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Health Risk Assessment of Heavy Metals in Imported Frozen Fish Sold in Damaturu and Maiduguri Fish Markets, Nigeria

[1*]Jajere, S. M., [1]Jidda, D., [2,3]Goni, M.D., [1]Sadiq, M.A., [1]Adamu, S. G., [1]Mohammed, S., [1]Saidu, A. S., [1]Malah, M., [1,4]Musa, H. I. and [1]Tijjani, A. O.

[1]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.
[2]Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, Pengkalan Chepa 16100 Kota Bharu, Kelantan, Malaysia.
[3]Institute for Artificial Intelligence and Big Data, Universiti Malaysia Kelantan, 16100 Kota Bharu, Kelantan, Malaysia.
[4]Department of Veterinary Laboratory Diagnostics, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia.

[*] *Author for Correspondence: drmsjajere@unimaid.edu.ng*

ABSTRACT

Heavy metals bioaccumulation in fish constitute an issue of public health concerns. This study aimed to evaluate the levels of Cadmium (Cd) and Lead (Pb) in the head and muscle tissues of imported frozen fish as well as health risk to man. A total of 30 frozen fish were sampled from different fish markets in Damaturu and Maiduguri in Yobe and Borno States, Nigeria respectively. Heavy metal concentrations were determined by atomic absorption spectrophotometer. The highest mean concentration levels (0.60 ± 0.32 mg/kg and 0.06 ± 0.10 mg/kg) of Pb and Cd were found in the head and the lowest (0.09 ± 0.02 and 0.01 ± 0.00) in the muscle tissues respectively. The mean concentration levels of Pb (0.60 ± 0.32 mg/kg) and Cd (0.06 ± 0.10 mg/kg) in fish markets A and D exceed the maximum allowable limits of 0.50 mg/kg and 0.05 mg/kg respectively. Fish markets had statistically significant ($p < 0.05$) effect on the concentration levels of Pb among the sampled fish. The assessment of potential health risk; estimated daily intake, the target cancer rate, target hazard quotient and hazard index values obtained were below the threshold, indicating consumption of head and muscle of imported frozen fish sold in the study areas is safe and hazardless. However, because of their non-degradable nature and tendency to accumulate in tissues and organs, we recommended that heavy metals contamination in imported frozen fish should be monitored regularly, and packages should contain concentration labels before releasing it into the market value chain.

Keywords: Frozen fish; Heavy metals; Hazard index; Risk assessment; Target cancer risk; Target hazard quotient

INTRODUCTION

Environmental pollution occurs when any substances contaminate the physical and biological components of the earth's atmospheric system to such an extent that normal environmental processes are adversely affected. These pollutants can be natural such as volcanic ash from a volcanic eruption, or artificial, created, consumed and discarded in an unsustainable manner (Nathanson, 2021). Runoff from this trash damages the quality of water, which is a significant concern for humankind as it is the most vital resource for subsistence (Brack *et al.,* 2017). One of the most critical water contaminants is heavy metal (HM); a global issue due to their long-term persistence on soils and water, increasing geo-ecological risks and disrupting natural biogeochemical cycles. Therefore, aquatic ecosystems, especially water bodies, are known as the main receptors of HMs, either directly or indirectly (Elkady *et al.,* 2015).

Elements with a density of more than 5 g/cm^3, atomic weight ranging from 63.546 to 200.590 AMU and a specific gravity greater than 4.0 are termed HMs (Guevara-García *et al.,* 2017). Water pollution with HMs has become a major environmental problem since the advent of agricultural, industrial, and modern technological revolutions and other man-made activities such as mining, excavations and construction (Vardhan *et al.,* 2019). HMs generally enters the environment, including aquatic habitats, through various means, constituting a significant potential threat to human and animal health (Konduracka, 2019).

Fish is generally and widely accepted due to its high palatability, low cholesterol and tender flesh. Globally, it is considered the cheapest source of animal protein (Maulu *et al.,* 2020; Maulu *et al.,* 2021). Similarly, in Nigeria, consumption of frozen fish is believed to be higher than other animal protein sources such as beef, mutton and chicken, among others. The massive importation of frozen fish has made Nigeria the largest importer of frozen fish in Africa (Olaoye *et al.*, 2022; Abubakar *et al.,* 2014) and ranking world's fourth largest importer—in volume terms (5.4% of global imports)—after China, Japan and The USA (Tall, 2023). Consequently, various frozen fish species are imported and sold in markets across northeastern Nigeria. The safety of fish and fish products and their quality assurance is among the main concerns in the public health and food industry today. Once HMs contaminates the environment, they persist for years, increasing the chances of exposure to humans and animals (Aladaileh *et al.,* 2020).

