



## **Elemental Study of Common Iced Fish Species Sold in Akure, Ondo State, Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Author VBS designed the study, managed the analyses of the study, wrote the protocol and wrote the first draft of the manuscript. Author AU managed the literature searches. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

There is a dearth of information on the mineral composition of essential, toxic elements of commonly consumed frozen fishes in Nigeria, hence, this study was conducted to determine the proximate, elemental composition and also estimate the daily intake of the minerals obtained in the frozen samples of Hake (*Merluccius merluccius*), Sardine (*Sardinella eba*), Chub Mackerel (*Scomber japonicus*), Atlantic horse Mackerel (*Trachurus trachurus*) and, Croaker (*Pseudolithus elongatus*) obtained from four markets in Akure, Ondo State, Nigeria. The fish species examined contained appreciable concentrations of protein which ranged from 15.19% in Chub mackerel to 21.75 % in Atlantic horse mackerel. The ash and moisture content suggest that the fish species are a good source of minerals and a veritable medium for microbial proliferation respectively, while, the crude fat value ranging between 0.16 % in Atlantic horse mackerel to 0.27 % in Hake showed that they are lean fat fishes. The Estimated Dietary Intake (EDI) of the macro and microelements analysed in the fish species (except for phosphorus) fell short of the Dietary Reference Intake (DRI) that were established by the Institute of Medicine. However, the concentrations of toxic elements such as lead, arsenic and cadmium exceeded the maximum limits set for these elements in foods, and this consequently poses a long term risk as a result of the bioaccumulation and biomagnifications of these toxic elements in the body.

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## 1. INTRODUCTION

Nigeria has a large number of public frozen seafood processing plants and retail markets are distributed around the country, where a considerable number of people buy their frozen seafood products daily. Fish and seafood constitute an important food component for a large section of world population [1]. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein [1]. Today, even more people are turning to fish as a healthy alternative to real meat [2]. The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids food in fatty pelagic fish species are extremely important aspect for health conscious people particularly in affluent countries where cardiovascular disease mortality is high [2]. Food and Agriculture Organization (FAO) [3] asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing world derives more than 30% of their animal protein from fish. Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids [4]. The major feature of the proximate composition of fish and shell fish is the great variability in lipid content [5]. Lean species contain typically 0.3-1.0% lipid, most of which is phospholipid and the remainder triglyceride, whereas the total amount in fatty species can be as much as 30% [5]. In fatty species, the amount of lipid varies seasonally within species and may fall to as low as 1%; these changes are accounted for entirely by changes in the proportion of triglycerides [5]. As the amount of lipid increases, the amount of water falls in almost linear proportion, while the amount of protein remains fairly constant. Food contains a wide range of elements such as sodium, potassium, iron, calcium, boron, magnesium, selenium, copper and zinc. These elements are essential in trace quantities for the maintenance of cellular processes. The majority of metals are natural components of the earth's crust. Metals and other elements can be naturally present in food or can enter food as a result of human activities such as industrial and agricultural processes [6]. Thus the World Health Organization (WHO) as well as the FAO of the United Nations state that monitoring eight elements in fish Mercury (Hg), Cadmium (Cd),

Lead (Pb), Arsenic (As), Copper (Cu), Zinc (Zn), Iron (Fe), Selenium (Sn) is obligatory while the monitoring of others though not obligatory may be useful [7]. Metals, particularly heavy metals such as lead, mercury, cadmium and arsenic constitute a significant potential threat to human health, both occupational and environmental [8]. Consequently, the Dietary Reference Intake (DRI), a system of nutrition recommendations from the Institute of Medicine of the National Academies (United States) was developed. It is a system grouped into Recommended Dietary Allowance (RDA) and Adequate Intakes (AI) [9]. An AI is the dietary intake used mostly for healthy breastfed infants but is also used for other health individuals [9]. A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population [9]. Unless otherwise specified, the UL represents total intake from food, water, and supplements [9]. The provisional tolerable weekly intake (PTWI), recommended by the Joint FAO/WHO Expert Committee on Food Additives [10], show appropriate safe exposure levels and is used to estimate the amount of contaminants, ingested over a lifetime without appreciable risk, correlatively, the target hazard quotient (THQ) which is the ratio between measured concentration and oral reference dose, weighted by the length and frequency of exposure, amount ingested and body weight has been utilized to determine the safety of ingested food substances [11]. The level of concern arising from ingestion of metals from the consumption of food is assessed by estimating the THQ [12]. The concern about the high levels of trace and heavy metals in foods has prompted several statutory bodies such as the WHO to establish maximum allowable concentrations for some of the metals in food [13].

## 2. MATERIALS AND METHODS

### 2.1 Study Area

This study was carried out in Akure, Ondo state of Nigeria. The market used were, Erekesan N7°15'10.1988" E5°11' 46. 5648", Nepa N7°14' 13.7952" E5°11'46. 5648", Isinkan N7°15'2.9916" E5°10'59. 3724" and, Futa N7°17'34.8036" E5°95. 0112". Ondo State is geographically located within the rainforest zone of Nigeria and has marked wet and dry seasons [14].

