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## HEAVY METALS DETERMINATION AND MICROBIAL ASSESSMENT OF SOME SPECIES OF FROZEN FISH SOLD AT UTAKO MARKET, ABUJA, NIGERIA

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### ABSTRACT

***This work assessed heavy metals [Copper (Cu), Iron (Fe), Cadmium (Cd), Zinc (Zn) and Lead (Pb)] concentrations and microbial load in the tissue (skin, fillet and gills) of some frozen fishes [Clupea harengus (herring), Scomber scombrus (Mackerel), Urophycis tenuis (White hake)] sold in Utako market using Flame Atomic Absorption Spectrophotometer (AAS) and standard microbiological procedures. The results obtained revealed that the concentrations of all the heavy metals determined except Zinc (Zn) were above the Food and Agriculture Organization (FAO) and World Health Organization (WHO) permissible limits in fresh water fish and fishery products. The total aerobic plate count (APC) was between  $2.15 \times 10^3$  cfu/g and  $47.6 \times 10^3$  cfu/g, total coliform count ranged from  $1.85 \times 10^3$  cfu/g to  $2.03 \times 10^3$  cfu/g and fungal counts ranged between  $6.02 \times 10^2$  cfu/g and  $18.3 \times 10^2$  cfu/g. The microbiological study showed that the skin had more load compared to other studied tissues and in all, the microbial load except APC also exceeded the FAO/WHO acceptable limits for frozen fish products. This study indicated that the products were not ideal for consumption due to bioaccumulation of these heavy metals and the issue of post-harvest contaminants that can multiply in case of defrosting which may impact negatively on the consumers. It is recommended that there should be proper handling and examination of frozen foods and they should be properly cooked before consumption.***

***Keywords: Heavy metals, frozen fishes, market, microbial load, consumers.***

### INTRODUCTION

Heavy metal refers to any metallic element whose density is relatively equal to or greater than 5 g/cm<sup>3</sup> and is toxic even at low concentration (Useh *et al.*, 2018). In recent times, the level of heavy metals concentrations in aquatic environment have been on the increase due to pollution from industrial wastes, changes in geochemical structure, agricultural and mining activities (Kareem *et al.*, 2016). Environmental pollution by heavy metals has become a worldwide problem due to the fact that heavy metals, unlike some other contaminants, are non-biodegradable (Useh *et al.*, 2018). Consequently, they are not detoxified but concentration can only increase through bioaccumulation especially in the aquatic environment. Pollution of water bodies with heavy metals poses a long term risk to aquatic lives and the ecosystem. Research have shown that fish which are often at the top of aquatic food chain assimilate these heavy metals through food materials, ingestion of suspended particulates and/or by constant ion exchange process of dissolved metals across lipophilic

membranes like the gills or adsorption of dissolve metals on tissues and membrane surfaces (Oluyemi and Olabanji, 2011; Kareem *et al.*, 2016; Łuczyńska *et al.*, 2022). The heavy metal is taken by the blood stream to either the liver or the bone for transformation or storage during adsorption (Igwemmar *et al.*, 2013). Substantial amounts of heavy metals tend to accumulate in the marine ecosystems and in fish muscle tissues by natural processes which subsequently get transferred to higher trophic levels via food chain. The contamination of fish with toxic heavy metals being an important link in the food chain causes a direct threat not only to the aquatic system, but to the primary consumers which are humans. Long-term effect of heavy metals exposure to human and higher animals includes mental lapse, kidney failure, and central nervous system disorder (Daniel *et al.*, 2013; Jasmina *et al.*, 2020). For example, chronic exposure to Cadmium can have harmful effects such as lung cancer, bone fractures, kidney dysfunction (Useh and Dauda, 2018).

A high consumption of copper, zinc and lead has been linked to Alzheimer's disease while zinc and iron are being linked to Parkinson's disease (Łuczyńska *et al.*, 2022).

Fish are important resource for humans, especially as food and commercial subsistence and have had a role in the culture through the ages. Fishing for food is an ancient practice that was found since the advent of mankind (Bowen *et al.*, 2016; Rodriguez *et al.*, 2019). Fishing has become an important part of human life such that the demand has led to involvement of both wild capture and aquaculture fisheries. However, the world is geared towards improvement of fish farming (aquaculture), although the wild capture fishing is still the leading sector that provides livelihoods and income for millions of people around the world (Abdullahi *et al.*, 2015). Consumption of imported frozen fish was found to supersede many source of animal protein such as beef and chicken among others in Nigeria. Massive importation of frozen fish in the country has ranked Nigeria the largest importer of frozen fish in Africa (Abubakar *et al.*, 2014). Fish is one of the main sources of easily digestible protein rich in long chain polyunsaturated omega-3 fatty acids, essential amino acids, fats, macro- and trace elements, and fat-soluble vitamins (Łuczyńska *et al.*, 2022). Fish and other seafood are unique dietary sources of cardioprotective docosahexaenoic (DHA) and eicosapentaenoic (EPA) fatty acids. Long chain polyunsaturated fatty acids are associated with improving health and preventing diseases of old age (Ekanem and Udoma, 2021). Thus, many public health authorities recommend regular fish consumption equivalent to at least 1–2 serving per week (quantities of approx. 300 g) in order to prevent diet-related chronic diseases (Australian Guidelines, 2013; WHO, 2018; Jasmina *et al.*, 2020). Studies have shown that n-3 polyunsaturated fatty acids (n-3 PUFA) which is found mostly in fish prevent or reduce the risk of cancer, neurological disorders, cardiovascular diseases and play an important role in the growth of foetus and the development of cognitive functions in children (Bowen *et al.*, 2016; Rodriguez *et al.*, 2019; Łuczyńska *et al.*, 2022).

