



Health risk assessments of some toxic metals in stockfish sold in Nigeria

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ABSTRACT

Aim: Stockfish is a popular delicacy in Nigeria but, because of high cost of the fish most consumers purchase and consume head bones, fins and gills. We hypothesized that fins and gills may contain higher concentrations of toxic metals. To evaluate some toxic metals levels in the body tissue, gills and fins of cod stockfish sold in three different cities in Nigeria. **Materials and Methods:** The concentrations of cadmium, lead, mercury and arsenic were determined in perchloric: nitric: sulphuric acid (1:2:2) dissolved fins, body tissue and gills of cod stockfish using atomic absorption spectrophotometry and Inductively Coupled Plasma Mass-Spectrophotometer. **Results:** The concentrations of cadmium and lead were significantly higher ($p < 0.001$) in gills compared to fins and body tissue of stockfish. There were no significant differences in the mean levels of mercury and arsenic between gills, fins and body tissues. **Conclusion:** The hazard indices of the measured toxic metals were higher in gills than body tissue and fin. The general public should be aware of the potential health hazard of eating non-muscle tissues of stockfish.

KEY WORDS: Toxic metals; Stockfish; Consumption; Nigerian markets.

INTRODUCTION

Stockfish is unsalted dried fish usually imported to Nigeria from some European countries particularly Norway and Iceland. In Nigeria the fish is rehydrated, sold and used in a number of different dishes. The fish is traditional dried in an open cold air and wind on wooden racks (hjell) which confers the characteristics flavor but also exposes the fish to very many other contaminants [1-2]. Stockfish also has regional names, including Okpoloko among Igbos, south east of Nigeria and Bazabaza among the Benins in south-south of the country. It is a popular source of digestible protein, vitamins and minerals. Studies have recommended the consumption of stockfish as source of omega-3 fatty acids which have some health benefits [3]. The most commonly sold species is cod (*Gardus morhua*) but others such as Ling (*Molva molva*), Haddock (*Melaogrammus aeglefinus*) and Tusk (*Brosme brosme*) are also found. Studies have reported that despite the quality control conducted by trained stockfish experts in the exporting countries, quality defects difficult to detect when the fish is dried may be observed when rehydrated [2]. The stockfish experts ensure that quality and food safety standards are maintained while low quality stockfish are used as supplemental food for pets [1]. Nigeria is one of the largest fish importing countries in Africa with per capita consumption of 7.5kg per annum. The total consumption was estimated to be 1.2 million metric tons [4]. Assessment of potential health hazard in food is vital in order to evaluate the consequences of human actions and their impact on public health [4].

Metals could enter fish either directly through the digestive tract due to consumption of contaminated water and food,

or non-dietary routes across permeable membranes such as gills [4]. Studies have indicated that fish are able to accumulate and retain heavy metals from their environment depending on the duration of exposure, concentration, temperature, salinity, hardness and metabolism of the animals [5]. Stockfish are prepared immediately after they are caught and are hung on the wooden rack from february to may every year. Stable cool weather (just above zero degree Celsius) protects the fish from insects and prevents an uncontrolled bacterial growth. The climate in Norway is excellent for stockfish production [1-3]. After three months hanging on the hjell, the fish are then matured for another 2-3 months indoors in a dry and airy environment [1]. The stockfish are then sorted by quality and exported in bales to Nigeria and other west African countries. Rehydration is done using drinking water and are sold in the markets.

There are conflicting reports on the levels of toxic metals in this important protein source. Whereas some have reported high levels of toxic metals thereby suggesting it could be a potential source of toxicity to humans [6], others observed low to undetectable levels of these toxicants, indicating no risk status from its consumption [7]. It was however reported that because of poor managerial policies of aquaculture in Nigeria, the consumption of stockfish and other sea foods may pose a potential health risk to consumers [4,8].

