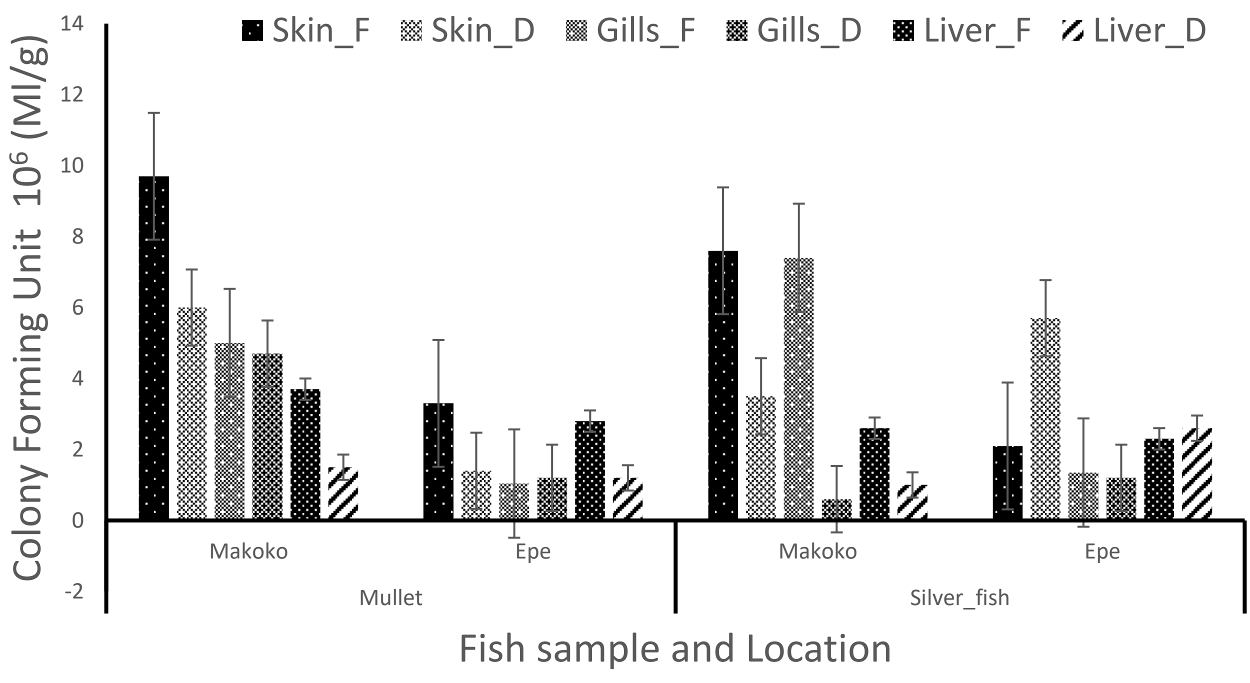

Figure 4.0: **Pseudomonas species count**

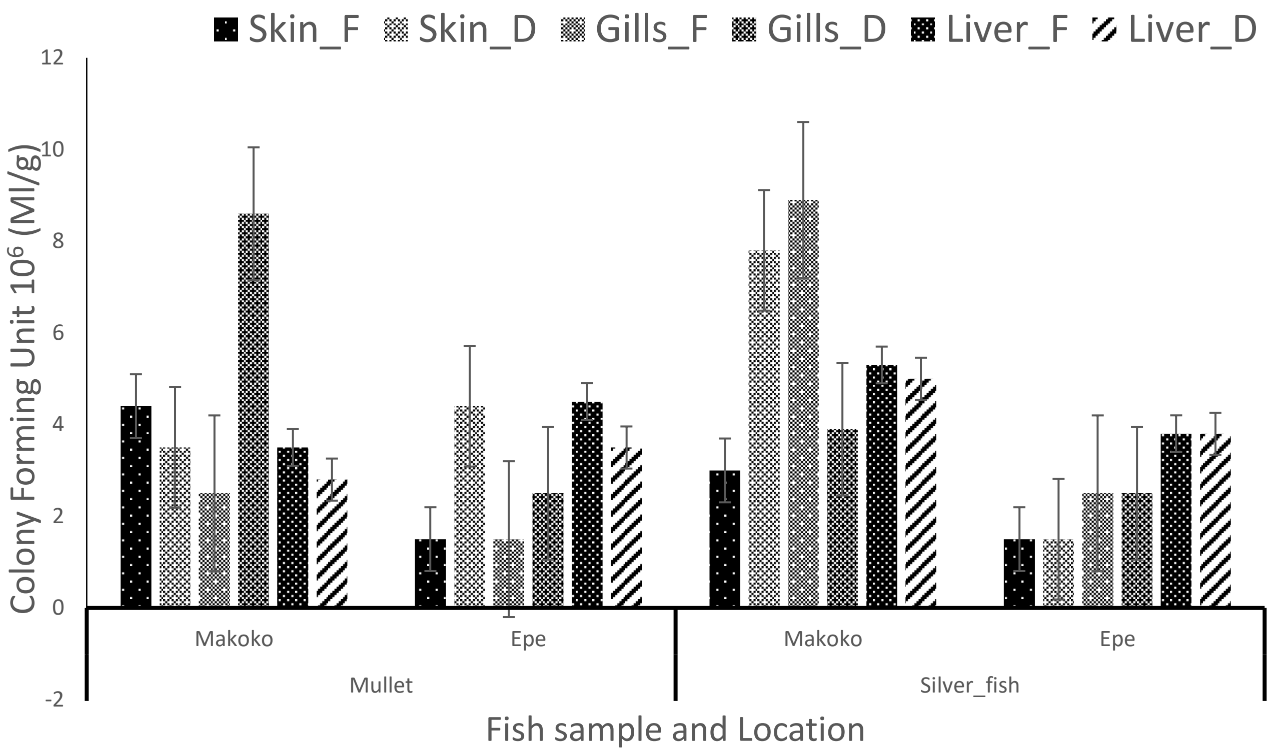

Figure 5.0: **Total fungi colony count**

Bacterial and fungi isolated, and identified from different organs; skin, gills and liver of Mullet and Silver catfish using morphological, microscopic features, biochemical characteristics and the Bergeys manual of determinative bacteriology are presented in Tables 1 and 2. Bacterial identified include *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Proteus morabilis Staphylococcus aureus, and Pseudomonas aeruginosa* in table 1 while fungi identified are *Aspergillus niger*, *Aspergillus flavus, Penicillium notatum, Rhizopus sp* and *Fusarium species* as indicated in table 2.

Table 1: **Bacterial isolated and identified from fresh and smoked dried Millet and Silver catfish**

Catalase	Citrate	Coagulase	Glucose	H2S	Indole	Lactose	Maltose	Mannitol	Methyl Red	Motility	Oxidase	Sucrose	Urea	VoghuesProkamer	Gram	Identified organism
+	+	N/A	+	-	-	+	-	-	-	+	-	-	-	+	-	*Enterobacter sp*
+	-	N/A	+	-	+	+	+	+	+	+	-	N/A	-	-	-	*Escherichia coli*
+	+	N/A	AG	-	-	+			-	-	-		+	+	-	*Klebsiella sp*
+	N/A	+	+	N/A	N/A	+	+	+	-	N/A	-	+	-	+	+	*Staphylococcus sp*
+	+	-	+	+	+	-	+	-	+	+	-		+	-	-	*Proteus sp*
+	+	-	-	+	-	+	-	-	+	+	-		+	-	-	*Pseudomonas sp*