The concern about the high levels of heavy metals in foods has prompted several statutory bodies such as the WHO to establish maximum allowable concentrations for some of the metals in food (WHO, 2011). Fish samples are considered as one of the most indicative factors, in freshwater systems, for the assessment of the potential of heavy metal pollution (Bowen *et al.*, 2016). Since the permissible limits of metals in seafood have been introduced in many parts of the world for the safe consumption of fish

species, monitoring programs investigating the levels of heavy metals in fish are gaining momentum, especially in developing countries like Nigeria where fish constitutes part of the healthy human diet because of their high nutritional quality such as providing the major source of protein. Thus the World Health Organization (WHO) as well as the Food and Agriculture Organization (FAO) of the United Nations state that monitoring eight elements (Hg, Cd, Pb, As, Cu, Zn, Fe, Sn) in fish is obligatory while the monitoring of others though not obligatory may be useful (Oluyemi and Olabanji, 2011; ). Several studies have shown that wild captured marine fish which might be brought from heavy metals contaminated waters are supplied mostly in frozen form, and they may bio-accumulate these heavy metals to a greater extent (Laila *et al.*, 2013; Abubakar *et al.*, 2014; Ibanga *et al.*, 2019; Ayanda *et al.*, 2019). Deep frozen method can only preserve it from decomposition by slowing down some biochemical activities, but do not have any impact on the presence of heavy metals contaminants (Laila *et al.*, 2013).

Quality and safety of the fish products and particularly frozen food is one of the main problems of food industry today. The presence or absence of food borne pathogens (particularly staphylococcus aureus and Escherichia coli) in a fish product is a function of harvest environment and sanitary conditions during capture, processing, distribution and/or storage (Hala *et al.*, 2017; Al-Sheraa, 2018). This suggests that fishes can be contaminated by both aquatic environment and post-harvesting conditions. Fish is a very perishable high protein food that typically contains substantial amount of free amino acids which are easily metabolized by microbes (Ismail and Belma, 2002). Marketing of fish in Nigeria is mostly carried out by local fishmongers at ambient temperature, a condition that favours contamination and spread of microorganisms. The qualities of frozen food have been affected by drip loss, product bleaching, rancidity and product dehydration (Ekanem and Udoma, 2021).

Freezing inhibits the growth of microbes by reducing their numbers but not destroying them. The survival of pathogens depends on the type of food, the category of the microbes, freezing range and thawing. Presence of these pathogenic microbes could cause foodborne disease such as cholera, campylobacteriosis, E. coli gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, etc. (Ismail and Belma, 2002). Due to the frequency of consumption of frozen foods within the Nigerian populace, there may be an opportunity of the extended hazard of contamination and change in

the exposures of the microorganisms and this could negatively impact the consumers (Emmanuel-Akerele and Uchendu, 2021). Microbiological test is equally imperative to public health as it points to the spoilage condition of fish which turns out to be the cause of food poisoning. Hence, this study was designed to determine the level of heavy metals concentration and carry out microbial assessment of some species of frozen fish sold at Utako market, Abuja, Nigeria.

## MATERIALS AND METHODS

### Description of the Study Area

Abuja, the capital of Nigeria is located in the central part of Nigeria, in the Federal Capital Territory (FCT) and was created in 1976. It lies between latitude 9°4'N of the equator and longitude 7°29'E of Greenwich Meridian. The territory is located just north of the confluence of the Niger River and Benue River. It is bordered by the States of Niger to the west and north, Kaduna to the northeast, Nasarawa to the east and south and Kogi to the southwest. It has a landmass of approximately 7,315 km<sup>2</sup>, with an estimated population of about 2.5 million and it is situated within the savannah region with moderate climatic conditions and also surrounded by abundant hills (Useh *et al.*, 2016). Utako District which is under Abuja Municipal Area Council (AMAC) is located in Phase 2 area of Abuja and this area is basically a residential area. Landmarks in this district include Utako Ultra-Modern Market, Arab Contractors, God is Good Motors, ThisDay Newspaper Complex amongst others (Useh *et al.*, 2016).

