

Nutrient and Trace Metal Profile of Smoked *Notopterus afer* from four Major Markets in Sierra Leone

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Abstract

The effects of smoking on the nutrient profile and trace metal levels in smoked knife fish (*Notopterus afer*) purchased from markets in four major districts of Sierra Leone was investigated. Concentrations of Cadmium (Cd), Lead (Pb), Zinc (Zn), Iron (Fe), Copper (Cu), and Manganese (Mn) in the smoked fish were determined. Fish samples were analyzed for proximate composition and heavy metal contents at the University of Ibadan, Nigeria. Proximate analysis was carried out using methods prescribed by AOAC while metal analysis was done using bulk scientific 200A atomic absorption spectrophotometer. Data was subjected to descriptive statistical analysis for easy understanding of results. ANOVA was used to test the multi interaction effect of locations, nutrients and metal levels. The Turkey's post-hoc test (HSD) was used to determine differences in the assessed variables. All statistical calculations were performed with SPSS 20.0 version for windows. The results of nutrient profile for *N. afer* showed that the fish furnishes enough nutrients, especially the protein content, which ranged from 43.73 ± 5.06 to 53.26 ± 11.33. The levels of all metals in the fish were above the limits permitted by Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO), European Community Regulation (EU) and European Commission (EC). Assessment of non-carcinogenic health hazard using Target Hazard Quotient (THQ) and Hazard Index (HI) suggested no concern from consumption of smoked *N. afer* bought from the selected markets. Cd level exhibited carcinogenic risk, while Fe and Zn showed significant health hazard risk (THQ > 1). The estimated daily intake (EDI) of the metals through fish consumption was below the permissible tolerable daily intake. It can be concluded that there is no likely potential human health risk by consuming smoked fish from the selected markets.

Keywords: Proximate composition**,** Trace metals, *Notopterus afer*, carcinogenic risk, hazard index, target hazard quotient.

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1. Introduction

A shift from animal protein to fish protein which has less cholesterol level (Shrivastava *et al*., 2011), has generally increased in recent decades (Wim *et al*., 2007). The fisheries sector is an important source of income, employment and food and nutrition security in Sierra Leone. The sector is one of the main contributors to the national economy, throwing in about 10% of the country GDP (Neiland *et al*., 2016). Fish according to Pasqualino *et al*. (2016) is the most important animal-source food in the diets of Sierra Leoneans as it provides close to 80% of animal protein intake. Fish is important for nutrition, and more importantly in Sierra Leone that ranks very low globally on poverty and nutrition indicators, with high incidence of malnutrition in under five children. Fish and other aquatic products are critical for economic activity, export earnings and employment in Sierra Leone, as well as food security and nutrition. Consumption of fish has significant health benefits; the omega-3-fatty acid in fish improves lipid profile and reduce cholesterol level which is a precursor to coronary heart disease, stroke and preterm disease (Burger and Gochfeld 2005; Musaiger and D'Souza 2008; Bashir *et al*., 2012). Bashir *et al*. (2012) noted that fish is a good source of vitamins and minerals. Musaiger and D'Souza (2008) reported that fish consumption has hypolipidemic/or antiatherogenic effects; lowering risk of prostate cancer, reduced occurrence of renal-cell carcinoma in women and reduced risk of dementia and Alzheimer disease in certain conditions.

Despite the plethora of benefits derived by human from a fish diet, the levels of toxic contaminants in fish are of considerable concern and have been implicated for serious health risks especially in human. Burger and Gochfeld (2005) and Bashir *et al*. (2012) noted that frequent fish consumption is a potential source of human exposure to toxic chemicals. There is therefore a global concern for the quality and safety of fish and fishery products as a major protein source (Huss *et al*., 2003) especially in the third world nations. Fish has been recognized as an important link for the transfer of toxic metals in humans (Shrivastava *et al*., 2011). Fish bio-accumulate considerable amounts of trace metals and organic pollutants that persist in their tissues for a long period. This metal in the long run enter the human metabolism through consumption causing dangerous health issues (Shrivastava *et al*., 2011). Metals have been noted to have double-edged roles in food which according to Iwegbue (2011) range from the nutritional requirement of essential elements to the toxicity associated with the excessive intake of both the essential and toxic metals; consequently, their determination is of necessary concern. Certain metals for example Cadmium, Lead and Mercury have been known to exhibit exceptional toxicity, even at low concentrations with significant health hazards. Metals like Cd and Hg are known to impair kidney function and reproductive capacity; cause hypertension, tumor and hepatic dysfunction, while Pb can cause renal failure, liver damage, impaired hearing or cause mental retardation, In women, at elevated levels may result in a shortened gestation period (Iwegbue, 2011).

Metals such as Cu, Fe, Mn, and Zn are essential and required for normal body function such as the synthesis of metalloprotein. Their deficiency in the body may lead to disease condition, while excessive intake may cause toxicity problems leading to premature aging and oxidative damage, a key component of chronic inflammatory disease and a suggested initiator of cancer (Naughton and Petroczi, 2008).

There are many pathways by which metals enter into fish especially through the food chains, cooking and smoking. Handling and processing practices have been known to modify fish proximate composition, amino acids and fat profiles, including their nutritional quality. Literature is replete with information on the assessment of the nutrient and heavy metal profiles of raw or uncooked fish, with scanty information on the nutrient and metal profiles of cooked or processed fish. A lot of studies has been carried out in other clime on the effect of processing on the concentration of heavy metals in fish and seafoods for example in African catfish (Ersoy and Ozeren, 2009; Ersoy, 2011); sea bass (Ersoy *et al*., 2006), seabass, and red seabream (He *et al*., 2010), sardine, hake, and tuna (Perello' *et al*., 2008); rainbow trout (Gokoglu *et al*., 2004), finfish and shellfish (Kalogeropoulos *et al*., 2012).