The concern about the high levels of HM contaminants in foods has led to the establishment of maximum allowable limits (MALs) by statutory bodies such as the World Health Organization (WHO) for certain heavy metals in food including seafoods and fish (WHO, 2011). Metals such as Cd, Pb and Hg are classified as potentially toxic when exceeding the MALs (Makedonski *et al.,* 2017; Rai, 2018; Rai *et al.,* 2019). Unlike organic contaminants, HMs are not degraded over time, and concentration may increase through bioaccumulation. Fish are often at the top of the aquatic food chain, and studies have shown that HMs can bio-accumulate in fish through direct absorption from the water via their gills and/or skin as well as through ingestion of contaminated food or particles during processing or storage (Mziray and Kimirei, 2016). Therefore, fish and fish products need to be carefully screened for safe consumption and to provide public health benefits.

Despite the occurrence of heavy metals being reported in imported Mackerel and Sardine available in some Nigerian markets with Cd values ranging from 0.016 - 0.105 mg LG1 and Pb values ranging from 0.013 - 0.105 mg LG1 (Burger *et al.,* 2017), there is still a need to expand the concentration levels of some heavy metals and update the status at various intervals and locations. Therefore, this work aimed to assess the safety of heavy metals in frozen fish sold in Maiduguri and Damaturu fish markets. Additionally, the study aimed to determine the concentrations of contaminant levels of two selected non-essential heavy metals namely Cd and Pb, in imported frozen fish and to compare them with WHO MALs.

MATERIALS AND METHODS

Study Area

This study was conducted in the Northeastern States of Yobe (Damaturu) and Borno (Maiduguri) of Nigeria. Damaturu, the capital city of Yobe State, is between Latitude 11^0 44' 40"N and Longitude 11^0 57'40"E, covering a landmass of 2,366km^2. The population of Damaturu was estimated at 88,014 in the 2006 National Population Census. Borno State is located at latitude 11^030^1N and longitude 13^000^1E with a land area of 70,898 km^2. It shares borders with the Republic of Niger to the North, Cameroon Republic to the East and the Tchad Republic to the Northeast. Both states are in the semi-arid zone, characterized by climate conditions with a dry season from November to early June. During this period, the daily temperature range between 30ºC to 41ºC, particularly from March to June (Eresanya, 2018). The rainy season typically occurs from late June to October, with low relative humidity and a short-wet season. They consist mainly of farmers, animal herders, fishermen, traders and civil servants. The state is home to various water bodies such as rivers, streams, dams, and marketplaces for livestock, fish, and goods spread throughout the region.

Study Design and Sample Collection

A cross-sectional study was designed in which thirty (30) different batches of imported frozen fish samples were randomly sampled at different time periods from various retailers in four (4) different fish markets located in Damaturu and three (3) fish markets in Maiduguri Metropolis between June 2021 and August 2021. The fish samples were dissected into head and muscle specimens. The samples were grouped based on the location of purchase (markets); three fish each from four different fish markets in Damaturu, and six fish each were sampled from three different fish markets in Maiduguri. The samples were collected based on availability. Information on fish markets was obtained through verbal interaction with the retailers, and sampling from the carton or batch was by random sampling. Imported frozen fish were randomly purchased from four different locations with a one-week interval between each purchase to ensure complete randomization in Damaturu (fish market A, B, C and D) and from three different locations also with a one-week interval between purchases in Maiduguri (fish market E, F and G).

Quality Assurance and Quality Control

A random sampling technique was employed to collect the fish samples from a batch or carton. The samples were placed in polythen bags with ice blocks to prevent spoilage or external contamination. Subsequently, these samples were transported to the laboratory on the same day and stored in the refrigerator for preservation until they were processed.