## 2.2 Sample Collection and Treatment

Five different frozen fish species such as -Hake (*Merluccius merluccius*), Sardine (*Sardinella eba*), Chub Mackerel (*Scomber japonicus*), Atlantic horse Mackerel (*Trachurus trachurus*), and -Croaker (*Pseudolithus elongatus*) were each obtained from fish mongers from four markets in Akure, Ondo State. The markets are Erekesan, Isinkan, Nepa and FUTA. The choice of sampling locations was informed by their population densities and high commercial activities while the choice of fish species premised on the commonly consumed by the populace. The samples were collected in Ziploc bags and transported in ice packs to the laboratory for analyses. All refrigerated samples were allowed to thaw for 2 hours before being treated for analysis. Analyses carried out were proximate analysis and, mineral analysis. Proximate analysis involved the estimation of crude protein, crude fat, crude fibre, ash content and moisture content. Elements determined were Sodium, Potassium, Calcium, Phosphorus, Zinc, Cadmium, Lead, Arsenic, Copper, Zinc, Iron, Selenium and, Manganese, while the dietary intake levels and target hazard quotient of these elements in the fish samples were also estimated. The muscle tissues of the fish species were used for the determination of the mineral composition. They were cut out from the fish species using a non-metallic cutter to avoid steel mill contamination. The muscle tissues of each of the fish species were then kept under refrigeration conditions in Ziploc bags until needed.

## 2.3 Proximate Analysis of the Various Fish Species

The muscle tissues of the fish species were analysed for proximate analysis. Muscle tissues (2g) were cut out from the fishes on a clean chopping board after they have been left to thaw for 2 hours. They were then kept under refrigeration conditions in Ziploc bags until required for the analysis. Proximate composition (crude protein, crude fat, crude fibre, ash content and moisture content) of the fish samples were determined based on the experimental protocols described by Association of Official's Analytical Chemists (AOAC) [15,16,17,18].

## 2.4 Elemental Analysis

The samples were firstly digested [19] (APHA and WCPF, 1998), all digested sample of the fish

species were thus analysed for heavy metals (As, Pb and, Cd) and other minerals, using Atomic Absorption Spectrophotometers (AAS), Model 210 VGP respectively [20]. Flame photometer was used to determine Ca, Na, and K, while, Vanado-molybdate Colorimetric method was used for the determination of phosphorus [21].

## 2.5 Determination of Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ) of Essential and Toxic elements through Fish Consumption

The EDI of essential and toxic elements (Pb, As, Cd, Mn, Fe, Se, Cu, Zn, Na, Ca, P and, K) were calculated according to the method employed in notable researches [22,23] as follows:

$$EDI = C_m \times M_g$$

Where, EDI= the estimated daily intake (mg/day/individual or  $\mu\text{g/day/individual}$ ),  $C_m$ = the mean concentration of metals in the fish species ( $\mu\text{g/g}$  or  $\text{mg/kg}$ ) and,  $M_g$  = average mass of fish consumed daily by an individual. The per capita fish consumption in Nigeria is 7.6kg which is equivalent to 20.8 g/day (0.0208kg/day) based on the report of Food and Science Nutrition of Nigeria [12]. The EDI of the essential elements were compared to the dietary reference intakes (DRIs) obtained from the Food and Nutrition Board of the Institute of Medicine [9]. The EDI of the lead (Pb) was compared with the European Food Safety Authority [24] benchmark dose lower confidence limit (BMDL) while, EDI of cadmium (Cd) arsenic (As), were compared to the provisional tolerable daily intake (PTDI) value obtained from provisional tolerable weekly intake (PTWI) values based on European Food Safety Authority [25] and WHO guidelines [26] respectively. Furthermore, to ascertain the level of concern arising from ingesting six potentially toxic metals (Zn, Cu, Mn, Fe, Pb and, Cd) [12] from the consumption of these fish species, the target hazard quotient (THQ) values were calculated by using the formula established by the United States Environmental Protection Agency [27] and it's as follows:

$$THQ = \frac{EDI}{RFD} \times 10^{-3}$$

Where,

EDI= Estimated daily intake of the elements in  $\mu\text{g/kg}$  or  $\text{mg/kg}$ ; RFD= Oral reference dose ( $\text{mg/kg}$ ) used were, Zn (0.3), Cu (0.04), Fe (0.7), Mn (0.14), Pb (1.5) and, Cd (0.001) [27].

## 2.6 Data Analysis

Data were subjected to analysis of variance (ANOVA) and treatment means were separated using Student-newman-keul's (S-N-K) test. The ANOVA was performed with SPSS 21.0 software (SPSS, Inc. 2013).