Information on the effect of processing, especially smoking on the nutrient and trace metal profile of fishes sold in major markets in Sierra Leone is sketchy if at all they are available. This present study therefore seeks to analyze and document information on the proximate and mental contents of important commercial species *Notopterus afer*, and also determine the hazard levels of the metals in the fish.

2. Materials and Methods

2.1 Study Area

The study was carried out in Sierra Leone, West Africa. Samples used for the study were bought from markets in four selected district headquarters, which include Western urban (Freetown), Bombali (Makeni), Bo (Bo City) and Kenema (Kenema town).

2.2 Sample collection and preservation

The Knife fish (*Notopterus afer*) samples used for the study were purchased from four different markets in the four selected districts of Sierra Leone. The smoked fish samples were wrapped with foil and packaged in well-labeled transparent polyethylene bags and were taking to the Department of Aquaculture and Fisheries Management, Njala University laboratory for further processing. The samples were air and sun dried daily before they were finally sent to the University of Ibadan Nigeria where the digestion, nutrients and trace metal analysis were carried out.

2.3 Methods for analysis of metals in fish

Bulk Scientific 200A atomic absorption spectrophotometer was used for the determination of Pb, Cd and Cu. Hollow cathode lamps were used as the excitation source. Lamp intensity and band pass were used according to the manufacturer's recommendations. Acetylene and airflow rates for all elements were 2 to 4 litres per min.

2.4 Heavy metal analyses

Fish sample tissues were oven dried at 70 to 73°C until constant weight was obtained. The specimens were then ground to fine powder and stored in desiccators in order to avoid moisture accumulation before digestion. The digestion procedure was carried out as described by Kotze *et al.* (2006). Twenty milliliter (20 ml) of concentrated nitric acid (55%) and 10 ml of perchloric acid (70%) was added to approximately 5 g tissue (dry mass) in a 100 ml Erlenmeyer flask. The digestion was done on a hotplate (200 to 250°C) until the solutions were clear. The digested fish solutions were filtered through an acid resistant 0.45 mm filter paper and made up to 50 ml each with distilled water. The samples were stored in clean glass bottles prior to the determination of the metal concentration with Atomic Absorption Spectrophotometer (AAS).

Stock standard was prepared by dividing the molar mass of the compound of the element by the molar mass of the element. The standard solution prepared was used to calibrate Atomic Absorption Spectrophotometer (AAS). Metal readings were taken from the equipment in mg/g and the results were converted to mg/kg which is the actual concentration of the metal in the sample using the equation proposed by Aderinola, *et al.* (2009):

$$\text{Concentration of metal} = \frac{\text{Calibration reading x volume of digest}}{\text{Weight of sample}}$$

2.5 Estimation of daily dietary intakes (EDI) of metals

The estimated daily intakes (EDI) for the analyzed metals were calculated by multiplying the respective mean concentration of the metal determined in the targeted fish samples by the weight of fish consumed by an average individual in Sierra Leone. This was obtained from the fish consumption rate set to 91.2 g day^{-1} per person from the annual per capital fish consumption of 33.3 kg for Sierra Leone (FUS, 2015) and calculated by using the formula proposed by Ahmed (2015).

$$\text{EDI} = \text{DFC x MC}$$

Where

DFC = daily food (fish) consumption,
MC = mean metal concentration in fish sample.

2.6 Estimation of target hazard quotient (THQ)

The non-carcinogenic risk assessments are typically conducted to estimate the potential health risks of pollutants using the target hazard quotient (THQ). The THQ values obtained through the consumption of fish species by local inhabitants can thus be assessed for each heavy metal and calculations can be done using the standard assumption for an integrated USEPA risk analysis as follows (USEPA, 1989).

$$\text{THQ} = \frac{C \times EFr \times ED \times FIR}{BW \times TA \times RfD \times 10^{-3}}$$

Where,

C is the heavy metal concentration in fish (mg/kg wet weight),
FIR is the fish consumption rate set to 91.2 g day^{-1} per person from the annual per capital fish consumption of 33.3 kg for Sierra Leone (FUS 2015),
EFr is the exposure frequency (365 days/year),
ED is the exposure duration (30 years or 10,950 days) for non-cancer risk as used by USEPA,
RfD is the reference dose of individual metal as shown in Table 1 (USEPA 2000), BW is an average adult body weight (70 kg)
TA is the average exposure time for non-carcinogens (10,950 days) (USEPA, 2011).

Table 1: Oral reference doses of metals (RfD) and upper tolerable daily intakes (UL) of assessed metals

No.	Element	RfD (mg Kg^{-1} day^{-1}) [references]	UL (mg day^{-1}) [references]
1	Fe	0.700 (USEPA 2010)	45 (FDA 2000)
2	Mn	0.014 (USEPA 2010)	11 (FDA 2000)
3	Zn	0.300 (Khan *et al*., 2008, USEPA 2010)	40 (FDA 2000)
4	Cu	0.040 (Khan *et al*., 2008, USEPA 2010)	10 (FDA 2000)
6	Cd	0.001 (Khan *et al*., 2008, USEPA 2010)	0.064 (Garcia-Rico *et al*., 2007)
7	Pb	0.0035 (Khan *et al*., 2008)	0.240 (Garcia-Rico *et al*., 2007)

2.7 Hazard index (HI)

Hallenbeck, (1993) reported that exposure to two or more pollutants may result in additive and/or interactive effects. Thus, in this study, cumulative health risk was evaluated by summing the THQ values of individual metal and expressed as Hazard index (HI) as below.

$$HI = THQ (Pb) + THQ (Cd) + THQ (Mg) + THQ (Fe) + THQ (Cu) + THQ (ZN)$$

2.8 Determination of carcinogenic risk

Carcinogenic risk (CR) indicates an incremental probability of an individual developing cancer over a lifetime due to exposure to a potential carcinogen. Cancer risk over a lifetime exposure to Pb were obtained using cancer slope factor (CSF), provided by USEPA (USEPA, 2000). The equation used for estimation of the cancer risk is as below:

$$CR = CSF \times EDI \text{ carcinogens}$$

Where,

CSF is the carcinogenic slope factor of 0.0085 (mg kg/day) for Pb (USEPA, 2010).
EDI is the estimated daily intake of heavy metals.