### Reagents/Apparatus

All chemicals and reagents, media and media ingredients were of analytical grade and of highest purity possible. They were supplied by BDH Labs (UK). BDH Chemicals Limited Poole England. Dissecting surgical blades, plastic containers, and trays were washed with distilled water. All glass wares were soaked in 10 % HNO<sub>3</sub> for 2 h and later rinsed with distilled de-ionized water prior to use for metal analysis. The glass wares for microbial analysis after being plugged with cotton wool were sterilized in a hot air oven at 160°C for 1 hour.

### Sample Collection and Preparation

A total of forty five (45) samples from fifteen (15) frozen fish [*Clupea harengus* (herring), *Scomber scombrus* (Mackerel), *Urophycis tenuis* (White hake)] samples (5 of each species) purchased at random from Utako main market were used for the analyses. These species were chosen due to their availability and patronage by

the residents of Abuja. These purchased samples were labelled and placed in sterile polyethylene bags and transported in a cold pack to the laboratory for analyses. In the laboratory, the fish samples were thawed at room temperature and dissected to separate the skin, fillet and gills (Abdullahi *et al.*, 2015; Hala *et al.*, 2017). The separated organs for heavy metals analysis were neatly placed in labelled prewashed petri dishes and dried to constant weight at 80°C for 2 days (Kareem *et al.*, 2016). The dried fish samples were pulverized using porcelain mortar and pestle and stored in amber bottles in vacuum desiccators before digestion.

### Digestion of Samples for Heavy Metals Analysis

About 0.5 g of each blended fish sample (skin, fillet and gills separately) was weighed into a Teflon beaker, 10 ml of 2:1 HNO<sub>3</sub> /H<sub>2</sub>O<sub>2</sub> was added and covered with watch glass. The mixtures were swirled gently and allowed to digest on a hot plate in a fume chamber for 2 h at 80°C until the brown fumes disappears (Igwemmar *et al.*, 2013; Kareem *et al.*, 2016). The digests were allowed to cool and filtered into 25 mL volumetric flasks with Whatman No. 1 filter paper and made up to mark with de-ionized water. Sample blanks were carried out throughout the digestion processes. All digestions were carried out in triplicates for each sample and the amounts of trace metals recorded as the mean value. The extracts were analyzed for heavy metals (Cu, Fe, Cd, Zn and Pb) using atomic absorption spectrophotometer (AAS) iCE 3000 Series 3000 at their respective wavelength (324.8, 248.3, 228.8, 213.9 and 283.3 nm) according to APHA method (2009).

### Preparation of Media and Samples for Microbiological Analysis

The media used for microbiological analysis include nutrient agar, macConkey agar, potato dextrose agar, each were prepared according to manufacturers' instruction. Swabs were taken from the skin of each fish sample and then 10 g of the fillet and gill tissues (of each fish sample measured separately) were collected aseptically using a dissecting set and placed into 90 ml of peptone water (0.85% NaCl w/v and 0.1% peptone w/v), then homogenized in a stomacher blender (Seward, UK) for 1 minute. A serial dilution of the homogenate was carried out in sterile universal bottles using 9 ml of 0.1 % sterile peptone water up to 10<sup>-10</sup> dilution. 1 ml from the dilutions were inoculated on Nutrient agar, MacConkey agar, Potato dextrose agar for the enumeration of total aerobic plate count, coliform count and fungal count respectively and then incubated at 37°C for 24 h,

while inoculated plates containing Potato dextrose agar was incubated at  $28\pm 3^{\circ}\text{C}$  for 3 to 5 days. All analyses were carried out in triplicates. Culture plates were examined at the end of incubation period for colony counts. The observed colony growth were counted using colony counter (Stuart Scientific, UK), counts were expressed as colony forming units per gram (cfu/g) of the samples (Sanjee and Karim, 2016; Olagbemide and Akharaiyi, 2021; Ekanem and Udoma, 2021).

#### Statistical Analysis

All the determinations were conducted in triplicates and data generated were analyzed statistically by one-way analysis of variance (ANOVA) technique using the Statistical Package for Social Sciences (SPSS) version 25.0.

### RESULTS AND DISCUSSION

The results of the studied heavy metals (Cu, Fe, Cd, Zn and Pb) recorded from the fish samples are summarized in Table 1. The highest concentration of Copper,  $1.49\pm 0.30$  mg/kg was recorded in the skin of *Urophycis tenuis* followed by the gills of *Scomber scombrus* ( $1.26\pm 0.11$  mg/kg) with the least value of Cu ( $0.58\pm 0.03$  mg/kg) recorded in the fillet of *Scomber scombrus*. The values of Cu recorded in this present study are lower than those reported by Kareem *et al.*, (2016) but higher than the values

