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Evaluation of Heavy Metals in *Gadus morhua* and the Associated Cancer Risks

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Abstract: *Gadus morhua* is a good protein source but some methods of its preservation can be a source of heavy metals contamination. *Gadus morhua* samples were collected from nine major markets for the identification and quantification of heavy metals. An Atomic Absorption Spectrophotometer (AAS) from an analytical and consultancy laboratory in the Department of Soil Resources Management, University of Ibadan was used for the heavy metal analysis after digesting the sample. The mean level of concentrations of the carcinogenic metals (Cd, Cr, Co, Pb, As, and Ni) identified are 0.110, 0.217, 0.440, 0.716, 0.574, and 0.284 mg/kg respectively, while for non-carcinogenic metals; Mn, Zn, Fe, and Cu the mean concentrations are 0.267, 0.636, 0.024 and 0.937 mg/kg respectively. Health risks for both adults and children were examined, which revealed that the risks for adults are greater than those for children. Also, the lifetime cancer risk was found to be higher than the recommended lifetime cancer risk. The mean level of concentrations of, Zn, Fe, Cu, Cd, Co, and As were lower than the recommended values; Ni is the same as the recommended value while the mean concentrations of Mn, Cr, and Pb are higher than their respective safe limits. The high concentrations of the carcinogenic metals Cr and Pb in *Gadus morhua* indicate therefore that consumption of *Gadus morhua* poses health risks to the public.

Keywords: *Gadus morhua*, Cancer Risk, Spectrophotometer, Exposure, Heavy Metals, Public Health

Introduction

Fishes are commonly consumed around the world; they are major sources of important nutrients such as protein, essential fatty acids, minerals, and vitamins. Protein is an essential part of human daily diet as it is necessary for body building and growth.

In human diets, various types of fish are consumed because these provide the necessary nutrients required in the body. In Nigeria, fish is one of the most consumed animal-source food (Byrd *et al*., 2021) and some of the commonly consumed types are Sardine, Croaker, Hake, Bonga, Catfish, Mackerel, Crayfish, Tilapia, Moonfish and Codfish. Codfish are obtained from Nigerian markets either as frozen or dried (known as stockfish) fish. Stockfish are either found as whole fish or already split along the backbone but joined by their tails.

Stockfish are found on both sides of the North Atlantic. They are mostly from the Scandinavian; they originate from Norway and are exported to different parts of the world. The Norwegian wild-caught stockfish are saltwater cod fish, naturally dehydrated with no added salt or preservatives (Song *et al*., 2009; Corby, 1999). Usually, cleaned fresh fish (head and entrails removed) are hung on wooden racks to dry in natural fresh cold air Fig. (1) for up to three months.

Fig. 1: Stockfish being air-dried for preservation

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The process then continues indoors for another 4-12 months. For the best quality, the space where the fish is stored must always be kept dry and have fresh air circulating, according to the processing protocol (Vrhovnik *et al*., 2013; Paul *et al*., 2019). The most common stockfish species are the cod family *Gadus morhua* and *Gadus* aeglefinus and others. Stockfish is said to be the richest source of protein, Calcium, Iron, Vitamins, and omega-3 fatty acids making it one of the highest protein-rich foods (Ogungbemi *et al*., 2022). One kilogram of Stockfish is said to be equal to approximately five kilograms of fresh fish (nutritionally) as water loss accounts for the difference in weight while all the nutrients are retained. Although the origin of Stockfish is not Africa, consumption of Gradus morhua is very popular in Africa, especially in Nigeria. It is prepared in different ways in different Nigerian delicacies.

Like all fishes, stockfish are susceptible to contaminants via various sources more so that they are exported and not consumed fresh. Contaminants are of either natural or man-made sources and these can elevate the concentrations of toxic metals in the aquatic environment (Murtala *et al*., 2012; Eke *et al*., 2008) and contaminants from polluted water can get into aquatic life through diffusion. Research works have established the fact that fishes are able to absorb and accumulate heavy metals from their ecosystem. Factors affecting the levels of accumulation of contaminants include duration of exposure, concentration of contaminant, temperature, salinity and hardness of natural habitat, and metabolism of fish (Eke *et al*., 2008).

