ASSESSMENT OF HEAVY METALS AND POLYCYCLIC AROMATIC HYDROCARBON CONCENTRATION IN SOME STOCKFISH SPECIES SOLD IN FIVE MAJOR MARKETS OF ABA SOUTH, ABIA STATE, NIGERIA

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ABSTRACT

The concentration of ten heavy metals (Cd, Cu, Fe, Ni, Pb, Cr, As, Hg, Zn and Mg), and poly aromatic hydrocarbons (PAHs) were determined in two species of imported stockfish samples namely: *Gardus morhua* (Cod) and *Molva molva* (Ling) retailed in five major markets located at Aba South Local Government Area, Abia state, Nigeria. The chemical analysis was carried out with the aid of ASTMD Gas Chromatography (GC) for PAHs and Atomic Absorption Spectrometer (AAS) for heavy metal. Prior to the AAS analysis, appropriate weights of the dried pulverized fish samples were digested with perchloric acid, nitric acid and sulphuric acid in the ratio [1:2:2]. The accuracy and precision of the determination in the study were evaluated using APHA, AWWA, WEF and ACCU standard. The result of the analyses showed that the accumulation of PAHs in Cod and Ling stockfish were present in very small or minute values ranging from 0.001-0.030µg/kg or are below detection limit (BDL). On the other hand, the result from the analysis of heavy metal showed that Ni, As, Hg, Pb, Cd and Cr were below detection limit (0.001mg/kg), whereas Fe, Cu, Zn and Mn were present in high amount with the concentration levels of the element ranging from 62.35-89.74mg/kg, 0.96-1.88mg/kg, 14.22-26.47mg/kg and 2.18-5.14mg/kg respectively. Finally, results of the potential health risk assessment showed that the total health hazard index of all the toxicants indicated no risk status from the consumption of the stockfish species studied.

INTRODUCTION

Stockfish is unsalted fish dried by cold air and wind on wooden racks on the foreshore, called 'hjell' (Kurlasky, 2014). Stockfish commonly referred to as "Okporoko" among the Igbos in Nigeria, is an important source of vitamin and protein which plays essential role in human development. Cod (*Gardus morhua*) is the most common fish used in stockfish production, while others such as Ling (*Molva molva*), Haddock (*Melaogrammus aeglefinus*), Tusk (*Brosme brosme*) etc. are used to a lesser degree (Koster *et al.*, 2009). The consumption of stockfish has been highly recommended because they are good sources of omega-3 fatty acids and other minerals associated with the health benefits due to their cardio-protective effect (Brawn, 2011).

However, the level of contamination in these stockfish and the poor managerial policies of our aquaculture are of particular interest, because of the potential risk to humans who consumes those (Ukoha *et al.*, 2014). The aquaculture industries are exposed to many chemical, biological and other pollution, mining, drilling and dumping of industrial wastes which have induced high level of polyaromatic hydrocarbons (PAHs) and heavy metals contamination in stockfish which may cause toxicity to humans (Amos-Tautau *et al.*, 2013).

Table 1: Common species of stockfish(Kurlansky, 2014)

S/N	Stockfish	Scientific name	English Name	Local name
1.	Cod	*Gadus morhua*	Cod	Okporoko
2.	Apama	*Sepia species*	Apama	Okporoko
3.	Haddock	*Melaogrammus aeglefinus*	Haddock	Okporoko
4.	Tusk	*Brosme brosme*	Tusk	Okporoko
5.	Ling	*Molva molva*	Ling	Okporoko

MATERIALS AND METHOD

This work was carried out at Golden Years Limited, Shell Agbada II F/S Road, Igwurunta, Port Harcourt, Rivers State from October, 2014 to December, 2014.

Sample collection: Specimens of two popular species of imported stockfish namely, Cod (*Gadus morhua*) *and* Ling *(Molva molva*) were purchased from retailers in five popular markets (Ahia Ohuru, Nkwo Ngwa, Ariaria, Shopping centre and Cemetry) in Aba south of Abia state, Nigeria. These species were imported from Norway into Nigeria and highly cherished as sources of protein especially by the "Igbos" of Eastern Nigeria. From each species, two stockfish were randomly collected and transported in sterile polyethene bags to the laboratory.

Sample preparation: The samples were separately dried in a laboratory oven at $65^{O}C$ for 12 hr to obtain a constant dry weight of 0.5g from each sample. Then dried samples were each ground to powder, using laboratory ceramic mortar and pestle, and sieved with 2mm sieve.

ANALYSIS OF HEAVY METALS

Digestion for Heavy Metals

Apparatus: Hot plate; 250cm^3 Pyrex conical flask; 100cm^3 volumetric flask; and Furnace
Reagents: $HClO_4$; HNO_3; and H_2SO_4

Digestion of sample: 10cm^3 of the acid mixture in the ratio (1:2:2) $HClO_4$, HNO_3, and H_2SO_4 was added into the sample and heated on a hot plate in a fume hood. The mixture was heated until a white fume was observed which signified that digestion was complete.

