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Heavy Metal Burden and Health Risk Assessment of Fresh, Frozen and Smoked Fish from a Local Market in Southwest Nigeria

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Abstract

Heavy metals load in fish environment and fish products had been an issue of public concern. The burden of some heavy metals in fresh tilapia (*Oreochromis niloticus*), frozen Herring (*Clupea harengus*), smoked herring, and associated risk to man was investigated. Atomic absorption spectrometry was used to determine metal concentrations. Standard risk measurement indices [daily metal intake (DMI), hazard quotient (HQ), and health risk index (HRI)] were used. Levels of Mn and Fe were significantly different ($p<0.05$) among the fish species, while the burden of other metals was low to not detected in the samples. The level of Cu (0.11±0.04), Zn (2.51±0.82), and Fe (8.72±4.41) were recorded in the bones of smoked herrings, while the highest levels of Mg were recorded in fresh tilapia. Gills recorded significantly ($p<0.05$) high levels of Mg (1.16±0.02), Fe (14.92±0.53), Cu (0.10±0.00), and Zn (1.35±0.04). The muscle of the frozen herring records the highest burden of these metals. Mn, Fe, Cu, and Cd revealed the highest HRI in all fish samples and age categories, especially for nine years old and below children. In conclusion, health fish indicators revealed that Mn, Fe, Cu, and Cd pose a risk to the populace and, with long time consumption, can do more damage to consumers, especially frozen herring.

***Keywords**:* Heavy metals, *Oreochromis niloticus*, *Clupea harengus*, Harzard quotient, Health risk index

Introduction

Pollution of water bodies is becoming an increasing course of concern due to industrialization and increased farming practices. This trend is directly or indirectly associated with a hike in urbanization and irresponsible agrochemical and industrial effluents discharged into aquatic systems [1]. Fish carry out all life processes in water, thus taking in metals by ingestion of suspended particulate materials in water, feeding, ionic exchange between fish and it's environment and adsorption on tissue and membrane surfaces [2].

Heavy metal burden in the fish environment directly relates to the burden in fish tissues [3]. According to Rajeswari and Sailaja [4], copper, manganese, cadmium, nickel, lead, and other stable metals with a density greater than 4.5 gcm-³ are considered heavy metals. Heidarieh *et al.* [5], in their study, mentioned the report by the World Health Organization (WHO) on the need to control heavy metal load in food sources to ensure the safety of the teeming populace. With the increase in the metal load of numerous aquatic biological systems comes increased worry about bioaccumulation of these toxic substances through the food chain and the potential risk to humans [6]. This worrisome situation posed by these substances necessitates regular monitoring of the burden of these metalloids in water bodies and river basins to guarantee the safety of human lives and the ecosystem in general. Also, public health education will serve to educate the masses on the importance of caution and the health implications of eating fish from polluted sources and promote good environmental management programs. Fish harvested are transported and sold in major markets as fresh, smoked, or frozen fish imported into the country.

In Nigeria, there are restricted reports on security appraisals of heavy metals in imported frozen fish. Some of these fish may have been harvested from heavily contaminated waters. Evidence exists that marine fish caught in the wild accumulates heavy metals in their tissues to the extent that it raises public health issues [7]. These wild caught fish are provided in frozen forms. The profound frozen technique can save it from decay by impairing some biochemical reactions; however, this does not affect the presence of heavy metals contaminants [8]. Likewise, there are numerous confirmations of bioaccumulation of heavy metal compounds to possibly lethal levels in fish and their products [9].

Heavy metal pollution is a genuine ecological concern not just on account of the direct lethal impact of metals on life forms yet on addition, the indirect impacts of the consumption of metal contaminated food [10]. Heavy metals, for example, Hg, Pb, Cd, and As are lethal to life forms due to their inference with cell forms [11]. The consequences have been connected to various pathological conditions, including neurological disorders, kidney damage, skin damage, circulatory framework problems, and an increased risk of cancer [12]. Heavy metals such as Zn, Fe, and Cu, which are essential for normal human cellular functions at a specific range of cellular concentrations, can also become toxic at elevated tissue concentrations or cause deficiency disorders at below normal tissue concentrations [13].