The source of contamination for stockfish exceeds their natural habitat, the environment for preservation, storage, and markets is also a source of contamination. During preservation, storage, exportation, and before being sold from the markets, environmental contaminants can easily attach themselves to the fish.

Common varieties of stockfish have been reported to be common pests of dried fish in Nigeria (Greenfield and Southgate, 2023). Nigeria is a tropical country with an annual average temperature of 26.9°C and 88% humidity around Lagos. This climatic condition is conducive to the breeding of fish insects. Fish vendors sometimes spray chemicals directly on stockfish to control the growth of these insects as insects reduce the quality of fish and subsequently the profits of the vendors. The application of chemicals on fish is a potential source of heavy metals exposure via foods. Therefore, it is important to assess the levels of toxic metal contamination in *Gadus morhua* sold in the market. This study therefore aims at identifying and quantifying different heavy metals in *Gadus morhua*.

Materials and Methods

Selecting a Template

These stockfish are as shown in Fig. (2). They come in different sizes, shapes, and weights, some already sliced into pieces, and the heads are also packed in different forms; hence all the parts are consumable.

Sampling and Preparation Techniques

Five samples each were collected from nine (9) major markets namely: Oyingbo, Jankara, Sandgross, Mushin, Yaba, Agege, Oshodi, Isolo, and Agboju markets at the pick hours of the market, this will enable the best of the fish to be collected. Since they come in dry form; the samples were pulverized using porcelain mortar and pestle. They were then sieved with a 1mm sieve so that they became homogenous samples.

Sample Analysis/Absorption Spectrometry

Samples pulverized were digested with the aid of concentrated hydrogen-trioxide-nitrate acid and hydrogen peroxide in a ratio of 1:1 (*v*/*v*) as recommended by FAO (Little *et al*., 2002). One gram of powdered sample was weighed and into a 250 mL round bottom chemical flask, then a mixture of 0.001L of the concentrated nitric acid (75%) with 0.00l mL of H_2O_2 (25%) was added.

Watch glass was used to cover the flask till vigorous reactions occurred. This was gently heated on a hot plate to about 130˚C till the volume was reduced to 3-4 mL and allowed to cool. The solution was filtered and then diluted up to 50 mL in a volumetric flask with deionized water. The resulting digests were kept in covered plastics until the time for analysis for toxic metal using an Absorption Spectrophotometer (AAS) from Perkin-Elmer model 306 Atomic.

Health Risk Parameters

The associated health risk indicators were evaluated using the concentrations of metals obtained from AAS analysis of the stockfish. These parameters are daily Ingestion (daI), hazard Quotient (hzQ), risk index (riX), target cancer Risk (tcR), and lifetime cancer Risk (ilcR).

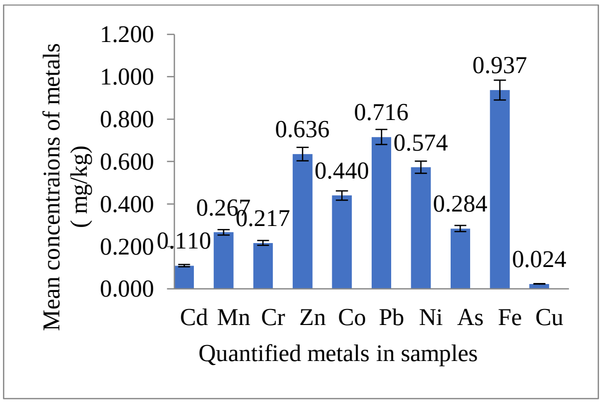

Fig. 2: Showing mean concentration of quantified metals in the sample

Daily Ingestion (daI)

