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SELECTED ELEMENTAL PROFILE OF IMPORTED *SARDINELLA AURITA*, *CLUPEA HARENGUS* AND *SCOMBER SCOMBRUS* SOLD IN ZARIA, NIGERIA

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ABSTRACT

Metals can be bio-accumulated and bio-magnified via the food chain and finally assimilated by human consumers resulting in health risk. Three species of imported marine frozen fishes namely; *Sardinella aurita*, *Clupea harengus* and *Scomber scombrus* were investigated for metallic elements using AA240FS Fast Sequential Atomic Absorption Spectrometer. Nine elements belonging to the groups of light metals (calcium and magnesium), biological essential heavy metals (copper, chromium, manganese, iron and nickel) and non-essential heavy metals (lead and cadmium) were evaluated. Calcium was the highest (290.75 ppm) concentrated light metal, Fe was the highest (2.587 ppm) concentrated biological essential heavy metal, while Pb and Cd were the highest (0.053 ppm) concentrated non-essential heavy metals. *Sardinella aurita* had significantly ($P \leq 0.05$) highest concentration of Cu (0.05) and Fe (2.59), while *S. scombrus* had the highest concentration of Mn (2.18 ppm). The concentrations of all biological essential heavy metals were below their Recommended Daily Allowance (RDA). *Scomber scombrus* had the highest concentration of Cd with mean values higher than the Tolerable Upper Intake Limit of 0.02mg/day. Therefore screening on importation of frozen fish, for non-essential heavy metals is recommended.

Keywords: heavy metals, frozen fish, light metals, non-essential heavy metals

INTRODUCTION

The pollution of the aquatic environment with heavy metals has become a worldwide problem over the years, because they are indestructible and most of them have toxic effects on organisms (MacFarlane and Burchett, 2000; Oksuz *et al*., 2009). Heavy metals are among the most harmful of the elemental pollutants and are of particular concern because of their toxicities to human (Boran and Altinok, 2010). The sources of heavy metals contamination mainly include natural occurrence derived from parent materials and human activities. Anthropogenic inputs are associated with industrialization as atmospheric deposition, waste disposal, waste incineration, urban effluents, traffic emissions, fertilizer application and long-term application of wastewater in agricultural lands (Wuana and Okieimen, 2011). Increase in population, urbanization, industrialization and agriculture practices have further aggravated the situation (Giguere *et al*., 2004; Gupta *et al*., 2009).

Living organisms require varying amounts of metals for biological functions. Trace metals are naturally found in the body at low concentrations and are essential for human health (Nhapi *et al.*, 2012). Iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn) are required by humans. Nhapi *et al.* (2012) stated that iron prevents anaemia, and zinc is a cofactor in over 100 enzyme-based reactions. Excessive levels of trace elements can be damaging to humans (Tuzen, 2003). Some metals such as mercury, plutonium and lead are toxic metals that have no known vital or beneficial effect on organisms. According to Tchounwou *et al*. (2012), the most toxic heavy metals are Cr, Ni, Pb, Cd, and As. Nhapi *et al.* (2012) stated that some heavy metals such as Cd, Cr and Pb have no known physiological activity, but they have proved detrimental beyond certain limit.

Toxicokinetics of heavy metals in the marine environment has been of major concern since they constitute a potential risk to a number of flora and fauna species (Boran and Altinok, 2010). According to Storelli *et al*. (2005), heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms. A profound problem associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification in the food chains (Zhuang *et al*., 2009). Therefore, heavy metals can be bio-accumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks (Agah *et al.*, 2009).