N/A; Note applicable, AG; Acid and gas

Presented in table 3 are metal concentrations (mg/kg) of different organs; skin, gills and liver of fresh and smoked dried Mullet and Silver catfish obtained from the Makoko fish market. The mean values of mineral concentrations; Zinc, Copper, Cadmium, Chromium, Lead, Manganese and Iron evaluated in this study varied significantly ($p < 0.05$) within and across the different organs (skin, gills and liver) of the fish samples and sample location. While Arsenic was not detected in all samples irrespective of sample location

Table 2: **Bacterial isolated and identified from fresh and smoked dried Millet and Silver catfish**

Sample_ code	Macroscopic and colony characteristics	Probable organism
SD_Skin	White colony with a raised centre having a dot at the centre. Slow-growing with a flat periphery	*Penicillium* sp
F_Gills	White becoming greyish brown, coenocytic, rhizoids ovoid sporangiospores with filamentous stolon	*Rhizopu*s sp
SD_Gills	Black colony, septate, conidia in chains and terminated *Aspergillus flavus* with vesicles	*Aspergillus niger*
SD_Liver	Dense felt yellow colony, septate, radiate conidia head and filamentous	*Aspergillus flavus*
SD_Gills	Whitish cotton aerial with elongated ovoid curved microconidia	*Fusarium sp*