Sample Preparation, Processing and Digestion

A total of 30 fish samples were purchased and placed on ice packs for immediate transportation to the Analytical Laboratory of the Department of Chemistry, Yobe State University. The collected samples were analyzed at the laboratory following the standard procedures described in the American Public Health Association 1020QC (2015) guidelines. Briefly, the fish samples were thawed at room temperature after being washed with distilled water in desiccators before dissection. The head and muscles were cut and removed using stainless sterile steel knife. Subsequently, the harvested parts were washed and placed in a well-labeled plastic container, indicating their respective sources (purchase area). The separate parts were then sun-dried for four days. The entire quantity of dried samples was then homogenized using a pestle and mortar in the laboratory.

The digestion of samples was based on microwave-assisted digestion using the Master 40 system (Digestion Microwave System, Buck Microwave Digestion System, Buck Scientific, USA). Briefly, 1g of tissue/organ sample with constant weight was placed in a polytetrafluoroethylene microwave test tube. A digestion reagents mixture consisting of 6mL of nitric acid at a 65% concentration and 2mL of hydrogen peroxide at a 35% concentration (used to reduce nitrous vapors and accelerate the digestion of organic matter by raising the temperature) was added in a ratio of 3:1. The mixture was allowed to sit for a while. The tube was then microwaved for 40 minutes at 180º C and cooled to room temperature in the oven. The supernatant was harvested and diluted with 40mL of distilled water in a volumetric flask for both head and muscle samples. Subsequently the solution was then filtered using a 90mm Whatman No. 1 filter paper to remove impurities. The metallic content of the digested samples and blanks was quantified using an Atomic Absorption Spectrophotometer (AAS Buck Scientific Model 210vgp, USA).

Health Risk Assessment

The potential health risks associated with the consumption of heavy metals from frozen fish were assessed based on the Estimated Daily Intake of metals (EDI), Target Hazard Quotient (THQ), Hazard Index (HI) and Target Cancer risk based on standard protocols.

Estimated Daily Intake (EDI)

The EDI of metals refers to the amount of a substance in food expressed on a body mass basis (usually in mg/kg body weight) that humans can ingest daily over a lifetime without significant health risks (Benard *et al.,* 2020). In this study, the EDI of Cd and Pb through the consumption of frozen fish in the study area were determined using the formula:

EDI = [C x QFC]/BW

This formula is described by the United States Environmental Protection Agency (USEPA) (2021), where C = mean concentration of Cd or Pb in the frozen fish (mg/kg), BW = average body weight in kg (Children 0-17 years = 30 kg; Adult ≥ 18 years = 60 kg) and QFC is the estimated quantity of frozen fish consumed daily (kg/person/day) = 0.0345 kg. The computed EDI values of Cd and Pb were then compared with their respective provisional tolerable daily intake (PTDI) values of 0.001 mg/kg/day and 0.002 mg/kg/day set by the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO) (2011).

Target Hazard Quotient (THQ)

The THQ represents the non-carcinogenic health risks posed by the consumption of heavy metals (Njoga *et al.,* 2021). The THQ of Cd and Pb through consumption of frozen fish in the study area was estimated following the recommendation of USEPA (2021); using the formula:

THQ = [(Ef x Ed x QFC x C) / (RfD x BW x Et)] x 10^{-3}

Here, Ef = exposure frequency in days (365), Ed = exposure duration (equivalent to the average life expectancy of Nigerian set at 55 years) (Benard et al., 2020), QFC = estimated quantity of frozen fish consumed daily (kg/person/day) = 0.0345 kg, C = mean concentration of Cd or Pb in the frozen fish (mg/kg), RfD = oral reference dose of 0.001 mg/kg/day for Cd and 0.004 mg/kg/day for Pb as was set by USEPA (2021), BW = average body weight in kg (Children 0-17 years = 30 kg; Adult ≥ 18 years = 60 kg) while Et (Ef x Ed) = exposure time (365 days x 55 years). The THQ has a benchmark value of 1. Therefore, the calculated THQ value of < 1 indicates little or no non-carcinogenic health risk. On the other hand, a value ≥ 1 was considered a possibility that non-carcinogenic adverse health effects may ensue following fish consumption (Benard *et al.,* 2020; Njoga *et al.,* 2021).