More worrisome is the consumption of stockfish head bone, fins and gills. Because of the cost of stockfish is beyond the reach of the poor, many consumers eat the head parts which are cheaper. We hypothesized that Stockfish fins and gills may contain higher concentrations of toxic metals than the muscle tissues. Therefore, this study was designed

to evaluate some toxic metals levels in the body tissue, gills and fins of cod stockfish sold in 3 geopolitical zones namely, south-west, south-east and south-south of Nigeria.

MATERIALS AND METHOD

The study was conducted at the Department of Medical Laboratory Science, University of Benin between April and November 2015. A total of 30 stockfish were purchased from 3 different markets in Lagos (south-west), Benin(south-south) and Onitsha (south-east) of Nigeria.

Health Risk Assessment: The potential health risk of consuming stockfish contaminated with the measured toxic metals was calculated using the method previously described [9]. This was assessed by calculating hazard quotient (HQ) for each toxic metal in the stockfish parts purchased from the markets. Hazard quotient refers to the ratio of a toxic metal exposure level over a period of time to a recommended or reference dose (contamination) for that toxic metal derived from a similar exposure [9]. Hazard index is the sum of the calculated hazard quotients of the measured toxic metal. The Food and Agriculture Organization (FAO) estimated the per capital consumption of fish in Nigeria to be 7.5kg per person per annum, which translated to daily per capital of 0.021kg/day [10]. Therefore, the mean daily exposure of each toxic metal for Nigerian population was calculated thus:

$HQ = \text{Intake/Reference dose (mg/kg/day)}$.

$\text{Intake (mg/kg/day)} = CC \times Ir \times Fs \times FE \times ED / (BWT \times AT)$

Where, HQ = Hazard quotient (unitless)

CC = Concentration of contaminant in Stockfish

Ir = Ingestion rate (kg/day)

Fs = Fraction ingested from contaminated Stockfish (unitless)

FE = Frequency of exposure (day/year)

DE= Duration of exposure (year)

BWT= Body weight (kg)

AT = Average time (period over which exposure is average in days).

Hazard index (HI) = Sum of all HQs.

A conservative body weight of 70kg and duration of exposure of 70years were used for the calculation [11]. The following variables Ir, FS and FE were estimated to be 0.02055kg/day, 100% and 365days/year respectively. Bioavailability factor (Fs) refers to the percentage of the total amount of toxic metals ingested that ultimately reached the blood stream and is capable of possibly causing harm to consumers, which is assumed to be 1(100%) for the purpose of screening [8]. The period over which exposure is averaged in days (conservative time) was estimated to be 25550days which is 365days multiply by 70years. A HQ greater than 0.2, indicates potential risk to human health while HI less than 1.0 indicates no adverse health effect.

But if intake is more than the oral recommended reference dose (RfD) and HI is ≥ 1.0 , it may indicate adverse health effects.

Sample Preparation: The fish muscles, fins, heads and gills were carefully removed with a plastic knife and oven dried at 65°C for 12-24hrs to obtain a constant dry weight of 1g from each specimen. The dried fish samples were homogenized using clean mortar and pestle. The mortar and pestle were soaked overnight in 20% (v/v) HNO₃ and rinsed with several changes of Milli-Q® water.

Digestion of Samples: Ten milliliters (10mL) of perchloric acid, nitric acid and sulphuric acid mixture in a ratio of 1:2:2 was poured unto the sample and heated on a hot plate in a fume cupboard. The mixture was heated until a white fume was observed which indicated that the digestion was complete. A triplicate of each sample was digested according to FAO/SIDA manual part 8 [12].

Determination of toxic metals: After digestion of the samples, the toxic metals cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) were initially analyzed using atomic absorption spectrophotometer (AAS) to ascertain the approximate levels of the metals. Inductively Coupled Plasma -Mass Spectrophotometer (ICP-MS) was there after used to determine the levels of the measured toxic metals. The range of the calibration curves used was 0-100mg/dL

ICP-MS is capable of detecting metals and several non-metals ions at concentrations as low as one part in 10¹² (part per trillion). Inductively coupled plasma is plasma that is energized (ionized) by inductively heating the gas with an electromagnetic coil, and contains a sufficient concentration of ions and electrons to make the gas electrically conductive. Quantification of an element in a sample is achieved with the inductively coupled plasma and then using a mass spectrometer to separate these ions according their mass to charge ratio and a detector receives an ion signal proportional to the concentration of the ions.

