http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(3), 201-210 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.3.07

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Aflatoxin, bacterial and heavy metal load in *Scomber scombrus* and *Clupea harengus* from two selected coldroom facilities in Kwara State, Nigeria

Nijerya Kwara eyaletinde seçilmiş iki soğuk oda işletmesinde *Scomber scombrus* ve *Clupea harengus*'taki aflatoksin, bakteri ve ağır metal birikimi

Oghenebrorhie Mavis Oghenochuko[1]* ● Rachael Oluwatosin Kolawole[2] ● Olasunkanmi Peter Olajide[3] ● Adeyinka Olamide Agbato[4]

[1]Department of Animal Science, Landmark University, Omu-Aran, 251103, Nigeria https://orcid.org/0000-0002-4622-3950
[2]Department of Animal Science, Landmark University, Omu-Aran, 251103, Nigeria https://orcid.org/0000-0002-5029-7530
[3]Landmark University SDG 2, Landmark University, Omu-Aran, 251103, Nigeria https://orcid.org/0000-0003-2195-6848
[4]Animal Care Laboratories, Abuja, 900001, Nigeria https://orcid.org/0009-0002-5767-0745

*Corresponding author: oghenochuko.oghenebrorhie@lmu.edu.ng
Received date: 07.03.2023 Accepted date: 13.08.2023

How to cite this paper:
Oghenochuko, O.M., Kolawole, R.O., Olajide, O.P., & Agbato, A.O. (2023). Aflatoxin, bacterial and heavy metal load in *Scomber scombrus* and *Clupea harengus* from two selected coldroom facilities in Kwara State, Nigeria. *Ege Journal of Fisheries and Aquatic Sciences, 40*(3), 201-210. https://doi.org/10.12714/egejfas.40.3.07

Abstract: Impurities found in fish have been a major cause of disease and illness to consumers. This study's objective was to evaluate the total aflatoxin, heavy metal and microbial load in two frozen fish: *Scomber scombrus* and *Clupea harengus* from two (2) cold room facilities in Omu-Aran and Ilorin. Heavy metals, aflatoxin and microbial load were investigated using standard methods. Health risk was also determined using health risk index (HRI), daily intake of metals (DIM), and health quotient (HQ), and total toxicity of metals (TTM). Cd, Cu, Ni, Pb, Mn and Cr did not differ significantly ($p>0.05$) in both species from both locations. Nickel was lower than the recommended limit by World Health Organization (WHO). Level of Mn and Cr were higher in both species. Mn load was higher in the muscles of the sampled fish than in the gills from September through to November with highest value of 1.26±0.08 and 1.30±0.12 obtained for *S. scombrus* and *C. harengus*. Highest concentrations of all metals was observed in the gills except manganese and copper [Cd = 0.03 (*S. scombrus*), Cr = 1.22±0.13 (*S. scombrus*), Ni = 0.025±0.04 (*S. scombrus*), Pb = 0.06±0.02 (*S. scombrus*)]. HRI was > 1 in the different age groups for the different metals. TTM was > 1 in both species. Total aflatoxin level was higher in the gills (4.25 – 5) ppb than in the muscle (1.5 - 3) ppb for both locations respectively. *Vibrio* spp. and non-coliform bacteria were high in both species from both locations. The study concludes that heavy metal loads (Mn, Cd, Cu, Cr, and Ni) were more than the permitted limitations imposed by FAO, WHO and EU legislation for fish and fish products placing consumers at health risk.

Keywords: Frozen fish, muscle, gill, heavy metal, bacterial load, aflatoxin

INTRODUCTION

In Nigeria, fish is recognized and cherished as a healthy, safe and a cheap source of protein (Amuneke et al., 2020) adding an average of 17% to the protein intake (Boyd et al., 2022), and per capita consumption of 20.2 kg per annum in 2020, and a total consumption of 157 million Mt (FAO, 2022). Fish importation as reported by the Minister of Agriculture and Rural Development accounts for about 75% of the total consumption. White croaker (*Umbrina canosai*), African mackerel (*Scomber scombrus* Linnaeus, (1758)), and herring (*Clupea harengus* Linnaeus, (1758)) are three popular frozen fish in the Nigerian markets, and they make up a sizable portion of the imported fish consumed in Nigeria. However, water body pollution due to the presence of heavy metal attributable to industrialization, and agricultural activities has grown to be a significant public health and environmental concern.

Nigeria has many public frozen fish sales outlets and retail marketplaces where both retailers and consumers regularly purchase frozen seafood items. Though, several investigations has been conducted on heavy metal loads of frozen foods in Nigeria market (Kareem et al., 2016; Ogundiran, et al., 2014), there are only a few of these studies that considered their sources (Abubakar et al., 2015). Frozen fish enters into the Nigeria market from different countries of which Russia, Netherlands, Chile are popular. Other countries are Mauritania, Faroe Island, Ireland, Japan, Norway, Peru, and Morocco. Besides the differences in source, the safety regulations in these countries varies, and the state of the water body also differ.

Although, fish is appreciated as one of the healthiest and cheapest source of protein, if contaminated fish are consumed, it can result in associated health risk hazard. According to Bintsis (2017), perhaps the most pervasive health issue of our time is foodborne disease, which also contributes significantly to lower economic output. Numerous elements, including Sodium (Na), Potassium (K), Iron (Fe), Calcium (Ca), Boron (B), Magnesium (Mg), Selenium (Se), Copper (Cu), and Zinc (Zn) are present in both the fish and its surroundings. The World Health Organization (WHO) as well as the Food and

Agriculture Organization of the United Nations (FAO) listed eight (8) elements that are present in fish that requires constant monitoring. They include; Mercury (Hg), Cadmium (Cd), Lead (Pb), Arsenic (As), Copper (Cu), Zinc (Zn), Iron (Fe), and Selenium (Sn), while screening of others—while not required—might be advantageous (Simpson and Uche, 2019).

This research was carried out in order to assess the amount of heavy metals present in two commonly consumed imported frozen fish bought from cold room facilities in Ilorin and Omu-Aran, Kwara State, and obtained from different countries/sources. Also, to examine the microbial load and total aflatoxin level of the species, and to assess the detrimental effects on human health from eating such fish.