Table 3: **Metal Concentration of fresh and dried Mullet and Silver catfish from Makoko fish market (mg/kg)**

S/n	Zinc	Copper	Cadmium	Chromium	Lead	Manganese	Iron
FLM	22.05±0.07^{c}	0.14±0.01^{c}	0.03±0.00^{a}	0.20±0.01^{d}	0.02±0.00^{ab}	1.17±0.01^{abc}	222.00±2.83^{h}
DLM	19.35±0.33^{c}	0.01±0.00^{a}	0.31±0.01^{c}	0.20±0.01^{d}	0.05±0.01^{c}	1.18±0.03^{abc}	293.00±4.25^{i}
FSM	11.90±0.14^{b}	0.06±0.01^{b}	BDL	0.08±0.01^{b}	0.03±0.02^{b}	0.91±0.01^{ab}	38.50±2.12^{b}
DSM	9.95±0.21^{b}	0.07±0.01^{b}	BDL	0.21±0.01^{d}	BDL	1.30±0.00^{bc}	70.50±0.71^{c}
FGM	50.80±0.28^{e}	0.55±0.04^{f}	BDL	0.15±0.01^{c}	0.09±0.01^{e}	1.80±0.02^{bc}	71.00±0.28^{c}
DGM	41.45±0.06^{d}	0.39±0.01^{e}	BDL	0.10±0.01^{b}	0.04±0.00^{bc}	1.40±0.00^{bc}	79.55±0.78^{d}
DSS	18.20±0.00^{c}	0.07±0.00^{b}	BDL	0.42±0.00^{f}	0.01±0.00^{a}	2.25±0.25^{c}	32.50±0.71^{a}
FSS	3.66±0.08^{a}	0.70±0.01^{g}	0.04±0.00^{b}	0.31±0.01^{e}	0.07±0.01^{d}	1.05±0.01^{ab}	297.00±1.41^{i}
FLS	122.05±0.71^{h}	0.14±0.01^{c}	0.03±0.00^{a}	0.20±0.01^{d}	0.02±0.00^{ab}	1.17±0.01^{abc}	222.00±2.83^{h}
DLS	108.50±6.36^{g}	1.50±0.01^{i}	BDL	0.03±0.00^{a}	0.01±0.00^{a}	0.09±0.01^{a}	198.00±2.83^{f}
FGS	112.80±3.11^{g}	1.19±0.01^{h}	BDL	0.95±0.01^{g}	0.36±0.01^{g}	1.48±0.31^{bc}	99.00±1.41^{e}
DGS	83.75±1.49^{f}	0.24±0.01^{d}	BDL	0.04±0.00^{a}	0.12±0.01^{f}	1.00±0.00^{ab}	210.50±2.12^{g}

Values are means of duplicate determinations. Means on the same column with different sets of superscripts are statistically different (Duncan Multiple Range Test) ($p<0.05$). FLM, Fresh liver Mullet; DLM, Dried liver; FSM, Fresh skin; DSM, Dried skin; FGM, fresh gills; DGM, Dried gill; DSS, Dried skin Silver catfish; FSS, Fresh skin; FLS, Fresh liver; DSS, Dried liver; FGS, Fresh gills; DGS, Dried gills. BDL, below the detectable limit.

FLS (122.05±0.71) has the highest mean values and was significantly different ($p < 0.05$) from the means of other samples for