## 3. RESULTS

The proximate composition of the fish species is displayed in Table 1. It shows the mean proximate composition (crude protein, crude fat, crude fibre, ash content and moisture content) as it varies with the fish samples with no significant difference ( $P>0.05$ ), it also shows that crude fibre was not determinable in the fish samples. Atlantic horse mackerel had the highest crude protein content ( $\approx 21.8 \pm 0.884\%$ ) and the lowest content of crude protein was in Chub mackerel ( $\approx 15.2 \pm 0.710\%$ ). For the crude fat, Hake had the highest content with  $\approx 0.27 \pm 0.015\%$  and Atlantic horse mackerel had the least crude fat content with  $\approx 0.16 \pm 0.017\%$ . In case of the ash content, Atlantic horse mackerel had the highest ash content with  $1.270 \pm 0.623\%$  and chub mackerel had the least ash content with  $1.076 \pm 0.526\%$ . While the ash content of 1.111%, 1.186% and 1.220% was obtained for sardine, hake and croaker respectively. The variations in the mean concentrations of micro, macro and toxic elements are presented in Figs. 1-3. There was no significant difference between the concentrations of each elements in the fishes (at  $P>0.05$ ). Fig. 1 shows the fish species have higher mean concentration of potassium than the other macroelements and also sodium concentration is lowest in all the fish samples. Sardine had the highest mean concentration of potassium ( $6.00 \pm 0.12\%$ ) while Hake had the least potassium concentration ( $5.55 \pm 0.07\%$ ) (Fig. 1). Also, Sardine is richer in sodium ( $2.54 \pm 0.11\%$ ) and Croaker the lowest ( $2.37 \pm 0.03\%$ ). For calcium, Atlantic horse

mackerel had the highest mean concentration ( $2.80 \pm 0.04\%$ ) while Croaker had the lowest mean concentration ( $2.50 \pm 0.72\%$ ). So also, Atlantic horse mackerel had the highest mean concentration of phosphorus ( $4.53 \pm 0.35\%$ ) while sardine had the lowest mean concentration ( $3.14 \pm 0.33\%$ ). Atlantic horse mackerel had the highest mean concentration of zinc ( $6.83 \text{ mg/kg}$ ) while Chub mackerel had the least zinc concentration ( $5.45 \text{ mg/kg}$ ) (Fig. 2). The trend was also observed in manganese concentration of the fish species as atlantic horse mackerel also had the highest mean concentration ( $3.80 \text{ mg/kg}$ ) while, Chub mackerel had the least ( $1.24 \text{ mg/kg}$ ). In the case of copper, selenium and iron, Atlantic horse mackerel ( $1.03 \text{ mg/kg}$ ), Hake ( $0.19 \text{ mg/kg}$ ) and Croaker ( $5.15 \text{ mg/kg}$ ) had the highest level respectively. Sardine had the least concentration ( $0.59 \text{ mg/kg}$ ) for copper while Chub mackerel had the least concentrations of  $0.08 \text{ mg/kg}$  and  $4.05 \text{ mg/kg}$  for selenium and iron respectively, although, Atlantic horse mackerel and hake had the same mean concentrations for iron ( $4.89 \text{ mg/kg}$ ). Fig. 3 shows that lead concentration was highest compared to the other toxic elements while cadmium was the lowest in all the fish samples. The lead concentration was highest in Sardine ( $0.40 \text{ mg/kg}$ ) while Chub mackerel had the least concentration in lead ( $0.19 \text{ mg/kg}$ ). It also showed that arsenic concentration was highest in Hake ( $0.08 \text{ mg/kg}$ ) and least in Sardine ( $0.06 \text{ mg/kg}$ ). Concentrations of cadmium were highest in Chub mackerel ( $0.03 \text{ mg/kg}$ ) and least in Hake ( $0.01 \text{ mg/kg}$ ). The Estimated Daily Intake (EDI) of the macroelements, microelements and toxic metals in the commonly consumed frozen fish samples in Akure, Ondo State of Nigeria and their Provisional Tolerable Daily Intake (PTDI), Dietary Reference Intake (DRI)/Tolerable Upper Intake Levels (UL) are presented in Tables 2-4. The target hazard quotient (THQ) of six potentially toxic elements was presented in Table 5.

**Table 1. Proximate composition (%) of frozen fish species sold in Akure**

Fish	Crude protein	Crude fat	Crude fibre	Ash content	Moisture content
CM	$15.19 \pm 0.710^{abc}$	$0.25 \pm 0.146^{ab}$	ND	$1.08 \pm 0.526^{abc}$	$69.21 \pm 0.299^{abcd}$
Sardine	$17.95 \pm 1.107^{abcd}$	$0.21 \pm 0.021^{abc}$	ND	$1.11 \pm 0.045^{ab}$	$69.55 \pm 0.620^{abcd}$
Hake	$18.32 \pm 1.449^{abc}$	$0.27 \pm 0.015^{ab}$	ND	$1.19 \pm 0.036^{abc}$	$70.02 \pm 0.124^{abc}$
Croaker	$20.80 \pm 0.841^{abcd}$	$0.18 \pm 0.160^{ab}$	ND	$1.22 \pm 0.267^a$	$69.86 \pm 0.066^{abc}$
AM	$21.75 \pm 0.884^{abc}$	$0.16 \pm 0.017^{ab}$	ND	$1.27 \pm 0.623^{ab}$	$70.08 \pm 0.077^{abc}$