The marine environments are the collection points for all the inland wastes discharged into the rivers, as they flow into seas and eventually oceans. The fish species under study are marine species. Ingestion of seafood contaminated by steady accumulation of these heavy metals has posed human health risks as in Minamata Bay case history. Metal pollution of the sea is less visible and direct than other types of marine pollution but its effects on marine ecosystems and humans are very extensive (Khayatzadeh and Abassi, 2010). Abou-Arab *et al*. (1996) reported that imported sardine and mackerel in Egypt contained levels of lead and chromium higher than permissible limits. The Nigerian fish markets are often flooded with Round sardinella *Sardinella aurita* Valenciennes, 1847, Atlantic herring *Clupea harengus* Linnaeus, 1758 and Atlantic mackerel *Scomber scombrus* Linnaeus, 1758 among other imported species of fish. Hence, the aim of this research is to assess the levels of heavy metal concentrations in imported frozen *Sardinella aurita*, *Clupea harengus* and *Scomber scombrus* sold within Zaria metropolis.

MATERIALS AND METHODS

Collection and preparation of samples

Six (6) samples of each experimental fish species (herring, mackerel and sardine) were obtained from the fish market and transferred in cold box to the Fisheries Laboratory, Department of Biology, Ahmadu Bello University, Zaria, for proper identification using identification keys from FishBase. The fish species were defrosted and rinsed with de-ionized water and weighed to the nearest mg with APOLLO/GF-A Multi-Functional Precision Balance, and both standard and total lengths were measured with metre rule. Samples were gutted and filleted using stainless steel knives and the entrails removed. The gills, liver and flesh were then separated, labelled appropriately, frozen and conveyed to the Multi-user Science Research Laboratory, Faculty of Life Sciences, Ahmadu Bello University, Zaria, for analysis.

Sample analysis

One gramme (1g) each of the defrosted fish tissue was digested separately using aqua regia digestion method. A mixture of HCl acid and HNO_3 in the ratio of 3:1, which is known as aqua regia mixture, was used for the digestion process. The sample in the digestion tube was mixed with 7ml of the aqua regia mixture and was left to sit overnight under reflux conditions. The mixture was then heated up to 180°C and the samples were processed until 1 ml of acid is remained. An additional 4 ml of aqua regia solution was added and evaporated for about one hour until the fumes coming off turned to white.

The digested sample was cooled and filtered through a Whatman 42 filter paper into 100 ml volumetric flask. Then15ml of distilled water was added to the filtrate and transferred into a labelled pre-treated sample bottle. The samples were analysed for metal content with AA240FS Fast Sequential Atomic Absorption Spectrometer, according to AOAC (1995). The metal concentration was read off a standard curve.

$$\text{Metal concentration (mg/l)} = \frac{A \times B}{C}$$

Where A = concentration of metal in digested solution (mg/l)

B = final volume of digested solution (ml)

C = sample size (ml)

Data analyses

The statistical relationships between samples were analysed. Tests for significant difference between means were carried out using analysis of variance (ANOVA) with values of $p \leq 0.05$ considered as significant. Significantly different means were separated using the Duncan's multiple range test. All statistical analyses were carried out using the superior performance statistical software package (IBM) version 20.

RESULTS AND DISCUSSION

Light metals in *Sardinella aurita*, *Clupea harengus* and *Scomber scombrus*

The mean concentrations of light metals obtained from the various fish species using the AA 240FS Fast Sequential Atomic Absorption Spectrometer is presented in Table 1. Two metals, Calcium (Ca) and Magnesium (Mg) were determined. Concentration of Ca and Mg ranged from 180.58 – 290.75 ppm and 8.87 – 14.36 ppm, respectively. *Sardinella aurita* had apparently higher concentration of Ca (290.75 ppm). The light metal concentrations in fish do not vary significantly across species (P > 0.05).

Table 1: Concentration of light metals in fish species

Element	*Sardinella aurita*	*Clupea Harengus*	*Scombrus scombrus*	P-value	RDA (mg/day)	TUIL (mg/day)
Ca	290.75 ± 105.91^a	180.58 ± 46.23^a	237.25 ± 126.40^a	0.736	1000*	2500#
Mg	13.94 ± 3.97^a	8.87 ± 1.40^a	14.36 ± 5.07^a	0.533	320-420*	65-350#

Means ± SEM are presented. Means with same superscripts across rows do not vary significantly (P > 0.05).