MATERIALS AND METHODS

Purchase of fish

Fish samples were purchased from two cold rooms in Ilorin and Omu-Aran, Kwara State, Nigeria. 5 kg fish samples each was bought per carton of 20 kg for both species (*S. scombrus* and *C. harengus*) per month. A total of three (3) cartons per species were examined (*S. scombrus* (n=70) and *C. harengus* (n=144). Sources of the fish samples used for the study were from Holland and Japan. Fish samples were kept in an ice chest and transported to the wet laboratory of the Department of Animal Science, Landmark University, Omu-Aran, Kwara State, Nigeria. The choice for using these fish species for the study, stemmed from consumers preference for these species in the country. Purchase was done once every third week of the month, for three months (September, October and November). This is because the cold rooms' operators informed that new batch of importation arrive at such interval most of the times.

Laboratory procedure

Using an electronic weigh balance, Camry (Model EK3250.5 kg), the weight of the fish samples was immediately determined and recorded. Five (5) whole fish were taken from *S. scombrus* and 10 from *C. harengus* monthly from each location for heavy metal determination.

Gills and muscles of *S. scombrus*(n=8) and *C. harengus* (n=14) were aseptically separated monthly for heavy metal analysis. Samples were preserved individually in well-marked plastic bags for digestion and microbiology study. Microbiology was investigated for the first and third months. For total aflatoxin determination, gill and muscle was collected from *S. scombrus* monthly. They were kept in the freezer until further use. Samples for heavy metal determination were then blended separately using an electronic blender (Binatone BLG-595 MK2). For the heavy metal determination, comparison was between the species: whole fish, gills, and muscles for the three months.

Digestion of fish samples

This study used a wet acid digestion method (Olalekan et al., 2019). From the milled samples, 0.5 g of each sample was weighed into a beaker. To the sample in the beaker, 4 ml of nitric acid was added. The beaker holding the mixture was placed on a hot plate for 15 minutes until the solution became clear, and was made up to 50 ml using distilled water and poured into sterile bottles, and left at room temperature until use.

Fish digest was exposed to Atomic Absorption Spectrophotometry (AAS) (Model 211 VGP Buck Scientific) for heavy metal analysis using the calibration plot technique (Adedire et al., 2021) at Afe Babalola University, Ado-Ekiti, Nigeria. The samples were analyzed with the concentration of the metals present being displayed in parts per million (ppm) after extrapolation from the standard curve.

Isolation, and identification of bacteria from experimental fish

Methods used in the process of bacteria isolation and identification are as described in the Cowan and Steel's manual for identification of medical bacteria (Barrow and Feltham, 1993). Composite samples of the whole fish samples from both species were grinded using sterile mortal and pestle. One gram of the composite whole fish sample was prepared for incubation in a suitable medium for culture. The cultures media used include nutrient agar (NA), McConkey agar (MA), nutrient broth (NB), blood agar (BA), phosphate buffer saline (PBS), Xylose lysine De-carboxylase Agar (XLD) agar and Thiosulfate–citrate–bile salts–sucrose agar (TCBS). All the fish samples were thoroughly homogenized in sterile water, cultured, and incubated for 24 hours at 37°C. Isolated bacteria were identified using cultural characteristics, cellular morphology, and biochemical test.

Cultural characteristics

Colonial cultural morphology of each isolates was examined according to their size, shape, colour, edges, elevation and haemolysis.

Gram staining

A drop of water was dispensed on the glass slide. Using the sterilized inoculating loop, a colony of organism was picked from the petri dish, and smeared on the glass slide and allowed to air-dry. This process was repeated for all the slides needed. The air-dried slide was then heat fixed and stained using crystal violet dye followed by grams iodine, acetone and safranine. Then the dried slide was examined under the microscope(100X) using immersion oil.

Biochemical identification

This was done to further characterize the bacteria isolates

according to World Health Organization (WHO) manual for laboratory investigation of bacteria organisms as described by Dawodu and Akanbi (2021).

a. Oxidase test

Electron transfer is demonstrated with this test, which is also used to distinguish enterobacteriacea from other bacterial species. In a petri dish, filter paper was covered with reagent using a inoculating loop. The suspected colony was applied to the moist filter paper using a plastic loop, and any color changes were checked. Within 10 seconds, purple coloration appeared over the streak, indicating electron transport. A change of colour is positive, if the colour remains the same, it is negative. Positive sample means they contain the enzyme "oxidase" that breaks down oxidase.

b. Catalase test

A drop of 3% H_2O_2 was dispensed on a glass slide. Using a heated and cooled wire loop, bacteria colony was picked from the petri-dish and smeared on the glass slide with H_2O_2 and observed for reaction. An effervescence reaction indicate positive for catalase.

c. Indole test

Gram negative bacilli are distinguished using this indole production test. Overnight, the organism grew on peptone water. The water culture was given a few drops of Kovac's reagent before being left for 24 hourst. Positive indole result was attested by the presence of a red ring above the peptone water.

d. Simmons citrate agar

This test is used to determine whether an organism can use citrate as a source of energy.

Citrate agar was prepared, heated and autoclaved, the test tubes were slanted after autoclaving. Inoculating loop was used to smear a colony of organism on the medium, which was then incubated for 24 hours at 37°C. A colony of organism was smeared on the agar and incubated for 24 hours. A positive test result was determined by a blue coloration, while the negative still retains the green colour of the agar .

e. Methyl red test

This is a method of enterobacteria differentiation. It determines when there is a enough amount of acid produced during the fermentation of glucose. A young culture of the organism was lightly injected into the medium, which was then incubated for 48 hours at 35°C. To the culture, five drops of methyl red indicator were added. A positive reaction was denoted by the color red.

Serial dilution of sample

Serial dilution was done for preparation of microbial load count of the intestine. A sample of the intestine sub sample (0.5g in 5ml of potassium buffer saline) was serially diluted using syringe in a sterile environment. The serial 10-fold dilution was prepared in 5ml dilution tubes for each of the samples, of which the first bottle containing the prepared sample and 9 sterile tubes containing 4.5ml of phosphate buffer solution each were placed on the rack. 0.1ml of the processed sample was mixed with 4.5ml of the first bottle of buffer solution, which produced the 10^{-1} dilution. Then 0.1ml from the first bottle was mixed with the second bottle solution and 0.1ml from the second to the third and serially in the same order until the last bottle, which is the 10^{-9} diluted sample.

Bacterial count of sample

Bacterial count was done as described by (Ogur, 2022). The samples obtained from serial dilution were inoculated on nutrient agar medium in petri dishes for microbial count. Firstly, 0.1ml was taken from the 10^{-1} dilution sample and seeded at a triangular distance on a nutrient agar media. This process is repeated until the 10^{-9} dilution sample, giving 9 inoculated plate with each plate from a dilution. After incubation at 37°C for 24hours three round shaped colonies from the nutrient agar medium were observed on the dish which were counted meticulously to determine the microbial load count.