Hazard Index (HI)

The HI determines the potential risk to human health from the combined effect of pollutants, as fish may be contaminated with multiple toxic heavy metals having similar adverse health effects. Therefore, it is appropriate to sum individual fish THQs of Cd and Pb detected in the study area using the formula:

HI = THQCd + THQPb

HI value < 1 depicts very little or no non-carcinogenic health risk, whereas value ≥ 1 foretells a possibility of non-carcinogenic adverse health effects (Njoga *et al.,* 2021).

Target Cancer Risk (TCR)

The TCR was calculated to assess the potential carcinogenic risks associated with lifetime exposure to Cd and Pb using the formula:

TCR = CSF x EDI as described by Naseri *et al.* (2021).

CSF (cancer slope factor) was set at 0.38 mg/kg/day for Cd and 0.008 mg/kg/day for Pb by USEPA (2021). Therefore, if the calculated TCR > 1 = carcinogenic risk.

Data Analysis

The data generated from this study were analyzed using the IBM® SPSS Statistics version 20 (IBM, Armonk, NY: IBM Corp.). Descriptive statistics (mean, standard deviation, Range, minimum and maximum) were used to analyze the obtained data. To compare the mean concentration values and determine the associations between the mean concentration levels of Cd and Pb and their respective WHO MALs of 0.05 mg/kg and 0.50 mg/kg, a one-sample t-test was employed. Similarly, Independent t-test; Kruskal-Wallis; Mann-Whitney test, and two-way ANOVA were performed where applicable in order to examine the effect of fish markets and parts of fish (head and muscle tissues) on the concentration levels of the heavy metals (Cd and Pb). Moreover, Levene's Test of Equality of Variances was used to test the assumption of homogeneity of variance for the dependent variables (Cd and Pb) mean concentration levels. In the univariate analysis, a *p-value* ≤ 0.05 was considered statistically significant.

RESULTS

Fish markets ranking sequences of Cd and Pb in Damaturu and Maiduguri Fish Markets

In Damaturu, the highest amount of Cd was 0.174 mg/kg in Fish Market D, followed by Fish Market A with 0.042 mg/kg, Fish Market C with 0.040 mg/kg and Market B with 0.017 mg/kg. However, the amount of Pb was found to be highest in Market A with 0.962 mg/kg, followed by Market D with 0.547 mg/kg, market B with 0.395 mg/kg and Market C with 0.138 mg/kg. In Maiduguri, the highest amount of Cd recorded was 0.140 mg/k in Market G, followed by Market E with 0.110 mg/kg and Market F with 0.108 mg/kg. However, the amount of Pb of 1.087 was found to be highest in Fish Market F with 0.108 mg/kg, followed by Market G with 0.824 mg/kg and Market E with 0.730 mg/kg.

Mean concentration levels (Range) of Cd and Pb concentration in Head and Muscle tissue samples

In Damaturu, the mean concentration level of Cd in the head was 0.174 mg/kg (Max. = 0.174, Min. = 0.000), and in the muscle, it was 0.100 mg/kg (Max. = 0.100, Min. = 0.000). The mean concentration level of Pb in the head was 0.893 mg/kg (Max. = 0.962, Min. = 0.069); in the muscle, it was 0.453 mg/kg (Max. = 0.522, Min. = 0.069). In Maiduguri, the mean concentration level of Cd in the head was 0.108 mg/kg (Max. = 0.108, Min. = 0.000), and in the muscle, it was 0.140

mg/kg (Max. = 0.140, Min. = 0.000), The mean concentration level of Pb in head was 1.087 mg/kg (Max. = 1.087, Min. = 0.000) and in the muscle was 1.040 mg/kg (Max. = 1.040, Min. = 0.000).

Fish markets specific mean concentration levels (mg/kg) of Cd and Pb in Head and Muscle tissues samples from Damaturu Fish Market

Table 1 shows the mean concentrations (mg/kg) of Cd and Pb in fish sampled from the four fish markets (A-D) from Damaturu. This table showed that the highest mean concentration of Cd (0.06mg/kg) was found in fish head samples from market D, while the lowest (0.01mg/kg) was found in fish muscle samples from market B. Similarly, the highest mean concentrations (mg/kg) of Pb (0.60 mg/kg) were found in fish head samples from market A, while the lowest (0.09mg/kg) was found in muscle samples from market C. The mean concentration levels of Cd (0.06 ± 0.10 mg/kg) in fish head from market D was not significantly (t (2) = 0.163, p = 0.886) higher than the MALs of 0.05 mg/kg. Similarly, the mean concentration of Pb (0.60 ± 0.32 mg/kg) in the fish head from market A was not significantly (t (2) = 0.541, p = 0.643) higher than the MALs of 0.5 mg/kg.

