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Assessment of heavy metal concentrations in imported marine fish species consumed in Ogun state, Nigeria

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ABSTRACT

Fish is an affordable protein source in developing countries such as Nigeria, however, human activities like mining, agriculture, and industrial discharges negatively impact these aquatic resources and their environment. Heavy metals leach into the ocean and accumulate in fish, potentially causing health issues for those who consume them. The present study evaluated the heavy metal concentrations in five commercially important and highly consumed fish species (Clupea pallasii, Merluccius productus, Gadus chalcogrammus, Scomber scombrus, Trachurus murphyi) in Nigeria. The study was conducted between August 2021 to January 2022, and Mn, Fe, Zn, Pb, Cd levels were sampled in a total of 50 fish using the AA 240 Fanst Sequential Atomic Absorption Spectrophotometer and expressed as mg/kg wet weight. Among the heavy metals estimated in all fish samples, Mn was the most prevalent, while Zn was relatively the lowest. Pb (6.39 mg/kg), Cd (1.39 mg/kg), and Fe (5.59 mg/kg) concentrations exceeded the FAO and WHO regulatory limits and may be deleterious to human health. Therefore, while essential as a protein source, the presence of heavy metals exceeding recommended limits in these commonly consumed imported fish species raises public health concerns. This study emphasizes the crucial need for stricter regulations and consistent monitoring of imported fish to guarantee their safety and consumer well-being in Nigeria.

Introduction

Heavy metal pollution has emerged as a significant environmental challenge since the onset of the industrial revolution (1760 to 1840). Anthropogenic activities such as agricultural, industrial, and mining activities have been on the increase and are recognized as a primary source of pollution in all aquatic environments (Bashir *et al.,* 2020; Gorur *et al.,* 2012; Daffonchio *et al.,* 2013; Akinsorotan *et al.,* 2023). These pollutants which include sewage effluents, industrial waste, plastics, and agrochemicals among others from point and non-point sources are being washed into the ocean (Talbot *et al.,* 2018). Though heavy metals are earth's natural constituents present in the oceans in trace concentrations through natural phenomena, anthropogenic activities are increasing their concentrations in the waters to levels dangerous to aquatic life and humans (Zahran and Willis, 2003).

These toxic elements adversely impact the physiological, and reproductive functions as well as the immunological systems of fish, exposing them to disease and increased mortality risk (Akgün *et al.,* 2007; Al-Weher, 2008). For example, aquatic fish species are known to suffer cytotoxic, mutagenic, and genotoxic harm due to heavy metals such as Mercury (Hg), Lead (Pb), Arsenic (As), and Cadmium (Cd). Similarly, in humans, the adverse effects encompass kidney damage, renal and hepatic system impairment, respiratory dysfunction, cancerous cell development,

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and other reproduction-related deficiencies have also been observed (Satarug *et al.,* 2017; Manea *et al.,* 2020). Heavy metals can be bioaccumulated and biomagnified in aquatic species through the process of absorption into their muscular tissue (Yilmaz *et al.,* 2007; Sarong *et al.*, 2015; Jamil *et al.,* 2023), as this occurs when the organisms absorb suspended particles and dietary components. (Arantes *et al.,* 2016).

Due to the increasing demand for fish in low-income countries like Nigeria, marine fish importation is often described as a solution to the country's widening fish demand-supply gap, with approximately 2.2 million tons of these fish, imported annually (Bruinsma, 2017). This makes these fish easily accessible in cold rooms in markets across the country, making them available for consumption in every household, as it supplements the input from artisanal fishery and Aquaculture. The marine environment where these fish are captured (such as the northern Atlantic and northwestern Pacific Ocean) has experienced rising contamination from heavy metals. These contaminations are produced from various sources such as sewage and industrial discharges, oil pollution, brine releases, coastal alterations etc. (Naser 2013; Pan *et al.,* 2019; Ho *et al.,* 2021). Marine fishes imported to Nigeria have been reported to contain high levels of toxic contaminant such as Pb (Uroko *et al.,* 2020), Hg and aluminium (Alfred, 2022) in their tissues, thus, negatively affecting their quality, thereby posing huge health risk to consumers (Mieiro *et al.,* 2012; Bashir *et al.,* 2013).