Note:

RDA - Recommended Daily Allowance

TUIL - Tolerable Upper Intake level

*IMNA - Institute of Medicine of the National Academies (1997)

#IM - Institute of Medicine (2006)

Calcium recorded the highest of all the metallic elements analysed in this study. Among the experimental fish species, *S. aurita* recorded the apparently higher concentration of Ca and significantly ($P \leq 0.05$) higher concentrations of Cu and Fe however, the Cu concentration for *S. scombrus* is lower than that reported by Biswas *et al*. (2017). The values recorded in this study are within the range reported by Oksuz *et al*. (2009) for Rose and Red shrimp in France.

According to Chen *et al*. (2017), not only does Mg in foods support a healthy immune system and improve bone health, they may help prevent the inflammation associated with certain cancers. Magnesium-rich foods have been found to increase heart health, help prevent stroke, and even cut your risk of dying from a heart attack (Altman, 1992). Also, Mg foods help support normal nerve and muscle function and keep your heartbeat in sync. Oksuz *et al*. (2009) reported Mg values which are significantly higher compared to the current findings. This could be due to the difference in species, season, area of catch and many other physical and environmental conditions.

Biological essential heavy metals in *Sardinella aurita*, *Clupea harengus* and *Scomber scombrus*

The mean concentrations of biological essential heavy metals obtained from the various fish species using the AA 240FS Fast Sequential Atomic Absorption Spectrometer is shown in Table 2. Five metals, copper (Cu), chromium (Cr), manganese (Mn), iron (Fe) and nickel (Ni) were determined. Concentrations of Cu, Cr, Mn, Fe and Ni ranged from 0.014 – 0.050 ppm, 0.00 – 0.0004 ppm, 0.286 – 2.180 ppm, 1.851 – 2.587 ppm and 0.00 – 0.023 ppm, respectively. Concentrations of some biological essential metals screened in the fish species vary significantly (P ≤ 0.05) among species; *Sardinella aurita* had significantly higher levels of Cu and Fe. Copper is a biological essential metal which enhances enzymatic activity of the body (Igwemmar *et al.*, 2013). It however poses health hazard when ingested in large amount (Alinnor and Obiji, 2010). Although in this study the mean value of Cu obtained fell within the RDA of 0.900 mg/kg.

Table 2: Concentration of biological essential heavy metals in fish species

Element	*Sardinella aurita*	*Clupea harengus*	*Scombrus scombrus*	P-value	RDA (mg/kg)	TUIL (mg/day)
Cu	0.050 ± 0.010^{a}	0.023 ± 0.010^{b}	0.014 ± 0.010^{b}	0.007	0.900♦	1000 -10000
Cr	0.0004 ± 0.0004^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.383	0.025-0.035♦	ID
Mn	0.286 ± 0.200^{c}	1.299 ± 1.100^{ab}	2.180 ± 1.770^{a}	0.054	1.800-2.300♦	2 - 11
Fe	2.587 ± 0.550^{a}	0.000 ± 0.360^{c}	1.862 ± 0.480^{b}	0.045	8.000-18.000♦	40 - 45
Ni	0.023 ± 0.010^{a}	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.080	0.100♣	0.2 - 1

Means ± SEM are presented. Means with same superscripts across rows do not vary significantly (P > 0.05)

Note:
RDA - Recommended Daily Allowance
TUIL - Tolerable Upper Intake level
ID - Insufficient data to set an Upper Limit
♦IMNA - Institute of Medicine of the National Academies (2001)
♣WHO - World Health Organization (2004)

Iron concentrations are significantly higher (P ≤ 0.5) in *Sardinella aurita* than the other fish species, but are however still lower than the Recommended Daily Allowance (RDA). Iron is a biological essential element in human diet. It forms part of the haemoglobin which allows oxygen to be carried from the lungs to the tissues. Iron deficiency causes anemia and fishes are the major dietary sources of this important element/metal. However, daily intake higher than 18 mg has toxic effects (IMNA, 2001). Mammals are not able to excrete excess Fe, and chronic Fe overload is associated with a slowly progressing failure of various organs (Ponka *et al*., 2007). Levels of Fe in canned fish show a wide variation around the world, the Fe levels between 4.83-29.2 µg/g and 21.0-88.8 µg/g have been reported in canned *S. aurita* in the USA and Brazil, respectively (Tarley *et al*., 2001; Ikem and Egiebor, 2005).