The instruments were calibrated using the standards of various concentrations.

Quality Control of Assay

Analytical grade of reagents was used throughout the research process. Certified reference materials (CRMs) from Le Centre de toxicologie du Quebec were analyzed to ensure accuracy and precision of assays.

Before being used all volumetric polyethylene (including the auto-sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO₃ during 24 hr. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container.

Statistical analysis

Statistical analysis was done using the Statistical Package for Social Scientists (SPSS, Chicago, IL, USA) version 16.0. All values were expressed as Mean \pm Standard Error of the

Mean. Results from all the specimens were compared using ANOVA. Level of significance was set at ($p < 0.05$).

RESULTS

Table 1 shows the concentrations of measured toxic metals in fins of Stockfish purchased from 3 different cities in Nigeria varied with locations and the parts of the fish. The concentrations of toxic metals in fins of Stockfish purchased from Lagos were $Pb > Hg$ and As while Cd was below the detectable limit. The fins of Stockfish purchased in Benin has mean concentrations of $Pb > Cd > As$ while Hg was below the detectable limit. The fins of those purchased from Lagos had mean concentrations of $Pb > Cd > As > Hg$. The concentration of Pb was highest in fins of Stockfish bought from Onitsha followed by Benin and Lagos and differences in the means were statistically significant ($P < 0.001$). The concentrations of Cd and As were below the limit recommended in food for human consumption (0.5mg/kg and 76mg/kg respectively).

The concentrations of measured toxic metals in muscle tissues of stockfish differ from one location to another (table 2). The mean concentrations of Pb were highest in the muscle tissue of stockfish from Onitsha followed by Benin and lowest in those from Lagos. The differences in the means was statistically significant ($P < 0.001$). The concentrations of Cd and As were below the concentration recommended in sea foods for human consumption while the concentration of Hg was above (0.001mg/kg) of intake

acceptable in food for human consumption.

The mean concentrations of Pb in gills were almost the same in the 3 locations, with those from Onitsha and Lagos higher than those purchased in Benin. The differences in the means were statistically significant ($P < 0.001$). The mean concentrations of Cd were highest in Lagos followed by Onitsha while those purchased in from Benin were below the detectable limit (table 3).

Table 4 shows the comparison of the mean concentrations of toxic metals in the different parts of Stockfish showed that gills contained the highest levels ($P < 0.001$) of Pb and Cd than fins and muscle tissues. The differences in means of Hg and As were statistically significant.

Table 5 shows the calculated intake estimate of toxic metals in the fins, muscle tissues and gills for the purpose of calculating the HQ and HI .

The calculated HQ for Cd was highest in gills followed by muscle tissue and fins. That of Pb was highest in the gills followed by fins and muscle tissues. While that of Hg was evenly distributed between the gills, muscle tissues and fins. The HQ of As was the same in gills and muscle tissues which was higher than in the fins. The HI was highest in the gills (17.4) followed by muscle tissues (13.5) and fins (12.0) (table 5).

Table 7 indicates the maximum acceptable limits of measured toxic metals according to international health organizations.

Table 1. Comparison of mean levels of toxic metals in the fins of stockfish purchased from markets in 3 different cities in Nigeria.

| Toxic metal (mg/kg dried weight) | Mean \pm SEM | | | P - Value |
|-------------------------------------|------------------------------|------------------------------|------------------------------|-----------|
| | Lagos | Benin | Onitsha | |
| Cadmium | 0.00 ^A \pm 0.00 | 0.16 ^B \pm 0.01 | 0.15 ^B \pm 0.00 | 0.0001 |
| Lead | 0.77 ^A \pm 0.02 | 0.49 ^B \pm 0.02 | 1.07 ^C \pm 0.02 | 0.0001 |
| Mercury | 0.01 ^A \pm 0.00 | 0.00 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.90 |
| Arsenic | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.02 ^A \pm 0.00 | 0.90 |

Superscript A and B are homologous subsets, with A,B and C showing the degree of closeness.

