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Determination of Heavy Metal Levels in Some Commonly Consumed Frozen Fish in Ibadan, Southwest, Nigeria

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ABSTRACT

There is a growing concern on the safety of frozen fish imported from various parts of the world where the levels of contamination of water bodies may not be well ascertained and regulatory measures not strictly adhered to. Concentrations of four heavy metals (Lead (Pb), Cadmium (Cd), Copper (Cu) and Mercury (Hg)) in the muscle, gill and liver were investigated for three most consumed frozen fish species (*Umbrina canosai*, *Clupea harengus* and *Scomber scombrus*) within Ibadan, metropolis. Samples were digested as described by FAO/SIDA. The concentrations of metals were quantified using a Varian AA240 Fast Sequential Flame Atomic Absorption Spectrophotometer (AAS). Heavy metals like Cd, Cu and Hg were significantly higher ($p < 0.05$) in the liver of the three species than in the muscle and gills. There was no significant difference in the Pb concentration for the three organs in *S. scombrus*, whereas, Pb was significantly higher in the liver of *U. canosai* (9.73 mg kg^{-1}) and *C. harengus* (4.40 mg kg^{-1}) than in the muscle (6.92 and 2.11 mg kg^{-1} , respectively) and gill (8.73 and 2.52 mg kg^{-1} , respectively). Copper concentration was marginally higher in *S. scombrus* (5.06 mg kg^{-1}) with the least value recorded in *U. canosai* (3.28 mg kg^{-1}). However, no significant variation ($p > 0.05$) was observed in the muscle Cd, Cu and Hg levels for the three species. Comparatively, *S. scombrus* has the highest concentration of heavy metals. This study revealed that the heavy metals investigated in the major organs and flesh was all above the limits recommended by World Health Organization.

Key words: Lead, cadmium, *Scomber scombrus*, *Umbrina canosai*, *Clupea harengus*

INTRODUCTION

Heavy metals are natural trace components of the aquatic environment, whose level have been reported to be on the increase in recent times due to pollution from industrial wastes, changes in geochemical structure, agricultural and mining activities (Singh *et al.*, 2007; Sprocati *et al.*, 2006). Heavy metals unlike organic contaminants are not degraded with time, but concentration can only increase through bio-accumulation (Aksoy, 2008). Fish are often at the top of aquatic food chain and studies have shown that they assimilate these heavy metals through ingestion of suspended particulates, food materials and/or by constant ion exchange process of dissolved metals across lipophilic membranes like the gills or adsorption of dissolve metals on tissues and membrane surfaces (Melville and Burchett, 2002). On adsorption, the pollutant is carried in the blood stream to either a storage point (bone) or to the liver for transformation or storage (Obasohan, 2008). With fish constituting an important link in the food chain, its contamination by toxic metals causes a

direct threat, not only to the entire aquatic environment, but also to humans that utilize it as food. From a human health perspective, mercury, arsenic, cadmium and lead have been identified as primary contaminants of concern (Burger *et al.*, 2007). These metals have no bio-importance in human biochemistry and physiology, but its consumption, even at very low concentration can be toxic (Nolan, 1983; Young, 2005). Even for metals with bio-importance such as zinc, nickel and chromium (Abduljaleel and Shuhaimi-Othman, 2011), dietary intake have to be maintained at the regulatory limits, as excesses will result in poisoning or toxicity (Young, 2005). This is because they combine with body biomolecules such as metal-binding protein and enzymes to form stable biotic compounds, thereby mutilating their structures and hindering them from performing the designated functions within the body system (Duruibe *et al.*, 2007).

Though the maximum permitted levels of metals in seafood have been introduced in many parts of the world for the safe consumption of fish species (Adams and McMichael, 1999) studies and monitoring programs examining heavy metal levels in fish are becoming more and more important, especially in developing parts of the world, where fish provide the major source of protein (Burger *et al.*, 1999). In Nigeria for instance, fish is appreciated as one of the healthiest and cheapest source of protein (Ukoha *et al.*, 2014) contributing about 55% of the protein intake, with per capita consumption of 7.52 kg per annum and a total consumption of 1.2 million Mt with import making up about 2/3 of the total consumption (Eletta *et al.*, 2003). Three prominent frozen fish in Nigerian markets include white croaker (*Umbrina canosai*), mackerel (*Scomber scombrus*) and herring (*Clupea harengus*, Linnaeus 1788) and constitute a large percentage of imported fish consumed.

White croaker (*Umbrina canosai*) is a species of croaker occurring in the Eastern Pacific. It has been taken from Magdalena Bay, Baja California to Vancouver Island, British Columbia, but are not abundant north of San Francisco (Walsh *et al.*, 1995). Meanwhile, the Atlantic mackerel (*Scomber scombrus*) is a coastal species found only in the north Atlantic (Uriarte *et al.*, 2001). However, herring (*Clupea harengus*) is found in shallow, temperate waters of the North Pacific and the North Atlantic oceans, including the Baltic Sea, as well as off the west coast of South America (FAO., 2012).