Zinc content. The least mean value was recorded in FSS (3.66±0.08). For copper content, DLS (1.50±0.01) has the highest mean value followed significantly by FGS (1.19±0.01) and FSS (0.70±0.01) while the least mean value was observed in DLM (0.01±0.00 mg/kg). FLM (0.03±0.00 mg/kg) and FLS (0.03±0.00) recorded the least mean values for Cadmium and were not significantly different ($p < 0.05$). DLM has the highest mean value and is significantly higher than the mean value of FSS (0.04±0.00). While Cadmium content was below the detection limit for other samples. Chromium content, FGS (0.95±0.01 mg/kg) has the highest mean value followed significantly by DSS (0.42±0.00) and FSS (0.31±0.01), while DGS (0.04±0.00) and DLS (0.03±0.00) recorded the least mean values and were not significantly different from each other. Further, DSS (0.01±0.00) and DLS (0.01±0.00) recorded the least mean values for Lead content while FGS (0.36±0.01 mg/kg) has the highest mean value and were significantly different from the mean values of others. Similarly, DLS (0.09±0.01) recorded the least mean value for Manganese and was different from other means, while DSS (2.25±0.25 mg/kg) has the highest mean scores. The mean values for iron content were generally higher compared to mean values for other minerals. DSS (32.50±0.71 mg/kg) and DLM (293.00±4.25 mg/kg) recorded the least and highest mean scores respectively for iron content.

Presented in table 4 are metal concentrations of fresh and smoked dried Mullet and Silver catfish from the Epe fish market. As expected, the mean values varied significantly across and within the different organs of the fish and mineral content considered.

Table 4: **Metal Concentration of fresh and dried Mullet and Silver catfish from Epe Fish Market (mg/kg)**

S/n	Zinc	Copper	Cadmium	Chromium	Lead	Manganese	Iron
FLM2	37.80±0.14^{g}	6.77±0.01^{g}	0.03±0.00^{a}	0.29±0.01^{e}	0.05±0.02^{d}	0.20±0.03^{f}	117.25±0.35^{j}
DLM2	20.35±0.21^{b}	2.40±0.00^{a}	0.01±0.00^{a}	0.16±0.01^{c}	0.03±0.00^{bc}	0.07±0.01^{bc}	72.85±2.82^{d}
FGM2	41.10±0.14^{i}	3.06±0.01^{c}	0.02±0.01^{a}	0.13±0.01^{b}	0.02±0.00^{ab}	0.04±0.02^{a}	59.95±0.21^{a}
DGM2	35.80±0.14^{f}	5.54±0.01^{d}	0.01±0.00^{a}	0.34±0.01^{g}	0.04±0.01^{cd}	0.13±0.01^{d}	97.85±0.01^{g}
FSM2	35.85±0.35^{f}	2.73±0.04^{ab}	BDL	0.09±0.01^{a}	0.08±0.01^{e}	BDL	61.45±0.07^{b}
DSM2	19.60±1.40^{a}	7.01±0.01^{i}	0.02±0.00^{a}	0.22±0.01^{d}	0.11±0.01^{f}	0.12±0.01^{d}	88.80±0.14^{f}
FSL2	39.55±0.21^{h}	6.88±0.01^{h}	0.02±0.00^{a}	0.29±0.01^{e}	0.05±0.00^{d}	0.15±0.01^{e}	68.60±0.14^{c}
DSL2	21.55±0.07^{c}	3.12±0.00^{cd}	0.01±0.00^{a}	0.11±0.01^{ab}	0.02±0.00^{ab}	0.08±0.00^{c}	104.30±0.28^{h}
FSS2	41.60±0.14^{j}	5.91±0.01^{e}	0.02±0.00^{a}	0.32±0.00^{f}	0.07±0.01^{e}	0.12±0.02^{d}	68.55±0.21^{c}
DSS2	22.65±0.07^{d}	3.02±0.01^{c}	BDL	0.32±0.01^{f}	0.23±0.01^{g}	0.04±0.00^{a}	113.25±0.28^{i}
FSG2	37.40±0.14^{g}	6.65±0.05f	0.01±0.00^{a}	0.29±0.01^{e}	0.67±0.01^{h}	0.16±0.01^{e}	60.60±0.85^{a}
DSG2	26.40±0.14^{e}	2.84±0.02^{b}	BDL	0.10±0.01^{a}	0.01±0.00^{a}	0.05±0.00^{ab}	86..95±0.35^{e}

Values are means of duplicate determinations. Means on the same column with different sets of superscripts are statistically different (Duncan Multiple Range Test) ($P<0.05$). FLM2, Fresh liver Mullet from Epe; DLM2, Dried liver; FSM2, Fresh skin; DSM2, Dried skin; FGM2, fresh gills; DGM2, Dried gill; DSS2, Dried skin Silver cat fish from Epe; FSS2, Fresh skin; FLS2, Fresh liver; DSL2, Dried liver; FSG2, Fresh gills; DSG2, Dried gills. BDL, below the detectable limit.