Each value is the mean  $\pm$  standard deviation of the replicates. Means within the same column followed by the same letter (in superscript) are not significantly different but are significantly different from others at  $P>0.05$  using Student-Newman-Keul's test; ND – Not determinable, CM= Chub mackerel, AM= Atlantic horse mackerel

**Table 2. Estimated daily intake (EDI) (mg/kg) of macroelements for 20.8 g (0.0208 kg) daily fish consumption compared to the dietary reference intake (DRI) for macroelements**

Fish species	Na	Ca	K	P
CM	517.71	1157.73	520.00	670.80
Sardine	527.49	1248.42	551.41	652.70
Hake	501.07	1155.02	566.80	795.60
Croaker	493.58	1198.08	519.17	834.70
AM	518.13	1158.14	581.98	941.20
Age group	AI	AI	RDA	RDA
1-3	1000 <sup>a</sup>	3000 <sup>a</sup>	700 <sup>a</sup>	460 <sup>a</sup>
4-8	1200 <sup>a</sup>	3800 <sup>a</sup>	1000 <sup>a</sup>	500 <sup>a</sup>
9-13	1500 <sup>bcde</sup>	4500 <sup>bc</sup>	1300 <sup>bc</sup>	1250 <sup>bc</sup>
14-18	1500 <sup>bcde</sup>	4700 <sup>bcd</sup> /5100 <sup>e</sup>	1300 <sup>bcde</sup>	1250 <sup>bcde</sup>
19-30	1500 <sup>bcde</sup>	4700 <sup>bcd</sup> /5100 <sup>e</sup>	1000 <sup>bcde</sup>	700 <sup>bcde</sup>
31-50	1500 <sup>bcde</sup>	4700 <sup>bcd</sup> /5100 <sup>e</sup>	1000 <sup>bcde</sup>	700 <sup>bcde</sup>
51-70	1300 <sup>bc</sup>	4700 <sup>bc</sup>	1000 <sup>b</sup> /1200 <sup>c</sup>	700 <sup>bc</sup>
>70	1200 <sup>bc</sup>	4700 <sup>bc</sup>	1200 <sup>bc</sup>	700 <sup>bc</sup>

The mean concentrations in % were converted to mg/kg based on the FAO/INFOODS guidelines for converting units, denominators and expressions, version 1.0. [28].

Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) are dietary recommendations established by Elements Food and Nutrition Board, Institute of Medicine, National Academies [29, 9]. Healthy individuals; a = males, b = females, c = pregnancy and, d = lactation established with different Recommended Dietary Allowances and Adequate Intakes by Food and Nutrition Board, Institute of Medicine, National Academies [29].

CM= Chub mackerel, AM= Atlantic horse mackerel.

**Table 3. Estimated Daily Intake (EDI) (mg/kg) of microelements for 20.8 g (0.0208 kg) daily fish consumption compared to the dietary reference intake (DRI) for microelements**

Fish	Zn	Cu	Se	Mn	Fe
CM	0.11	0.014	0.002	0.026	0.084
Sardine	0.12	0.012	0.002	0.038	0.102
Hake	0.14	0.016	0.004	0.051	0.102
Croaker	0.12	0.022	0.003	0.061	0.107
AM	0.14	0.021	0.003	0.079	0.102
Age group	RDA	RDA	RDA	AI	AI
1-3	3 <sup>a</sup>	0.34 <sup>a</sup>	0.02 <sup>a</sup>	1.2 <sup>a</sup>	7 <sup>a</sup>
4-8	5 <sup>a</sup>	0.44 <sup>a</sup>	0.03 <sup>a</sup>	1.5 <sup>a</sup>	10 <sup>a</sup>
9-13	8 <sup>bc</sup>	0.7 <sup>bc</sup>	0.04 <sup>bc</sup>	1.9 <sup>b</sup> /1.6 <sup>c</sup>	8 <sup>bc</sup>
14-18	11 <sup>b</sup> /9 <sup>c</sup> /12 <sup>d</sup> /13 <sup>e</sup>	0.89 <sup>bc</sup> /1 <sup>d</sup> /1.3 <sup>e</sup>	0.055 <sup>bc</sup> /0.06 <sup>d</sup> /0.07 <sup>e</sup>	2.2 <sup>b</sup> /1.6 <sup>c</sup> /2 <sup>d</sup> /2.6 <sup>e</sup>	11 <sup>b</sup> /15 <sup>c</sup> /27 <sup>d</sup> /10 <sup>e</sup>
19-30	11 <sup>bd</sup> /8 <sup>c</sup> /12 <sup>e</sup>	0.9 <sup>bc</sup> /1 <sup>d</sup> /1.3 <sup>e</sup>	0.055 <sup>bc</sup> /0.06 <sup>d</sup> /0.07 <sup>e</sup>	2.3 <sup>b</sup> /1.8 <sup>c</sup> /2 <sup>d</sup> /2.6 <sup>e</sup>	8 <sup>b</sup> /18 <sup>c</sup> /27 <sup>d</sup> /9 <sup>e</sup>
31-50	11 <sup>bd</sup> /8 <sup>c</sup> /12 <sup>e</sup>	0.9 <sup>bc</sup> /1 <sup>d</sup> /1.3 <sup>e</sup>	0.055 <sup>bc</sup> /0.06 <sup>d</sup> /0.07 <sup>e</sup>	2.3 <sup>b</sup> /1.8 <sup>c</sup> /2 <sup>d</sup> /2.6 <sup>e</sup>	8 <sup>b</sup> /18 <sup>c</sup> /27 <sup>d</sup> /9 <sup>e</sup>
51-70	11 <sup>b</sup> /8 <sup>c</sup>	0.9 <sup>bc</sup>	0.055 <sup>bc</sup>	2.3 <sup>b</sup> /1.8 <sup>c</sup>	8 <sup>bc</sup>
>70	11 <sup>b</sup> /8 <sup>c</sup>	0.9 <sup>bc</sup>	0.055 <sup>bc</sup>	2.3 <sup>b</sup> /1.8 <sup>c</sup>	8 <sup>bc</sup>