Acceptable risk levels for carcinogens range from 10−4 (risk of developing cancer over a human lifetime is 1 in 10,000) to 10−6 (risk of developing cancer over a human lifetime is 1 in 1,000,000).

2.9 Proximate Analysis of fish samples

In this study, moisture content (%), total ash (%), fat (%), fiber (%) and carbohydrate (%) were determined by the methods prescribed by Association of Official Analytical Chemists (AOAC, 2005). Crude protein content of the fish samples was determined according to the method prescribed by AOAC (2000).

2.10 Statistical analysis

Data obtained in this study was subjected to descriptive statistical analysis for easy understanding of results. ANOVA was used to test the multi interaction effect of locations, nutrients and metal levels. The Turkey's post-hoc test (HSD) was used to test if the means of two different groups under comparison are significantly different ($p<0.05$). All statistical calculations were performed with SPSS 20.0 version for windows.

3. Results and Discussion

3.1 Proximate composition of fish samples

The proximate composition of *Notopterus afer* are presented in Table 2. Proximate composition are useful for determining how the nutritive value of fish conforms to the range of dietary requirement and commercial specification.

Table 2. Proximate composition of smoked *Notopterus afer*

Parameters	Treatments±STD			
	Bo	Freetown	Kenema	Makeni
ASH	18.00±3.49[a]	18.23±3.60[a]	18.53±0.60[a]	20.33±2.35[a]
CP	46.60±3.33[a]	43.73±5.06[a]	44.39±1.47[a]	48.20±5.60[a]
EE	7.70±0.20[a]	8.47±0.51[a]	8.60±0.70[a]	8.60±0.50[a]
CF	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]	0.01±0.00[a]
DM	91.89±0.34[a]	89.41±0.89[b]	91.42±0.64[a]	91.37±0.48[a]
MC	8.11±0.34[b]	10.59±0.89[a]	8.58±0.64[b]	8.63±0.48[a]
CHO	22.15±2.39[a]	18.96±7.97[a]	19.89±0.78[a]	36.69±41.15[a]

CP – Crude protein; EE – Ether extract; CF – Crude fibre; DM – Dry matter; MC – Moisture content; CHO – Carbohydrate

The parameters measured for the fish were not significantly different from each other. The values obtained for the assessed variables are not significantly at variance with what other researchers reported (Abdullahi, 2001; Mumba and Jose, 2005; Effiong and Mohammed, 2008; Olayemi *et al*., 2011). Ash values which ranged from 18.00±3.49 to 20.33±2.35 were not significantly different for the four locations. Ash according to Adewumi *et al.* (2014) is a measure of the mineral content of food item. Ash is the inorganic residue that remains after the organic matter has been burnt off (Adewumi *et al.,* 2014). Fish with high ash values may be a good source of minerals such as calcium, potassium, zinc, iron and magnesium. The results for ash obtained in this study is at variance with the result Jolaoso *et al*. (2016) reported for smoked *Pseudotolithus elongatus* from Ologe and Lagos Lagoo, Nigeria.

The protein content obtained for the fish in this study ranged from 43.73±5.06% – 48.20±5.60% and were not significantly different from each other (Table 2). Smoking is believed to concentrate crude protein contents of red fish, (Kumolu-Johnson, 2010) and according to Koral *et al*. (2009) this concentration effect is basically as a result of loss of moisture and not the loss of protein nitrogen by the smoked fish. Adewole and Omotosho (1997) also reported that variations observed could be as a result of fish age, habitat, and feed consumption or absorption capability and conversion potentials of essential nutrients from their diets or their local environment into such biochemical attributes needed by the organism's body. The ranges of CP obtained in this study were higher than that of Pierestani *et al*. (2009), Musaiger and D`Souza (2008) and Bassey *et al*. (2014), and also higher than the values obtained by Jolaoso *et al*. (2016) for smoked *Sphyraena barracuda* (31.58%) at Ologe and Lagos Lagoon, Nigeria.

Ether extract for *N. afer* ranged from 7.70±0.20 to 7.70±0.20 and were not significantly different for all the four sampling locations. Fat or fatty acids is important as it is needed for the prevention of cardiovascular disorder and also for maintaining proper functioning of membrane in living cells. The fat content of this study were not different from what Fawole *et al*. (2007) and Bassey *et al*. (2014) reported in their studies. Fish according to Ackman (1989) can be grouped into four categories based on their fat contents: lean fish (< 2%), low fat (2-4%), medium fat (4-8%) and high fat (>8%). Hence, based on these categorization *N. afer* can be regarded as high in fat. The fat values reported in this study are not far from the 10.62% reported by Daniel (2015) for *Chrysichthys nigrodigitatus* obtained from Cross River, Nigeria. The value is however below the value of 25.31±2.78 reported by Msuku and Kapute (2018) for smoked Diplotaxodon fish in Malawi. The results of this study is also at variance from the 0.40% fat that Jolaoso *et al*. (2016) obtained for smoked *Pseudotolithus elongatus* in Ologe and Lagos Lagoo, Nigeria. Traditional method of fish preservation, smoking and salting have been said to reduce crude fat content of fish due to oxidation and crude fat breakdown into other components; oxidation of poly-unsaturated fatty acids (PUFA) contained in the fish tissue to products such as peroxides, aldehydes, ketones and the free fatty acids (Holma and Maalekuu, 2013).