For Zinc content, FSS2 has the highest mean score followed significantly by FGM2 (41.10±0.14 mg/kg) and FSL2 (39.55±0.21 mg/kg) while DSM2 (19.60±1.40 mg/kg) recorded the least mean value. In the same vein, DLM2 (2.40±0.00 mg/kg) recorded the least mean score and significantly varied from the mean score of other samples for copper. The highest mean value was observed in DSM2 (7.01±0.01 mg/kg) followed by FSL2 (6.88±0.01 mg/kg), FLM2 (6.77±0.01 mg/kg) and FSG2 (6.65±0.05 mg/kg) respectively. Further, no significant difference ($p < 0.05$) was observed for Cadmium in all samples except FSM2, DSS2 and DSG2 with Cadmium concentration below the detection level. DGM2 (0.34±0.01) recorded the highest mean value and significantly differ from the mean scores of other samples while FSM2 (0.09±0.01) and DSG2 (0.10±0.01) have the least mean scores for Chromium. Furthermore, FSG2 (0.67±0.01) and FLM2 (0.20±0.03) recorded the highest mean values, while DSG2 (0.01±0.00), and DSS2 (0.04±0.00) and FGM2 (0.04±0.02) have least mean scores for Lead and Manganese respectively. Similarly, the least mean score was observed in FGM2 (59.95±0.21 mg/kg) and significantly different ($p < 0.05$) from other means for iron content while the highest mean score was observed in FLM2 (117.25±0.35 mg/kg).

DISCUSSION

Microbiological analysis of fresh and smoked dried Mullet and Silver catfish recorded high microbial load in both locations. The microbial load recorded in this study are within the range earlier reported for smoked *Chrysichthys nigrodigitatus* by Adelaja *et al.* (2013) who reported $3.1 \times 10^6 - 4.9 \times 10^6$ and $6.8 \times 10^6 - 13.8 \times 10^6$ cfu/g respectively. Similarly, Udochukwu *et al.* (2016) reported microbial load within the range for both fresh and smoked dried Merluza, Scombia and Sese. Contrarily, several studies have recorded lower microbial loads in fresh and smoked dried fish, fish products and shrimps (Akinwumi and Adegbehingbe, 2015; Zaki *et al.,* 2021). The high microbial load observed in this study may be attributed to the microbiological population of the aquatic environments where the fish are cut, fish gut microbiomes, exposure of the fish to polluted air, unhygienic handling during and after processing (Jami *et al.,* 2014; Ringo *et al.,* 2016; Yukgehnaish *et al.,* 2020).

Variations in microbial load for fresh and smoked fish samples, and different organs of fish; skin, liver and gills were recoded. Adelaja *et al.* (2013) reported variations in bacterial accounts in smoked *Chrysichthys nigrodigitatus* for skin, liver and gills. Generally, a lower microbial load has been reported for smoked fish and fish products compared to their raw/fresh counterpart (Anihouvi *et al.,* 2019; Zaki *et al.,* 2021). The variations of microbial load observed in this study could be owed to several factors; sources of the fish samples, quality of the raw material, sediments, handling and hygiene practices, and long exposure to high temperatures (Xiong *et al.,* 2015; Sheng and Wang, 2021; Zaki *et al.,* 2021). Also, all microbes are killed by a hot smoking process with the fish being cooked and dried at high temperatures except a few pathogenic bacteria (Dutta *et al.,* 2018).