Recommended Dietary Allowances (RDAs) and Adequate Intakes (AIs) are dietary recommendations established for Elements by Food and Nutrition Board, Institute of Medicine, National Academies [30,31].

Healthy individuals; a = children, b = males, c = females, d = pregnancy and, e = lactation established with different Recommended Dietary Allowances and Adequate Intakes by Food and Nutrition Board, Institute of Medicine, National Academies [29].

CM= Chub mackerel.

AM= Atlantic horse mackerel

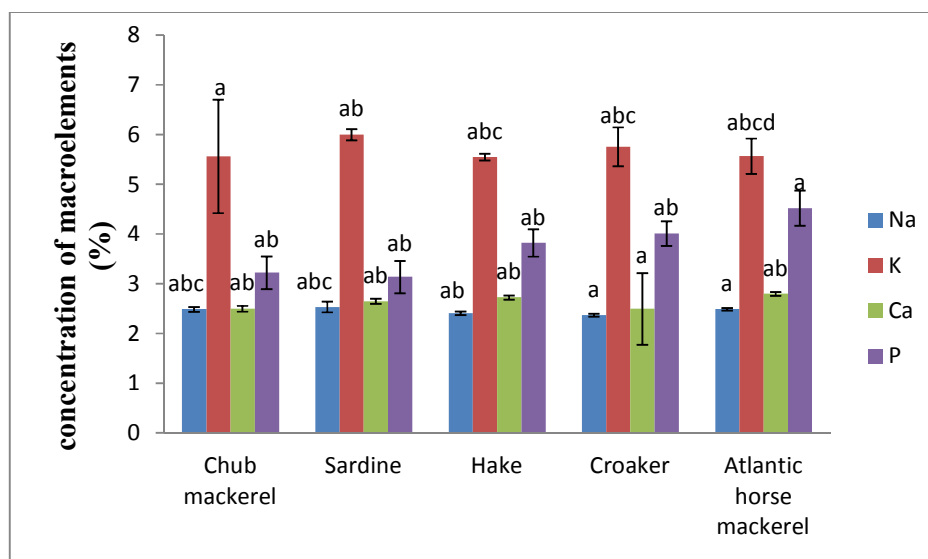
**Table 4. Estimated Daily Intake (EDI) ( $\mu\text{g}/\text{kg}$ ) of toxic elements for 20.8 g (0.0208 kg) daily fish consumption compared to the dietary reference intake (DRI) for toxic elements**

Fish	As	Pb	Cd
Chub mackerel	1	4	0.6
Sardine	1	8	0.4
Hake	2	8	0.3
Croaker	1	5	0.4
Atlantic horse mackerel	2	7	0.4
PTDI/BDML	150 <sup>a</sup>	35 <sup>b</sup>	70 <sup>a</sup>