Crude fibre recorded for *N. afer* showed similarity and were not significantly different for all the locations (0.01±0.0). Fishes are not known to have capacity to sufficiently digest crude fibre. According to Stickney and Shumway, (1974), many monogastric animals, of which fish is one have intestinal microflora that is incapable of degrading dietary fibre. Fish do not possess endogenous enzymes that can catalyse the hydrolysis of cellulose and other fibrous constituents of the diet (Stickney and Shumway, 1974). Information on the nutritional value and dietary requirement of fibre for farmed fish is limited (Halver, 1972). Consequently, the inclusion of crude fibre in fish feeds is limited to about 7 percent, in order to limit the amount of undigested materials entering the culture system (Gatlin III, 2010). Smoking has been associated with increase in fish fibre contents, by oxidizing poly-unsaturated fatty acids (PUFA) components, contained in their tissues to products such as peroxides, aldehydes, ketones and free fatty acids (Daramola *et al*., 2007).

Dry matter content of the formulated diets ranged from 89.41±0.89 to 91.89±0.34; these values are not statistically different except for Freetown location that vary from others ($P<0.05$). Dry matter of feed is the final product after elimination of moisture through drying. It is an indicator of the amount of nutrients that are available to the animal in a particular feed. According to Kiczorowska, *et al*. (2019) smoking process increased the concentration of nutrients, including the content of dry matter, crude ash, and crude protein, and reduced the fat content.

The Moisture Content of the fish (MC) exhibited some level of significance ($p < 0.05$) as shown in Table 2. Moisture contents of a fish according to Olagunju *et al.* (2012) are a good indicator of its relative energy level, protein and lipid. Smoking, salting and frying have been observed to reduce moisture content of fishes (Holma and Maalekuu, 2013). Fish with low fat are known to have high water in their body and tend to be white in color. For example Pierestani *et al.* (2009) reported *that Liza aurata, Cyprinus carpio* and *Sander lucioperca* had fat contents lower than 5% and were classed as low/lean fish with moisture contents above 75%. The moisture values obtained in this study although are above the 6 – 8% safe level for dried fish, are not significantly varied from what Msuku and Kapute (2018) obtained for Diplotaxodon from Lake Malawi.

The carbohydrate determined for the fish in this study was not significantly different for the four locations. Gatlin III, (2010) noted that fish do not have a specific dietary requirement for carbohydrate. High carbohydrate values in the fish according to Adewole and Omotosho (1997) could be due to the presence of elements like calcium and potassium in the fish species diets.

3.2 Heavy metal concentrations in sampled fishes

The mean heavy metal contents determined for *Notopterus afer* at the four sampling locations are presented in Tables 3, while Table 4 shows the permissible levels of metal in food as a comparison to the value obtained in this study. Heavy metal contents of the fish samples varied in their concentrations with locations but were not significant different, except for lead (Pb) that showed significant differences ($P<0.05$) in Bo and Freetown. The levels of metals in *Notopterus afer* showed some levels of elevation when compared to allowable thresholds.

The order of metal concentration in the two fish species was Fe > Zn > Mn > Pb > Cu > Cd. Lakshaman et al (2009) noted that accumulation of metals in different species is the function of their respective membrane permeability and enzyme system. The highest concentration of Pb was detected in *Notopterus afer* with concentration of 3.72 ± 1.24 – 14.2 ± 1.03 mg/kg. According to Crosby, (1977) ammunition, cable sheaths, type metals pigments, storage batteries, solder and anti-knock compounds in petrol are the sources of lead. Higher concentration of lead is known to inhibit active transport mechanisms involving ATP and may also suppress cellular oxidation-reduction reactions and even inhibit protein synthesis (Adeyeye *et al.,* 1996). Lead is nonessential element and a great threat to life if present in substantial quantity. It is toxic even at low concentrations and has no known function in biochemical processes (Burden *et al*., 1998). The Pb values recorded for *N. afer* in this study were quite high and were above the maximum standard level of 0.5 mg/kg dry weight recommended by FAO (1983). The researchers because of these abnormally high level presence in the smoked fish have decided to

a comprehensive survey and analysis using a different laboratory in order to validate the veracity of these results. Zinc has the second highest concentration among the metals in this study (66.00±12.68 – 89.67±24.48). Zinc is one of the essential elements as copper and cobalt for both animals and humans. Zinc has been reported to be necessary for embryo development in fish (Carpene *et al*., 1994). Smoking of fish is believed to increase the bio-concentration of Zinc in fish. Zinc serves as cofactors to some important enzymes and also involved in most metabolic pathway in humans and its deficiency can lead to loss of appetite, growth retardation, skin changes and immunological abnormalities; however, at higher concentration they tend to be toxic (Fawole *et al*., 2013). Fish takes up Zn directly from water, especially via mucous and gills. The varying concentration of zinc in the muscle of fish samples could be due to the presence of large number of fishing vessels and trawlers, which use galvanized metal coatings to prevent rusting, and this ultimately find its way into the ambient media through leaching (Lakshamanan, *et al*., 2009). The values of Zn obtained were above the 50 mg/kg maximum zinc level permitted for fish according to Food Codex. The values of Zn in this study are higher than the value recorded in the tissues of *Oreochromis niloticus* from Ologe lagoon by Kumolu-Johnson and Ndimele (2012).
Copper concentration reported in this study for *N. afer* ranged from 1.08±0.75 – 11.35±17.45 and were not significantly different. These values were however above what Rejomon *et al*. (2010), Olowu *et al*. (2010), Yilmaz (2009) and Daniel *et al*. (2013) reported. The values reported for copper here was also well above the 3.0 and 1.0 – 3.0 mg/kg prescribed limits for food fish by WHO (1994) and FEPA (2003) for copper in food and fish respectively.

