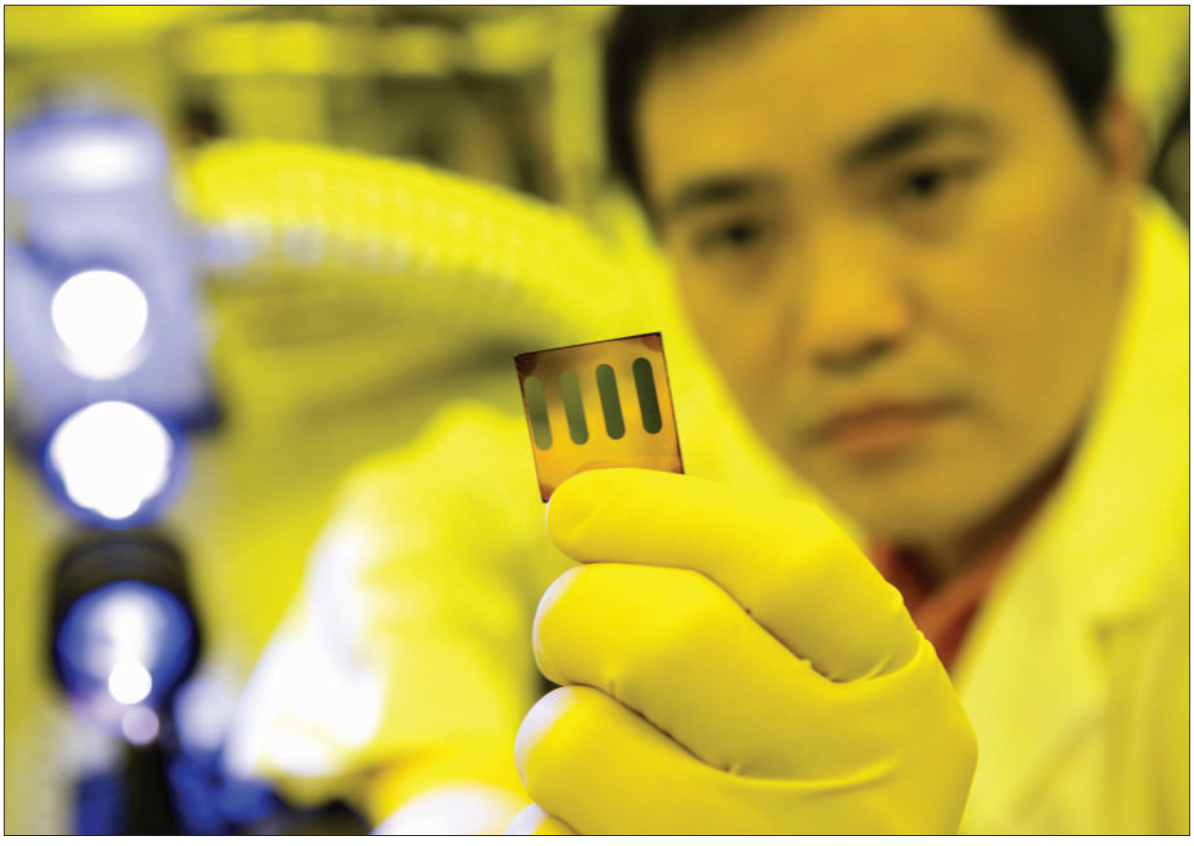

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Research Article
Heavy Metal Analysis of Consumer Fish Sold in Three Selected Markets in Awka, Anambra State, Nigeria

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Abstract

Background and Objective: There is a growing concern on the safety of consumer fish procured from various markets in Nigeria. This study investigated the concentrations of five heavy metals; cadmium (Cd), manganese (Mn), mercury (Hg) lead (Pb) and zinc (Zn) in the muscle of three types of consumer fish (smoked fish, fresh fish and imported frozen fish) sold in three selected markets in Awka, Anambra State. **Materials and Methods:** The samples analyzed were: Fresh fish (*Clarias gariepinus*), imported frozen fish (*Scomber scombrus*) and smoked fish (*Clarias gariepinus* and *Oreochromis niloticus*). The concentrations of the metals were quantified using a Varian AA240 fast sequential Flame Atomic Absorption Spectrophotometer (AAS). **Results:** The results obtained showed that metal concentrations in the muscles of the different fish samples were in the range of zinc, 0.660-6.830 mg kg^{-1}; manganese, 0.043-3.230 mg kg^{-1}; cadmium, 0.002-0.068 mg kg^{-1}; lead, 0.045-0.463 mg kg^{-1} and mercury, 0.097-0.403 mg kg^{-1}. This study revealed that cadmium, mercury and zinc in the fish samples were all below the limits recommended by Food and Agricultural Organization (FAO)/World Health Organization (WHO) except lead and manganese whose levels exceeded the FAO/WHO standard in smoked fish and fresh fish obtained from Nkwo Amenyi market. **Conclusion:** Fish sold in Awka metropolis is safe for human consumption except smoked fish and fresh fish procured from Nkwo Amenyi Market. This study recommends continuous monitoring and proper quality control of the consumer fish sold in Awka, Anambra State, Nigeria for public safety.