In recent years, there has been a growing body of research on the contamination of aquatic species by heavy metals (Kumolu-Johnson and Ndimele, 2011; Kareem *et al.,* 2016). However, these studies have mainly focused on fish species from inland waters such as lakes, rivers, dams etc., and there has been a paucity of similar research on imported marine species which are highly demanded and are easily obtained in cold rooms in both rural and urban communities in Nigeria. This therefore necessitates this study as frequent fish health, quality and heavy metal toxicity monitoring is crucial in seafood quality assessment.

The five most demanded fish found in Nigeria cold rooms are Atlantic mackerel (*Scomber scombrus*), pacific herring (*Clupea pallasii*), Alaska pollock (*Gadus chalcogrammus*), jack mackerel (*Trachurus murphyi*) and pacific hake (*Merluccius productus*), these fish species account for a significant percentage of imported fish consumed in the country (Kareem *et al.,* 2016). Considering the potential risk posed to the people of Ogun state, Nigeria with a population of over 6.3 million, (NPC, 2022), investigating the metals levels in these commonly consumed fish species is crucial. Hence, this research seeks to assess the toxicity of heavy metals in the tissues of five commercially significant marine fish species acquired from cold storage facilities in Ogun State, Nigeria. Additionally, it aims to estimate the potential health risks to humans resulting from the consumption of these fish species by employing the Exposure Daily Intake (EDI) and the Target Hazard Quotient (THQ) for all the metals studied.

Materials and Methods

Sample collection

Fifty samples of five different fish species (10 samples of each) were bought in cold rooms within the Ijebu-ode metropolis, Ogun state, Nigeria between August 2021 to January 2022. The number of samples used was due to the limited funding available. To prevent deterioration, the fish samples were promptly placed in ice packs and transported to the laboratory. All fish specimens were kept below -10 ^{0}C in a refrigerator until required for analysis (Ademoroti, 1996).

Sample preparation

Fish specimens were allowed to thaw and rinsed thoroughly with clean water. The total length and body weight were measured using a measuring board and a digital weighing scale. prior to the digestion and heavy metals assessment. Each of the fish samples was washed with deionised water, sliced into smaller pieces, 15 g of muscle tissue was collected, and oven dried at 65 °C until a constant weight was obtained (Abubakar *et al.,* 2015). The dried sample of each of the fishes was pulverised, weighed, and stored under clean sample bottles ready for digestion (Ismaeel and Kusag, 2015).

Digestion of fish specimens

Each sample was digested using a concentrated acid mixture (HNO_3 and $HClO_4$ in a 5:1 ratio). A 0.5 g fish sample was digested with the acid mixture until a transparent solution was obtained, following the method described by Allen *et al.* (1986). The resulting solution was filtered and diluted to 50 ml and 25 ml, respectively, using deionized water to create working standard solutions for each metal ion.

Heavy metal detection

The concentrations of Pb, Cd, Zinc (Zn), Iron (Fe), and Manganese (Mn) in the fish samples were analyzed utilizing the AA 240 Fanst Sequential Atomic Absorption Spectrophotometer. The results obtained were expressed in mg/kg wet weight (Cunniff, 1995).

FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2010).

$$EDI = \frac{MS \times C}{BW}$$

Where MS = adult meal size (227 g in EDI and 0.227 kg in THQ); BW = average human body weight (adults, 70 kg); and C = the metal concentration.

Target hazard quotient (non-carcinogenic health risk index)

The THQ for heavy metals resulting from the consumption of commercial fish was determined by calculating the ratio of daily metal intake to the oral reference dose for each heavy metal, following USEPA (2013).

$$THQ = \frac{EF \times ED \times MS \times C}{RfDo \times BW \times AT} \times 10^{-3}$$

Where EF = exposure frequency (365 days/year), ED = exposure duration (adults, 70 years); MS = adult meal size (227 g in EDI and 0.227 kg in THQ); C = metal concentration; RfDo = oral reference dose (mg/kg/day) specific for each heavy metal; AT = average exposure time (it is equal to EF x ED). THQ was estimated using oral reference doses of 0.014 mg/kg/day for Mn, 0.7 mg/kg/day for Fe, 0.3 mg/kg/day for Zn, 0.004 mg/kg/day for Pb and 0.001 mg/kg/day for Cd (EPA, 2010; USEPA, 2015).