recorded by Łuczyńska *et al.*, (2022). Copper is an essential element that promotes the activities of enzymes in the body. In all the tissues of the different species of fish studied, the concentrations of Cu were above the FAO/WHO permissible limits of 0.4 mg/kg. The gills of all studied species had the highest concentrations of Iron ranging from  $36.48\pm 9.62$  mg/kg (*Scomber scombrus*) through  $30.12\pm 5.10$  mg/kg (*Urophycis tenuis*) to  $25.71\pm 0.01$  mg/kg (*Clupea harengus*) with the lowest value ( $13.58\pm 2.13$  mg/kg) recorded from the skin of *Clupea harengus*. The concentrations of Fe recorded in this study are lower than the values reported by Abubakar *et al.*, (2014) but higher than the values recorded by Łuczyńska *et al.*, (2022). According to Abubakar *et al.*, (2014), the concentration of iron in the gills would fairly entail the level of iron present in the surrounding water of the fish. The lowest level of iron recorded from the skin could be due to the fact that they are not active tissues. Generally, the value levels of iron recorded in the entire tissues of fish species studied exceeded the safety limits of 0.8 mg/kg recommended by CCFAC, (2011). Fe is an essential element in human diet and fish contains relatively high amounts of readily absorbable iron. Iron forms part of haemoglobin which allows oxygen to be carried from the lungs to the tissues (Daniel *et al.*, 2013).

**Table 1: Concentrations of Heavy Metals in the Analysed Fish Samples (mg/kg)**

Species	Tissues	Cu	Fe	Cd	Zn	Pb
<i>Clupea harengus</i>	Skin	$0.74\pm 0.01$	$13.58\pm 2.13$	$3.84\pm 1.01$	$5.47\pm 0.16$	$0.48\pm 0.00$
	Fillet	$0.64\pm 0.10$	$20.26\pm 4.01$	$1.95\pm 0.20$	$5.28\pm 0.40$	$2.17\pm 1.01$
	Gills	$0.86\pm 0.00$	$25.71\pm 0.01$	$4.47\pm 2.13$	$8.37\pm 2.11$	$2.26\pm 0.00$
<i>Scomber scombrus</i>	Skin	$0.72\pm 0.10$	$15.29\pm 3.12$	$1.53\pm 0.00$	$7.81\pm 0.02$	$2.03\pm 0.10$
	Fillet	$0.58\pm 0.03$	$17.24\pm 0.00$	$1.87\pm 0.10$	$5.82\pm 0.11$	$1.25\pm 0.04$
	Gills	$1.26\pm 0.11$	$36.48\pm 9.62$	$1.90\pm 0.04$	$6.35\pm 1.42$	$3.16\pm 1.10$
<i>Urophycis tenuis</i>	Skin	$1.49\pm 0.30$	$27.03\pm 0.49$	$2.73\pm 0.01$	$8.62\pm 1.37$	$1.74\pm 0.15$
	Fillet	$1.22\pm 0.00$	$23.57\pm 2.73$	$2.46\pm 0.10$	$7.83\pm 2.05$	$2.55\pm 0.10$
	Gills	$1.18\pm 0.32$	$30.12\pm 5.10$	$2.81\pm 0.03$	$8.09\pm 0.10$	$3.67\pm 0.02$
FAO/WHO limits		0.4	0.8	0.1	30.0	0.4

*The results are means of triplicate determination  $\pm$  standard deviation*

The concentration of Cadmium was highest ( $4.47\pm 2.13$  mg/kg) in the gills of *Clupea harengus* followed by the skin ( $3.84\pm 1.01$  mg/kg), and the least concentration of Cd ( $1.53\pm 0.00$  mg/kg) was recorded in skin of *Scomber scombrus*. From the results, the values of Cd obtained from all the studied samples were above the maximum acceptable standard of 0.1 mg/kg set by the FAO/WHO indicating that the sampled species with evidence of cadmium pollution carries attendant health consequences. The values of Cd in this study are higher than those recorded by Oluyemi and Olabanji, (2011) and Hala *et al.*, (2017) but

lower than values recorded by Abdullahi *et al.*, (2015). Although the absorption of cadmium is low, it has a long half-life because it accumulates in the body and it may bioaccumulate in all levels of aquatic and terrestrial food chains (Oluyemi and Olabanji, 2011). Industrial processes such as smelting or electroplating and the addition of fertilizers can increase the concentration of Cd in the environment. Cadmium can be found in all foodstuff and particularly high amounts occur in organs of cattle, seafood and some mushroom species (Amin *et al.*, 2021).

Cadmium at high exposure levels is associated with nephrotoxic effects and long-term exposure may cause bone damage (Jasmina *et al.*, 2020). The concentration of Zinc from this study for all the fish species studied ranged from  $5.47 \pm 0.16$  mg/kg (*Clupea harengus*) to  $8.62 \pm 1.37$  mg/kg (*Urophycis tenuis*) in skin,  $5.28 \pm 0.40$  mg/kg (*Clupea harengus*) to  $7.83 \pm 2.05$  mg/kg (*Urophycis tenuis*) in fillet and  $6.35 \pm 1.42$  mg/kg (*Scomber scombrus*) to  $8.37 \pm 2.11$  mg/kg (*Clupea harengus*) in gills which were all within the FAO/WHO permissible limit of 30.0 mg/kg. Thus, this indicated that the fishes examined were free from Zn related toxicity. The concentrations of Zn from this study were similar to the values recorded by Daniel *et al.*, (2013) and Łuczyńska *et al.*, (2022) but lower than the values obtained by Ibanga *et al.*, (2019). Zn is an essential metal known to play important roles in human metabolic pathways and its shortage can cause appetite loss, retarded growth, skin changes and dysfunction of the immune system (Ayanda *et al.*, 2019). Zinc has been reported to be necessary for embryo development in fish (Daniel *et al.*, 2013).