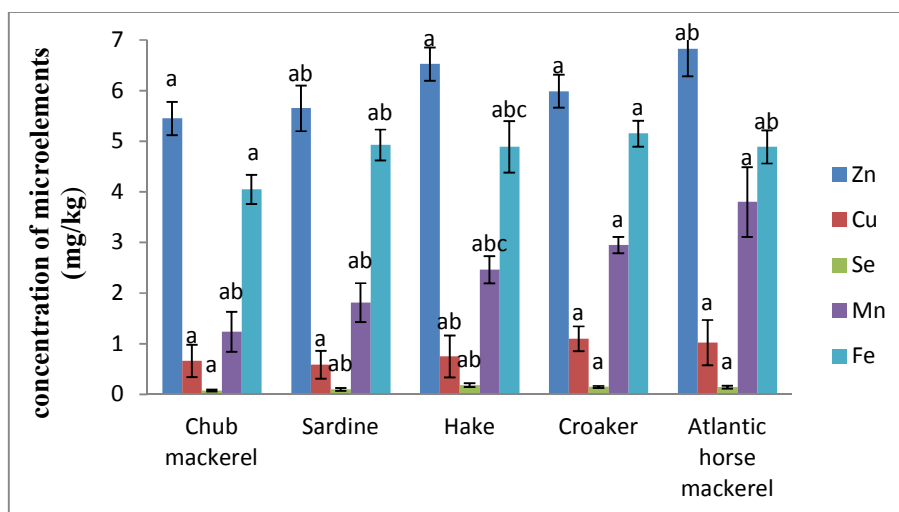
PTDI= Provisional Tolerable Intake

Notes: a= PTDI, Provisional Tolerable Daily Intake for toxic elements based on WHO guidelines [26].

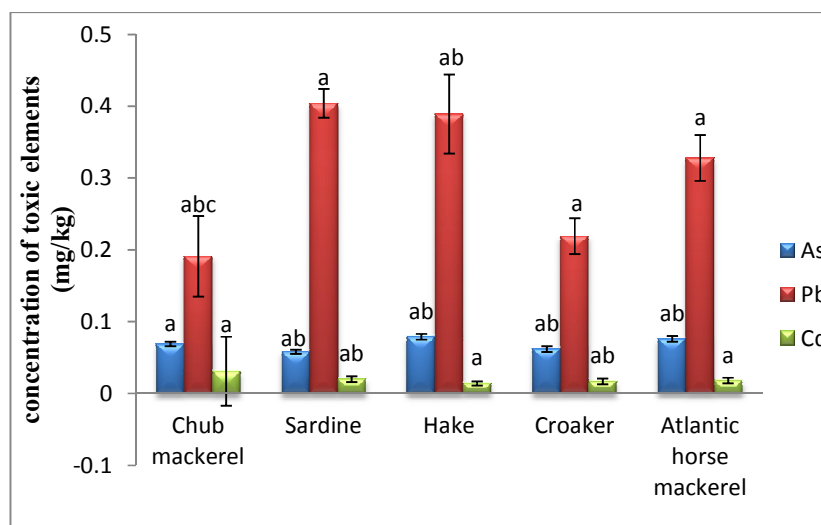
b= Corresponding benchmark dose lower confidence limit (BMDL) for lead were obtained by multiplying the BMDL value 0.5  $\mu\text{g}/\text{kg}/\text{day}$  [24]



**Fig. 1. Mean concentration of macroelements in frozen fish species sold in Akure**  
Na-Sodium; K- Potassium; Ca- Calcium; P- Phosphorus



**Fig. 2. Mean concentrations of Microelements in frozen fish species sold in Akure**  
Zn- Zinc; Cu- Copper; Se- Selenium; Mn- Manganese; Fe- Iron



**Fig. 3. Mean concentration of toxic elements in frozen fish species sold in Akure**  
As- Arsenic; Pb- Lead; Cd- Cadmium

**Table 5. Target hazard quotient for consumption of 20.8 g per day (0.0208 kg/day) of the fish species**

Fishes	Zn	Cu	Mn	Fe	Pb	Cd
CM	0.0004	0.0004	0.0002	0.0001	0.000003	0.6
Sardine	0.0004	0.0003	0.0003	0.0001	0.000005	0.4
Hake	0.0005	0.0004	0.0004	0.0001	0.000005	0.3
Croaker	0.0004	0.0006	0.0004	0.0002	0.000003	0.4
AM	0.0004	0.0005	0.0004	0.0001	0.000005	0.4

Oral reference dose (mg/kg) used were, Zn (0.3), Cu (0.04), Fe (0.7), Mn (0.14), Pb (1.5) and, Cd (0.001) [40].  
THQ is either >1 or <1, where THQ > 1 indicates a reason for health concern [12].

CM= Chub mackerel.

AM= Atlantic horse mackerel.