Daily intake of heavy metals

The EDI of heavy metals, as calculated using the following equation as described by Rattan *et al.* (2005), was compared to the provisional permissible tolerable daily intake (PTDI) values set by the Joint

Statistical analysis

Heavy metal concentrations were analyzed using R statistical software version 4.2.1, and the data were presented as means ±standard deviation (Mean ±SD) of the triplicates. The normality of the metal concentrations data was assessed through the Shapiro-Wilk test and a Q-Q plot. Differences among the means of heavy metal concentrations were determined using the Kruskal-Wallis test. Post-hoc analysis, specifically the Dunn Test, was employed to identify significant differences among the species. The mean concentration of each heavy metal was compared to the maximum recommended permissible limits set by FAO/WHO. Additionally, Spearman correlation was utilized to examine the relationship between the metals in the fish. The study further estimated the potential human health risks associated with consuming these fish species.

Results

Descriptive analysis

The results show that *Merluccius productus* had the highest average weight (453.9 g) while *Gadus chalcogrammus* recorded the least weight (288.34 g) among the fish sampled. The mean total length of the sampled fish species ranged from 25 cm in *Clupea pallasii* to 54 cm in *Merluccius productus* as shown in Table 1. The summary statistics of lengths and weights are presented in Table 1.

Table 1. Minimum, maximum, Mean ±SD of the total length and weight of all fish spp. sampled.

Species	Local name	Total length (cm)			Weight (g)			ORIGIN
		Min	Max	Mean±SD	Min	Max	Mean±SD	
Scomber scombrus	Atlantic mackerel	27	36	31.2±2.87	260	350	303.9±31	Pacific, Peru
Clupea pallasii	Pacific herring	25	33.5	29.6±2.96	200	252	233.8±17.15	Russia
Gadus chalcogrammus	Alaska pollock	27	38.5	33.5±3.61	130	250	201.8±38.23	USA
Trachurus murphyi	Chilean jack mackerel	26	37	30.2±3.78	221	300	248.32±25.2	Chile
Merluccius productus	North Pacific hake	39	54	47.4±5.03	380	500	453.9±40.58	USA

Heavy metal concentrations

The statistical analysis of Pb, Cd, Zn, Fe, and Mn concentrations (mg/kg) in all sampled fish, including the mean±SD are summarized in Table 2. Lead concentrations were highest in *Trachurus murphyi* (10.38 mg/kg), with no significant difference in the levels found in *Merluccius productus* (8.37 mg/kg). However, Pb concentration was significantly lower ($P<0.05$) in *Clupea pallasii* (4.04 mg/kg) and *Scomber scombrus* (2.25 mg/kg), which had the lowest Pb concentration level. Lead levels found in all fish sampled are above the regulatory tolerable FAO/WHO limit of 0.4 mg/kg (FAO/WHO, 2011). Manganese concentration in all fish sampled ranged from 0.19 mg/kg in *Trachurus murphyi* to 1.08 mg/kg in *Clupea pallasii* and are all below the permissible limits. *Trachurus murphyi* and *Merluccius productus* had significantly lower ($P<0.05$). There are no significant differences in Mn levels detected between *Clupea pallasii* and *Scomber scombrus*. Iron concentration in all fish sampled was well above the tolerable limits with a minimum value found to be 4.59 mg/kg in *Scomber scrombus* and levels as high as 7.0 mg/kg detected in *Gadus chalcogrammus*, with its level differing statistically to all other species. In contrast to Fe levels, Zn concentration found in all fish species is well below the FAO/WHO threshold, with the maximum value recorded being 3.46 mg/kg in *Clupea pallasii*. Following the same trend with Fe, all Cd levels were statistically higher than the recommended limits in all fish spp. The lowest Cd concentrations were found in *Trachurus murphyi* (0.60 mg/kg) and maximum concentration of 2.18 mg/kg was found in *Merluccius productus*.

Table 2. Summary statistics of heavy metal concentrations (mg/kg) in the tissues of fish sampled.

Fish Species	Mn (Mean±SD)	Fe (Mean±SD)	Zn (Mean±SD)	Pb (Mean±SD)	Cd (Mean±SD)
Scomber scombrus	0.39 ± 0.12^{bd}	4.59 ± 0.72^{b}	2.44 ± 0.21^{b}	2.25 ± 0.82^{d}	1.62 ± 0.23^{bc}
Clupea pallasii	1.08 ± 0.15^{c}	5.56 ± 0.41^{ac}	3.46 ± 0.86^{b}	4.04 ± 0.84^{cd}	0.91 ± 0.21^{d}
Gadus chalcogrammus	0.53 ± 0.06^{cd}	7.0 ± 0.27	1.37 ± 0.10	6.92 ± 0.95^{ac}	1.66 ± 0.43^{ac}
Trachurus murphyi	0.19 ± 0.05^{a}	5.82 ± 0.79^{c}	1.86 ± 0.27^{a}	10.38 ± 0.91^{b}	0.60 ± 0.16^{d}
Merluccius productus	0.30 ± 0.06^{ab}	4.98 ± 0.16^{ab}	1.76 ± 0.19^{a}	8.37 ± 0.23^{ab}	2.18 ± 0.75^{ab}
FAO/WHO*	0.4	0.8	30	0.4	0.1