Lead concentrations ranged between  $0.48 \pm 0.00$  mg/kg (*Clupea harengus*) and  $2.03 \pm 0.10$  mg/kg (*Scomber scombrus*) in the skin,  $1.25 \pm 0.04$  mg/kg (*Scomber scombrus*) and  $2.55 \pm 0.10$  mg/kg (*Urophycis tenuis*) in the fillet,  $2.26 \pm 0.00$  mg/kg (*Clupea harengus*) and  $3.67 \pm 0.02$  mg/kg (*Urophycis tenuis*) in gills which were higher than the safety standards of 0.4 mg/kg recommended by FAO/WHO. The highest concentration of Pb ( $3.67 \pm 0.02$  mg/kg) was recorded from the gills of *Urophycis tenuis* while the least concentration ( $0.48 \pm 0.00$  mg/kg) was obtained from the skin of *Clupea harengus* and this disparity may be attributed to the feeding habit of this species as well as the level of

habitat contamination (Kareem *et al.*, 2016). The result of Pb concentrations recorded here were lower than those reported by Ayanda *et al.*, (2019) but higher than the report of other studies of Kareem *et al.*, (2016), Hala *et al.*, (2017) and (Oluyemi and Olabanji, 2011). Lead could contaminate aquatic environment from industrial and agricultural discharges, high ways or motor traffic and from mine (Ayanda *et al.*, 2019). Lead is known to induce reduced cognitive development and intellectual performance in children and increased blood pressure and cardiovascular disease in adults. Its residues could result in haematological, gastrointestinal and neurological dysfunction. Sever or prolonged exposure to Pb may also cause chronic nephropathy and reproductive impairment (Hala *et al.*, 2017; Ibanga *et al.*, 2019). Fish are unique among vertebrates due to their ability to acquire metals in two different ways: from water through the gills and from diet through the gut (direct and trophic uptake routes). The direct uptake route is more important, because the gills are the main target organ for metal toxicity in fish (Łuczyńska *et al.*, 2022). Garai *et al.*, (2021) mentioned that heavy metals may enter the fish body directly from the water and sediments, through the gills/skin and from its food/prey through the digestive tract and then get accumulated in their tissues which will then be introduced into the food chain, which is a problem for humans. Considering the fact that practically each of the fish species studied comes from different ecosystem, we cannot define the exact relationships ecologically among all the species studied in the sense that the differences among them may come from both biotic and abiotic factors.

**Table 2: Mean Aerobic Plate Count from the Analysed Fish Samples (cfu/g)**

Tissues	<i>Clupea harengus</i>	<i>Scomber scombrus</i>	<i>Urophycis tenuis</i>
Skin	$47.6 \times 10^3$	$28.3 \times 10^3$	$41.3 \times 10^3$
Fillet	$2.23 \times 10^3$	$2.46 \times 10^3$	$2.15 \times 10^3$
Gills	$4.27 \times 10^3$	$5.02 \times 10^3$	$3.64 \times 10^3$

Table 2 summarized the mean aerobic plate count (APC) in the different parts of the fish samples analysed. From the results, it was observed that the skins of the three species of fish (*Clupea harengus*, *Urophycis tenuis*, *Scomber scombrus*) studied had more aerobic plate counts ( $47.6 \times 10^3$  cfu/g,  $41.3 \times 10^3$  cfu/g,  $28.3 \times 10^3$  cfu/g) respectively, compared with the fillets and gills. The count was highest in the skin of *Clupea harengus* with a load of  $47.6 \times 10^3$  cfu/g and lowest in the fillet of *Urophycis tenuis* with a load of  $2.15 \times 10^3$  cfu/g which was within the maximum internationally acceptable

microbiological limit for the APC,  $5 \times 10^5$  cfu/g (CCFAC, 2011). This implied that all the samples of each species of the fish met the acceptable limit specified by CCFAC. But there was a highly significant difference ( $p < 0.05$ ) of APC between the examined skin and other tissues. Although, the APC of any food articles are not sure indicator of their safety for consumption, yet it is one of the important source of judging the hygienic condition under which food has been produced, handled and stored (Fatin *et al.*, 2016).