Manganese levels recorded in the study ranged from 3.50 ± 2.18 – 33.83 ± 36.96. Manganese functions as an essential constituent for bone structure, for reproduction and for normal functioning of the nervous system. It is also part of the enzyme system (Fleck, 1976). It is obvious that the fish investigated will complement the supply of zinc and manganese in the food of the people who eat the fish. Iron in meat or fish plays an important role in the prevention of iron deficiency. Half of the iron in meat and fish is present as haeme iron (in haemoglobin). Fe is an essential element in human diet and forms part of hemoglobin, which allows oxygen to be carried from the lungs to the tissues. This is well absorbed at about 15.33%, a figure that can be contrasted with other forms of iron such as that from plant foods at 1-10% (Bender, 1992). Iron from animal sources also enhances the absorption of iron from other sources and so contributes significantly to the prevention of anaemia, which is so widespread in developing countries (Wheby 1974). Iron level recorded in this study was however on the high side compared to the permissible levels of 43 mg/kg recommended by WHO/FAO.

Cadmium is widely known to be a highly toxic non–essential heavy metal and it does not have a role in biological process in living organisms. Thus, even at its low concentration, cadmium could be harmful to living organisms (Tsui and Wang, 2004). The maximum concentration of Cadmium detected in the muscle of *N. afer* indicated high potential health risk to the majority of the patronizing fish consumer population in the study areas. The grand mean concentration of Cadmium in the fish which was above the WHO/FAO maximum permissible limit of 0.2mg/kg for food samples could not be tolerated and the consumers might raise alarm if they were actually aware of its potential health risks.

Table 3. Heavy metal concentrations of smoked *Notopterus afer*

Parameters	**Treatments±STD**			
	Bo	**Freetown**	**Kenema**	**Makeni**
Pb	3.72±1.24[b]	5.40±1.52[b]	12.25±4.51[a]	14.20±1.03[a]
Cd	0.27±0.29[a]	0.08±0.10[a]	0.12±0.10[a]	0.13±0.08[a]
Mn	33.83±36.96[a]	3.50±2.18[a]	8.67±10.40[a]	8.83±11.47[a]
Fe	348.17±332.99[a]	160.33±52.57[a]	175.83±78.69[a]	400.17±481.16[a]
Cu	1.53±0.80[a]	1.08±0.75[a]	1.12±1.17[a]	11.35±17.45[a]
Zn	66.00±12.68[a]	77.33±14.37[a]	75.12±14.42[a]	89.67±24.48[a]

Table 4. Metal maximum permissible limit (mg/kg)

Metals	***N. afer*** **(mg/kg)**	**Maximum permissible limit (mg/kg)**
Pb	8.892	2.0 (MAFF 1995)
Cd	0.150	0.2 (WHO/FAO)
Mn	13.708	5.5 (WHO/FAO)
Fe	271.125	43 (WHO/FAO)
Cu	3.771	20 (MAFF 1995)
Zn	77.029	50 (MAFF 1995)

3.3 Estimated daily intakes (EDI) and carcinogenic risk (CR)

The estimated dietary intakes and carcinogenic risk of metals based on per capital consumption of 33.3 kg fish for *N. afer* are displayed in Table 5 while Table 6 provides information on the THQ of metals from consumption of the fish. There was no carcinogenic risk recorded for any of the fish while THQ of Fe (0.505) and Zn (0.335) indicated significant health risk. THQ of < 0.2 for any given pathway is considered acceptable. For preliminary quantitative risk assessment, THQ of 0.2 is regarded negligible. If THQ is > 0.2, the risk assessment should either be redefined and or risk management measures need be taken. The results in this study revealed that Copper contributed the highest daily intake and Pb contributed the lowest daily intake. The EDI revealed that the investigated fish samples were below the recommended values hence no risk to people's health associated with the intake of the studied metals through the consumption of the fish. Carcinogenic risk associated with the consumption of the fish species is minimal with the exception of cadmium that exhibited tendency towards carcinogenic risk. Generally, the values of CR lower than 10^{-6} are considered as negligible, above 10^{-4} are considered to be unacceptable and lying in between 10^{-6} and 10^{-4} are considered as acceptable range, Cancer and non-cancer risks estimate threshold is 10^{-4}. CR is used as a bright line for setting goals for decision-making. 10^{-4} should however not be considered as cancer risk benchmark. This threshold can be 32 months for children younger than 6 years and 98 months for adults (NEHC, 2001). 10^{-6} is set as a point of departure for cancer risk as a regulatory decision. Cd was close to cancer risk threshold of 10^{-4} and as such should be monitored closely.

Table 5. Estimated daily intakes (EDI) and Carcinogenic risk (CR) for Knife fish

Metals	***Notopterus afer*** **(Knife fish)**		
	Concentration mg/kg	EDI µg/kgbw/day	Carcinogenic risk (CR)
Pb	8.892	11.585	0.927 na
Cd	0.150	0.195	0.002 na
Mn	13.708	17.860	0.117 na
Fe	271.125	353.237	3.003 na
Cu	3.771	4.913	0.042 na
Zn	77.029	100.358	0.853 na

Legend: EDI – estimated daily intake; CR – carcinogenic risk; na – not applicable.

Table 6. THQ for smoked *N. afer*

Metals	**Oral Reference Dose - Rfd (mg/kg per day)**	***Notopterus afer* (Knife fish)**	
		Concentration (mg/kg)	**THQ**
Pb	1.5 (0.005 mg/g)	8.892	0.008
Cd	1×10^{-3}	0.150	0.065
Mn	1.4×10^{-1}	13.708	0.128
Fe	7.0×10^{-1}	271.125	0.505
Cu	4.0×10^{-2}	3.771	0.123
Zn	3.0×10^{-1}	77.029	0.335
HI = (∑THQ Pb…..THQ Zn)			

THQ: Total Hazard Quotient

3.4 Hazard index - HI

The hazard index (HI) recorded for *Notopterus afer* in this study was 1.164. Hazard index (HI) of < 1.0 is considered acceptable while HI > 1 need to be redefined or be subjected to monitoring (NEHC, 2001). Hazard index (HI) measured for *N. afer* was above 1, an indication of human health risk.