The results of the Spearman correlation between the heavy metals assessed in the fish species are summarized in Table 3 below. The EDI values for Cd ranged between (0.002 – 0.007 mg/kg), Fe ranged between (0.015 – 0.023 mg/kg), Mn ranged between (0.001 – 0.004 mg/kg), Pb ranged between (0.007 – 0.034 mg/kg) and Zn between (0.006 – 0.011 mg/kg) in all fish sampled (Table 4). Only Pb intake in *Trachurus murphyi* (0.034 mg/kg) and *Merluccius productus* (0.027 mg/kg) exceeded the FAO and WHO-recommended PTDI maximum regulatory limits. The THQ values for heavy metals calculated for each fish species are shown in Table 5. The THQ for Cd and Pb were greater than 1 in all fish species as it ranged from 1.946 – 7.069 mg/kg in Cd and 1.824 – 8.415 mg/kg in Pb respectively.

Table 3. Spearman correlation matrix of heavy metals in all five samples. Bold numbers indicate significance.

Spearman correlation	*Mn*	*Fe*	*Zn*	*Pb*	*Cd*
Mn	1				
Fe	0.084	1			
Zn	0.652	-0.319	1		
Pb	-0.500	0.287	-0.499	1	
Cd	-0.211	-0.111	-0.317	-0.117	1

Table 4. Estimated daily intake in mg/kg/day calculated for each metal by average human body weight.

Sample	Cd	Fe	Mn	Pb	Zn
Scomber scombrus	0.005	0.015	0.001	0.007	0.008
Clupea pallasii	0.003	0.018	0.004	0.013	0.011
Gadus chalcogrammus	0.005	0.023	0.002	0.022	0.004
Trachurus murphyi	0.002	0.019	0.001	0.034	0.006
Merluccius productus	0.007	0.016	0.001	0.027	0.006
PTDI	0.007	5.6	2	0.025	7

Table 5: Target Hazard Quotient calculated in mg/kg/day for each metal by average human body weight.

Sample	Cd	Fe	Mn	Pb	Zn
Scomber scombrus	5.253	0.021	0.090	1.824	0.026
Clupea pallasii	2.951	0.026	0.250	3.275	0.037
Gadus chalcogrammus	5.383	0.032	0.123	5.610	0.015
Trachurus murphyi	1.946	0.027	0.044	8.415	0.020
Merluccius productus	7.069	0.023	0.069	6.786	0.019

Discussion

This study investigated and compared the heavy metals toxicity in muscle tissue of the five most consumed marine fish species, obtainable in wholesale fish cold rooms in the western region of Nigeria. Unlike previous similar studies where research was conducted on inland captured freshwater fish such as rivers, lakes, and dams (Obasohan *et al.,* 2006; Kumolu-Johnson and Ndimele, 2011; Wangboje and Ikhuabe, 2015), consumption of imported fish has increased geometrically to more than 67% of fish consumption in Nigeria (Liverpool-Tasie *et al.,* 2021), thus, this study focuses on imported marine fish. A larger population of the country (76%) depends on these fish for their source of protein according to a National Average Annual Consumption per Capital report in 2015 and less research has been done to assess their toxicity levels and the risk posed to consumers.

Lead concentrations in all fish sampled in this study were above the maximum FAO and WHO permissible limit (0.84 mg/kg). This finding is comparable to reports of Pb levels higher than the regulatory limits in some imported marine fish such as *Umbrina canosai*, *Clupea harengus*, freshwater sardinella (*Sardinella tawilis*) and Atlantic salmon (*Salmo solar*), imported to Nigeria (Oluyemi, 2011;

Kareem *et al.,* 2016). Iron is an essential element in fish but may be toxic at high concentrations. Similar to Pb, Fe concentrations in all fish sampled in this study exceeded the maximum FAO and WHO permissible limit (0.8 mg/kg). This corroborates the findings of Oyelowo *et al.* (2020) who reported Fe level higher (1.73 mg/kg) than the permissible limit in some Nigeria imported marine fish which include *Gadus chalcogrammus*.