Total aflatoxin in *Scomber scombrus* from different location

Total aflatoxin test protocol was carried out as described in the Romer labs test kits with slight modification (Avrameas, 1969). Five (5) g was weighed from the composite fish gill and muscle separately and was placed in beaker containing 25 mls of 70% methanol and left for 10 minutes for extraction of aflatoxin. Then the mixture was filtered using a No. 1 Whatman filter paper. A one-tenth dilution was then made by adding 100 µl of the filtrate to 900 µl of 70% methanol. 50 µl of the diluent and 100 µl of conjugate: fumonisin was then dispensed into the green-bordered well. 100 µl from the filtrate-conjugate mixture was taken and dispensed into the antibody coated wells and incubated at room temp for 15 minutes. The content of the well was then discarded and washed with distilled water 3 times after which 100 µl of urea peroxidase (substrate) was added to the well and incubation was done for 5 minutes. It was then observed for colour change (different shades of blue to colourless) after which a 100 µl of stop solution (sulphuric acid (1 mol/dm^3)) was added and the plates were read with ELISA plate reader at 450 nm wavelength. Optical density of the samples was recorded and multiplied by 10.

On Microsoft Excel, a graph was plotted of the standard concentrations versus optical densities. From this graph, extrapolations were made to determine levels of total aflatoxin in fish samples.

Risk assessment

Using hazard quotient (HQ) (Khan et al., 2015), health risk

index (HRI) (Abubakar et al., 2015), total toxicity of mixtures (TTM) index (Anzecc and Armcanz, 2000), and daily intake of metal (DIM) (Okunola et al., 2011), risk evaluation was conducted to measure the danger presented by human consumption of tainted fish samples.

Hazard quotient (HQ) fomulae

HQ was determined using the equation;

$$\mathrm{HQ} = \frac{Wfish * Mfish}{RfD * Bo})$$

Where,

Wfish = daily dry weight of fish that is eaten (gd-1). For nutritional needs, adults with body weight 79.96 kilograms and more, consume 20.9 grams of fish daily, children weighing 49.7 kilograms and below, consume 10.1 grams daily, and 6.2 grams per person (0 years – 9 years) weighing 17.3 kilograms was advised.

Mfish ($mgkg^{-1}$) = metal concentration in fish,

RfD ($mgkg^{-1}d^{-1}$) = metal reference dose used; Iron (0.7), Manganese (0.014), Zinc (0.3), Copper (0.04), Nickel (0.02), Cadmium (0.001).

Bo (kg) = average body weight

Daily intake of metals (DIM)

The DIM formula was developed to estimate the daily loading of metals into the human system from fish intake.

$$\mathrm{DIM} = (Cmetal\frac{Dfish}{Bo})$$

Where,

Cmetal = concentration of heavy metals in the fish ($mgkg^{-1}$),

Dfish = daily nutritional intake of fish (gday-1),

Bo = average body weight (Kg)

Health risk index (HRI)

The Health Risk Index (HRI) was calculated using the formula below.

$$\mathrm{HRI} = \frac{DIM}{RfD}$$

A Health Risk Index (HRI) value of less than one (1) denotes a safe exposure to such a heavy metal and is regarded as acceptable; otherwise, the fish may be at danger for exposure to heavy metals.

Total toxicity of mixtures (TTM)

Total Toxicity of Mixtures (TTM) for heavy metals was calculated using TTM index.

$$\mathrm{TTM} = \Sigma(1\frac{Ci}{GVi})$$

Where,

Ci = Concentration of the 'ith' component of mixture

GVi = Value to use as a guide for the 'ith' component. Values that should be used as triggers for low-risk livestock water consumption. Iron not enough hazardous, Lead 0.1 mg/L, Manganese not sufficiently toxic, Nickel 1 mg/L, Zinc 20 mg/L, Cadmium 0.01 mg/L, Chromium 1 mg/L, Copper 0.4 - 5 mg/L.

TTM >1= The mixture was shown to be above the Guideline value

Statistical analysis

Weight of *S. scombrus* and *C. harengus* and heavy metal data in the whole fish, gills, and muscles of sampled fish were analysed using simple descriptive statistics on the Statistical Package for Social Sciences version (SPSS) version 20. Health Risk index were calculated from the mean of the heavy metals concentration in the whole fish.

RESULTS

Weight of sampled *Scomber scombrus* and *Clupea harengus*

Average weight of *S. scombrus* and *C. harengus* used for the study are represented in Table 1. *S. scombrus* purchased from Ilorin (385.22 ± 88.04) were heavier than those gotten from Omu-Aran. *C. harengus* gotten from Omu-Aran, recorded highest weight (214.33 ± 31.09).

Table 1. Weights of sampled *Scomber scombrus* and *Clupea harengus*

Species	Location	N	Mean weight (g)	Min. (g)	Max. (g)
S. scombrus	Omu-Aran	39	375.00 ± 62.75	205.01	442.78
S. scombrus	Ilorin	39	385.22 ± 88.04	225.0	657.88
C. harengus	Omu-Aran	72	214.33 ± 31.09	155.32	281.80
C. harengus	Ilorin	72	197.37 ± 40.98	115.50	277.41

Heavy metals in sampled fish

Mean concentrations of specific metals in the gills, muscle and whole *S. scombrus* and *C.* harengus for the three (3) consecutive months is shown in Tables 2, 3, 4. Mn and Cr were higher in both species than other metals. Mn load was higher in the muscles of the sampled fish than in the gills from September through to November with highest value of 1.26 ± 0.08 and 1.30 ± 0.12 obtained for *S. scombrus* and *C. harengus*. Highest concentrations of all metals was observed in the gills except manganese and copper [Cd = 0.03 (*S. scombrus*), Cr = 1.22 ± 0.13 (*S. scombrus*), Ni = 0.025 ± 0.04 (*S. scombrus)*, Pb = 0.06 ± 0.02 (*S. scombrus*)].