Though much work had been carried out on contamination of these metalloids in various water bodies and their corresponding health risk assessed, not much comparison had been carried out between different states of fish, that is, fresh, frozen, and smoked fish obtained from the study area. This study was therefore designed to compare the heavy metal concentrations in fresh, frozen, and smoked fish commonly consumed in the study area and to assess their corresponding health risk indices.

Materials and Methods

The study involved different forms of fish bought from a local fresh fish market in Abeokuta, Nigeria. 24 fish samples each for the various forms were used for the study. The fish samples were transported to the wet laboratory of the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Nigeria. The choice of fish species used for the study stemmed from the preference of the populace. Fish species purchased for the investigation were:

fresh Tilapia (*Oreochromis niloticus*), frozen and smoked herring (*Clupea harengus).*

The morphological character of the fish (total length and standard length) was measured using a measurement board to the nearest 0.1 cm. Weights of fish samples were taken using Camry electronic weighing scale (Model EK3250. 5kg) to the nearest 0.1 g. Fresh fish were cleaned with deionised water. Dried fish samples were crushed into fine powder by porcelain mortar and pestle and stored in amber colored bottles in vacuum desiccators. Frozen fish samples were thawed and then allowed to attain room temperature before dissection. Muscle, gills, and bone of frozen samples were removed following *Tru-cut* method [14]. The outer barrel was inserted to a depth of about 1 cm into the fish muscle tissue beneath the scale at an oblique angle (to minimize penetration depth). The needle was withdrawn and the sample (muscle) was placed as mentioned above. Fish gills, bones, and muscles were removed, and each specimen was kept separately in well tagged plastic bag and oven dried before digestion.

All oven-dried muscles, bones and gills specimens were crushed, sieved, ground, and homogenized. Wet acid digestion was adopted for this study [15]. The samples were digested with a mixture of nitric acid and hydrochloric acid. One g of each ground sample was weighed into a beaker; 17 mL of nitric acid and 8 mL of hydrochloric acid were added to each weighed sample. The mixture was then placed in a microwave oven and heated at 25^{o}C for 15 min till the solution became clear before diluting with 25 mL distilled water for muscles, bones, and gills, then filtered using Whatman filter paper (90 mm).

The digested fish samples were analyzed for heavy metals contamination using atomic absorption spectrophotometer (AAS) (Model 210 VGP Buck scientific) at FATLAB, Nigeria Company Ibadan. Two certified reference materials (CRM), fish muscle (ERM-BB422) and tuna fish tissue (BCR-627) (Institute for Reference Material and Measurement, IRMM), were used.

Risk assessment was evaluated to determine the threat posed by intake of contaminated fish by a man using hazard quotient (HQ), health risk index (HRI) according to methods [16], total toxicity of mixtures (TTM) index [17], and daily intake of metal (DIM) [18]. Category A was designated for adults (aged 20 years and above), category B designated for children (aged range from 10- 19 years), and the last category C designated for (age range 0 – 9 years) [19].

The hazard quotient was determined using the equation

$$HQ = \frac{Wfish \times Mfish}{RfD \times Bo}$$

Where,

- W_{fish} is the dry weight of edible fish consumed per day (gd^{-1}).
- The daily intake of fish for the nutritional requirement was 20.9 g intake rate for adults with an average body weight of 79.96 kg (aged 20 years and above), 10.1 g intake rate for children with an average body weight of 49.7 kg (aged range from 10- 19 years) and 6.2 g intake rate for individuals with a body weight of 17.3 kg (aged range 0 – 9 years).
- M_{fish} is the concentration of metal in the fish ($mgkg^{-1}$).
- R_fD is the metal reference dose ($mgkg^{-1}d^{-1}$); R_fD used; Fe (0.7), Mn (0.014), Zn (0.3), Cu (0.04), Ni (0.02), Cd (0.001).
- Bo is the average body weight (kg)

The DIM was calculated to determine the daily loading of metals into the human system with the consumption of fish

$$DIM = \frac{Cmetal \times Dfish}{Bo}$$

Where,

- C_{metal} is the concentration of heavy metals in the fish ($mgkg^{-1}$),
- D_{fish} is the daily nutritional intake of fish ($gday^{-1}$),
- B_o is the average body weight (Kg)

The following formula was used for the calculation of HRI.