Determination of Heavy Metals: After the digestion the sample was allowed to cool and 20cm^3 of distilled water was added to bring the metals into solution. Sample was allowed to cool to room temperature and filtered using ashless Whatman filter into a 100cm^3 volumetric flask and made to mark with distilled water for AAS analysis (AAS Phoenix 986, Biotech, UK) (APHA, AWWA and WEF). Analysis of Hg and As were carried out by means of Cold Vapour and Hydride ion generation respectively.

Table 3: Instrument Conditions/ Operating parameters of AAS machine

S/N	Element	Operating Current (mA)	Maximum Current (mA)	Sensitivity Line (nm)
1	Zinc	6	15	213.9
2	Iron	8	25	248.3
3	Chromium	5	20	357.9
4	Cadmium	4	15	228.8

5	Nickel	8	25	232.2
6	Lead	-	10	329.8
7	Mercury	-	8	253.7
8	Copper	-	15	324.7
9	Manganese	-	20	279.5
10	Copper	8	15	193.7

ANALYSIS OF POLY AROMATIC HYDROCARBONS (PAHs)

Procedure for PAH Analysis (ASTM 4657-92): 5g of the sample was weighed into an extraction bottle and 20ml of dichloromethane was added and sonicated in an ultrasonic sonicator for 2 hrs. The extract was concentrated to 20cm^3 in a rotary evaporator. 20ml 0.5 KOH in 100ml of methanol was added and the mixture was refluxed for 1hr in a water bath at 60^{o}C. 20cm^3 Deionized water was added and extracted with hexane (20cm^3). The extract was dried over anhydrous sodium sulphate and the extract was concentrated at 60^{o}C in a rotary evaporator to 20cm^3. The extract was passed through silica gel column which had been pre-conditioned with hexane. The extract was eluted with 20cm^3 of hexane for aliphatic fractions. To some column, 20cm^3 dichloromethane was added for the elution of PAHs and the eluent was concentrated to 1cm^3 and solvent exchanged with 1cm^3 of actonitrile. 1µl of the extract was injected into a pre-programmed HP 5890 G-FID. The concentration of the PAHs was calculated from the peak area of the calibration standards.

GC Operating Conditions for PAHs

- Initial oven temp-100^{O}C
- Initial hold time-0.5 min.
- Ramp – 15^o C/min to 200^{o}C/min to 300^{o}C
- Final oven temp- 300^{o}C
- Detector temp- 340^o C
- Injector temp- 250^o C
- Carrier gas- He
- Ignition gas- H_2 and air

RESULTS AND DISCUSSION

Results of Heavy metal analyses

The result of the concentrations of ten heavy metals in Cod and Ling stockfish species collected from the five major markets are shown as follows:

Table 4: Concentrations of heavy metals in the cod and lings stockfish samples from Aba in (mg/kg)

Parameter	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Nickel	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Iron	69.78	76.03	68.55	82.77	71.25	93.48	80.12	80.12	81.07	62.35
Lead	0.002	BDL	BDL	0.003	0.002	BDL	BDL	BDL	BDL	0.003
Chromium	0.004	BDL	0.003	0.005	0.006	0.003	BDL	BDL	BDL	0.008
Arsenic	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Mercury	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Copper	0.98	1.56	1.88	1.40	1.56	1.33	0.96	0.96	1.26	0.98
Zinc	14.22	18.49	19.27	16.38	18.23	20.44	20.07	20.07	20.28	16.38
Manganese	3.56	4.26	3.05	5.14	2.66	3.19	5.01	5.01	5.01	2.18
Cadmium	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

Key: BDL – Below Detection Limit (0.001mg/kg)

S1-NkwoNgwa cod, **S2-** AhiaOhuru Cod, **S3-** Ariaria, **S4-** Shopping centre Cod, **S5-** Cemetary Cod, **S6-** NkwoNgwa Lings, **S7-** AhiaOhuru Lings, **S8-** Ariaria Lings, **S9-** Shopping centre Lings, **S10-** Cemetary Lings.

Result of PAH analysis

The summary of the concentrations of various PAHs present in Cod and Lings stockfish samples collected from the five markets in Aba South of Abia state, Nigeria is shown in Table 5.