Table 2. Mean concentration of heavy metals in sampled fish in the first month (September)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.018 ± 0.003	0.214 ± 0.01	1.07 ± 0.15	0.41 ± 0.05	0.009 ± 0.01	0.06 ± 0.02
		Muscles	0.001 ± 0.001	0.19 ± 0.03	0.50 ± 0.01	1.05 ± 0.07	0.001 ± 0.00	0.001 ± 0.001
		Whole	0.012 ± 0.01	0.21 ± 0.01	0.81 ± 0.43	0.66 ± 0.25	0.005 ± 0.01	0.04 ± 0.03
	C. harengus (n = 24)	Gills	0.008 ± 0.01	0.32 ± 0.01	0.75 ± 0.12	0.61 ± 0.01	0.000 ± 0.001	0.02 ± 0.08
		Muscles	0.007 ± 0.001	0.21 ±0.003	0.39 ± 0.02	1.04 ± 0.07	0.001 ± 0.00	0.015 ± 0.002
		Whole	0.008 ± 0.001	0.26 ± 0.05	0.58 ± 0.18	0.81 ± 0.21	0.001 ± 0.001	0.02 ± 0.01
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.003 ± 0.00	0.28 ± 0.01	0.89 ± 0.02	0.48 ± 0.01	0.005 ± 0.001	0.03 ± 0.001
		Muscles	0.001 ± 0.001	0.15 ± 0.01	0.35 ± 0.01	1.205 ± 0.02	0.004 ± 0.003	0.006 ± 0.001
		Whole	0.001 ± 0.001	0.148 ± 0.005	0.35 ± 0.007	1.205 ±0.02	0.004 ± 0.003	0.006 ± 0.001
	C. harengus (n = 24)	Gills	0.01 ± 0.00	0.29 ± 0.02	0.524 ± 0.01	0.56 ± 0.01	0.01 ± 0.001	0.04 ± 0.002
		Muscles	0.002 ± 0.001	0.12 ± 0.01	0.53 ± 0.01	0.87 ± 0.14	0.003 ± 0.001	0.02 ± 0.002
		Whole	0.006 ± 0.005	0.199 ± 0.09	0.52 ± 0.01	0.73 ± 0.17	0.006 ± 0.004	0.03 ± 0.02

Means are presented as mean ± SD.

Table 3. Mean concentration of heavy metals in sampled fish in the second month (October)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.02 ± 0.01	0.29 ± 0.05	0.93 ± 0.28	0.41 ± 0.07	0.009 ± 0.01	0.06 ± 0.02
		Muscles	0.002 ± 0.003	0.22 ± 0.03	0.38 ± 0.05	1.26 ± 0.08	0.004 ± 0.002	0.02 ± 0.02
		Whole	0.017 ± 0.01	0.26 ± 0.05	0.66 ± 0.32	0.74 ± 0.44	0.009 ± 0.01	0.04 ± 0.03
	C. harengus (n = 24)	Gills	0.008 ± 0.01	0.34 ± 0.07	0.81 ± 0.26	0.51 ± 0.08	0.000 ± 0.001	0.03 ± 0.008
		Muscles	0.002 ± 0.01	0.21 ±0.05	0.35 ± 0.09	1.23 ± 0.29	0.001 ± 0.001	0.006 ± 0.002
		Whole	0.014 ± 0.001	0.28 ± 0.1	0.57 ± 0.29	0.88 ± 0.42	0.001 ± 0.001	0.02 ± 0.01
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.01 ± 0.00	0.32 ± 0.04	0.96 ± 0.28	0.57 ± 0.11	0.006 ± 0.01	0.04 ± 0.007
		Muscles	0.002 ± 0.003	0.204 ± 0.08	0.28 ± 0.09	1.06 ± 0.03	0.003 ± 0.003	0.005 ± 0.01
		Whole	0.006 ± 0.004	0.22 ± 0.1	0.52 ± 0.43	0.85 ± 0.25	0.005 ± 0.004	0.02 ± 0.02
	C. harengus (n = 24)	Gills	0.01 ± 0.005	0.29 ± 0.05	0.67 ± 0.05	0.57 ± 0.11	0.011 ± 0.01	0.06 ± 0.01
		Muscles	0.00 ± 0.00	0.14 ± 0.03	0.42 ± 0.03	1.30 ± 0.12	0.004 ± 0.004	0.006 ± 0.003
		Whole	0.007 ± 0.007	0.21 ± 0.09	0.54 ± 0.14	0.92 ± 0.38	0.009 ± 0.007	0.03 ± 0.03

Means are presented as mean ± SD.

Table 4. Mean concentration of heavy metals in sampled fish in the third month (November)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.03 ± 0.01	0.38 ± 0.18	1.22 ± 0.13	0.65 ± 0.17	0.025 ± 0.04	0.05 ± 0.01
		Muscles	0.005 ± 0.003	0.34 ± 0.06	0.3 ± 0.1	0.69 ± 0.06	0.001 ± 0.000	0.01 ± 0.01
		Whole	0.02 ± 0.02	0.33 ± 0.1	0.84 ± 0.54	0.66 ± 0.13	0.022 ± 0.05	0.03 ± 0.02
	C. harengus (n = 24)	Gills	0.22 ± 0.11	0.34 ± 0.06	0.91 ± 0.29	0.58 ± 0.12	0.006 ± 0.01	0.05 ± 0.1
		Muscles	0.007 ± 0.003	0.31 ±0.008	0.31 ± 0.008	1.15 ± 0.07	0.005 ± 0.001	0.001 ± 0.001
		Whole	0.14 ± 0.15	0.32 ± 0.1	0.63 ± 0.36	0.87 ± 0.27	0.005 ± 0.001	0.04 ± 0.12
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.02 ± 0.006	0.27 ± 0.02	0.61 ± 0.07	0.37 ± 0.07	0.005 ± 0.002	0.03 ± 0.002
		Muscles	0.006 ± 0.003	0.48 ± 0.05	0.23 ± 0.02	0.83 ± 0.04	0.005 ± 0.002	0.008 ± 0.002
		Whole	0.014 ± 0.01	0.39 ± 0.12	0.36 ± 0.18	0.64 ± 0.25	0.005 ± 0.002	0.02 ± 0.02
	C. harengus (n = 24)	Gills	0.02 ± 0.008	0.36 ± 0.06	0.98 ± 0.24	0.59 ± 0.15	0.013 ± 0.002	0.03 ± 0.004
		Muscles	0.02 ± 0.01	0.42 ± 0.03	0.304 ± 0.02	0.77 ± 0.13	0.009 ± 0.005	0.01 ± 0.007
		Whole	0.016 ± 0.006	0.38 ± 0.06	0.65 ± 0.38	0.65 ± 0.18	0.01 ± 0.002	0.02 ± 0.005

Means are presented as mean ± SD.

Risk assessment index of metals

Tables 5, 6, 7 showed calculated health quotient (HQ), daily intake of metal (DIM), and health risk index (HRI) for different age groups. HRI was >1 in all the age categories for all metals excluding nickel for all purchases in the two species of fish sampled.

Total toxicity of metals (TTM) was >1 for *S. scombrus* and *C. harengus* (Table 8).