Heavy metal occurrence have been reported in Mackerel and Sardine available in some Nigerian markets (Beetseh and Abraham, 2013; Ukoha *et al.*, 2014) with values for cadmium ranging from 0.016-0.105 mg L⁻¹ and lead values ranging from 0.013-0.105 mg L⁻¹. Though these values fall within the recommended tolerable limits, there is need to expand the species examined and update the status at various intervals and locations. This study investigates the concentration of some heavy metals in the gills, flesh and liver of some commercially consumed frozen fish in Ibadan metropolis (Largest city in West Africa) to ascertain their safety for human consumption.

MATERIALS AND METHODS

Study site: The study site, Ibadan is located on longitude 350' East of the Greenwich meridian and latitude 723' North of the equator with a population of 1,991,367 (Areola, 1994).

Reagents/apparatus: All reagents used were of analytical grade. Deionized water was used for solutions preparation and dilutions. All glass wares were soaked in 10% HNO₃ for 2 h and later rinsed with distilled de-ionized water prior to use for metal analysis. Ultrapure standard solutions of 1000 mg L⁻¹ of the metals (Merck, Darmstadt, Germany) were used for calibration. Nitric acid (65%) and hydrogen peroxide (30%) of ultrapure quality for digestion were from same source.

Sample collection and preparation: Two major fish storage facilities were selected within the metropolis using purposive sampling and three most prominent fish species in the market namely, white croaker (*Umbrina canosai*), mackerel (*Scomber scombrus*) and Herring (*Clupea harengus* Linnaeus, 1788) were collected bi-monthly for 12 months (Each species from each facility were in triplicate groups per sampling period). Samples were placed in prewashed polyethylene bags and taken to the laboratory in icebox.

In the laboratory, the fish were thawed at room temperature, weight and the total length measured, before dissecting for analysis. Fish samples were dissected to separate the muscle, gills and liver as recommended by UNEP/FAO/IOC/IAEA (1984). The separated organs were neatly placed in labeled acid washed petri dishes and dried to constant weight at 80°C for 2 days (Abubakar *et al.*, 2015). Teflon mortar was used for pulverization and homogenization of tissues before digestion.

Digestion of samples: The labeled samples were digested (in triplicate) according to methods described by FAO/SIDA (1983). The 0.5 g of each sample was weighed into a dry digestion tube. Five milliliter of 2:1 Nitric per chloric acid (H_2O_2/HNO_3) was added. The mixtures were swirled gently and allowed to digest on a hot plate in a fume chamber for 2 h at 80°C until the brown fumes disappears (Igwegmar *et al.*, 2013). The digests were allowed to cool and filtered into 25 mL volumetric flasks with Whatman No. 1 filter paper and made up to mark with de-ionized water. Sample blanks were carried out throughout the digestion processes. The metallic content of the digested samples and the blanks were quantified using a Varian AA240 Fast Sequential Flame Atomic Absorption Spectrophotometer (AAS) and vapour generation accessory (Varian VGA 77) with closed end cell for mercury. The quality of analytical procedure was confirmed by analyzing standard reference materials of mussel. Concentration of Pb, Cd, Cu and Hg were expressed as mg kg⁻¹.

Statistical analysis: Data resulting was subjected to analysis of variance (ANOVA) to determine the differences in the heavy metal concentration of the three fish species and organs. Duncan Multiple Range Test was used to separate differences between means. Statistical significance level was set at p<0.05 using SPSS version 17.

RESULTS AND DISCUSSION

The mean total length and weight of sampled fish are shown in Table 1. *Umbrina canosai* had the highest average weight of 848.75±5.50 g while *S. scombrus* and *C. harengus* had 420±6.20 and 339.17±5.25 g, respectively. Mean total length ranged from 34.08 in *C. harengus* to 41.17 cm in *U. canosai*. Lead, mercury, copper and cadmium were detected in all the fish samples collected.

Lead was significantly lower (p<0.05) in the muscle of *C. harengus* with a value of 2.11 mg kg⁻¹, followed by 6.92 and 9.45 mg kg⁻¹ in *U. canosai* and *S. scombrus*, respectively. In gills, Pb concentration were significantly higher (p<0.05) in *U. canosai* (8.73 mg kg⁻¹) and *S. scombrus* (10.38 mg kg⁻¹) whereas, concentration in the liver ranged from 4.4 mg kg⁻¹ in *C. harengus* to

Table 1: Mean total length and Mean wet weight of experimental samples

Fish species	Mean total length (cm) (±SD)	Mean wet-weight (g)
<i>Umbrina canosai</i>	41.17±5.24	848.75±218.95
<i>Scomber scombrus</i>	36.88±1.92	420.00±65.64
<i>Clupea harengus</i>	34.08±2.37	339.17±52.34