4. Conclusion

Tradition process of fish preservation such as smoking has the capacity to reduce nutritional composition of fishes and also bioconcentrate heavy metals in fishes. The nutrient profiles of the fish species assessed in this study are appropriate to supply nutrients needs of consumers. The metal levels recorded were however beyond recommended permissible levels; consequently, in order to assure public safety these metals must be controlled in food sources. Reason for their control is owing to the fact that increased concentration of food heavy metals is associated with the etiology of a number of diseases (WHO 1995), especially cardiovascular, renal, neurological and bone diseases. In view of the importance of fish in human diet, it is necessary that biological testing and monitoring of the fish meant for consumption be carried out regularly to ensure human safety and health. Monitoring and evaluation should be carried out periodically in order to ascertain when the levels of heavy metals is above the acceptable limit for safe consumption as these metals could be passed to humans and predispose the consumers to possible health hazards. Safe disposal of domestic wastes and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment.

Conflict of interest

No conflict of interest declared.

5. References

Abdullahi, S.A. (2001). Investigation of Nutritional Status of *Chrysichthys nigrodigitatus, Barus filamentous* and *Auchenoghats occidentals*, Family Bangdae. *J. Arid Zone Fish*. 1:39-50.

Ackman, R.G. (1989). Nutritional composition of fats in seafoods. Progress *in Food Nutrition Science*. 13:161-241.

Aderinola, O.J., Clarke, E.O., Olarinmoye, O.M., Kusemiju, V & Anetekhai, M.A. (2009). Heavy metals in surface water, sediments, fish and periwinkles of Lagos Lagoon. *American-Eurasian Journal of Agricultural and Environmental Sciences.* **5**(5): 609-617.

Adewole, S.O. & Omotosho, J.S. (1997). Nutrient Composition of some freshwater Fishes in Nigeria. *BioScience Research Community* **11**(4): 333-336.

Adewumi, A.A., Adewole, H.A., & Olaleye, V.F. (2014). Proximate and elemental composition of the fillets of some fish species in Osinmo Reservoir, Nigeria. Agriculture and biology journal of North America, **5**(3): 109-117.

Adeyeye, E.T., Akinyugha, N.J., Fesobi, M.E. & Tenabe, V.O. (1996). Determination of some metals in *Clarias gariepinus* (Cuvier and Vallenciennes), *Cyprinus carpio* (L.) and *Oreochromis niloticus* (L.) fishes in a polyculture fresh water pond and their environments. Aquaculture, 147: 205-214.

Ahmed, M.K., Shaheen, N., Islam, M.S., Habibullah-al-Mamun, M., Islam, S., Mohiduzzaman, M. & Bhartacharjee, L. (2015). Dietary intake of trace elements from highly consumed cultured fish (Labeorohita, *Pagasius pangasius* and *Oreochromis mossambicus*) and human health risk implications in Bangladesh. Chemosphere. 128:284–292.

AOAC (2000). Official Methods of Analysis. 17th ed. Gaithersburg, Maryland, USA, AOAC International.

Bashir, F. A., Shuhaim-Othman, M. & Mazlan A.G. (2012). Evaluation of trace metal levels in tissues of two commercial fish species in Kapar and Mersing coastal waters, Peninsular Malaysia. *J. Environ. Public Health*: 352309. Article ID352309, 10 pages, doi: 10.1155/2012/352309.

Bassey, F.I., Oguntunde, F.C., Iwegbue, C.M.A., Osabor, V.N. & Edem, C.A. (2014). Effects of processing on the proximate and metal contents in three fish species from Nigerian coastal waters. *Food Science and Nutrition*; **2**(3): 272–281. doi: 10.1002/fsn3.102.

Bender, A. (1992). Meat and Meat products in human nutrition in developing countries, *F.A.O Nutrition Paper* 53, FAO, Rome, 91pp.

Burden, V.M., Sandheinrich, M.B. & Caldwell, C.A. (1998). Effects of lead on the growth and alpha aminolevulinic acid dehydrates activity of juvenile rainbow trout, *Oncorhynchus mykiss*. *Environmental Pollution*, 101: 285-289.

Burger, J. & Gochfeld, M. (2005). Heavy metals in commercial fish in New Jersey. *Environ. Res*. 99:403–412. doi: 10.1016.02.001.

Carpene, E., Gumiero, B., Fedrizzi, G. & Serra, R. (1994). Trace elements (Zn, Cu, Cd) in fish from rearing ponds of Emilia-Romagna region (Italy). *Sci. Total Environ*. 141: 139-146.

Crosby. N.T. (1977) Determination of metals in food – a review Analyst 102 – 268.

Daniel, E.O., Ugwueze, A.U. & Igbegu, H.E. (2013). Microbiological Quality and Some Heavy Metals Analysis of Smoked Fish Sold in Benin City, Edo State, Nigeria. World Journal of Fish and Marine Sciences **5** (3): 239-243.

Daniel, I.E. (2015). Proximate composition of three commercial fishes commonly consumed in Akwa Ibom State, Nigeria. International Journal of Multidisciplinary Academic Research, **3**(1). Available online at download (psu.edu).

Daramola, J.A., Fasakin, E.A. & Adeparusi, E.O. (2007). "Changes in Physicochemical and Sensory Characteristics of Smoke-Dried Fish Species Stored at Ambient Temperature". *African Journal of Food, Agriculture Nutrition and Development*. **7**(6).

Effiong B.N. & Mohammed, I. (2008). Effect of Seasonal Variation on the Nutrient Composition in Selected Fish species in Lake Kainji-Nigeria. *Nature Sci*., **6**(2).