Daily ingestion (*daI*) is the amount of toxic metals intake per consumption of a daily recommended quantity of stockfish. (Paul *et al*., 2019; Corby, 1999) This is obtained by measuring the mean body weight (*Abw*) in kg, children are 15 kg, and adults 65 kg. The frequency of exposure (*feE*) to the toxic metal for both children and adults is 365 days/year. The rate of Ingestion (*raI*) for children is 250 mg/day and for adults 450 mg/day, and the exposure period (*exD*) is 12 years for children and for adults 70 years. However, the average time (*avT*) for carcinogenic in both children and adults is the same (365×30 days/year); for non-carcinogenic 365×12 days/year for children and for adults 365×70 days/year:

$$daI = \frac{A_c \times raI}{Abw}$$

A_c is the mean concentration of heavy metals (mg/kg); raI is the arithmetic mean of rate ingestion of metals per person taken to be $250\times 10^{-3}\ kg$ for children and $450 \times 10^{-3}\ kg$ for adults (Speedy, 2003; Doe *et al*., 2012) while the mean weight *Abw* is 35 kg for the children and 65 kg for the adults.

Hazard Quotient (HQ)

In estimating the hazard quotient due to heavy metals, USEPA recommended relationships between the parameters are as stated:

$$hzQ = \frac{feE \times exD \times raI \times Cof \times A_c}{Abw \times avT \times RfD} \times 10^{-3}$$

feE is the exposure rate (365 days/year); *exD* is the frequency of exposure (30 years for non-cancer risk as suggested by USEPA (World Health Organization and Food and Agriculture Organization)), *raI* is the ingestion rate which is 250×10^{-3} kg for the children and 450×10^{-3} kg for the adults. *Cof* whose value is 0.208 is the conversion factor and A_c is the concentration of the heavy metal.

Table (1) shows the reference oral dose *RfD* for different metals, oral reference dose *RfD* which is the estimation of the daily exposure over lifetime in $mgkg^{-1}day^{-1}$.

Risk Index (riX)

This a function of the hazard quotient and the arithmetic sum of all the individual metals identified:

$$riX = \sum_{n=1}^{n=K} hzQ_n$$

n is an individual metal identified in the samples.

Cancer Risk Targeted (TCR)

Important parameter for carcinogenic risk indication (USEPA, 2011).

$$tcR = \frac{feE \times daI \times raI \times Cps \times A_c}{Abw \times avT_c} \times 10^{-3}$$

Table 1: Reference dose and cancer slope factor value for evaluation associated health risk

Identify elements	Reference Dose RfD (mg\kgBw /day)	Cancer slope factor (*CST*) (mg\kg bw /day)$^{-1}$	References
Pb	3.5×10^{-3}	8.5×10^{-3}	Little *et al*. (2002)
Ni	2×10^{-2}	9.10×10^{-1}	Doe *et al.* (2012); Kumar and Thakur (2018)
Cd	1×10^{-3}	5.00×10^{-1}	
Cr	3×10^{-4}	5.00×10^{-1}	IRIS (Kumar and Thakur, 2018; Food and Agriculture Organization of the United Nations, 2011)
As	3×10^{-4}	1.5	U.S.E.P.A. (2016)
Mn	0.046		Kumar and Thakur (2018)
Fe	7.0×10^{-1}		USEPA (2012)
Co	4.3×10^{-2}		Nutrition Today (1998); Integrated Risk Information System (1988)
Zn	3.0×10^{-1}		
Cu	4.0×10^{-2}		USEPA (2012)

Cps-carcinogenic potency slope for the oral route (mg/kg bw/day)$^{-1}$ for different metals as shown in Table (1).

avT_c -averaging time of carcinogens.

Lifetime Cancer Risk (il)

The lifetime cancer risk is used to evaluate the probability of an individual developing cancerous cells due to that individual exposure to carcinogenic metals in a lifetime by consumption of stockfish over a lifetime:

$$ilcR = \sum_{n=1}^{n=K} (daI \times csT)_n$$

With *CST* as a cancer potency factor.

The allowable limits of *ilcR* for heavy metals are $10^{-6}<$ $ilcR <10^{-4}$. It has been recommended by the US EPA that the safe limit for cancer risk is below about 1 chance in 1,000,000 lifetime exposure ($ILCR <10^{-4}$).