Key words: Heavy metals, consumer fish, markets, Awka, aquatic ecosystem, pollutants, public health

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Several water bodies throughout the world are being contaminated by anthropogenic activities. The pollutants are in form of pesticides, heavy metals, personal care products, pharmaceuticals etc. However, among these pollutants, heavy metals pose serious threats to the ecology of water body primarily influencing one of the major protein sources that is fish[1]. Heavy metal contamination of aquatic ecosystem constitutes a major public health problem[2]. Fish are one of the most widely distributed organisms in the aquatic environment and are considered as one of the main protein sources of food for human[3]. Nutritionally, fish consumption is widely encouraged due to its high content of omega-3-polyunsaturated fatty acid: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[4]. Fish provides an important source of protein, polyunsaturated fatty acids (PUFA), liposoluble vitamins and essential minerals, which are associated with health benefits and normal growth[5]. According to FAO statistics, fish accounted for about 16% of the global population's intake of animal protein and 6% of all protein consumed[6]. The American Heart Association recommended eating fish at least twice a week in order to reach the daily intake of omega-3 fatty acids[7].

Heavy metals are important group of chemical pollutants, whereby food is the main route for entry into human body; some heavy metals irreversibly are bound to human body tissues e.g. cadmium to kidney and lead to bones[8]. Fish constitute a major source of heavy metals in food[9]. High level of metals in the environment may lead to an excessive accumulation which cause problem to human, animals and plants[10]. Consequently, researchers and regulatory agencies carry out a periodic assessment of the aquatic ecosystem to ascertain the quality of fish consumed by the populace[11]. Anthropogenic activities continuously increase the amount of heavy metals in the environment, especially in the aquatic ecosystem[4]. Pollution of heavy metal in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem[12]. Increase in population, urbanization, industrialization and agricultural practices have further aggravated the situation[13,14]. The threat of toxic metals in the environment is more serious due to their non-biodegradable nature and their long biological half-lives when accumulated[15]. As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals[16,17] and thus causing heavy metal pollution in water bodies[12]. Heavy metals have the ability to bio accumulate and biomagnify in the food chain and finally be assimilated by human consumers resulting in health risks[18].

The objectives of this work was to identity and quantify the heavy metals in consumer fish sold in Awka main market, Nkwo Amenyi and Eke Amawbia markets in Awka and to detect whether the heavy metal contents are within the recommended safe limits for human consumption.

MATERIALS AND METHODS

Study area: The study was carried out in November, 2018 in the Applied Biochemistry departmental laboratory in Nnamdi Azikiwe University Awka by some staff of the Department of Fisheries and Aquaculture Management as a staff development project.

The study area lies in the tropical region of Anambra state of Nigeria. Awka is within the latitude 6°20` and 6°94`N of the equator and longitude 7°06` and 7°78` of the Greenwich meridian and flows Southwest direction (Fig. 1). Awka lies below 300 m above sea level in a valley on the plains of the Mamu river. Two ridges or cuestas, both lying in a North-South direction, form the major topographical features of the area. Wooded savannah grassland predominates primarily to the north and east of the city. The climate is the tropical rainforest zone of Nigeria and experiences two distinct seasons brought about by the two predominant winds that rule the area: the southwestern monsoon winds from the Atlantic Ocean and the northeastern dry winds from across the Sahara Desert.

The temperature in Awka is generally 27-30°C between June and December but rises to 32-34°C between January and April, with the last few months of the dry season marked by intense heat with annual relative humidity of 69-96%.

Sample collection: The consumer fish samples were procured from three selected markets in Awka, Anambra State of Nigeria. The three local markets that were selected for the study in Awka metropolis were Awka main market, NkwoAmenyi and Eke Amawbia markets.