Table 5. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples (mgkg^{-1}) in the first month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories)			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.012 ± 0.01	0.003	0.002	0.004	3	2	4	3.1366	2.4386	4.3006
		Cu	0.21 ± 0.01	0.055	0.043	0.078	1.375	1.0 75	1.95	1.3723	1.0669	1.8815
		Cr	0.81 ± 0.43	0.2117	0.1646	0.2903	70.5667	54.8667	96.7667	70.5727	54.8692	96.7630
		Mn	0.66 ± 0.25	0.1725	0.1341	0.2365	12.3214	9.5786	16.8929	12.3222	9.5803	16.8951
		Ni	0.005 ± 0.01	0.0013	0.001	0.0018	0.065	0.05	0.09	0.0654	0.0513	0.0895
		Pb	0.04 ± 0.03	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
	C. harengus	Cd	0.008 ± 0.001	0.0021	0.21	0.029	2.1	2.0	2.9	2.0911	1.6257	2.8671
		Cu	0.26 ± 0.05	0.068	0.0528	0.0932	1.7	1.32	2.33	1.6990	1.3209	2.3295
		Cr	0.58 ± 0.18	0.1516	0.1179	0.2079	50.5333	39.3	69.3	50.5336	39.2891	69.2871
		Mn	0.81 ± 0.21	0.2117	0.1646	0.2903	15.1214	11.7571	20.7357	15.1227	11.7577	20.7349
		Ni	0.001 ± 0.001	0.003	0.002	0.0004	0.015	0.01	0.02	0.1307	0.1016	0.1792
		Pb	0.02 ± 0.01	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
Ilorin (Japan)	*S. scombrus*	Cd	0.001 ± 0.001	0.0003	0.0002	0.0004	0.3	0.2	0.4	0.2614	0.2032	0.3584
		Cu	0.148 ± 0.005	0.0387	0.0301	0.0530	0.9675	0.7525	1.325	0.9671	0.7519	1.3260
		Cr	0.35 ± 0.007	0.915	0.0711	0.1254	30.5	23.7	41.8	30.4944	23.7089	41.8112
		Mn	1.205 ±0.02	0.315	0.2449	0.4319	22.5	17.49	30.85	22.4974	17.4914	30.8464
		Ni	0.004 ± 0.003	0.0011	0.0008	0.0014	0.055	0.04	0.07	0.0523	0.0406	0.0717
		Pb	0.006 ± 0.001	0.0016	0.0012	0.0022	0.4	0.3	0.55	0.3921	0.3048	0.5376
	C. harengus	Cd	0.006 ± 0.005	0.0016	0.0012	0.0022	1.6	1.2	2.2	1.5683	1.2193	2.1503
		Cu	0.199 ± 0.09	0.0520	0.0404	0.0713	1.3	1.01	1.7825	1.3004	1.0110	1.7829
		Cr	0.52 ± 0.01	0.1360	0.1057	0.1864	45.3333	35.2333	62.1	45.3090	35.2247	62.1195
		Mn	0.73 ± 0.17	0.1908	0.1483	0.2616	13.6286	10.5929	18.6857	13.6291	10.5964	18.687
		Ni	0.006 ± 0.004	0.0016	0.0012	0.0022	0.08	0.06	0.11	0.0784	0.0610	0.1075
		Pb	0.03 ± 0.02	0.0078	0.0061	0.0108	1.95	1.525	2.7	1.9604	1.5242	2.6879

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 6. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples (mgkg^{-1}) in the second month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.017 ± 0.01	0.0044	0.0035	0.0061	4.4	3.5	6.1	4.4435	3.4547	6.0925
		Cu	0.26 ± 0.05	0.0680	0.0528	0.0932	1.7	1.32	2.33	1.6990	1.3209	2.3295
		Cr	0.66 ± 0.32	0.1725	0.1341	0.2365	72.4167	44.7	78.8333	57.5038	45.3469	78.8439
		Mn	0.74 ± 0.44	0.1934	0.1504	0.2652	13.8143	10.7429	18.9429	13.8158	10.7416	18.9430
		Ni	0.009 ± 0.01	0.0024	0.0018	0.0032	0.12	0.009	0.16	0.1176	0.0915	0.1613
		Pb	0.04 ± 0.03	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
	C. harengus	Cd	0.014 ± 0.001	0.0037	0.0029	0.0050	3.7	2.9	5.0	3.6593	2.8451	5.7103
		Cu	0.28 ± 0.1	0.0732	0.0569	0.1004	1.83	1.4225	2.51	1.8297	1.4225	2.5087
		Cr	0.57 ± 0.29	0.1490	0.1158	0.2043	49.6667	38.6	68.1	49.6623	38.6117	68.0925
		Mn	0.88 ± 0.42	0.2300	0.1788	0.3154	16.4286	12.7714	22.5286	16.4296	12.7327	22.5268
		Ni	0.001 ± 0.001	0.0003	0.0002	0.0004	0.015	0.01	0.02	0.0131	0.0102	0.0179
		Pb	0.02 ± 0.01	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
Ilorin (Japan)	*S. scombrus*	Cd	0.006 ± 0.004	0.0016	0.0012	0.0022	1.6	1.2	2.2	1.5683	1.2193	2.1503
		Cu	0.22 ± 0.1	0.0575	0.0447	0.0789	1.4375	1.1175	1.9725	1.4376	1.1177	1.9711
		Cr	0.52 ± 0.43	0.1360	0.1057	0.1864	45.3333	35.2333	62.1333	54.0187	41.9987	74.0655
		Mn	0.85 ± 0.25	0.2222	0.1727	0.3046	15.8571	12.3357	21.7571	15.8695	12.3383	21.7589
		Ni	0.005 ± 0.004	0.0013	0.0010	0.0018	0.065	0.05	0.09	0.0653	0.0508	0.0896
		Pb	0.02 ± 0.02	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
	C. harengus	Cd	0.007 ± 0.007	0.0018	0.0014	0.0025	1.8	1.4	2.5	1.8297	1.4225	2.5087
		Cu	0.21 ± 0.09	0.0550	0.0430	0.0780	1.375	1.075	1.95	1.3723	1.0669	1.8815
		Cr	0.54 ± 0.14	0.1412	0.1097	0.1935	47.0667	36.5667	64.5	49.0485	36.5795	64.5087
		Mn	0.92 ± 0.38	0.2405	0.1869	0.3297	17.1786	13.014	23.55	17.1765	13.3544	23.5508
		Ni	0.009 ± 0.007	0.0024	0.0018	0.0032	0.12	0.09	0.16	0.1176	0.0915	0.1613
		Pb	0.03 ± 0.03	0.0005	0.0004	0.0007	0.125	0.1	0.175	1.9604	1.5242	2.6879