#### 4. DISCUSSION

The high crude protein detected (15.19% in chub mackerel to 21.75% in Atlantic horse mackerel) may be attributed to the fact that fishes are good source of animal protein [32], but the qualitative differences for each fish species could be as a result of fish consumption or absorption capability and conversion potentials of essential nutrients from their diets or their local environment into such biochemical attributes needed by the organisms body [33]. The observed range of ash content in the fishes indicates that the species is a good source of minerals such as calcium, potassium, zinc and, iron [11]. Ash is a measure of the mineral content of food item [11]. Generally, fish can be grouped into four categories according to their fat content: lean fish (< 2%), low fat (2 to 4%), medium fat (4 to 8%) and high fat (> 8%) [34]. These fishes had a low lipid content; hence, their classification as

lean fishes. This indicates that the fishes are good for consumption especially for persons with high fat content in the body. No crude fibre was detected. However, it is noteworthy that, high moisture content detected in the fish species generally provides good media for the growth and proliferation of microorganisms [35]. The consumption of these fishes especially sardine and Atlantic Horse mackerel can be encouraged as the EDIs of these fishes constitute between 35.2 to 52.8%, 26.6 to 41.6% and 44.8 to 83.1%, of the DRIs established for the daily intake of sodium, potassium and calcium respectively in foods. This postulation is conceived from the health benefits these elements provide for the human body as sodium is an important macroelement that regulates blood volume, blood pressure, osmotic equilibrium and pH, although when the threshold is exceeded could lead to hypertension [36], calcium is an essential component of the bones and cartilage

(phosphorus alike) playing an important role in blood clotting, by stimulating the release of thromboplastin from the blood platelets [23]. It will suffice to note that although these fishes might have come short of the Dietary Reference Intakes (DRI) of these macroelements (Ca, Na, and K) established by the institute of medicine, judging by the amount in percentage the EDIs make of the DRIs, the regular consumption of these fishes can be advised as the remaining percentage can be obtained from other food items (vegetables and fruits) rich in these elements. The EDI values of phosphorus in some fishes were however observed to be higher than the RDAs set by the Food and Nutrition Board of the Institute of Medicine, National Academies [9] for different age groups of those classified as healthy individuals by the Food and Nutrition Board of the Institute of Medicine, National Academies [9]. For instance, an adult between the ages 19 to 70 years is recommended to ingest 700 mg/day of phosphorus, while for children (1-8 years) it is 460-500 mg/day. However, persons between the ages of 9 to 18 years (especially females between the ages of 14-18 years who are of child bearing age [9] and could either be pregnant or lactating) are expected to take 1250mg/day of phosphorus. Hence, the consumption of Hake, Atlantic horse mackerel and Croaker should be minimal amongst persons between the ages of 1-8years and 19-70 years due to their EDI values of phosphorus (795.60 mg/day, 941.20mg/day and 834.70mg/day respectively) compared to the RDA value set for these age groups to avoid impairing calcium homeostasis especially in older women [37]. Consequently, a person between the ages 1-8 years should take little or none of the fish to avoid the risk of been exposed to hazards that occur as a result of excessive intake of phosphorus. But, individuals between the ages of 9-18 can consume all the fish species analysed as they are good source of phosphorus for their age groups. The Zinc levels ranged from 5.460±0.325 mg/kg in Chub mackerel to 6.525±0.328 mg/kg in Hake which was different from what was reported by Ogundiran et al. [6], that a lower concentration of zinc at a range of 0.023mg/kg in Croaker to 0.032 mg/kg in Atlantic horse mackerel obtained in Ibadan and Lagos. The mean values obtained for zinc shows that the fish species are polluted with respect to zinc when compared with WHO standard of 1 mg/kg [38] and FEPA standards of 0.075 mg/kg [39]. The calculated EDIs however, adequately fell short of the Tolerable Upper Intake Level of zinc

established by the Food and Nutrition board, Institute of medicine, National academies [31] of 40mg/d, which makes the fishes safe for consumption. Furthermore, previous studies observed that, zinc an essential biological mineral which is regulated and maintained at certain concentration in fish is due to physiological requirements for survival or homeostatic regulation [40]. Zinc levels were generally higher in the frozen fish species than the corresponding copper, manganese, selenium and iron (Fig. 2) as it was also reported by Ogundiran et al. [6]. However, it must be noted that copper becomes harmful when taken in large quantity and its harmful toxicity is largely attributed to cupric ( $\text{Cu}^{2+}$ ) form which is commonly found in fish [41], resulting in various cellular abnormalities like oxidative stress, Huntington's disease and osmo/ionregulatory impairments [42,43,44]. Deficiency of copper although, in humans can result in albinism and anaemia [45]. The high value (5.150mg/kg in croaker to 4.050mg/kg in chub mackerel) recorded for iron in the fish muscles may be due to its availability in the water and feeds they consume [11]. This trend agrees with other studies where elevated amount of iron was found in fish tissues [46,47,48]. It was reported that iron concentrations in the range of 3.10–4.76 mg/kg in fish samples from Nworie Rivers, Nigeria [49]. However, people suffering from iron overload disorder called hemochromatosis can be advised to consume regularly these fish species as they are relatively low in iron. Arsenic, lead and cadmium are the toxic heavy element analysed in frozen fishes sold in Akure. In the present study, these toxic metals were observed in the tissues of the various fish in this study albeit at different rates. Such pattern has been observed in a number of other studies, covering several fish species [47,50,51,52]. Muscle has been considered to have metal accumulating potential [46]. The EDIs of arsenic (4-8  $\mu\text{g}/\text{d}$ ) in these fish species are below the PTDI value (150 $\mu\text{g}/\text{day}$ ) set by WHO (2003) which makes them safe for consumption, the level of concentrations (0.058 mg/kg in sardine to 0.079 mg/kg in hake) are higher than the maximum level of arsenic (0.0005 mg/kg) set for food by JECFA [53]. The fish species can be seen as a source of long-term arsenic-induced risks which are carcinogenic due to the cumulative nature of arsenic. The major biochemical effects of arsenic are complexation with coenzymes, uncoupling of phosphorylation and coagulation of protein [54]. The contamination of these fish species with