Authors have reported metal concentration results in fish similar to those obtained in this study. Chromium and Ni were recorded only in *S. aurita*. This could be attributed to the available contaminants in the abiotic component of their habitats, their feeding habits, ecological needs, metabolism, age and size (Adeyeye and Ayoola, 2012). Nickel concentrations are comparatively elevated in aquatic plants and animals in the vicinity of nickel smelters, nickel-cadmium battery plants, electroplating plants, petroleum industries and stainless-steel industries. It is also used in nickel alloys, jewelry, paint, spark plugs, catalysts, ceramics, disinfectants, magnets, batteries, inks, dyes, vacuum tubes, sewage outfalls, coal ash disposal basins, and found to be elevated in populated areas (USFDA, 1993). Food uptake as well as food processing is the major uptake route of Ni for humans apart from other natural sources.

Non-essential heavy metals in *Sardinella aurita*, *Clupea harengus* and *Scomber scombrus*

The mean concentrations of non-essential heavy metals obtained from the various fish species using the AA 240FS Fast Sequential Atomic Absorption Spectrometer is shown in Table 3. Two metals, Lead (Pb) and Cadmium (Cd) were determined. Low level of Pb was recorded only in *S. scombrus*. Concentration of Pb and Cd ranged from 0.00 – 0.053 ppm and 0.019 – 0.053 ppm, respectively. The concentration of Cd in fish varied significantly (P ≤ 0.05), being highest in *Scomber scombrus*.

Table 3: Concentration of non-essential heavy metals in fish species

Element	*Sardinella aurita*	*Clupea harengus*	*Scombrus scombrus*	P-value	TUIL(mg/day)
Pb	0.000 ± 0.000^{a}	0.000 ± 0.000^{a}	0.053 ± 0.053^{a}	0.383	0.50•
Cd	0.019 ± 0.004^{b}	0.019 ± 0.005^{b}	0.053 ± 0.006^{a}	0.000	0.02•

Means ± SEM are presented. Means with same superscripts across rows do not vary significantly (P > 0.05).

Note:
RDA - Recommended Daily Allowance
TUIL - Tolerable Upper Intake level
• FAO/WHO - Food and Agricultural Organization/World Health Organization (2010)

Cadmium accumulates primarily in the kidneys and has long biological half-life of up to 35 years (WHO, 2003). Apart from Ca, Cr, Mg, Ni and Pb the concentrations of metals in the experimental fish species do not vary significantly (P > 0.05) even though numerical differences were recorded. These could be because the three fish species feed at the same trophic level of the food chain. Iwegbue (2015) however opined that the difference in the concentrations of metals may be due to the differences in their origin and environmental conditions which influences the endogenous concentrations of the metals in these fish species as a result from difference in feeding habits, age, sex, physiology and duration of exposure of the fish to environmental contaminants.

CONCLUSION

Sardinella aurita had relatively higher concentration of calcium (290.75 ppm), while *S. scombrus* had relatively higher concentration of Magnesium (14.36 ppm). Their concentrations are both lower than their recommended daily allowance. *Sardinella aurita* had significantly (P < 0.05) highest concentration of Cu (0.05) and Fe (2.59), while *S. scombrus* had the highest concentration of Mn (2.18 ppm). The concentrations of all the biological essential heavy metals are below their RDA. The consumption of imported frozen *Sardinella aurita* especially as a dietary source of Cu and Fe is recommended. *Scomber scombrus* had the highest concentration of Pb and Cd, with the value of 0.053 for Cd being higher than the TUIL of 0.02 mg/day. Therefore, this suggests that *Scomber scombrus* is not safe for consumption elemental-wise; specifically with respect to Cd. Therefore screening on importation of frozen fish, for non-essential heavy metals is recommended.

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