There was no statistically significant difference in the mean scores for Cd in the head (0.323) and muscle tissues (0.228) (t (22) = 0.616, p = 0.544). Similarly, there was no statistically significant difference in the mean scores for Pb in the head (0.314) and muscle tissues (0.267) (t (22) = 0.536, p = 0.597).

Kruskal-Wallis test was performed to analyze the Cd and Pb scores of the four markets (A, B, C and D). The differences between the rank totals for Cd in market A (14.83), B (7.42), C (16.50) and D (11.25) were not statistically significant (H (3, n = 24) = 5.867, p = 0.118). However, the differences between the rank totals for Pb in market A (18.33), B (9.33), C (5.17), and D (17.17) were statistically significant (H (3, n = 24) = 14.360, p = 0.002). The Post-Hoc Test (Bonferroni correction) showed that there was a significant difference in the observed mean concentration levels between fish market C with D (U ($n_1 = n_2$ = 6) = -12.000, p = 0.020) and A (U ($n_1 = n_2$ = 6) = 13.167, p = 0.008). None of the other comparisons were significant after the Bonferroni adjustment.

Fish markets specific mean concentrations (mg/kg) of Cd and Pb in Head and Muscle tissues samples from Maiduguri Fish Market

Table 2 shows the mean concentrations (mg/kg) of Cd and Pb in fish sampled from Maiduguri's three fish markets (E-G). This table showed that the highest mean concentration of Cd (0.03mg/kg) was found in the fish head and muscle samples from markets F and G, respectively, while the lowest (0.01mg/kg) was found in fish head samples from markets E and G. Similarly, the highest mean concentrations (mg/kg) of Pb (0.39 mg/kg) was found in fish muscle samples from market F, while the lowest (0.25 mg/kg) was found in head samples from market E.

A two-way ANOVA was performed to compare the mean values of Cd to examine the effect of fish markets and fish parts (head and muscle) on concentration. The analysis revealed no statistically significant interaction between the effects of fish markets and fish parts (F (2, 30) = 0.829, p = 0.446). The simple main effects analysis showed that fish markets did not have a statistically significant (p = 0.794) effect on the concentration of Cd. Similarly, fish parts did not have a statistically significant (p = 0.688) effect on the concentration of Cd. The mean values of Pb were compared using a two-way ANOVA to examine the effect of fish markets and parts (head and muscle) on concentration. The analysis revealed no statistically significant interaction between the effects of fish markets and fish parts (F (2, 30) = 0.081, p = 0.922). The simple main effects analysis showed that fish markets did not have a statistically significant (p = 0.643) effect on the concentration of Pb. Similarly, fish parts did not have a statistically significant (p = 0.995) effect onu8 the concentration of Pb.

Health Risk Assessment of Cd and Pb Specific to Children and Adult Consumers of Frozen Fish in Damaturu, Yobe State, Nigeria

The Health Risk Assessment of Cd and Pb specific to children and adult consumers of frozen fish in Damaturu is shown in Tables 3 & 4. The computed EDI for Cd in children and adult populations ranged between 5.75E-06 and 6.90E-05 mg/kg/day. Similarly, the EDI values of Pb in children and adults ranged between 5.18E-06 and 6.90E-04 mg/kg/day (Table 3). The calculated EDI values were generally higher in children than in the adult population and were lower than the WHO-recommended PTDI.

The TCR for Cd in children and adult populations ranged between 2.19E-06 and 2.62E-05. Similarly, the TCR values of Pb in children and adults ranged between 4.14E-07 and 5.52E-06, which were lower than the TCR > 1 = carcinogenic risk (Table 3). The THQ of all the HMs and in all organs for both children and adult populations ranged between 5.75E-06 and 1.15E-04. The HI value < 1 = very little or no non-carcinogenic health risk (Table 4). The HI value for the two metals was less than unity for children and adult populations (Table 4).