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

HRI value of less than 1 implies safe exposure from such heavy metal and considered acceptable, otherwise the fish may pose heavy metals risk

TTM index for heavy metals was determined by applying the TTM index.

$$TTM = \sum\left(\frac{Ci}{GVi}\right)$$

Where:

Ci = Concentration of the 'ith' component of mixture

GVi = Guideline value for the 'ith' component. Trigger guideline values for livestock drinking water at low risk. Cd 0.01 mg/L, Cr 1 mg/L, Cu 0.4 – 5 mg/L, Fe not sufficiently toxic, Pb 0.1 mg/L, Mn not sufficiently toxic, Ni 1 mg/L, Zn 20 mg/L.

TTM >1= The mixture has exceeded the Guideline value.

All data were subjected to multivariable statistical programmes. One-way analysis of variance (ANOVA) was used to determine the differences in the heavy metal concentration of the three fish species. Duncan Multiple Range Test was used to separate differences between means. The statistical significance level was set at $p<0.05$ using SPSS version 17. Pearson correlation was used to test the relations between the metal concentrations in the fish organs using the Student t-test (2-tailed).

Results and Discussion

This section reported and discussed the research findings on the heavy metal load in the sampled fish. Findings were compared with previous studies on the subject matter.

Heavy Metals in Fish Samples

Table 1 shows the concentrations of selected metals in the sampled fish. Levels of Mn and Fe were significantly different ($p<0.05$) among the fish species. Fresh tilapia has the highest value of iron which was statistically the same as the value recorded for frozen herrings but varied significantly at 95% probability from that of smoked herrings. High level of copper was recorded in frozen herring compared to that recorded in smoked herring. No marked variation was recorded in Zn, Cr, Cd, Pb, and Ni levels among the fish samples.

The concentrations of Fe presented for fresh *Oreochromis niloticus*, smoked *Clupea harengus*, and frozen *Clupea harengus* in this investigation corroborated the findings of [20] in the liver of *Salmo salar* and that of [21] on *Silurus triostegus* and *Liza abu*. Fe exists naturally in water, like other metals, can also be introduced into the water from natural deposits and via the anthropogenic pathways such as effluent from industrial wastes, refining of iron ores, and corrosion of ironcontaining metals [22]. The load of Fe recorded in the gills of samples is an indicator of the metal concentrations present in the fish environment as this organ is the major organ used by fish to absorb water and ions from their environment [23]. The values of HRI of Fe were calculated to be > 1 in the three fish samples, and all age categories of the population in this study indicated that eating of Fe contaminated fish can cause danger to human health and coincides with a heavy burden of the metal in samples. However, this finding disagreed with that reported [18], which reported lower HRI values for frozen *Scomber scombrus* sold in Zaria metropolis. Also, the findings of this study did not corroborate that of [24], who recorded a higher estimated daily intake of Fe in *C. gariepinus* but HQ values <1. Reasons for this dissimilarity are likely due to location and also species differences.

***Table 1*. Heavy metals in the fish species.**

Metals (mg/kg)	Fresh Tilapia	Smoked Herring	Frozen Herring	Permissible limits
Mn	0.71±0.14[a]	0.33±0.12[b]	0.25±0.03[b]	0.0000001
Fe	11.29±1.11[a]	5.80±1.57[b]	8.36±1.78[ab]	0.00001
Cu	0.10±0.00	0.08±0.01	24.11±23.99	0.000003
Zn	1.10±0.12	1.17±0.42	0.91±0.12	0.00001
Cr	nd	nd	nd	-
Cd	nd	nd	nd	-
Pb	nd	nd	nd	-
Ni	nd	nd	nd	0.000007

Mean±SD with a different superscript in the same row are significantly different (P<0.05). nd- Not detected

Levels of Cu in the fresh *O. niloticus* and smoked *C. harengus* aligned with that of [25]. However, the metal concentration for frozen *C. harengus* was extremely high and implied that the environment from which the fish was taken was contaminated, further confirming the study [26]. This agrees with the mean Cu values in the muscles of the tilapia species recorded by [27] but was lower than that observed in *O. niloticus* by [28]. Reasons could be attributed to the different geographical locations and water sources from which species were collected. Also, mean Cu values of 1.33 ± 0.06 mgkg^{-1} have been observed in sampled *Tilapia zilli* from the freshwater ecosystem in Southeastern Nigeria [29]. HRI values < 1 in fresh *O. niloticus* and smoked *C. harengus* corroborated the report of Osakwe *et al.* [24] posed no threat to human health.