Results and Discussion

Mean concentrations levels of each of the 10 metals in *Gadus morhua* were as reported in Fig. (3) with a marginal error of about 5%. The highest concentrations are found in Fe, Pb, and Zn. While the lowest values obtained are in Cu and Cd.

These metals are classified into carcinogenic and non-carcinogenic metals for easy analysis of the risk indices and the hazard quotient. Cd, Cr, Co, Pb, Ni, and as are the carcinogenic metals found in the samples; and the mean concentrations of these metals are 0.110, 0.217, 0.440, 0.716, 0.574, and 0.284 mg/kg respectively. Non-carcinogenic metals detected in the samples are Mn, Zn, Fe, and Cu with which mean concentrations are 0.267, 0.636, 0.937, and 0.024 mg/kg respectively. The results of the concentrations from the nine major markets are shown in Table (2).

Table 2: Concentration of toxic metals from the samples

	Concentrations of the elements in each sample (mg/kg)									
Samples	Cd	Mn	Cr	Zn	Co	Pb	Ni	As	Fe	Cu
SP1	0.194 ±0.08	0.312 ±0.11	0.072 ±0.01	0.691 ±0.19	0.378 ±0.18	0.753 ±0.31	0.723 ±0.20	0.568 ±0.13	1.485 ±0.21	0.023 ±0.01
SP2	0.159 ±0.10	0.302 ±0.08	0.308 ±0.12	0.703 ±0.15	0.609 ±0.21	1.043 ±0.02	0.724 ±0.17	0.257 ±0.09	1.168 ±0.22	0.029 ±0.01
SP3	0.132 ±0.07	0.327 ±0.17	0.216 ±0.10	0.825 ±0.12	1.229 ±0.39	1.021 ±0.11	0.761 ±0.21	0.401 ±0.11	0.989 ±0.21	0.012 ±0.01
SP4	0.146 ±0.09	0.421 ±0.19	0.277 ±0.09	0.653 ±0.21	0.122 ±0.07	0.248 ±0.14	0.446 ±0.07	0.322 ±0.02	0.939 ±0.23	0.041 ±0.11
SP5	0.087 ±0.01	0.206 ±0.01	0.287 ±0.09	0.543 ±0.16	0.459 ±0.15	0.932 ±0.11	0.681 ±0.19	0.311 ±0.10	1.064 ±0.31	0.018 ±0.01
SP6	0.107 ±0.03	0.341 ±0.11	0.202 ±0.12	0.329 ±0.08	0.394 ±0.07	0.603 ±0.21	0.720 ±0.21	0.262 ±0.07	0.121 ±0.10	0.006 ±0.01
SP7	0.032 ±0.01	0.162 ±0.13	0.191 ±0.02	0.402 ±0.07	0.302 ±0.16	0.426 ±0.10	0.512 ±0.21	0.134 ±0.02	0.721 ±0.21	0.029 ±0.01
SP8	0.021 ±0.07	0.064 ±0.04	0.181 ±0.01	0.941 ±0.41	0.028 ±0.08	0.701 ±0.21	0.021 ±0.01	0.019 ±0.01	1.005 ±0.22	0.031 ±0.01
Mean	0.110	0.267	0.217	0.636	0.440	0.716	0.574	0.284	0.937	0.024

Table 3: Health risk assessment parameters due to carcinogenic metals

Toxic metals	*hzQ* (adult) $x10^{-3}$	*hzQ* (children) $x10^{-3}$	*Targeted cancer risk* $x10^{-10}$	*daI* (mg/kg BW/day) $x10^{-3}$	Cancer risk $x10^{-3}$
Pb	0.687	0.304	0.065	5.113	0.044
Ni	0.096	0.043	4.581	4.096	3.728
Cr	2.428	1.073	0.189	1.548	0.774
As	3.184	1.408	2.059	2.030	3.046
Cd	0.369	0.163	0.056	0.784	0.392

Health risk parameters have been evaluated and presented in Table (2). Since both children and adults consumed *Gadus morhua*, the health risk assessments have been analyzed in this study for both children and adults.