Table.2. Comparison of mean levels of toxic metals in the muscle tissue of stockfish purchased in markets from 3 cities in Nigeria.

| Toxic metal (mg/kg dried weight) | Mean \pm SEM | | | P - Value |
|----------------------------------|------------------------------|------------------------------|------------------------------|-----------|
| | Lagos | Benin | Onitsha | |
| Cadmium | 0.26 ^B \pm 0.02 | 0.07 ^A \pm 0.04 | 0.30 ^B \pm 0.01 | 0.0001 |
| Lead | 0.60 ^A \pm 0.03 | 0.72 ^B \pm 0.02 | 0.92 ^C \pm 0.03 | 0.0001 |
| Mercury | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.90 |
| Arsenic | 0.02 ^A \pm 0.00 | 0.03 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.80 |

Superscript A and B are homologous subsets, with A,B and C showing the degree of closeness.

Table 3. Comparison of mean levels of toxic metals in the gills of stockfish purchased from markets in 3 different cities in Nigeria.

| Toxic metal (mg/kg dried weight) | Mean \pm SEM | | | P - Value |
|-------------------------------------|------------------------------|------------------------------|------------------------------|-----------|
| | Lagos | Benin | Onitsha | |
| Cadmium | 0.35 ^B \pm 0.03 | 0.00 ^A \pm 0.00 | 0.27 ^B \pm 0.01 | 0.0001 |
| Lead | 0.97 ^C \pm 0.04 | 0.65 ^A \pm 0.09 | 0.97 ^B \pm 0.03 | 0.0001 |
| Mercury | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.00 ^A \pm 0.00 | 0.0001 |
| Arsenic | 0.03 ^A \pm 0.00 | 0.02 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.0001 |

Superscript A and B are homologous subsets, with A,B and C showing the degree of closeness.

Table 4. Comparison of mean levels of toxic metals in fin, muscle tissue and gill of stockfish purchased from 3 different cities in Nigeria.

| Toxic metals (mg/kg dried weight) | Mean \pm SEM | | | P - Value |
|--------------------------------------|------------------------------|------------------------------|------------------------------|-----------|
| | FIN | BODY | GILL | |
| Cadmium | 0.16 ^A \pm 0.01 | 0.21 ^B \pm 0.02 | 0.31 ^C \pm 0.03 | 0.001 |
| Lead | 0.78 ^B \pm 0.03 | 0.75 ^A \pm 0.03 | 0.86 ^C \pm 0.03 | 0.001 |
| Mercury | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 0.01 ^A \pm 0.00 | 1.00 |
| Arsenic | 0.01 ^A \pm 0.00 | 0.02 ^A \pm 0.00 | 0.02 ^A \pm 0.00 | 0.16 |

Superscript A and B are homologous subsets, with A,B and C showing the degree of closeness.

Table 5. Estimated intake of toxic metals in fin, muscle tissue and gill of stockfish purchased from 3 different cities in Nigeria based on per capital consumption of 7.5kg/person/year

| Measured toxic metals (mg/kg/day) | Fin | Muscle tissue | Gill |
|--------------------------------------|--------|------------------|--------|
| Cadmium | 0.0048 | 0.0063 | 0.0093 |
| Lead | 0.0234 | 0.0225 | 0.0258 |
| Mercury | 0.0003 | 0.0003 | 0.0003 |
| Arsenic | 0.0003 | 0.0006 | 0.0006 |

Table 6. Hazard Quotients and Hazard indices of measured toxic metals in Fin, Muscle tissue and Gill of Stockfish purchased from 3 different cities in Nigeria.