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 7. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples ($mgkg^{-1}$) in the third month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories)			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.02 ± 0.02	0.0052	0.0041	0.0072	5.2	4.1	7.2	5.2276	4.0644	7.1676
		Cu	0.33 ± 0.1	0.0862	0.0671	0.1183	2.155	1.6775	2.9575	2.1564	1.6766	2.9567
		Cr	0.84 ± 0.54	0.2196	0.1707	0.3011	73.2	56.9	100.3667	73.1866	56.9014	100.3468
		Mn	0.66 ± 0.13	0.1725	0.1341	0.2365	12.3214	9.5786	16.8929	12.3222	9.5803	16.8951
		Ni	0.022 ± 0.05	0.0052	0.0041	0.0072	0.26	0.205	0.36	0.2614	0.2032	0.3584
		Pb	0.03 ± 0.02	0.0078	0.0061	0.0108	1.95	1.525	2.7	1.9604	1.5242	2.6879
	C. harengus	Cd	0.14 ± 0.15	0.0366	0.0285	0.0508	36.6	28.5	50.8	36.5933	28.4507	50.1734
		Cu	0.32 ± 0.1	0.0865	0.0650	0.1147	2.1625	1.625	2.8675	2.0911	1.6258	2.8671
		Cr	0.63 ± 0.36	0.1647	0.1280	0.2258	54.9	42.6667	75.2667	54.8900	42.6761	75.2601
		Mn	0.87 ± 0.27	0.2274	0.1768	0.3118	16.2429	12.6286	22.2714	16.2429	12.6286	22.2709
		Ni	0.005 ± 0.001	0.0013	0.0010	0.0018	0.065	005	0.09	0.0653	0.0508	0.0896
		Pb	0.04 ± 0.12	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
Ilorin (Japan)	*S. scombrus*	Cd	0.014 ± 0.01	0.0037	0.0029	0.0050	3.7	2.9	5.0	3.6593	2.8451	5.0173
		Cu	0.39 ± 0.12	0.1020	0.0793	0.1398	0.3	1.9825	3.495	2.5485	1.9814	3.4942
		Cr	0.36 ± 0.18	0.0941	0.0835	0.1290	31.3667	27.8553	43	31.3657	24.3863	43.0058
		Mn	0.64 ± 0.25	0.1673	0.1301	0.2294	11.95	9.2929	16.3857	11.9488	9.2900	16.3832
		Ni	0.005 ± 0.002	0.0013	0.0010	0.0018	0.065	0.05	0.09	0.0653	0.0508	0.0896
		Pb	0.02 ± 0.02	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
	C. harengus	Cd	0.016 ± 0.006	0.0042	0.0033	0.0057	4.2	3.3	5.7	4.1821	3.2515	5.7341
		Cu	0.38 ± 0.06	0.0993	0.0772	0.1362	2.4825	1.93	3.405	2.4831	1.9306	3.4046
		Cr	0.65 ± 0.38	0.1699	0.1321	0.2330	56.6333	44.0333	77.6667	56.6325	44.0309	77.6493
		Mn	0.65 ± 0.18	0.1699	0.1321	0.2330	11.9214	9.4357	16.6429	12.1355	9.4352	16.6391
		Ni	0.01 ± 0.002	0.0026	0.0020	0.0036	0.13	0.10	0.18	0.1307	0.1016	0.1792
		Pb	0.02 ± 0.005	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 8. TTM for individual responses to heavy metal accumulation in pooled fish samples

Species	Metals	Mean±SD ($mgkg^{-1}$)	Guideline value (mg/L)	Ci/Gvi	TTM
S. scombrus	Cd	0.010 ± 0.01	0.01	1.00	
	Cu	0.23 ± 0.07	5.0	0.046	
	Cr	0.64 ± 0.35	1	0.64	3.536
	Mn	0.77 ± 0.35	0.5	1.54	
	Ni	0.01 ± 0.01	1	0.01	
	Pb	0.03 ± 0.03	0.1	0.3	
C. harengus	Cd	0.01 ± 0.01	0.01	1.00	
	Cu	0.24 ± 0.01	5.0	0.048	
	Cr	0.55 ± 0.19	1.0	0.55	3.622
	Mn	0.86 ± 0.34	0.5	1.72	
	Ni	0.004 ± 0.01	1.0	0.004	
	Pb	0.025 ± 0.02	0.1	0.3	

Total aflatoxin in sampled organs of *Scomber scombrus*

Aflatoxin levels in the organs of *Scomber scombrus* from different coldrooms and sources revealed that there was no significant difference. Highest levels of aflatoxin were recorded in the gills of the fish from both sources Table 9.

Table 9. Aflatoxins level in sampled organs of *Scomber scombrus*

Location	Gills (ppb)	Muscles (ppb)
Omu-Aran (Holland, n = 24)	5.00	1.50
Ilorin (Japan, n = 24)	4.25	3.00

Microbial load in sampled fishes

Table 10 shows the bacteria and fungi found in the muscles of the experimental fish. 2 cfu/100µl and 80 cfu/100µl of the coliform and non-coliform bacteria were present in *S. scombrus* bought from Omu-Aran market in the month of September Table 10. Other bacteria identified include *Enterobacter intermedius* and *Shigella sonnei*. Coliform and non-coliform bacteria load in the month of November were observed to be higher to too numerous to count in both species. Other bacteria identified are *Citrobacter diversus* and *Shigella sonnei* Table 11.

DISCUSSION

Mn concentration which was noticed in this study to have exceeded the recommended permissible limit for fish and fish products 0.000025 $mgkg^{-1}$ according to (Skovgaard, 2003) is an indication of the water pollution levels from which the fish was captured. In addition, the activities ongoing around the fish environment and the effluents deposited into the water body could be a factor. Dissimilar report was document in the study by (Benzer et al., 2013).