The daily intake of each of the carcinogenic metals due to consumption of the nutritionally recommended amount of *Gadus morhua* has been shown in Table (3). The hazard quotient of these metals in adults has been found to be greater than those of children in each of the metals. The highest hazard quotient occurs in both the adults and the children, while the lowest hazard quotient was found in Ni.

The targeted cancer risk due to these metals is the risk of developing cancer in adults because adults are more prone to develop cancerous cells due to their exposure to carcinogenic metals in the consumption of *Gadus morhua*. However, one or more risk factors may increase the probability of developing cancer-related problems. In the sample studied, Cr is more likely to increase the risk of developing cancer while consuming *Gadus morhua* for a long period of time.

One of the most important health risk assessment parameters is the risk index Fig. (3) presents the risk indices in both adults and children.

From the nine major markets visited for sampling, the health risk index in adults is greater than that of the children in all the samples as indicated in Fig. (3). This may be the result of long-time consumption of *Gadus morhua* by adults as compared to the children, nevertheless, the cancer risk per the million populations still remained reasonable. Depending on the living habits of an individual, the rate of developing cancer due to *Gadus morhua* consumption may be accelerated by other factors such as smoking, etc., that may be habitual in an individual. Some of the targeted metals are agents of cancer hence the cancer risk due to these metals has been evaluated.

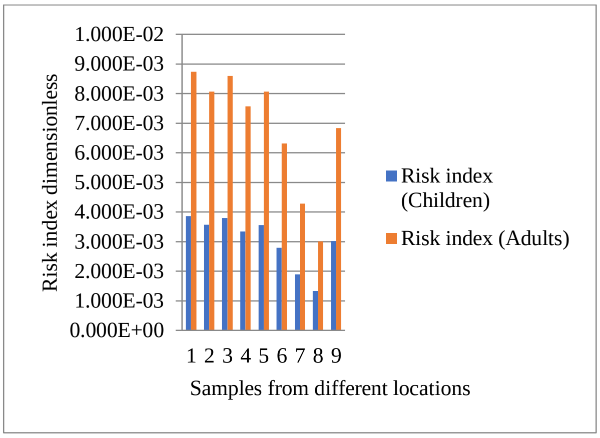

Fig. 3: Comparison of the health risk index in consuming *Gadus morhua* by both adults and children

From Fig. (4); Ni, has the greatest cancer risk in the samples, for the adult a value is 2.381E-10 for children 4.581E-10; while as has a value of 1.074E-10 for the adults and 2.059E-10 for the children. Cd and Pb both have the lowest value of cancer risk in this sampling. Fig. (5) shows the comparison of the incremental lifetime cancer risk due to the consumption of *Gadus morhua* for both the Adults and the children.

The lifetime cancer risk is an indicator of the probabilistic factors of cancer occurrences per a given population in an environment in a lifetime. The obtained values for the lifetime cancer risk in the case of the children are slightly higher than those of the adults in this study.

Nevertheless, the lifetime cancer risk is slightly higher than the recommended permissible values. The IAEA and WHO allowable value of the lifetime cancer risk for these carcinogenic metals are such that $10^{-6} < ilcR < 10^{-4}$. Here in this study, the mean incremental lifetime cancer risk obtained is in the 4.213×10^{-5} and 3.728×10^{-3}.

Various regulatory bodies in the world have levels of heavy metal tolerance in human food; this depends on the geochemical structure of the area of interest. For this reason; this study compared the level of heavy metal concentrations in *Gadus morhua* to that of the work done from different parts of the world and the results are as indicated in Table (3).

UNEP-United Nations Environmental Programme. IAEA-International Atomic Energy Agency

TFC-Turkish Food Codes. EC-European Commission. FAO/WHO-Food and Agriculture Organization/World Health Organization

For Cd, the obtained level of concentration is lower than most of the recommended values expected for TFC. In this research work the value of Mn concentration is higher than the tolerated value by UNEP, the Cr value obtained here is higher than the recommended value from FAO/WHO, Pb value obtained in this study is higher than most values of the regulatory bodies as seen in Table (3), while Ni value is about the same value with that of IAEA-407 recommended value.