Ersoy, B. (2011). Effect of cooking methods on the heavy metal concentrations of the Africa Catfish (*Clarias gariepinus*). *J. Food Biochem*. 35:351–356.

Ersoy, B. & Ozeren, A. (2009). The effect of cooking methods on mineral and vitamin contents of African catfish. *Food Chem*. 115:419–422.

Ersoy, B., Yanar, Y., Kucukgulmez, A. & Celik, M. (2006). Effects of four cooking methods on heavy metal concentrations of sea bass fillets (Dicentrarchus labra finne, 1785). *Food Chem*. 99:748–751.

Fawole, O.O., Ogundiran, M.A., Ayandiran, T.A. & Olagunju, O.F. (2007). Mineral composition in some selected fresh water fishes in *Nigeria. J. Food Saf*. 9:52–55.

FAO (1983). Compilation of legal limits for hazardous substances in fish and fishery products, Food and Agricultural Organization, Fishery circular No. 464, pp. 5–100.

FDA (2001). Fish and fisheries products hazards and controls guidance (3rd Ed.). Center for Food Safety and Applied Nutrition, US Food and Drug Administration.

FEPA (2003). Guideline and standards for environmental pollution and control in Nigeria. Federal Environmental Protection Agency Nigeria.

Fleck, H. (1976). Introduction to Nutrition. 3rd Eds. New York, USA: Macmillan, pp. 207-19.

FUS (2015). Per capita consumption. Retrieved April 06, 2022 from https://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus11/08_percapita2011.pdf

Garcia-Rico, L, Leyva-Perez, J, Jara-Marini, M.E. (2007). Content and daily intake of copper, zinc, lead, cadmium, and mercury from dietary supplements in Mexico. *Food Chem Toxicol*., 45: 1599-1605. 10.1016/j.fct.02.027.

Gatlin III, D.M. (2010). Principles of Fish Nutrition. SRAC Publication No. 5003. Retrieved from https://fisheries.tamu.edu/files/2013/09/SRAC-Publication-No.-5003-Principles-of-Fish-Nutrition.pdf (tamu.edu).

Gokoglu, N., Yerlikaya, P. & Cengiz, E. (2004). Effects of cooking methods on the proximate composition and mineral contents of rainbow trout (*Oncorhynchus mykiss*). *Food Chem*. 84:19–22.

Hallenbeck, W.H. (1993). Quantitative Risk Assessment for Environmental and Occupational Health. Lewis, Chelsea.

Halver, J.E. (1972) Components of plant and animal foodstuffs other than nutrients, in *Fish Nutrition.* Academic Press, New York, pp. 184–6.

He, M., Ke, C.H. & Wang. W.X. (2010). Effects of cooking and subcellular distribution on the bioaccessibility of trace elements in two marine fish species. *J. Agric. Food Chem*. 58:3517–3523.

Holma, K.A. & Maalekuu, B.K. (2013). Effect of traditional fish processing methods on the proximate composition of red fish stored under ambient room conditions. *American Journal of Food and Nutrition*, **3**(3):73-82.

Huss, H. H., Ababouch, L. & Gram, L. (2003). *Assessment and Management of Seafood Safety and Quality*. Food and Agriculture Organization of the United Nations. FAO Fisheries Technical Paper 444. Rome.

Iwegbue, C. M. A. (2011). Concentrations of selected metals in candies and chocolates consumed in Southern Nigeria. *Food Addit. Contam. Part B* 4:22–27.

Jolaoso, A.O., Njoku, K.L., Akinola, M.O., Adesuyi, A.A., & Adedokun, A.H. (2016). Heavy Metal Analyses and Nutritional Composition of Raw and Smoked Fishes from Ologe and Lagos Lagoon, Lagos, Nigeria. *J. Appl. Sci. Environ. Manage*. **20** (2) 277 – 285, **http://dx.doi.org/10.4314/jasem.v20i2.7.**

Kalogeropoulos, N., Karavoltsos, S., Sakellari, A., Avramidou, S., Dassenakis, N. & Scoulla, M. (2012). Heavy metals in raw, fried and grilled Mediterranean finfish and shellfish. *Food Chem. Toxicol*. 50:3702–3708.

Khan S, Cao Q, Zheng, Y.M, Huang, Y.Z. & Zhu, Y.G. (2007). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, *China. Environ Pollut*., 152: 686-692. 10.1016/j.envpol.06.056.

Kiczorowska, B., Samoli ́nska, W., Grela, E.R. & Bik-Małodzi ́nska, M. (2019). Nutrient and Mineral Profile of Chosen Fresh and Smoked Fish. Nutrients, 11, 1448; doi:10.3390/nu11071448.

Kotze, P.D., Preez, H.H. & Van-Vuren, J.H.J. (2006). Bioaccumulation of copper and Zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. Dept. of Zoology, Rand Afrikans University press ISSN 0378 4738. Water SA Vol. 25 No. 1, South Africa.

Kumolu-Johnson C.A. & Ndimele, P.E. (2012). Some Aspects of the Limnology and Heavy Metal Content of Water, Sediment and *Oreochromis niloticus* (Linnaeus, 1758) from Ologe Lagoon, Lagos, Nigeria. *Research Journal of Environmental Toxicology,* 6:210-221.

Kumolu-Johnson, C.A., Aladetohun, N.F. & Ndimele, P.E. (2010). The effects of smoking on the nutritional qualities and shelf-life of *Clarias gariepinus*. *African Journal of Biotechnology* **9**(1): 073-076.

Koral, S., Köse, S. & Tufan, B. (2009). Investigating the Quality Changes of Raw and Hot Smoked Garfish (*Belone belone euxini*, Günther, 1866) at Ambient and Refrigerated Temperatures. *Turkish Journal of Fisheries and Aquatic Sciences*, 9:53-58.