Table 10. Microbial load and identification in sampled fishes from both sources in September

Source	Species	Coliform cfu/100µ	Non-Coliform cfu/100µl	*E. coli*	*Salmonella* spp.	*Vibrio* spp. cfu/100µl	*Streptococcus* spp.	Other bacterial isolated
Holland	*S. scombrus*	2 x 10^4	8 x 10^5	-	-	0	-	*Enterobacter intermedius*
	C. harengus	0	0	-	-	0	-	*Shigella sonnei*
Japan	*S. scombrus*	0	0	-	-	0	-	
	C. harengus	0	0	-	-	0	-	

Key: TNTC - Too numerous to count, CFU – Colony forming unit

Table 11. Microbial load and identification in sampled fishes from both sources in November

Source	Species	Coliform cfu/100µ	Non-Coliform cfu/100µl	*E. coli*	*Salmonella* spp.	*Vibrio* spp. cfu/100µl	*Streptococcus* spp.	Other bacterial isolated
Holland	*S. scombrus*	4.9x10^5	TNTC	-	-	4.7x10^5	-	*Citrobacter diversus*
	C. harengus	5.6x10^5	TNTC	-	-	4.4 x10^5	-	*Shigella sonnei*
Japan	*S. scombrus*	2.2x10^5	4.96x10^6	-	-	2.9 x10^5	-	
	C. harengus	2.1x10^5	TNTC	-	-	1.20 x10^6	-	

Key: TNTC - Too numerous to count, CFU - Colony forming unit

Differences could be attributable to the differences in location from which the sampled species were obtained. Mn levels in the analyzed *S. scombrus* and *C. harengus* are beyond the prescribed risk allowance for consumers (HRI value of Mn across the different ages was > 1) and therefore, a source of danger to people's health.

Cr levels also reported in this study to exceed the permissible limit for fish and fish products, though, play a key function in glucose metabolism in its biological usable form, is also a notable hazardous metal. Daily, intake of chromium would be regarded as adequately taken if its 35 µg/day and 25 µg/day for young men and women, and could be less for younger persons (Trumbo et al., 2001). That means, the level reported in this study could be hazardous to the consumers of the fish when it accumulates in the body overtime. In addition, risk assessment indices calculated in this study was high for this metal across the different age classification (DIM, HQ, HRI, and TTM). Findings of this study corroborates with the report of Hothem et al. (2007).

Cu, Pb, Cd, which were also higher than the permissible levels for fish and fish products in the current study aligns with (Abubakar et al., 2015). Though, the essentiality of Cu in maintaining good health cannot be overruled, but if taken above the permissible level can result in liver and kidney damage (Ahmad et al., 2022). The mean concentrations of copper reported here as opposed to previous studies, was lower (Frías-Espericueta et al., 2014; Kareem et al., 2016). In addition, the HRI of Cu and Cd were >1 making the consumption of the fish sampled hazardous to the consumers.

Aflatoxin, produced by fungi in agricultural crops such as maize, cottonseed, etc. could have found its way into the water body due to runoff of wastewater from livestock feed mills and food producing industries into the high seas and oceans. The total aflatoxins recorded in the gills of sampled fish from Holland and Japan reported to have exceeded the permissible limits (4 ppb) could be due to its direct contact with the water and thus, the major organ of accumulation of aflatoxins. The gill is the first barrier of defense and the first organ to be exposed to suspended particles in water (Ahmed et al., 2015). Though, recorded to be lower in the muscle of the fish than the permissible limit, it is too close to the maximum aflatoxin level for human food (4ppb) according to the Commission of the European Communities (2001). Aflatoxins are immunosuppressive, mutagenic, teratogenic, and carcinogenic, if found in large quantities in foods, it can cause several health hazards (nausea, vomiting, abdominal pain, convulsions, and other signs of acute liver injury) to consumers (Dhakal et al., 2022; Azizi and Rouhi, 2013).

The discrepancy in the microbial load recorded for coliform, and non-coliform bacteria, and fungi during the study period could be due to the source of the fish, handling during processing and by buyers, contamination introduced from the fishing vessel, and during storage. It could also be due to the sample collection method. The total coliform bacteria count observed were within the standard recommended by NAFDAC for public health (5.0 × 10^5 and 1.0 × 10^6 cfu g-1) (Taiwo et al., 2021). Total coliform bacteria being indicator of sewage contamination of the fish sample could be a reflection of the source of the fish, and especially if the source of water flowing into the water body comes from human residents and livestock farms. *Vibrio* spp. observed to be present in the experimental fish species especially in the last purchase is the aetiology of diarrhea and are not to be present in fresh and frozen fish in accordance with the International Association of Microbiology Society's guidelines (Sanjee and Karim, 2016). *Vibrio cholerae* is reported to be the third-highest cause of shellfish-related diseases, next to noncholera *Vibrio* spp. (Wittman and Flick, 1995).

CONCLUSION

The heavy metal loads in this study were respectively above the permissible limits set by FAO, WHO and EU legislation for fish and fish products. Thereby posing a risk to the final consumer of the fish species as revealed by the risk indices of DIM, HQ, HRI, and TTM, and this calls for serious

public health concern. Total aflatoxin levels in the fish muscle sampled did not pose any threat to consumers as it was below the permissible limit. The study recommends that government should provide screening centres at the various entry points to ensure proper monitoring and screening of imported frozen fish before entry into the country.

ACKNOWLEDGEMENTS AND FUNDING

The authors acknowledge the efforts of Professor O.B. Akpor of the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria for his support, and guidance during the study.

AUTHORSHIP CONTRIBUTION

Oghenebrorhie Mavis Oghenochuko, Adeyinka Olamide Agbato: Conceptualization, idea, design. Rachael Oluwatosin Kolawole, Olasunkanmi Peter Olajide, Adeyinka Olamide Agbato: Data curation. Rachael Oluwatosin Kolawole: Writing-Original draft preparation. Oghenebrorhie Mavis Oghenochuko, Adeyinka Olamide Agbato, Olasunkanmi Peter Olajide: Revised first draft. Oghenebrorhie Mavis Oghenochuko, Rachael Oluwatosin Kolawole, Olasunkanmi Peter Olajide, Adeyinka Olamide Agbato: Revised final draft. Oghenebrorhie Mavis Oghenochuko: Supervision.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ETHICS APPROVAL

No specific ethical approval was necessary for this study as frozen fish were used.

DATA AVAILABILITY

All relevant data is in the article.