Unexpectedly, higher microbial loads in a few instances were recorded for coliform, *Pseudomonas* sp, *Staphylococcus* sp, and total aerobic bacterial and fungi count in dry gills or dry liver or dry skin of dry fish compared to fresh fish samples in this study. The higher microbial load observed in dry gills, liver and skin of fish samples could be attributed to moisture reabsorption from the environment that encourages the speedy growth of microorganisms. Several factors determine the quality of smoked fish products; type of the smoking procedure and the nature of the wood used, preparation of the raw material, the fish quality at the time of smoking, poor hygiene of processors, poor packaging and storage technique (da Silva, 2002; Udochukwu *et al.,* 2016). Other studies have reported post–harvest handling and processing as a major source of microbial contamination for smoked fish (Basti *et al.,* 2006; Christianah *et al.,* 2010). In this study, bacterial; *Enterobacter* sp, *Escherichia coli*, *Klebsiella* sp, *Staphylococcus* sp, *Proteus* sp and *Pseudomonas* sp while fungi; *Penicillium* sp, *Rhizopus* sp, *Aspergillus niger, Aspergillus flavus* and *Fusarium sp* were identified. The bacterial and fungal species identified in fresh and smoked dry Millet and Silver catfish have been reported in fresh and dry fish, and fish products (Akinwumi and Adegbehingbe, 2015; Udochukwu *et al.,* 2016). Pathogenic and spoilage microorganisms can be introduced into fish and fish products from pre-harvesting, processing, handling and post-harvesting (Okonko *et al.,* 2008; Udochukwu *et al.,* 2016). Studies have also attributed the presence of these pathogenic microorganisms in fish samples to the microbial flora associated with the fish, aquatic niche, and sediments (Xiong *et al.,* 2015; Yukgehnaish *et al.,* 2020). Several studies have reported the presence of pathogenic bacteria in fresh and smoked dry fish, and fish products (Akinwumi and Adegbehingbe, 2015; Sheng and Wang, 2021). Also, the presence of *Penicillium* sp, *Rhizopu*s sp, *Aspergillus* sp and *Fusarium* sp in the smoked dry fish samples has been attributed to moisture reabsorption from the environment and consequently increase the growth of microorganisms in the fish.

The occurrence of these pathogenic bacteria and fungi particularly aflatoxigenic moulds in the fish samples examined in this study posed serious safety concerns given that this may harm the health of the consumers (Huss *et al.,* 2000; Okonko *et al.,* 2008). The presences of these pathogenic microorganisms in food when consume could results in the following health implications; gastroenteritis, fish-borne diseases of human, food poisoning, diarrhoea and kidney damage, bacteremia, pneumonia, mycotoxins and liver destruction (Adelaja *et al.,* 2013; Udochukwu *et al.,* 2016)

As expected, the level of metals varies within the different organs of fish, fish species, and sources of fish and across the metals. Similar variations in levels of metal were observed in fresh and smoke-dry fish samples. These observed variations of metals concentrations in fish samples could be attributed to several factors; temperature and salinity of the water, nutrient availability, season, location, metal concentrations and chemical characteristics of water, species, size and part of fish analyzed (Tuzen, 2003; Rauf *et al.,* 2009; Bashir *et al.,* 2012). Also, studies have shown that heavy metals in fish can be obtained from sediments, food and surrounding waters (Nussey *et al.,* 2000; Wangboje and Ikhuabe, 2015). Further, the concentration of heavy metals at different levels in different organs of fish has been attributed to the affinity between metals and different organs of fish during heavy metals uptake (Karadede *et al.,* 2004; Peakall and Burger, 2003).

Zinc values recorded in this study in both sample locations are within permission limit in fish except for FLS (122.05±0.71), FGS (112.80±3.11) and DLS (108.50±6.36). For human consumption in fish, the maximum permissible Zn is 100mg/Kg. Higher metals concentrations in the gills may be attributed to breathing and feeding processes which essentially occur in the gills inside the head of the fish and consequently exposed the gills to high concentrations of metals (Sadiq *et al.,* 1982; Olusola and Festus, 2015). Studies have reported that some tissues in fish particularly, the liver and gills have shown active metabolic activity in their physiological role compared to other tissues (Marcovecchio *et al.,* 1991; Alhemmali *et al.,* 2018). Furthermore, lower Zn values were observed in all organs of smoked dry fish samples and across sample locations in this study. The lower Zn values in smoked dry fish samples may be attributed to evaporation loss through the effect of applied heat on the metal concentrations of fresh fish during smoked drying (Abu and Eli, 2081; Adamu-Governor *et al.,* 2023).