arsenic may not be far-fetched as sea water which homes these species contains 2 to 5ppb of arsenic and its concentration may even be higher in public water supplies which might have been used to wash these species after harvesting and before storage [54]. The major source of lead in these fish species could be as a result of industrial effluent into their habitat by industries that use lead in the manufacturing their products. The mean level of lead obtained (0.404 mg/kg - 0.191 mg/kg in sardine and chub mackerel respectively) differs from the range of the mean level of concentration of the same fish species in Ibadan and Lagos (0.002 mg/kg in hake to 0.012 mg/kg in chub mackerel) reported by Ogundiran et al. [11]. In other studies [54,56,57,38,39] on metal concentration in fish, values reported were lower to that obtained in the present study. The EDIs obtained for lead in the fish species (which ranges from 4µg/day in chub mackerel to 8 µg/day in both sardine and hake) is low when compared with the corresponding Benchmark Dose lower confidence Limit (BMDL) for lead (35 µg/day). It could be said that the fish species are wholesome for the consumers. However, based on the maximum level of 0.0002 mg/kg that lead must have in food, then the fish species might be a source of lead poisoning to the consumers of these fishes overtime since its concentration in these fishes exceed that established by EFSA [24]. The EDI value deduced for cadmium in the fish species used in this study ranged from 0.3 µg/day in Hake to 0.6 µg/day in Chub mackerel, while it was 0.4 µ/day in Sardine, Croaker and Atlantic horse mackerel. These values are lower than the PTDI established for cadmium in foods (70 µg/day) by EFSA [25], hence the consumers of these fish species are not at risk of consuming this fish samples. However, comparing the level of concentration in the fish species (0.031 mg/kg in chub mackerel, 0.020 mg/kg in sardine, 0.018 mg/kg in atlantic horse mackerel, 0.017 mg/kg in croaker, 0.014 mg/kg in hake) to the maximum level of cadmium that is expected to be in foods (0.0004 mg/kg; EFSA [24]), the consumers of these fish species sold in Akure are at a long term risk of cadmium poisoning. Cadmium poisoning can cause aminoaciduria (urinary excretion of aminoacids), hypocalciurea (urinary excretion of excessive Ca) and formation of kidney stones [54]. A disease specifically associated with cadmium poisoning was reported in Japan called *itai itai* (or ouch-ouch disease) which arose from the bioaccumulation of cadmium through the ingestion of cadmium while consuming rice [54]. An increased Cd levels in

these fishes is worrisome, especially considering the fact that it could be very hazardous to the fish itself, as cadmium has been reported by Alibabic et al. [58] to alter their genetic material. It is also one of the most toxic heavy metal, even at relatively low concentrations [47,48]. The estimated THQ values as presented in Table 5 of the six heavy metals (Zn, Cu, Mn, Fe, Pb and Cd) in the fish species reported in this study indicated no level of concern as they were less than 1. Target hazard quotient (THQ) value is not a measure of risk but indicates a level of concern.

## 5. CONCLUSION

This study has shown that, Hake (*Merluccius merluccius*), Sardine (*Sardinella eba*), Chub Mackerel (*Scomber jopanicus*), Atlantic horse Mackerel (*Trachurus trachurus*), Croaker (*Pseudolithus elongatus*) which are the commonly consumed iced fish species in Akure, Ondo state of Nigeria are rich source of protein. Atlantic horse mackerel in this study was seen to be the richest source of the essential nutrients analysed making up about 44.4% (i.e. 4 out of 9 essential nutrients) which was the highest compared to the other fish species. Generally, all the fish species had high concentration of lead (Pb), arsenic (As) and, cadmium (Cd). Although, Sardine, Hake and, Chub mackerel were fish types with the highest concentrations of Pb, As and, Cd respectively. Upon estimating the EDIs of these toxic elements and comparing them with the PTDIs established as the standard, the study revealed that the consumption of the fish species do not pose a threat to the lives of the consumers but due to the high level of these elements in the fish they could be at a long-term risk due to the accumulative properties of these elements. Furthermore, according to the guideline for interpretation of THQ calculated for some of the heavy elements (zinc, copper, manganese, iron, lead and cadmium) analysed, there is no health concern arising from the consumption of these fish species. Consequently, from the investigation of the proximate composition, there was high moisture content which serves as good condition for the proliferation of microorganisms [35] and hence the deterioration of these fish species.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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