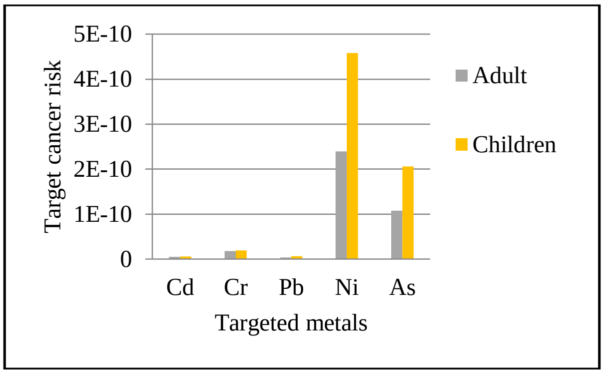

Fig. 4: Comparison of the carcinogenic risks due to targeted metals in *Gadus morhua* for both adults and children

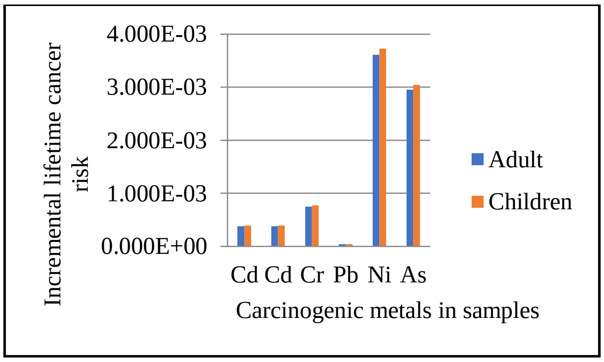

Fig. 5: Comparing the adult and children's lifetime cancer risk index in consuming *Gadus morhua*

Table 4: Shows the recommended levels of tolerance of some of these metals as recommended by various regulatory bodies from different parts of the word

Organization	Metals (mg/kg)										References
	Cd	Mn	Cr	Zn	Co	Pb	Ni	As	Fe	Cu	
FAO/WHO	0.50	0.50	0.05	5.00		0.50					World Health Organization and Food and Agriculture Organization (2003)
IAEA-407	0.18		0.73			0.12	0.60				Wyse *et al*. (2004)
TFC	0.05					0.20					T.F.C. (2002)
UNEP	0.30	0.02		5.00		0.30					I.A.E.A. (1985)
Directive 2005/78/EC	0.50					0.20					Commission Regulation (EC) Amending Regulation (EC) No 466/2001 as Regards Heavy Metals (Text with EEA Relevance) 2005
Present work	0.11	0.27	0.22	0.64	0.44	0.72	0.57	0.28	0.94	0.02	Present study

Conclusion

In the study on *Gadus morhua* a stockfish species widely consumed all over the world, both the carcinogenic metals and non-carcinogenic metals were identified and the levels of concentrations were analyzed. From these results, the levels of concentrations of some of these carcinogenic metals were far above the permissible levels recommended by UNEP and EC.

Comparing the cancer risk indicators in both the children and adults it was observed that the cancer risk index in children is far greater than in adults, thus adults may be advised to take less *Gadus morhua* than children. Another cancer risk indicator used is the lifetime cancer risk which is also slightly higher in *Gadus morhua* than the recommended values by USEPA one of the regulatory bodies especially in Nickel and Arsenide. Finally, *Gadus morhua* has some level of toxic metal contamination.

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Author's Contributions

Kayode Idowu Ogungbemi: Formulates the research work, supervises the data collection and drafts the first documents called the manuscripts.

Margret Bose Adedokun: Supervised sample collections and proofread the final write-up of the manuscript before submission.

Josephine Nneamaka Onyeka-Ubaka: Contributed to data analysis, software administrator and corrected the draft.

Ethics

There is no ethical issue with this study, because we have not use human or life animals for this study.

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