As indicated in both fresh and smoked dry Mullet and Silver catfish, Cu values recorded in Epe were much higher than in the Makoko fish market in all fish organs examined, Bioavailability of slightly high concentrations of metals especially copper to fish in water may be responsible for the higher Cu values in fish samples from Epe. Some of the main sources of copper; are pesticides, alloys, cooking utensils, piping and wiring, fossil fuel and wood preservatives (Wangboje and Ikhuabe, 2015). Studies have reported higher concentrations of copper in the liver of fish and attributed it to the role of metallothioneins in metabolism, storage and detoxification leading to an increase in the accumulation of essential metals in the liver (Gorur *et al.,* 20121; Abarshi *et al.,* 2017). Further, copper values recorded for all organs examined were higher than copper values reported for fresh and smoke-dry shrimps, Oyster, and different organs of different species of fish in previous studies (Wangboje and Ikhuabe, 2015; Abu and Eli, 2018; Alhemmali *et al.,* 2018) but lower than values reported for smoked dried shrimps (Adamu-Governor *et al.,* 2023). The mean scores of Cu in fish organs recorded in this study were far lower than the maximum permissible limit of 120 mg/kg in food (Olusola and Festus, 2015).

Cadmium (Cd) was only detected in FLM, DLM, FSS and FLS in samples from Makoko while Cd was detected in all samples from Epe except for FSM2, DSS2 and DSG2. The concentration of Cd could be probably below detection limits in the river where the fish samples were harvested and consequently reflected the concentrations of Cd in fish tissues (Abarshi *et al.,* 2017). As demonstrated in the mean scores for Cd, organs of fish samples from Makoko showed higher concentrations of Cd compared to tissues of fish samples from Epe. The values of Cd obtained in this study are within the range reported by Abu and Eli (2017) for fresh and smoked Oysters (0.04 – 0.21 mg/kg), Wangboje and Ikhuabe, (2015) for different species of fish (0.04 – 0.25 mg/kg), Lawal *et al* (2020) tilapia and catfish (0.03 – 0.28 mg/kg). Other studies have reported higher concentrations of Cd in fresh and smoked shrimps (0.30 – 0.90 mg/kg), and different tissues in different species of fish (Abu and Eli, 2018; Abarshi *et al.,* 2017). The concentration of the Cd recorded in this study except for DLM (0.31 mg/kg) was lower than the maximum acceptable limit of 0.05 mg/kg as specified by the European Union.

As demonstrated in this study, Chromium (Cr) was detected in all tissues in both fresh and smoked Mullet and Silver catfish. Studies have reported the presence of Cr in smoked dried shrimps, and different organs in different species of fresh fish (Olusola and Festus, 2015; Adamu-Governor *et al.,* 2023). Gills have the highest concentrations of Cr and are followed by the skin as indicated in the distribution pattern of chromium in the fish tissues. The presence of high concentrations of chromium in the gills of fish has been reported by Olusola and Festus (2015) and other organs (Alhemmali *et al.,* 2018; Lawal *et al.,* 2020). In addition, the Cr values recoded for tissues in this study were higher than the range (0.02 – 0.09 mg/kg) reported by Alhemmali *et al*. (2018) for *Discentrarchus punctatus* and lower than the values for different organs in different species of fish reported in several studies (Olusola and Festus, 2015; Lawal *et al.,* 2020). The maximum permissible limit of Chromium is 8 mg/kg in food and the concentrations of Cr recorded were lower than the acceptable limit.

Lead (Pb) concentrations were detected in the tissues of both fresh and smoked dry Mullet and Silver catfish except DMS. Similarly, gills showed the highest concentrations of Pb in fresh Silver catfish irrespective of the two locations. The Pb concentrations recorded in different organs of fish in this study correlate with Pb concentrations in different organs of *Arius latisculata* (0.01- 0.12 mg/kg), *Cynoglosus browni* (0.05 – 0.11 mg/kg) by Olusola and Festus, (2015). Several studies have reported Pb concentrations in different organs of *Discentrarchus punctatus* (Alhemmali *et al.,* 2018), different species of fresh fish (Wangboje and Ikhuabe, 2015), fresh and smoked Oysters, and Shrimp (Abu and Eli, 2018). Contrarily, others studies have reported higher concentrations of Pb in different organs, and different species of fish (Abarshi *et al.,* 2017; Lawal *et al.,* 2020). The value of Pb in the tissues of Mullet and Silver catfish recorded is below the maximum permissible limit of 2 mg/kg (Cronin *et al.,* 1998; Abarshi *et al.,* 2017).