The most serious problem related to fish product safety is the contamination with microbial pathogens. More so, fish products are highly sensitive to spoilage because of their high moisture content, neutral pH, high amount of

amino acids and naturally present autolytic enzymes. Cooling and freezing are the usual methods for fish conservation and the quality of stored fish inevitably deteriorates with shelf-life expiration (Deyan *et al.*, 2015).

**Table 3: Mean Coliform Count from the Analysed Fish Samples (cfu/g)**

Tissues	<i>Clupea harengus</i>	<i>Scomber scombrus</i>	<i>Urophycis tenuis</i>
Skin	$2.03 \times 10^3$	$1.97 \times 10^3$	$1.96 \times 10^3$
Fillet	$1.94 \times 10^3$	$1.88 \times 10^3$	$1.90 \times 10^3$
Gills	$1.87 \times 10^3$	$1.85 \times 10^3$	$2.00 \times 10^3$

From Table 3, it was observed that coliform bacteria were present in all the fish samples with counts that ranged from  $1.85 \times 10^3$  cfu/g in the gills of *Scomber scombrus* to  $2.03 \times 10^3$  cfu/g in the skin of *Clupea harengus* which exceeded the acceptable limit of  $\leq 100$  cfu/g total coliforms (TC) for fresh and frozen fish. Coliforms are indicator organisms signifying contamination of a product by faecal matter. The presence of total coliform is a pointer of sewage contamination which may also occur during different processing

steps such as transport and handling (Sanjee and Karim, 2016). Freezing only retard the growth and proliferation of contaminating organisms, it seldom destroys/kills the organism. The presence of coliform calls for concern since the presence of bacteria in this group indicates the possibility of the presence of disease organisms in the fish samples (Oranusi *et al.*, 2014). However, there was no significant difference ( $p < 0.05$ ) of coliform counts among the examined tissues of all species.

**Table 4: Mean Fungal Count from the Analysed Fish Samples (cfu/g)**

Tissues	<i>Clupea harengus</i>	<i>Scomber scombrus</i>	<i>Urophycis tenuis</i>
Skin	$15.5 \times 10^2$	$13.7 \times 10^2$	$18.3 \times 10^2$
Fillet	$8.04 \times 10^2$	$7.24 \times 10^2$	$7.39 \times 10^2$
Gills	$6.45 \times 10^2$	$6.02 \times 10^2$	$7.61 \times 10^2$

Fungal counts were recorded in all the studied fish samples, with counts ranging from  $6.02 \times 10^2$  cfu/g to  $18.3 \times 10^2$  cfu/g. The skin of *Urophycis tenuis* had the highest fungal load of  $18.3 \times 10^2$  cfu/g, followed by *Clupea harengus* with the load of  $15.5 \times 10^2$  cfu/g and the least count of  $6.02 \times 10^2$  cfu/g was recorded from the gills of *Scomber scombrus* (Table 4). The fungal counts recorded indicated the presence of fungal species in the fish samples which can cause serious health concern because of their mycotoxigenic potentials (Moon *et al.*, 2018). Fungi spp are common environmental contaminants of food products and they are

observed as opportunistic pathogens in fresh and salt water fishes and have also been implicated in veterinary and human diseases (Oranusi *et al.*, 2013). Essien *et al.*, (2005) reported that *Aspergillus flavus* and *Aspergillus fumigatus* which are fungal species produced aflatoxins, which destroys the liver and kidney in man resulting to death. The composition of fish shows that it is highly nutritious and as a result, humans source for it as a healthy diet. But its nutritional contents highly encourage the growth of microorganisms which makes man as a primary consumer susceptible to infections if he consumes fish contaminated by pathogens.

## CONCLUSION

Contamination of fish with heavy metals could be from water pollution through domestic and industrial anthropogenic activities. When fish are exposed to contamination from heavy metals, their organs accumulate the metals in varying concentrations. This disparity may be credited to different rates of metabolism. According to Ayanda *et al.*, (2019), organisms differ in their metabolic rates, amount of food they consume and food requirements. Any of these could have played a role in the differences observed in

metal accumulation by the studied fish species. Contaminated fish could be dangerous and this study indicated that the products were not ideal for consumption due to bioaccumulation of these heavy metals and the issue of post-harvest contaminants that can multiply in case of defrosting which may impact negatively on the consumers. Therefore, it is vital to undergo effective and efficient control of hygiene through regular chemical and bacteriological examination to ensure acceptable contamination levels and prevention of food intoxications.