Metal Load in Organs of Sampled Fish

Table 2 shows the burden of these substances on the gills, bones, and muscles of sampled fish. Significant variations at 95% probability were observed in Mn and Zn load among samples, but no significant variation was observed for Fe, Cu, Cr, Cd, Pb, and Ni.

***Table 2*. Metal load in gills, bones and muscles.**

Metals (mg/kg)	Bone	Gill	Tissue
Mn	0.61±0.09[a]	0.49±0.17[ab]	0.19±0.03[b]
Fe	8.16±1.29	7.87±1.97	9.42±1.75
Cu	0.10±0.01	0.08±0.01	24.12±23.99
Zn	1.64±0.32[a]	0.89±0.15[b]	0.64±0.10[b]
Cr	nd	nd	nd
Cd	nd	nd	nd
Pb	nd	nd	nd
Ni	nd	nd	Nd

Mean±SD with a different superscript in the same row are significantly different (P<0.05). nd- Not detected

The concentrations of these substances in the bones, gills, and muscles of the sampled species are presented in Table 3. No statistical variation (P>0.05) was observed in the levels of Fe, Cu, Zn, Cr, Cd, Pb, and Ni in the sampled fish bones. Fresh tilapia has the highest significant (p<0.05) concentration of Mn, while frozen herring has the least value.

Statistical variations ($p<0.05$) were observed in Mn, Fe, and Zn concentrations in the gills and muscles of sampled fish. There was no record of Cr, Cd, Pb, and Ni found in the sampled fish muscles.

The concentrations of Zn reported for all samples corroborated the levels recorded for Tilapia species obtained from Masinga reservoir and lower than 28.00 – 76.33 mg kg^{-1} obtained in Athi River system [30]. Anim *et. al.* [31] observed lower mean Zn levels in *C. gariepinus* muscles but comparable levels in *Tilapia zilli* sampled from Densu River, Ghana. Mean Zn levels recorded [32] in Tilapia (*Oreochromis niloticus*) muscle tissues (51.20 ±3.90 mg kg^{-1}) obtained from aquaculture ponds in Kolkata wetlands, India was higher than levels observed in this study. The value of HRI of Zn in all age categories is > 1, and this implies consumption of fish species with similar levels of this metal poses a threat to the health of the population.

The Mn concentrations as reported here in the sampled fish were within the WHO permissible limit for fish and fish products at 2.50 mg/kg [25]. This report is however at variance with the previous study [33]. Differences could be attributable to the water source and pollution state of the various water bodies. However, the concentrations compared well with Mn levels in muscles of *C. gariepinus* recorded by [34] respectively. The feeding habits of this species from the same water body mentioned above could be responsible for the lower levels of the metal. The HRI value of Mn was > 1 in all age category, and this implies that the levels of Mn in the sampled fish is above the risk limit for humans and thus, poses a threat to human health.

***Table 3*. Heavy metals in the organs of sampled fish.**

Organs	Species	Metals (mg/kg)							
		Mn	*Fe*	*Cu*	*Zn*	*Cr*	*Cd*	*Pb*	*Ni*
Bones	FT	0.77±0.06a	7.81±0.52a	0.09±0.01a	1.29±0.09a	nd	nd	nd	nd
	SH	0.74±0.19a	8.72±4.41a	0.11±0.04a	2.51±0.82a	nd	nd	nd	nd
	FH	0.31±0.01b	7.94±0.19a	0.09±0.00a	1.11±0.03a	nd	nd	nd	nd
Gills	FT	1.16±0.02a	14.92±0.53a	0.10±0.00a	1.35±0.04 a	nd	nd	nd	nd
	SH	0.15±0.02b	5.67±0.46b	0.05±0.01a	0.70±0.09b	nd	nd	nd	nd
	FH	0.16±0.08b	3.03±2.62b	0.09±0.04a	0.63±0.31b	nd	nd	nd	nd
Muscle	FT	0.19±0.04 b	11.14±1.25a	0.12±0.01a	0.64±0.04b	nd	nd	nd	nd
	SH	0.10±0.00c	3.00±1.27b	0.09±0.00a	0.29±0.01c	nd	nd	nd	nd
	FH	0.28±0.02a	14.12±0.58a	72.15±71.93a	0.98±0.05a	nd	nd	nd	nd