Health Risk Assessment of Cd and Pb Specific to Children and Adult Consumers of Frozen Fish in Maiduguri, Borno State, Nigeria

The Health Risk Assessment of Cd and Pb specific to the children and adult consumers of frozen fish in Maiduguri is shown in Tables 5 and 6. The computed EDI values for Cd in children and adult populations ranged between 5.75E-06 and 3.45E-05 mg/kg/day, while EDI values of Pb in both children and adult populations ranged between 1.44E-04 and 4.49E-04 mg/kg/day (Table 5). The calculated EDI values were generally higher in children than in the adult population and lower than the WHO recommended PTDI.

The TCR for Cd in children and adult populations ranged between 2.19E-06 and 1.31E-05, and TCR values of Pb in both populations ranged between 1.15E-06 and 3.59E-06, which were lower than the TCR > 1 = carcinogenic risk (Table 5). The THQ of all the HMs and in all the organs for both children and adult populations ranged between 5.75E-06 and 1.12E-04. The HI value < 1 = very little or no non-carcinogenic health risk (Table 6). The HI value for the two metals was less than unity for children and adult populations (Table 6)

Table 1: Fish markets specific mean concentrations of Cd and Pb in Head and Muscle tissues samples (n=3 per markets) from Damaturu market, Yobe State, Northeastern Nigeria.

Fish market	Fish parts	Mean Concentrations ± SD (mg/kg)*	
		Cd	Pb
Fish market A	Head	0.03±0.01	0.60±0.32
	Muscle	0.02±0.01	0.36±0.12
Fish market B	Head	0.01±0.01	0.18±0.12
	Muscle	0.01±0.00	0.22±0.16
Fish market C	Head	0.03±0.01	0.11±0.04
	Muscle	0.02±0.01	0.09±0.02
Fish market D	Head	0.06±0.10	0.36±0.17
	Muscle	0.04±0.05	0.40±0.13

* SD, Standard deviation; Cd, Cadmium; Pb, lead

Table 2: Fish markets specific mean concentrations (mg/kg) of Cd and Pb in Head and Muscle tissues samples (n=6 per markets) from Maiduguri market, Borno State, Northeastern Nigeria.

Fish market	Fish parts	Mean Concentrations ± SD (mg/kg)*	
		Cd	Pb
Fish market E	Head	0.01±0.01	0.25±0.26
	Muscle	0.02±0.04	0.27±0.20
Fish market F	Head	0.03±0.05	0.35±0.38
	Muscle	0.02±0.04	0.39±0.36
Fish market G	Head	0.01±0.01	0.38±0.36
	Muscle	0.03±0.05	0.33±0.21

* SD, Standard deviation; Cd, Cadmium; Pb, lead

Table 3: Estimated daily intake (EDI) and target cancer risk (TCR) specific to children and adult consumers of frozen fish sold in Damaturu, Yobe State, Northeastern Nigeria.

Fish market	Metals	Fish parts	EDI *		TCR	
			Children	Adult	Children	Adult
Fish market A	**Cd**	Head	3.45E-05	1.73E-05	1.31E-05	6.56E-06
		Muscle	2.30E-05	1.15E-05	8.74E-06	4.37E-06
	Pb	Head	6.90E-04	3.45E-04	5.52E-06	2.76E-06
		Muscle	4.14E-04	2.07E-04	3.31E-06	1.66E-06
Fish market B	**Cd**	Head	1.15E-05	5.75E-06	4.37E-06	2.19E-06
		Muscle	1.15E-05	5.75E-06	4.37E-06	2.19E-06
	Pb	Head	2.07E-04	1.04E-04	1.66E-06	8.28E-07
		Muscle	2.53E-04	1.27E-04	2.02E-06	1.01E-06
Fish market C	**Cd**	Head	3.45E-05	1.73E-05	1.31E-05	6.56E-06
		Muscle	2.30E-05	1.15E-05	8.74E-06	4.37E-06
	Pb	Head	1.27E-04	6.33E-05	1.01E-06	5.06E-07
		Muscle	1.04E-04	5.18E-05	8.28E-07	4.14E-07
Fish market D	**Cd**	Head	6.90E-05	3.45E-05	2.62E-05	1.31E-05
		Muscle	4.60E-05	2.30E-05	1.75E-05	8.74E-06
	Pb	Hm ibjgjuead	4.14E-04	2.07E-04	3.31E-06	1.66E-06
		Muscle	4.60E-04	2.30E-04	3.68E-06	1.84E-06

EDI, Estimated daily intake; TCR, Target cancer risk; * EDI Unit, mg/kg bw/day, bw, body weight

Table 4 : Target hazard quotient (THQ) and hazard index (HI) specific to children and adult consumers of frozen fish sold in Damaturu, Yobe State, Northeastern Nigeria.