Cadmium concentrations detected in all sampled fish exceeded the recommended permissible limits of 0.1 mg/kg. The elevated levels of Cd in fish can be attributed to its strong coordination with metallothionein protein, as suggested by studies (Uzairu *et al.,* 2009; Boominathan *et al.,* 2014). Numerous investigations on fish have indicated that Cd absorption, primarily through the gills or gastrointestinal tract, results in its accumulation in muscle tissue, liver, and kidneys (Fazli *et al.,* 2009; Kalay and Canli, 2000). The heightened levels of these heavy metals in these species may stem from the bioaccumulation of toxic effluents resulting from anthropogenic activities in the environments where they are harvested (Afshan *et al.,* 2014).

Consumption of these fish species may expose consumers to Cd, Fe and Pb toxicity which could lead to several detrimental effects. For instance, Cd toxicity may alter glucose levels and antioxidant defense in humans (Celik and Oehlenschlager, 2004).

Mn levels were found in very low concentrations compared to the permissible limits in all fish sampled except in *Clupea pallasii* (1.08 mg/kg) and *Gadus chalcogrammus* (0.53 mg/kg). The precise reason for the high levels found in these two species is unclear. The high bioaccumulation might probably be species-specific whereby these species might be more efficient in absorbing and retaining Mn or maybe due to their similar habitat and diet preference. *Clupea pallasii* and *Gadus chalcogrammus* feed mostly on crustaceans such as copepods (Gray, 2019) and this may enhance the levels of Mn in their tissues. This result however contradicts Uroko *et al.* (2020) who also reported a low Mn level for *Clupea pallasii* and other marine spp. imported to the country.

Zinc levels were found in very low concentrations compared to the permissible limits (30 mg/kg) with maximum values of 3.46 mg/kg in *Clupea pallasii.* Oyelowo *et al.* (2020) where all fish samples also had Zn levels lower than the maximum limit. The low Zn levels might probably be due to its negative correlation with metals found in very high concentrations i.e., Fe (-0.319), Pb (-0.499) and Cd (-0.317).

Correlations between various heavy metals have previously been identified for several fish species (Carvalho *et al.,* 2005; Rahman *et al.,* 2012). The negative correlation found in these species may be due to the metal competition for binding sites in fish. However significant positive correlations found between Mn and Zn, and between Fe and Pb metals show the synergistic relationships that may occur between heavy metals in their host. This could be evidenced with generally high levels of both Fe and Pb and low levels of both Mn and Zn in this study.

The EDI values for Pb in both *Trachurus murphyi* (0.034 mg/kg) and *Merluccius productus* (0.027 mg/kg) were higher than the maximum permissible FAO limit of 0.025 mg/kg in this study. This means that daily consumers of these two marine species might be at a high risk of Pb toxicity since they will be ingesting more than the regulatory tolerable daily intake of this toxic chemical in their body. These two species must be consumed with caution and preferably less than the average meal size (i.e., 227 g) should be recommended to reduce the exposure to Pb toxicity.

The THQ values for Cd and Pb in all fish species were > 1 indicating potential health concerns, implying that prolonged consumption of these fish species may lead to health risks through continuous bioaccumulation over extended exposure periods. As recommended above, a reduction in the intake of this species to no more than once a week could potentially reduce the likely risk of adverse effects from these heavy metals.

Conclusions

This study aimed to assess the safety of the top five imported frozen fish varieties sold in Ijebu-ode metropolis, Nigeria. The evaluation assessed heavy metal toxicity (Cd, Pb, Zn, Fe, and Mn). The findings revealed negative allometric growth patterns and significantly low condition factors in *G. chalcogrammus* and *M. productus*, suggesting potential implications for fishery stocks. Heavy metal levels in the imported fish exceeded the limits recommended by FAO and WHO, posing a significant public health risk to consumers. It is imperative for the Federal Department of Fisheries in Nigeria to intensify toxicity assessments of imported fish for consumption. The concentrations of Pb, Fe, and Cd in all species analyzed exceeded the recommended maximum limits by FAO and WHO, while Mn levels in some species were also elevated. Only Zn concentration remained well below the maximum limit. Continuous consumption of these fish may pose risks to human health due to their bioaccumulative effects on the body. In conclusion, this research contributes valuable data for ongoing investigations into the import of frozen marine fish species in Nigeria.

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