Table 2: Mean concentration of heavy metals in fish

Species	Organ	Pb	Cd	Cu	Hg
<i>Umbrina canosai</i>	Muscle	6.92±0.59 ^a	0.60±0.15 ^a	3.28±0.80 ^a	0.46±0.00 ^a
	Gill	8.73±0.32 ^{ab}	0.59±0.21 ^a	2.89±0.61 ^a	0.07±0.01 ^a
	Liver	9.73±0.74 ^b	1.62±0.32 ^b	9.20±0.64 ^b	0.85±0.10 ^b
<i>Clupea harengus</i>	Muscle	2.11±0.28 ^a	0.62±0.19 ^a	3.63±0.80 ^a	0.02±0.01 ^a
	Gill	2.52±0.28 ^a	0.78±0.17 ^a	3.93±0.60 ^a	0.06±0.01 ^a
	Liver	4.40±0.48 ^b	1.66±0.34 ^b	7.62±0.24 ^b	0.92±0.07 ^b
<i>Scomber scombrus</i>	Muscle	9.45±1.38 ^a	0.61±0.27 ^a	5.06±0.36 ^a	0.05±0.01 ^a
	Gill	10.38±0.91 ^a	0.91±0.12 ^a	5.77±0.98 ^a	0.09±0.00 ^a
	Liver	13.18±2.03 ^a	2.18±0.71 ^b	9.74±0.37 ^b	1.31±0.32 ^b
Maximum acceptable limits (FAO/WHO)		0.40	0.10	0.40	0.5-1.0

Values are Means±SEM, Values in each column with the same superscript are not significantly different at $p < 0.05$

13.18 mg kg⁻¹ in *S. scombrus*. Lead is described as a classical chronic or cumulative poison, which may result in neurological, hematological, behavioral, renal, cardiovascular and reproductive effects at levels above the tolerable limit (FAO/WHO., 2011). Lead concentrations were highest in all organs of *S. scombrus* (Table 2) compared to other species and this may be attributed to the feeding habit of this specie as well as the level of habitat contamination. Romeo *et al.* (1999) stated that the concentrations of metals in gills reflect their concentration in water where the fish lives, whereas the concentration in the liver represents storage of metals in water. High concentration of Pb in the liver is in agreement with various authors working on other species (Storelli *et al.*, 2012; Bashir *et al.*, 2013). This is because the liver is the primary organ responsible for the detoxification, transportation and storage of toxic substances (Uzairu *et al.*, 2009).

Cadmium concentration in fish muscles ranged from 0.60 in *S. scombrus* to 0.63 mg kg⁻¹ in *U. canosai* as shown in Table 2. Cadmium concentrations were highest in fish liver, with the highest value recorded in *S. scombrus* (2.18 mg kg⁻¹) and least in *U. canosai* (1.62 mg kg⁻¹). The results obtained in this study were lower than values reported by Abubakar *et al.* (2015) for *S. scombrus*. Compared with the other organs, the muscle had the lowest levels of cadmium. This is because of the inactive nature of this tissue which is responsible for its inability to accumulate heavy metals to a large extent (Eneji *et al.*, 2011).

Copper is an essential element which enhances enzymatic activity of the body (Igwemmar *et al.*, 2013). The concentration of copper in the gills of species were statistically similar ($p < 0.05$), ranging from 2.89 mg kg⁻¹ in *U. canosai* through 3.93 in *C. harengus* to 5.77 mg kg⁻¹ in *S. scombrus*. Values followed the same trend for muscles. However, copper concentration was significantly higher in the liver of *U. canosai* and *S. scombrus* than *C. harengus*. The values recorded in this present study are higher than that reported for *S. scombrus* and *U. canosai* (Igwemmar *et al.*, 2013), but they fall far below the recommended safety limit of 30 mg kg⁻¹.

Mercury is a natural occurring metallic element which can be present in foodstuffs by natural causes. Fish ingest contaminated mercuric food which passes through the gastrointestinal tract and gets distributed, accumulated or detoxified by the liver (Mieiro *et al.*, 2011). The concentration of mercury in muscles ranged from 0.02 in *C. harengus* to 0.05 mg kg⁻¹ in *S. scombrus*. This metal is absorbed by the gills and carried by the blood stream to the liver where it is detoxified and excreted as bile before it gets to the flesh, leading to the less toxicity of heavy metals in the flesh (Romeo *et al.*, 1999). Mercury level in the liver range from 0.85 in *U. canosai* to 1.31 mg kg⁻¹ in *S. scombrus*.

Except in the muscle of *U. canosai* where Hg concentration was higher, *S. scombrus* recorded the highest concentration of all heavy metals in the muscles, gills and livers examined in this study. The pattern of metal content in *S. scombrus* and *U. canosai* is Pb>Cu>Cd>Hg, while it is Cu>Pb>Cd>Hg for *C. harengus*.

CONCLUSION

This study showed that the levels of heavy metals in gill, liver and muscle of the three species (*S. scombrus*, *U. canosai* and *C. harengus*) are higher than the acceptable limit recommended by FAO and WHO except mercury level in *S. scombrus* liver. This accumulation of heavy metals especially in fish muscle is of great health concern to human. It is therefore, become pertinent for government to monitor frozen fish importation more effectively and create public awareness on the implication of heavy metal accumulation in food on human health.

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