Lakshmanan, R., Kesavan, K., Vijayanand, P., Rajaram, V. & Rajagopal, S. (2009). Heavy metals accumulation in five commercially important fishes of Parangipettai, Southeast Coast of India. *Advance Journal of Food Science and Technology*, 1: 63-65.

Msuku, L. & Kapute, F. (2018). Effect of smoking and sun drying on proximate composition of *Diplotaxodon* fish species (Ndunduma) from Lake Malawi, Malawi. Ajfand, **18**(1). DOI: 10.18697/ajfand.81.16960.

Mumba, P.P. & Jose, M. (2005). Nutrient composition of selected fresh and processed fish species from Lake Malawi: a nutritional possibility for people living with HIV/AIDS. *International Journal of Consumer Studies*; **29**(1): 72-77.

Musaiger, A. O. & D'Souza. R. (2008). The effects of different methods of cooking on proximate, mineral and heavy metal composition of fish and shrimps consumed in the Arabian Gulf. Arch. Latinoam. *Nutr*. 58:103–109.

National Research Council (US) Committee on Toxicology (2001). Review of the US Navy's Human Health Risk Assessment of the Naval Air Facility at Atsugi, Japan. Washington (DC): National Academies Press (US). INTERPRETATION OF RISK-ASSESSMENT RESULTS. Available from: https://www.ncbi.nlm.nih.gov/books/NBK223271/

Naughton, D. P., & Petroczi, A. (2008). Heavy metal ions in wines: meta-analysis of target hazard quotient reveals health risk. Chem. Cent. J. 2:22. doi: 10.1186/1752-153x-2-22. Available from http://journal.chemistrycentral.com/content/ 2/1/22.

Neiland, A.E., Cunningham, S., Arbuckle, M., Baio, A., Bostock, T., Coulibaly, D., Gitonga, N.K., Long, R. & Sei, S. (2016). Assessing the potential contribution of fisheries to economic development: The case of post-Ebola Sierra Leone *Natural Resources* 7:356–76. http://dx.doi.org/10.4236/nr.2016.76031.

Official Methods of Analysis. (2005). Association of Official Analytical Chemists. 18th Edition. Washington D.C., USA.

Olagunju, A., Muhammad, A., Mada, S.B., Mohammed, A., Mohammed, H.M. & Mahmoud, K.T. (2012). Nutrient Composition of *Tilapia zilli*, *Hemisynodontis membranacea*, *Clupea harengus* and *Scomber Scombrus* Locally Consumed in Africa. *World Journal of Life Sciences and Medical Research*; 2:16-19.

Olayemi, F.F., Adedayo, M.R., Bamishaiye, E.I. & Awagu, E.F. (2011). Proximate composition of catfish (Clarias gariepinus) smoked in Nigerian stored products research institute (NSPRI): Developed kiln. International Journal of Fisheries and Aquaculture **3**(5), pp. 96-98.

Olowu, R.A., Ayejuyo, O.O., Adewuyi, G.O., Adejoro, I.A., Denloye, A.A.B., Babatunde, A.O. & Ogundajo, A.L. (2010). Determination of heavy metals in fish tissues, water and sediment from Epe and Badagry lagoons, Lagos, *Nigeria. E- J. Chem*., 71: 215-221.

Pasqualino, M.M., Thilsted, S.H., Phillips, M.J. & Koroma, A.S. (2016). Food and nutrition security in Sierra Leone with a focus on fish in Tonkolili District. Penang, Malaysia: WorldFish. Program Report: 23.

Perello', G., Marti-Cid, R., Llobet, J.M., & Domingo, J.L. (2008). Effect of various cooking processes on the concentrations of arsenic, cadmium, mercury and lead in foods. *J. Agric. Food Chem*. 56:11262–11269.

Pierestani, S., Ali-Sahari, M., Barzegar, M. and Seyfabadi, S.J. (2009). Chemical composition and minerals of some commercially important fish species from the south Caspian Sea. *Int. Food Res. J.* 16:39–44.

Rejomon, G., Nair, M. & Joseph, T. (2010). Trace metal dynamics in fishes from the southwest coast of India. *Environ. Monit. Assess*., 167: 234-255.

Shrivastava, V. S., Indrajit, S. & Shandil, A. (2011). Study for determination of heavy metal in fish species of the River Yamuna (Delhi) by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Shriram Institute for Industrial Research, University Road, Delhi, India.

Stickney, R.R. & Shumway, S.E. (1974). Occurrence of cellulose activity in the stomachs of fishes. *J. Fish. Biol.*, 6, pp. 779–90.

Tsui, M. T. K. & Wang, W. V. (2004). Uptake and elimination routes of inorganic mercury and methyl mercury in Daphnia manga. *Environ.Sci.Tecno*.38, 808-816.

USEPA (2010). *Risk-based Concentration Table*. United State Environmental Protection Agency, Washington, DC.

USEPA, (1989). Risk assessment guidance for superfund, Human Health Evaluation Manual. EPA/540/1-89/002 vol I. Office of Emergency and Remedial Response, Washington, DC.

USEPA. (2000). *Risk-based concentration table: Philadelphia PA; Washington, DC, USA*.

USEPA (2011). USEPA Regional Screening Level (RSL) Summary Table: November 2011.

Wheby, M.S. (1974. Systemic effects of iron deficiency. In: W.H. Crosby (Editor), Iron. Medicom, Inc., New York, pp. 39-45.

WHO, (1994). Guidelines for drinking water quality recommendation. World Health Organization Geneva, pp: 130.

WHO (1995). Lead Environmental Health Criteria. WHO, Geneva.

Wim,V., Issabelle, S. K., Stefan, D. H. & John, V. C. (2007). Consumer perception versus scientific evidence of farmed and wild fish: exploratory insights for Belgium. *Aquaculture International* 15:121-136.

Yilmaz, F. (2009). The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Koycegiz lake-Mugla. *Turkish Journal of Science and Technology*, 4: 7-15.