The samples analyzed in this study were three consumer fish: fresh fish (*Clarias gariepinus*) imported frozen fish (*Scomber scombrus*) and smoked fish (*Clarias gariepinus* and *Oreochromis niloticus*). Three samples of each fish species were purchased directly from each of the selected markets making a total of twenty seven samples (Table 1). The fish are commonly consumed by the local populace in Awka

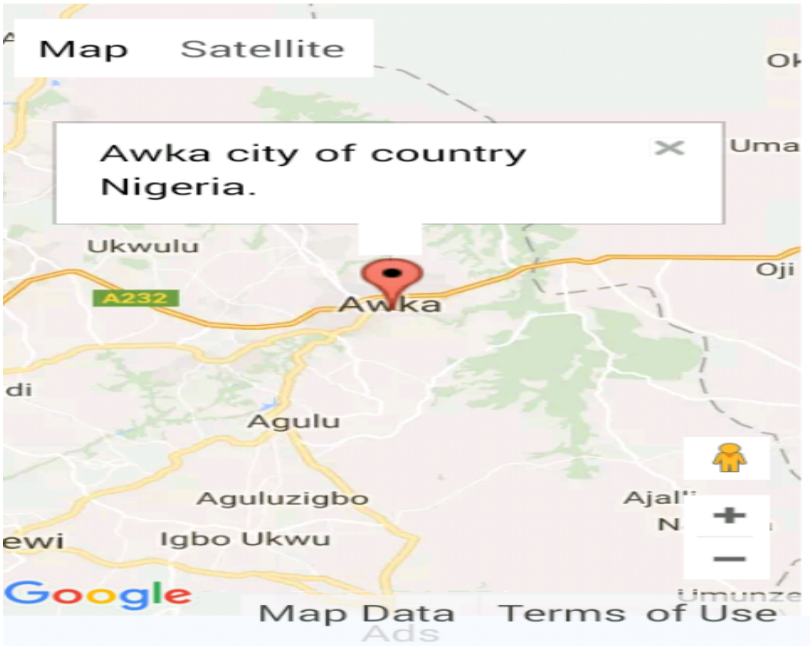

Fig. 1: Map showing the study area adapted from Google map

Table 1: Purchasing points of the fish samples

Market	Smoked fish	Imported frozen fish	Fresh fish
Awka main market	SF1 (*Clarias gariepinus*)	IF1 (*Scomber scombrus*)	FF1 (*Clarias gariepinus*)
Eke Amawbia market	SF2 (*Oreochromis niloticus*)	IF2 (*Scomber scombrus*)	FF2 (*Clarias gariepinus*)
Nkwo Amenyi market	SF3 (*Clarias gariepinus*)	IF3 (*Scomber scombrus*)	FF3 (*Clarias gariepinus*)

All imported frozen fish came from Chile, South America

metropolis. Fish were placed in isolated containers during transportation and immediately taken to the laboratory. The fish samples obtained from each of the markets are coded as follows (for identification purposes) in addition to the fish species and their sources.

Experimental work: Muscle tissue of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and it is the most edible part of the fish. A wet digestion method was used. The reagents used for the wet digestion of the fish samples were concentrated tetraoxosulphate (VI) acid (H_2SO_4) and Hydrogen peroxide (H_2O_2). The samples were collected and ground in electric blender and transferred into nine different beakers for each sample. Three grams of each samples were weighed and transferred into a boiling tube. Three milliliter of each conc. Tetraoxosulphate (VI) acid and hydrogen peroxide were added to each boiling tube using a pipette, the mixtures were allowed to stand for 10-15 min. The mixtures were swirled gently and allowed to digest on a heating mantle with the boiling tube clamped on a retort stand for 1 h at 80°C until the brown fumes disappeared and samples allowed to cool. The sample was further treated with 1 mL of hydrogen peroxide in order to obtain a clear sample digestion. The digested samples were filtered through Whatman filter papers and each solution made up to 25 mL mark with deionised water. One milliliter of the solution was injected into a Varian AA 240 Atomic Absorption Spectrophotometer and the reading for each of the metals was recorded at 540 nm.

Statistical analysis: Statistical data was analyzed to determine the differences in the heavy metal concentration of the fish samples of the three studied sites. This was done using two-way Analysis of Variance ANOVA. The statistical significance level was at 5%. $LSD_{0.05}$ was also run to ascertain the fish sample with the highest level of heavy metal content. All test procedures were performed in a computer program using Sigmaplot version 13.x for scientific graphing and data analysis.