REFERENCES

Abubakar, A., Uzairu, A., Ekwumemgbo, P.A., & 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*, 11 p. https://doi.org/10.1155/2015/303245

Adedire, C.O., Adeyemi, J.A., Owokoniran, G.O., Adu, B.W., & Ileke, K.D. (2021). Effects of application of copper-based fungicides in cocoa plantations on the abundance and diversity of macroinvertebrates in adjacent rivers in Southwestern Nigeria. *Ife Journal of Science, 23*(2), 1-13. https://doi.org/10.4314/ijs.v23i2.1

Ahmad, S., Ärnlöv, J., & Larsson, S.C. (2022). Genetically Predicted circulating copper and risk of chronic kidney disease: A mendelian randomization study. *Nutrients, 14*(3), 509. https://doi.org/10.3390/nu14030509

Ahmed, Q., Bat, L., Yousuf, F., Ali, Q.M., & Nazim, K. (2015). Accumulation of heavy metals (Fe, Mn, Cu, Zn, Ni, Pb, Cd and Cr) in tissues of narrow-barred Spanish mackerel (Family-Scombridae) fish marketed by Karachi Fish Harbor. *Open Biological Sciences Journal*, *1*, 20-28. https://doi.org/10.2174/2352633501501010020

Amuneke, K.E., Igbodiegwu, G.C., Okeke, P.A., & Adibe, A.C. (2020). Bacteriological profile of selected fish species and water sample from Otuocha River Anambra State. *Journal of Agriculture and Food Sciences,* 2015(1), 20-28. https://doi.org/10.4314/jafs.v18i1.2

Anzecc, & Armcanz (2000). Australian and New Zealand Guidelines for Fresh and Marine Water Quality - Aquatic Ecosystems - Rationale and Background Information- Volume 2. *Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand*. 314 p

Avrameas, S. (1969). Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry*, *6*(1), 43 48. https://doi.org/10.1016/0019-2791(69)90177-3

Azizi, G.I., & Rouhi, S. (2013). The comparison of total fumonisin and total aflatoxin levels in biscuit and cookie samples in Babol City, Northern Iran. *Iranian Journal of Public Health*, *42*(4), 422-427.

Barrow, G., & Feltham, R. (1993). *Cowan and Steel's Manual for the Identification of Medical Bacteria* (3rd Ed.). Cambridge, Cambridge University Press. 319 p. https://doi.org/10.1017/CBO9780511527104

Benzer, S., Arslan, H., Uzel, N., Gül, A., & Yilmaz, M. (2013). Concentrations of metals in water, sediment and tissues of *Cyprinus carpio* L., 1758 from Mogan Lake (Turkey). *Iranian Journal of Fisheries Sciences*, *12*(1), 45 - 55.

Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, *3*(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529

Boyd, C.E., McNevin, A.A., & Davis, R.P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food Security*, *14*, 805–827. https://doi.org/10.1007/s12571-021-01246-9

Commission of the European Communities (2001). Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, OJ L 77, 1 p.

Dawodu, O.G., Akanbi, R.B. (2021) Isolation and identification of microorganisms associated with automated teller machines on Federal Polytechnic Ede campus. *PLoS ONE*, *16*(8):,e0254658. https://doi.org/10.1371/journal. pone.0254658

Dhakal, A., Hashmi, M.F., & Sbar, E. (2022). Aflatoxin Toxicity. In StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557781/

FAO. (2022). The State of the World Fisheries and Aquaculture. Towards Blue Transformation. Rome, FAO. 266 p. https://doi.org/10.4060/cc0461en

Frías-Espericueta, M.G., Zamora-Sarabia, F.K.G., Osuna-López, J.I., Muy-Rangel, M.D., Rubio-Carrasco, W., Aguilar-Juárez, M., & Voltolina, D. (2014). Cadmium, copper, lead, and zinc contents of fish marketed in NW Mexico. *The Scientific World Journal*, 4 p. https://doi.org/10.1155/2014/546897

Hothem, R.L., Bergen, D.R., Bauer, M.L., Crayon, J.J., & Meckstroth, A.M. (2007). Mercury and trace elements in crayfish from northern California. *Bulletin of Environmental Contamination and Toxicology*, *79*, 628–632. https://doi.org/10.1007/s00128-007-9304-6

Kareem, O.K., Orisasona, O., & 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. https://doi.org/10.3923/rjet.2016.82.87

Khan, S., Shah, I.A., Muhammad, S., Malik, R.N., & Shah, M.T. (2015). Arsenic and heavy metal concentrations in drinking water in Pakistan and risk assessment: A case study. *Human and Ecological Risk Assessment*, *21*(4), 1020-1031. https://doi.org/10.1080/10807039.2014.950925

Ogundiran, M.A., Adewoye, S.O., Ayandiran, T.A., & Dahunsi, S.O. (2014). Heavy metal, proximate and microbial profile of some selected commercial marine fish collected from two markets in south western Nigeria. *African Journal of Biotechnology*, *13*(10), 1147-1153. https://doi.org/10.5897/ajb2013.13240

Ogur, S. (2022). Pathogenic bacteria load and safety of retail marine fish. *Brazilian Journal of Biology*, *82*, e262735. https://doi.org/10.1590/1519-6984.262735

Okunola, O.J., Alhassan, Y., Yebpella, G.G., Uzairu, A., Tsafe, A.I., Abechi, E.S., & Apene, E.(2011). Risk assessment of using coated mobile recharge cards in Nigeria. *Journal of Environmental Chemistry and Ecotoxicology*, *3*(4), 80-85.

Olalekan, R.M., Adedoyin, O., & Odubo, T.V. (2019). Measures of harm from heavy metal contents (lead and cadmium) in women's lipstick and lipgloss in Yenagoa Metropolis, Bayelsa State, Nigeria. *SSRN Electronic Journal*, *2*(3), 236-242. https://doi.org/10.2139/ssm.3326545

Sanjee, S. Al, & Karim, M.E. (2016). Microbiological quality assessment of frozen fish and fish processing materials from Bangladesh. *International Journal of Food Science*, 2016(ID 8605689), 6 p. https://doi.org/10.1155/2016/8605689

Simpson, V.B.,& Uche, A. (2019). Elemental study of common iced fish species sold in Akure, Ondo State, Nigeria. *European Journal of Nutrition & Food Safety*, *10*(1), 31-42. https://doi.org/10.9734/ejnfs/2019/v10i130094

Skovgaard, N. (2003). Safety and quality issues in fish processing. *International Journal of Food Microbiology*, *85*(1–2), 204-205. https://doi.org/10.1016/s0168-1605(02)00488-9

Taiwo, I.O., Olopade, O.A., & Bamidele, N.A. (2021). Microbial load of some imported frozen fish species in Lagos, Nigeria. *Nigerian Journal of Animal Production*, *44*(3), 152-160. https://doi.org/10.51791/njap.v44i3.756

Trumbo, P., Yates, A.A., Schlicker, S., & Poos, M. (2001). Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *Journal of the American Dietetic Association*, *101*(3), 294-301. https://doi.org/10.1016/S0002-8223(01)00078-5

Wittman, R.J., & Flick, G.J. (1995). Microbial contamination of shellfish: Prevalence, risk to human health, and control strategies. *Annual Review of Public Health*, *16*, 123-140. https://doi.org/10.1146/annurev.pu.16.050195.001011