Mean±SD with a different superscript in the same row are significantly different ($p<0.05$). nd- Not detected

***Table 4.* Pearson correlation matrix between heavy metals concentrations in bones, gills and muscles of sampled fishes.**

		Fresh Tilapia				Frozen Herring				Smoked Herring			
		Mn	***Fe***	***Cu***	***Zn***	***Mn***	***Fe***	***Cu***	***Zn***	***Mn***	***Fe***	***Cu***	***Zn***
Bone	Mn	1				1				1			
	Fe	-0.543	1			0.169	1			-0.293	1		
	Cu	0.416	.-0.323	1		cnc	Cnc	cnc		0.673*	0.092	1	
	Zn	0.043	-0.459	0.438	1	0.326	0.487	cnc	1	0.342	-0.529	-0.275	1
Gills	Mn	1				1				1			
	Fe	0.411	1			-0.082	1			-0.426	1		
	Cu	Cnc	cnc	cnc		0.521	0.302	1		0.287	0.124	1	
	Zn	-0.179	-0.841**	cnc	1	-0.692*	0.395	0.319	1	-0.027	-0.202	-0.063	1
Muscle	Mn	1				1				Cnc			
	Fe	-0.273	1			0.342	1			Cnc	1		
	Cu	-0.089	-0.354	1		0.106	-0.026	1		Cnc	Cnc	cnc	
	Zn	0.736*	-0.211	-0.133	1	-0.540	0.351	-0.519	1	Cnc	0.059	cnc	1

Cnc – Cannot be computerized. * - Correlation is significant at the 0.05 level. ** - Correlation is significant at the 0.01 level

With regard to the high correlation in the levels of Zn and Mn, especially in the muscle of fresh tilapia, it's an indication that when the species is obtained from a water body high in these metals and consumed, these metals can easily be transferred to the consumer.

Pearson Correlation Matrix between heavy metals of sampled fish

The correlation analysis between the heavy metals in the different organs of samples showed a positive correlation ($r = 0.736$, $p = 0.05$) between Zn and Mn in the muscle of fresh tilapia. Similar positive correlation ($r = 673$, $p = 0.05$) was observed between Cu and Mn in the bones of smoked herring Table 4.

Risk assessment index of metals

The risk indices for these toxic substances (mgkg^{-1}) in fresh *O. niloticus*, frozen and smoked *C. harengus* measured using the HQ, DIM and HRI of different age groups are summarized in Table 5. The Mn concentration observed to be highest in fresh tilapia of 0.71 mgkg^{-1} gave rise to DIM of 0.185, 0.144, and 0.254 in categories A, B, and C, respectively, and HRI greater than 10 in all age categories with the highest risk potential observed in children of 9 years and below (Table 5). Frozen *C. harengus* with the highest Cu concentration of 24.11 mgkg^{-1} gave rise to DIM of 6.30, 4.90, and 8.64 and HRI of 157.5, 122.4, and 160 for categories A, B, and C, respectively (Table 5). Smoked *C. harengus* with concentrations of Cu as low as 0.01 mgkg^{-1} gave rise to DIM of 0.0026, 0.0020, and 0.0035 in categories A, B, and C, respectively, and HRI greater than 2 in all age categories with highest risk potential observed in children of 9 years and below (Table 5). TTM for individual responses to heavy metal accumulation in fish samples (Table 6).

The detection of Cd only in smoked *C. harengus* cannot be scientifically explained as fuel wood for smoking has not been documented as a source of Cd. The study of Cieślik *et al.* [35] documented a significant decrease in common smoked carp (*Cyprinus carpio*) but an insignificant increase in northern pike (*Exos lucius*) and rainbow trout (*Oncorhynchus mykiss*). However, the

Absence of the metal from *O. niloticus* and frozen *C. harengus* did not agree with previous research on heavy metal accumulation in the fresh tilapia and frozen herring [36, 37]. The Cd accumulation with a different species has been found with higher concentrations than this study revealed [38]. This indicated that Cd accumulations in fish tissues could largely be associated with the nature of the fish environment, the types of the fish species, feeding habits, and differences in tissue accumulation. The value of HRI of Cd is > 1 in all age category, and this implies that Cd in the examined fish poses a threat to human health or that the level recorded in this study is not safe for human consumption.