**REFERENCES**

- Abdullahi, A., Adamu, U., Ekwumemgbo, P. A. and Okunola, O. J. (2015). Risk Assessment of Heavy Metals in Imported Frozen Fish *Scomber scombrus* Species Sold in Nigeria: A Case Study in Zaria Metropolis. *Advances in Toxicology*, Volume 2015, <http://dx.doi.org/10.1155/2015/303245>.
- Abubakar, A. Uzairu, A., Ekwumemgbo, P. A. and Okunola O. J. (2014). Evaluation of Heavy Metals Concentration in Imported Frozen Fish *Trachurus Murphyi* Species Sold in Zaria Market, Nigeria. *American Journal of Chemistry*. 4(5): 137-154. DOI: 10.5923/j.chemistry.20140405.02
- Al-sheraa, A. S., 2018. Microbial quality of three imported fresh locally produced marine fishes in Al-Faw City, Basrah, Iraq. *Journal of Aquaculture Research and Development*, 9 (4), <https://doi.org/10.4172/2155-9546.1000531>
- American Public Health Association. Standard methods for the examination of water and wastewater. APHA, AWWA, WEF/2009, APHA Publication. 20th ed. Washington DC; 2009.
- Amin, A. N., Ahemd, A.M. and Ahmed, O.M. (2021). Chemical and bacteriological risks of shrimp and clams (Gandofly) from Suez Gulf. *Food Research*, 5 (3): 281 – 288. DOI: [https://doi.org/10.26656/fr.2017.5\(3\).635](https://doi.org/10.26656/fr.2017.5(3).635).
- Australian Dietary Guidelines Summary, Eat For Health. (2013). 1st ed. Australian Government National Health and Medical Research Council, Department of Health and Aging. Available at, [https://www.eatforhealth.gov.au/sites/default/files/fles/the\\_guidelines/55a\\_australian\\_dietary\\_guidelines\\_summary\\_book](https://www.eatforhealth.gov.au/sites/default/files/fles/the_guidelines/55a_australian_dietary_guidelines_summary_book)
- Ayanda, I. O., Ukinebo, I. E. and Oluwakemi A. B. (2019). Determination of selected heavy metal and analysis of proximate composition in some fish species from Ogun River, Southwestern Nigeria. *Heliyon*, <https://doi.org/10.1016/j.heliyon.2019.e02512>
- Bowen, K. J., Harris, W.S. and Kris-Etherton, P.M. (2016). Omega-3 Fatty Acids and Cardiovascular Disease: Are There Benefits? *Curr. Treat. Options Cardiovasc. Med.* 18: 69.
- CCFAC (Codex Committee on Contaminants in Foods) (2011). Food Standards Programed: Joint FAO/WHO. Fifth Session, The Hague, The Netherlands; 21 – 25th March.
- Daniel, E. O., Ugwueze, A. U. and Igbegu, H. E. (2013). Microbiological Quality and Some Heavy Metals Analysis of Smoked Fish Sold in Benin City, Edo State, Nigeria. *World Journal of Fish and Marine Sciences*, 5 (3): 239-243. DOI: 10.5829/idosi.wjfm.2013.05.03.7149
- Deyan, S., Ivan, V. and Hristo, D. (2015). Microbiological status of fish products on retail markets in the Republic of Bulgaria. *International Food Research Journal*, 22(1): 64-69.
- Ekanem, J. O and Udoma, I. G. (2021). Bacteriological Assessment of Some Frozen Fishes Sold at Ator Market in Ikot Ekpene Metropolis. *Global Journal Of Pure And Applied Sciences*, Vol. 27, 2021: 355-359. DOI: <https://dx.doi.org/10.4314/gjpas.v27i4.1>
- Emmanuel-Akerele, A. H. and Uchendu, F. C. (2021). Microbial Assessment of Frozen Foods sold in Ayobo, Lagos. *Bacterial Empire*, Vol. 4, No. 4, e305. <https://doi.org/10.36547/be.305>
- Essien, J. P., Ekpo, M. A. and Brooks, A. A. (2005). Mycotoxigenic and proteolytic potential of moulds associated with smoked shark fish (*Chlamydoselachus anguincus*). *J App Sci Enviro Management*, 9: 53-57.
- FAO (2012). The state of world review of fisheries and aquaculture. Part 1. [www.fao.org/docrep/016/i2727e/i2727e01](http://www.fao.org/docrep/016/i2727e/i2727e01).
- Fatin, M. H., Rasha, A. E., Marionet, Z. N., Mohammed, S. R. (2016). Bacterial and Chemical quality of Frozen Chicken Meat Received at Governmental Hospital modern. *Benha Veterinary Medical Journal*, 30(1): 109-117.
- Garai, P., Banerjee, P., Mondal, P. C. and Saha, N. (2021). Effect of heavy metals on fishes: Toxicity and bioaccumulation. *J. Toxicol. Clin. Toxicol.* 11, 18.
- Hala, A. M., Gehan, I.E. and Shereen, A. Y. (2017). Assessment of the Bacterial Quality and Toxic Heavy Metal Residues of Frozen Fish Fillet In Kaferelsheikh Markets. *Alexandria Journal of Veterinary Sciences*. Vol. 54:108-116. DOI: 10.5455/ajvs.261613
- Ibanga, I. J., Moses, E. A., Edet, E. J. and Moses, A. E. (2019). Microbial and some heavy metals analysis of smoked fishes sold in urban and rural markets in Akwa Ibom State, Nigeria. *Calabar Journal of Health Sciences*, 3(2):73-79.