Fish market	Fish parts	THQ				HI	
		Cd		Pb			
		Children	Adult	Children	Adult	Children	Adult
Fish market A	Head	3.45E-05	1.73E-05	1.73E-05	8.63E-05	2.07E-04	1.04E-04
	Muscle	2.30E-05	1.15E-05	1.04E-04	5.18E-05	1.27E-04	6.33E-05
Fish market B	Head	1.15E-05	5.75E-06	5.18E-05	2.59E-05	6.33E-05	3.16E-05
	Muscle	1.15E-05	5.75E-06	6.33E-05	3.16E-05	7.48E-05	3.74E-05
Fish market C	Head	3.45E-05	1.73E-05	3.16E-05	1.58E-05	6.61E-05	3.31E-05
	Muscle	2.30E-05	1.15E-05	2.59E-05	1.29E-05	4.89E-05	2.44E-05
Fish market D	Head	6.90E-05	3.45E-05	1.04E-04	5.18E-05	1.73E-04	8.63E-05
	Muscle	4.60E-05	2.30E-05	1.15E-04	5.75E-05	1.61E-04	8.05E-05

THQ, Target hazard quotient, HI, Hazard index

Table 5 Estimated daily intake (EDI) and target cancer risk (TCR) specific to children and adult consumers of frozen fish sold in Maiduguri, Borno State, Northeastern Nigeria.

Fish market	Metals	Fish parts	EDI*		TCR	
			Children	Adult	Children	Adult
Fish market E	Cd	Head	1.15E-05	5.75E-06	4.37E-06	2.19E-06
		Muscle	2.30E-05	1.15E-05	8.74E-06	4.37E-06
	Pb	Head	2.88E-04	1.44E-04	2.30E-06	1.15E-06
		Muscle	3.11E-04	1.55E-04	2.48E-06	1.24E-06
Fish market F	Cd	Head	3.45E-05	1.73E-05	1.31E-05	6.56E-06
		Muscle	2.30E-05	1.15E-05	8.74E-06	4.37E-06
	Pb	Head	4.03E-04	2.01E-04	3.22E-06	1.61E-06
		Muscle	4.49E-04	2.24E-04	3.59E-06	1.79E-06
Fish market G	Cd	Head	1.15E-05	5.75E-06	4.37E-06	2.19E-06
		Muscle	3.45E-05	1.73E-05	1.31E-05	6.56E-06
	Pb	Head	4.37E-04	2.19E-04	3.50E-06	1.75E-06
		Muscle	3.80E-04	1.90E-04	3.04E-06	1.52E-06

EDI, Estimated daily intake; TCR, Target cancer risk; * EDI Unit, mg/kg bw/day, bw, body weight

Table 6: Target hazard quotient (THQ) and hazard index (HI) specific to children and adult consumers of frozen fish sold in Maiduguri, Borno State, Northeastern Nigeria.

Fish market	Fish parts	THQ				HI	
		Cd		Pb			
		Children	Adult	Children	Adult	Children	Adult
Fish market E	Head	1.15E-05	5.75E-06	7.19E-05	3.59E-05	8.34E-05	4.17E-05
	Muscle	2.30E-05	1.15E-05	7.76E-05	3.88E-05	1.01E-04	5.03E-05
Fish market F	Head	3.45E-05	1.73E-05	1.01E-04	5.03E-05	1.35E-04	6.76E-05
	Muscle	2.30E-05	1.15E-05	1.12E-04	5.61E-05	1.35E-04	6.76E-05
Fish market G	Head	1.15E-05	5.75E-06	1.09E-04	5.46E-05	1.21E-04	6.04E-05
	Muscle	3.45E-05	1.73E-05	9.49E-05	4.74E-05	1.29E-04	6.47E-05