| Measured toxic metals | Fin | Muscle tissue | Gill |
|-----------------------|------|------------------|------|
| Cadmium | 4.8 | 6.3 | 9.3 |
| Lead | 6.6 | 6.3 | 7.2 |
| Mercury | 0.3 | 0.3 | 0.3 |
| Arsenic | 0.3 | 0.6 | 0.6 |
| Hazard Index | 12.0 | 13.5 | 17.4 |

Table 7. Maximum acceptable limits of the measured toxic metals in Seafood and fish

| Measured toxic metals | Maximum limit(mg/kg) | References |
|-----------------------|-------------------------|-----------------------|
| Cadmium | 0.5 | FAO/WHO(1983) [13] |
| Lead | 0.5 | FAO/WHO(1983) [13] |
| Mercury | 0.001 | FAO/WHO(1983) [13] |
| Arsenic | 76 | USFDA(1993)[14] |

DISCUSSION

The data presented in this study indicated that the concentrations of measured toxic metals differ from one part of the stockfish to another. The concentrations of metals were highest in the gills than body tissues and fins. The toxic assessments indicated by the high levels of HQs and HI which were higher in the gills and body tissues and fins. It was suggested that $HQ > 0.2$ could pose potential health risk to humans [11,15]. From the data presented above, all the calculated HQs for the measured toxic metals ranged from 0.3 to 9.3. The observed levels of toxic metals are consistent with that reported in some species of fish [15]. The accumulation of high levels of toxic metals in food may result in serious system health effects, hence various organizations and regulatory bodies of some countries have established the maximum limits of concentrations of toxic metals that could be acceptable [16-17]. Previous studies have reported lower concentrations than we observed in some species of stockfish [7,18-19]. It was observed that cod and similar species can accumulate toxic metals which could be hazardous to consumers [19]. They reported lower levels of toxic metals and concluded that the observed levels though low, were higher than the maximum limit recommended by the regulatory bodies. Our observation completely disagreed with that reported by Eze and Ogbuehi [7]. Who reported that Cd, Pb, Hg and As were below detectable limit (0.001mg/kg) and concluded that the health assessment does not suggest risk status from their consumption. Even though the above mentioned studies evaluated toxic metal concentration in body tissue of stockfish, the concentrations we observed in this present study were higher and could represent potential health risks in consumers. More so, the habit of consuming head bones and gills should be discouraged because of the

higher concentrations of Cd and Pb, high HQ and HI in the gills. Higher concentrations of Cd and Pb were previously reported in the gills than muscle tissues of other species of fish [20]. The higher toxic metal levels observed in gills may be due to the important roles gills play in the normal physiology of aquatic animals. The gills are the sites of gas exchange, ion regulation, acid balance and waste excretion [21-22]. Studies have shown high levels of toxic metals in other species of fish commonly consumed in Nigeria [8,20]. Babatunde et al. [8] reported that the concentrations of nickel and chromium found in 5 different species of fresh water fish were higher than the maximum permissible limits for human consumption but the levels of cd, Pb and cobalt were lower. Other sources of contamination could be through air, environment and food.

Toxic metal levels observed in the fins and body muscle of stockfish ranged between 0.01-0.02 for Hg and 0.01-0.03 for As which were within the maximum permissible limit by WHO and FAO as suggested by WHO/FAO [13]. The mean Pb levels ranged between 2.31-9.73mg/kg which were above the safe limit set by WHO/FAO [13] and USFDA[14](Table 6). The bio-toxicological effects of heavy metals refer to the harmful effects of toxic metals to the body when consumed above the recommended limits which may lead to learning disabilities, impaired protein and hemoglobin synthesis, severe anemia, renal failure depending on the toxic metal involved. Accumulation of Cd could lead renal impairment, fragile bones and male reproductive challenges [23].

CONCLUSION

The concentrations of Cd and Pb in fins, muscle tissues and gills of stockfish were higher than the maximum levels recommended by various regulatory bodies, while the concentrations of Hg and As were below the acceptable limits. Hazard quotients and HI of the measured toxic metals in gills were higher than muscle tissues and fins. There are indications of potential health risk to consumers. The general public should be aware of the potential health hazard of eating non-muscle tissues of stockfish.

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