- Igwemmar, N. C., Kolawole, S. A. and Odunoku, S. O. (2013). Heavy metal concentration in fish species sold in Gwagwalada market, Abuja. *International Journal of Scientific Research*, 2: 7-9.
- Ismail, K. and Belma, D. (2002). Microbiological investigations on some of the commercial frozen meat in Izmir. *Turk. Elec. J. of Biotech*, 35(1): 18-23.
- Jasmina, D. A., Marjanovic, D., Tahirovic, K., Caklovica, A., Turalic, A., Lugusic, E., Omeragic, M. and Caklovica, F. (2020). Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Scientific Report*, 10:13238, <https://doi.org/10.1038/s41598-020-70205-9>
- Jasmina, D., Marjanovic, A., Tahirovic, D., Caklovica, K., Turalic, A., Lugusic, A., Omeragic, E., Sober, M. and Caklovica, F. (2020). Heavy metals in commercial fish and seafood products and risk assessment in adult population in Bosnia and Herzegovina. *Scientific Reports*, 10:13238 | <https://doi.org/10.1038/s41598-020-70205-9>.
- Kareem, O. K. Orisasona, O. and Olanrewaju, A.N. (2016). Determination of Heavy Metal Levels in Some Commonly Consumed Frozen Fish in Ibadan, Southwest, Nigeria. *Research Journal of Environmental Toxicology*, 10 (1): 82-87. DOI: 10.3923/rjet.2016.82.87.
- Laila, D. L., Wahidu, Z. and Tajul, A. Y. (2013). Effect of chilled-frozen storage on the physic-chemical, microbial and sensory quality of farmed bighead carp (*Hypophthalmichthys Nobilis*). *Journal of Fisheries and Aquatic Science*, 2, (3): 1 – 7. <http://dx.doi.org/10.3923/jfas.2013>.
- Łuczynańska, J., Pietrzak-Fiećko, R., Purkiewicz, A. and Łuczynański, M.J. (2022). Assessment of Fish Quality Based on the Content of Heavy Metals. *Int. J. Environ. Res. Public Health*, 19, 2307. <https://doi.org/10.3390/ijerph19042307>.
- Moon, D., Priyanka, R. M., Rakeb-Ul-Islam, M. and Debasish, S. (2018). Bacterial and Fungal Population Assessment in Smoked Fish during Storage Period. *Journal of Food: Microbiology, Safety & Hygiene*, 3(1):127. DOI: 10.4172/2476-2059.1000127
- Olagbemide, P. T. and Akharaiyi, F.C. (2021). Evaluation of microorganisms associated with vended frozen fish in Ado Ekiti locality. *Food Research*, 5 (4): 21 – 28.
- Olagbemide, P. T. and Akharaiyi, F. C. (2021). Evaluation of microorganisms associated with vended frozen fish in Ado Ekiti locality. *Food Research*, 5 (4) : 21 – 28.
- Oluyemi, E. A. and Olabanji, I. O. (2011). Heavy Metals Determination in Some Species of Frozen Fish sold at Ile-Ife Main Market, South West Nigeria. *Ife Journal of Science*, 13(2) : 355 – 362.
- Oranusi, S., Obioha, T. U. and Adekeye, B. T. (2014). Investigation on the microbial profile of frozen foods: Fish and Meat. *International Journal of Advanced Research in Biological Sciences*, 1(2): 71-78.
- Rodriguez, M., Rebollar, G.P., Mattioli, S. and Castellini, C. (2019). n-3 PUFA Sources (Precursor/Products): A Review of current Knowledge on Rabbit. *Animals : Multidisciplinary Digital Publishing Institute*, 9(10): 806. Doi:10.3390/ani9100806
- Sanjee, S. A. and Karim, M. E. (2016). Microbiological Quality Assessment of Frozen Fish and Fish Processing Materials from Bangladesh. *International Journal of Food Science*, Volume 2016, <http://dx.doi.org/10.1155/2016/8605689>
- Useh, M. U. and Dauda, M. S. Heavy. (2018). Heavy Metals Contamination and their Potential Toxicity in Petroleum Sludge Impacted Soils from Itsekiri Communities, Delta State, Nigeria. *Chemical Science International Journal*, 24(1): 1-15. DOI: 10.9734/CSJI/2018/42973.
- Useh, M. U., Onwuazor, O. P., Orijajogun, J. O., Samuel, C. J., Uzama, D. and Dauda, M. S. (2016). Physicochemical studies of potable water resources within the Kubwa Vicinity of Bwari Area Council, Abuja. *Journal of Applied Life Sciences International*, 8(4):1-7, DOI: 10.9734/JALSI/2016/28462
- World Health Organization (WHO). (2018). Population nutrient intake goals for preventing diet-related chronic diseases. Available at, [https://www.who.int/nutrition/topics/5\\_population\\_nutrient/en/index.html](https://www.who.int/nutrition/topics/5_population_nutrient/en/index.html).