THQ, Target hazard quotient, HI, Hazard index

DISCUSSION

In this study, the heavy metal concentrations in fish's head and muscle tissue samples showed that Pb and Cd were present in almost all the samples, suggesting contamination with Pb and Cd. The observed distribution of Pb and Cd could result from contamination from human activities in the source countries where most frozen fish is usually imported. This is supported by the findings of Perera *et al.* (2015) and Yedjou *et al.* (2016), who linked contamination of Cd and Pb to anthropogenic activities such as cement production and battery mining. The concentrations of Cd and Pb in the head samples were higher than those in the muscle tissues. This could be attributed to the fact that gaseous exchange in fish takes place in the gills. Heavy metals like Cd and Pb cannot easily penetrate the skin barrier; they are inhaled or ingested, absorbed and transported to various tissues, including muscle (Schoeters *et al.,* 2016). Another plausible explanation for the lower concentration levels of the heavy metals in muscle tissues of fish compared to the other organs examined could be attributed to the fact that muscle tissue has low metal binding protein (metallothioneins). Zhang *et al.* (2018) and Latif *et al.* (2022) observed similar findings.

The mean concentration levels of Cd and Pb in the head and muscle tissues of most sampled fishes were lower than the maximum permissible limits set by World Health Organization which are 0.05 mg/kg and 0.5 mg/kg respectively. However, in a few samples, the concentrations exceeded these limits. In Damaturu, although not statistically significant, the mean concentration levels of Pb (0.60 mg/kg, $p = 0.643$) and Cd (0.06 mg/kg, $p = 0.886$) in fish markets A and D, respectively, were found to be higher than the maximum allowable limits. However, in Maiduguri, the estimated mean concentration levels were within the permissible limits. Some studies have reported that even at lower concentrations, Cd and Pb could be detrimental to human and animal health (Garai *et al.,* 2021). Additionally, the net result of uptake is bioaccumulation; therefore, long-term exposure to heavy metals can cause death or impairment of vital organs such as the central nervous system, kidney and liver and could be genotoxic (Garai *et al.,* 2021; Olayinka-Olagunju, 2021; Dou *et al.,* 2022).

In Damaturu, the mean concentration levels of Pb in fish market A was significantly higher than in Market C. Similarly, there was a statistically significant difference between markets D and C respectively. Although the Pb pollutant occurs naturally in the environment, the observed differences could be attributed to anthropogenic activities in the area emitting high concentrations. Therefore, the ranking sequences of Pb based on the fish markets were A > D > B > C in descending order. Although there was no significant statistical difference, the ranking sequences of Cd based on fish markets were D > A > C > B in descending order. However, in Maiduguri, the ranking arrangements of Pb and Cd based on the fish market were F > G > E and G > E > F, respectively, in descending order and this was not statistically significant.

The estimated health risk assessment indexes for frozen fish samples sourced from both Damaturu and Maiduguri fish markets revealed that the EDI and TCR for Cd and Pb values obtained were very low when compared with the PTDI and carcinogenic risk (>1) values set by the FAO/WHO and USEPA, respectively. Similar findings were observed by Benard *et al.* (2020). The THQ and HI values recorded less than one, indicating that the consumption of frozen fish in the study areas carries very little or no non-carcinogenic risk. This finding contrasts with the likely health risk from the consumption of shellfish asserted by Benard *et al.* (2020), who reported values above the threshold of 1.

Conclusions

In conclusion, heavy metals (Cd and Pb) are present in most of the fish samples tested and were within allowable limits set by WHO, except in a few cases that exceeded the maximum limits. The assessment of potential health risk; estimated daily intake, the target cancer rate, target hazard quotient and hazard index values of Cd and Pb obtained were below the threshold, indicating consumption of head and muscle of imported frozen fish sold in the study areas is safe and hazardless. Because of their non-degradable nature and tendency to accumulate in tissues and organs, we recommended that heavy metals contamination in imported frozen fish should be monitored regularly, and packages should contain concentration labels before releasing it into the market value chain. Moreover, the consumers of frozen fish in the study area should be enlightened about the harmful effects of heavy metal toxicity as well as screened for any incidence of the traces of heavy metals in their body

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Conflict of Interest

The authors have no conflict of interest to declare.

Authors' Contribution

SMJ, DJ and MAS contributed to the study concept and design. Material preparation, data collection and analyses were performed by MM, DJ, MDG, SGA, SM and ASS. The draft manuscript was prepared by SMJ and DJ. MAS, HIM and AOT read, corrected and approved the manuscript for publication. All authors have read and approved the final manuscript.

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