Table 5: Polyaromatic Hydrocarbon (PAHs) concentrationtockfishs of cod and lings s of samples in (µg/kg)

Parameter	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Naphthalene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2 Methylnaphthalene	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Acenapthylene	BDL	0.001	BDL	BDL	BDL	BDL	BDL	BDL	0.001	BDL
Acenaphthene	BDL	0.001	BDL	BDL	BDL	BDL	0.002	BDL	0.001	BDL
Fluorene	BDL	0.002	BDL	BDL	0.005	BDL	0.001	BDL	0.001	BDL
Phenanthrene	0.003	0.003	0.002	0.001	0.002	0.006	0.001	0.005	0.002	0.003
Anthracene	0.004	0.003	0.001	0.001	0.004	0.002	0.002	0.002	0.001	0.002
Fluoranthene	0.015	0.014	0.008	0.009	0.021	0.012	0.009	0.012	0.014	0.014
Pyrene	0.003	0.004	0.002	0.003	0.001	0.002	0.006	0.002	0.002	0.003
Benzo(a)anthracene	0.001	BDL	0.002	BDL	0.002	0.001	0.001	0.001	BDL	0.002
Chrysene	0.002	0.006	BDL	0.006	BDL	0.001	0.008	BDL	0.003	0.001
Benzo(b)fluoranthene	0.001	BDL	0.002	BDL	BDL	0.002	BDL	0.001	BDL	0.001
Benzo(k)fluoranthene	0.003	0.006	0.002	0.006	0.008	BDL	0.007	0.004	0.004	0.004
Benzo(a)pyrene	BDL	BDL	0.001	BDL	BDL	0.002	BDL	BDL	BDL	BDL
Dibenzo(a,h)anthracene	0.002	BDL	0.005	0.0003	0.006	0.001	BDL	0.002	BDL	0.002
Benzo(g,h,i)perylene	0.003	0.001	0.004	0.002	0.002	0.001	0.002	0.003	0.002	0.001
Indeno(1,2,3-d)pyrene	0.001	0.002	0.002	0.001	0.002	0.002	0.002	0.002	0.002	0.001
Total	**0.038**	**0.043**	**0.031**	**0.032**	**0.053**	**0.031**	**0.041**	**0.034**	**0.033**	**0.034**

DISCUSSION

Nickel (Ni), Iron (Fe), Lead (Pb), Chromium (Cr), Arsenic (As), Mercury (Hg), Copper (Cu), Zinc (Zn), Manganese (Mn) and Cadmium (Cd) were analysed and the levels of the heavy metals concentration were measured in each of the stockfish purchased from the Five markets in Aba South Local Government of Abia state, Nigeria using Atomic Absorption

Spectrophotometer. The results of the analysis were presented in Table 1 - 11. The concentration of Fe ranged between 62.35 - 89.74 mg/kg. The highest concentration, 62.35-93.48 mg/kg was recorded in Ling (*Molva molva*) with lowest 69.78-82.77 mg/kg been recorded in Cod (*Gardus morhua*). Fe is an essential element in human diet. It forms part of haemoglobin, which allows oxygen to be carried from the lungs to the tissues. Severe Fe deficiency causes anaemia in humans. Zn range was from 14.22 – 26.47 mg/kg which was below acceptable limit set as 30 mg/kg in stockfish by regulatory agencies (FAO, 1983). Cu ranged from 0.96 - 1.88 mg/kg which was slightly above the standard set as 0.1 mg/kg by the regulatory agencies (FAO, 1983). Mn concentration ranged from 2.18 – 5.14 mg/kg, thus is within the maximum acceptable limit set for stockfish at 5.0 mg/kg by regulatory agencies (WHO, 1985). All other trace elements such as As, Hg, Pb, Ni, Cd and Cr were below detection limit (0.001 mg/kg).

Table 6: Maximum acceptable limits of some Heavy metals in Stockfish

Heavy metal	Maximum limit	Reference
Fe	-	-
Mn	0.5	WHO (1985)
Cu	30	FAO (1983)
Zn	30	FAO (1983)
Ni	70-80	USFDA (1993)
Ca	-	-
Cd	0.5	FAO (1983)
Pb	0.5	FAO (1983)
Co	-	-
Cr	12-13	USFDA (1993)

The summary of the concentrations of various PAHs present in both Cod and Lings stockfish samples retailed in these 5 markets of Aba metropolis were shown in Table 12. All the 17 targeted PAHS were detected in insignificant quantity in both the Cod and Lings stockfish sold at the five markets sampled.

Finally, results of the potential health risk assessment showed that the total health hazard index of all the toxicants indicated no risk status from the consumption of the stockfish species studied.

CONCLUSION

This study has shown that concentration of some heavy metals such Fe, Mn, Zn and Cu in Cod and Ling stockfish (*Gadus mohua* and *Molva molva* respectively) sold in five major markets (Nkwo-ngwa, Ahia ohuru, Ariaria, Shopping centre and Cemetery) in Aba south of Abia State, Nigeria were high and as such exceeded the maximum permissible limits (FAO, UNEP, FEPA and WHO). On the other hand, the polyaromatic hydrocarbons were below the risk level, which indicates no risk status from the consumption of the stock fish species studied.

It seems evident from our results that the Cod and Lings stockfish sold in the five markets studied as presently sold and consumed by the people of Aba in Abia State, in South Eastern Nigeria may not create significant health risk and continuous monitoring will be necessary to forestall any significance increase in future.

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