Toxicity mixture of heavy metals that is relatively high in the fresh *C. harengus* is extremely dangerous to the health of consumers. This depicts the state of the water body from which the fish was caught. Though, not much has been done on TTM of frozen seafood, few works by [39-41] revealed > 1 TTM for the different fish species they examined.

***Table 5*. HQ, DIM and HI for individual responses to heavy metal accumulation in fish samples.**

Species	Metals	Mean±SD (mgkg-1)	DIM (Age categories)			HQ (Age categories)			HRI (Age categories)		
			A	*B*	*C*	*A*	*B*	*C*	*A*	*B*	*C*
Fresh *O. niloticus*	Mn	0.71±0.14	0.19	0.14	0.25	13.26	10.31	18.18	13.25	10.29	18.14
	Fe	11.29±1.11	2.95	2.29	4.04	4.22	3.28	5.78	4.21	3.28	5.78
	Cu	0.10±0.00	0.03	0.02	0.04	0.65	0.51	0.90	0.65	0.51	0.88
	Zn	1.10±0.12	0.29	0.22	0.39	0.96	0.75	1.31	0.96	0.73	1.31
Frozen *C. harengus*	Mn	0.25±0.03	0.065	0.51	0.09	4.67	3.63	6.40	4.64	3.64	6.40
	Fe	8.36±1.78	2.19	1.69	2.99	3.12	2.43	4.28	3.12	2.43	4.28
	Cu	24.11±23.99	6.30	4.90	8.64	157.5	122.5	216.0	157.5	122.5	216.0
	Zn	0.91±0.12	0.23	0.18	0.326	0.792	0.62	1.09	0.77	0.61	1.09
Smoked *C. harengus*	Mn	0.33±0.12	0.086	0.067	0.118	6.2	4.8	8.45	6.16	4.79	8.43
	Fe	5.80±1.57	1.52	1.17	2.08	2.17	1.68	2.97	2.14	1.67	2.97
	Cu	0.08±0.01	0.02	0.02	0.03	0.52	0.41	0.72	0.52	0.41	0.75
	Zn	1.17±0.42	0.31	0.24	0.42	1.02	0.79	1.40	1.03	0.79	1.40
	Cd	0.01±0.1	0.003	0.002	0.004	2.60	2.03	3.58	2.61	2.01	3.58
	Ni	0.01±0.01	0.003	0.002	0.004	0.13	0.10	0.18	0.13	0.10	0.18

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

***Table 6*. TTM for individual responses to heavy metal accumulation in fish samples.**

Species	Metals	Mean±SD (mgkg-1)	Guideline value (mg/L)	Ci/GVi	TTM
Fresh *O. niloticus*	Cu	0.10±0.00	5.0	0.02	0.08
	Zn	1.10±0.12	20	0.06	
Frozen *C. harengus*	Cu	24.11±23.99	5.0	4.84	4.89
	Zn	0.91±0.12	20	0.05	
Smoked *C. harengus*	Cu	0.08±0.01	5.0	0.02	1.09
	Zn	1.17±0.42	20	0.06	
	Cd	0.01±0.1	0.01	1.0	
	Ni	0.01±0.01	1	0.01	

Guideline values used here are as documented for livestock [17]

Conclusion

The study successfully measured and ascertained the levels of Mn, Fe, Cu, Zn, Cr, Cd, Pb, and Ni in fresh tilapia species, smoked, and frozen herring purchased from a named market in southwest Nigeria. Conclusively, the Mn, Fe, Cu, and Zn levels in the sampled fish are higher than the permissible levels. All fish sampled place the consumers at risk as levels of Mn, Fe, and Cd were higher than the permissible limit, which could become chronic by long time consumption and can cause damage to the consumer, especially to frozen herring. TTM of heavy metals in frozen and smoked herring was higher than the guideline value documented for livestock. Considering the result of the risk assessment indicators (DIM, HQ and HRI) and for the health of the populace, *C. harengus* and other seafood coming into the country should be properly screened before they are allowed into the country.

Conflict of Interest

The authors declare no conflict of interest.

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