Manganese (Mn) was present in all the organs of fresh and smoked dry Mullet and Silver catfish examined except FSM2. The values of Mn recorded for fish samples from Makoko are much higher compared to the values recorded for organs of fish from Epe. Abarshi *et al.* (2017) reported the presence of Manganese in different organs of fish obtained from Bonny (9.34-11.02 µg/g) and Finima (43.72-323 µg/g) rivers which are lower than the values recorded in this study. These authors deduced the higher values of Mn recorded particularly tissues of fish from Finima River to frequent crude oil spills and other industrial discharge into the water body. Also, another study has reported manganese values (0.08-0.38 mg/kg) within the range obtained in this study from different organs of fish (Alhemmali *et al.,* 2018).
Finally, Iron (Fe) was detected in the tissues of fresh and smoked dry Mullet and Silver catfish in this study. Fe values recorded for tissues of fish from Makoko were much higher compared to values recorded for Epe. A similar trend was also observed in Fe values recorded for smoked dry tissues compared to fresh fish tissues irrespective of fish species. The values of Fe recorded in this study are higher than the values reported for different organs of tilapia (0.74-1.93 mg/kg) and catfish (0.95-1.26 mg/kg) (Lawal *et al.*, 2020), different organs (0.24-1.59 mg/kg) of *Discentrarchus punctatus* (Alhemmali *et al.,* 2018). Also, much lower values of Fe were recorded for organs of fishes from Bonny (102-216 µg/g) and Finima (565-801 µg/g) rivers (Abarshi *et al.,* 2017). Based on the values of Fe recorded, 62.5% of the tissues have Fe content within permissible limit and the maximum detectable limit for Fe in fish is 100 mg/kg as specified by WHO (WHO, 1989).

Metals are used by fishes for enzymatic and normal physiological processes at different concentrations in aquatic environments. These metals; Cu, Zn, Fe and Mn are regarded as essential elements given that these metals useful in; enzymes activity, synthesis of haemoglobin, as a cofactor for many enzymes, biochemical and physiological function in blood cells, a structural component of regulatory proteins among other functions (Sivaperumal *et al.,* 2007; Edward *et al.,* 2013). While other metals; Pb, Cr, As and Cd are found in the environment with scanty knowledge about their specific biological function to aquatic organisms and humans. Studies have reported that for glucose metabolism, chromium is required as a trace metal (Singh and Kalamdhad, 2011; Sivakumar and Xiaoyu, 2018). However, the presence of these metals in the tissues of fish serves as an indicator of the level of metal pollution in aquatic environments. These metals have found their way into the water bodies via refuse, pesticides, alloys, fossil fuels, batteries, industrial effluent discharge, sewage discharge and wood preservatives (DPR, 2010; Wangboje and Ikhuabe, 2015; Abarshi *et al.,*2017). Excessive intake and exposure of these metals to fishes in aquatic environments over a long period will lead to bioaccumulation of these metals in the fish tissues (Zhao *et al.,* 2012; Ofudje *et al.,* 2014) and consequently may increase metal concentrations that exceed the maximum permissible limits in the fish organs. The effect of excessive exposure and bioaccumulation of these metals include; changes in the distribution and abundance of the population, impaired metabolic functions, low productivity and reproductive defect (Adedeji and Okocha, 2011; Wangboje and Ikhuabe, 2015). Consumption of these fishes with low concentrations of heavy metals, fish tissues with bioaccumulation of other metals by man, and over a long period could result in the following health complications; neurotoxicity, nephrotoxicity, hepatotoxicity, testicular degeneration and chronic lung disease (Garcia-Leston *et al.,* 2010; Mason *et al.,* 2010; Stancheva *et al.,* 2013).

In conclusion, this study revealed the presence of higher microbial load for the total aerobic count in (smoked dry live and fresh gills), coliform count (smoked dry gills and skin), Pseudomonas sp (fresh and dry skin), fungi count (smoked dry and fresh gills) for Makoko only while Staphylococcus sp (fresh and dry skin) for Makoko and Epe irrespective of fish species. The study also identified *Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus,* and *Pseudomonas aeruginosa* while *Aspergillus niger, Aspergillus flavus, Penicillium notatum, Rhizopus sp* and *Fusarium species* as bacteria and fungi respectively. Varying concentrations of Pb, Cu, Zn, Fe, Cr, Cd and Mn were observed in both fresh and smoked dry Mullet and Silver catfish, while As was not recorded.

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