RESULTS

The results of the three species of consumer fish sampled for heavy metals from three selected markets in Awka metropolis are shown in Table 2.

The smoked fish *Clarias gariepinus* (SF1) sampled from Awka main market contained all the tested heavy metals; Zn, Mn, Pb, Cd and Hg in the following increasing order; Cd, Pb, Mn, Hg and Zn ranging from 0.006±0.13 to 1.105±0.20 mg kg^{-1}. The smoked *Oreochromis niloticus* (SF2) from Eke Amawbia market also contained all the tested heavy metals in the following increasing order; Cd, Pb, Mn, Hg and Zn ranging from 0.025±0.10 to 0.660±0.10 mg kg^{-1}. The results also showed that smoked *Clarias gariepinus* (SF3) from Nkwo Amenyi contained all tested heavy metals in the following increasing order; Cd, Hg, Pb, Mn and Zn ranging from 0.020±0.10 to 6.189±0.30 mg kg^{-1}.

The imported frozen fish samples *Scomber scombrus* from Awka main market, (IF1) IF2 contained all the tested heavy metals in the following increasing order; Cd, Hg, Pb, Mn and Zn ranging from 0.019±0.02 to 8.820±0.10 mg kg^{-1}. Imported frozen fish sample 2 *Scomber scombrus* (IF2) from Eke Amawbia market contained all tested heavy metals in the following increasing order; Cd, Pb, Hg, Mn and Zn ranging from 0.009±0.10 to 0.890±0.10 mg kg^{-1}. Imported frozen fish sample 3 *Scomber scombrus* (IF3) from NkwoAmenyi market contained all tested heavy metals in the following increasing order; Mn, Cd, Hg, Pb and Zn ranging from 0.043±0.12 to 6.830±0.10 mg kg^{-1}.

Fresh *Clarias gariepinus* samples from Awka main market (FF1) contained all the tested heavy metals in the following increasing order; Cd, Pb, Hg, Mn and Zn ranging from 0.002±0.10 to 0.959±0.10 mg kg^{-1}. Fresh *Clarias gariepinus* samples from Eke Amawbia market (FF2) contained all tested heavy metals in the following increasing order; Cd, Hg, Pb, Mn and Zn ranging from 0.044±0.10 to 2.530±0.50 mg kg^{-1}. Fresh *Clarias gariepinus* samples from Nkwo Amenyi (FF3) also contained all tested heavy metals in the following increasing order; Cd, Hg, Pb, Mn and Zn ranging from 0.065±0.10 to 3.606±0.70 mg kg^{-1}.

The results of the heavy metal analysis of consumer fish samples from three markets in Awka showed that zinc levels were consistently higher in all fish samples than the other heavy metals tested.

DISCUSSION

Cadmium concentration in the analyzed fish samples ranged from 0.002 mg kg^{-1} in imported frozen fish procured from Awka main market to 0.068 mg kg^{-1} in smoked fish sold in the same market as shown in Table 2. The maximum permissible limit of cadmium in fish for human consumption specified by FAO/WHO is 1.0 mg kg^{-1}. The observed concentration of cadmium (0.002-0.068 mg kg^{-1}) in this study is lower than the maximum allowable limits. The samples analyzed in this study do not present any health risk considering cadmium contents since they contained lower amount of this metal than many other studies. The results obtained from these samples were slightly lower than those reported by other researchers[2,13]. Cadmium has been shown to accumulate mainly (about 75%) in kidney, liver and gills of freshwater fish but it can also be deposited in the hearts and other tissues and cause pathological changes of varying severity in the above-mentioned organs[14]. Moreover, cadmium inhibits calcium uptake in the gills and may alter the metabolism of essential trace element by affecting normal tissue distribution of trace elements such as zinc and copper[18].

The concentration of lead recorded in the fish samples is lower than the permissible limit of 0.4 mg kg^{-1} in fish except for smoked fish and fresh fish obtained from Nkwo Amenyi market. In this study, the levels of lead showed a similar trend compared to other findings[13,17,19]. Lead is a confirmed toxic metal with no known beneficial effect on the human body. Aquatic-organisms bio accumulate lead from water and diet,

Table 2: Concentration of heavy metals in the fish samples in mg kg^{-1} (Mean±standard error of mean)

Fish samples	Zinc	Manganese	Lead	Cadmium	Mercury
SF1	1.015±0.20^{a}	0.164±0.10^{a}	0.121±0.20^{a}	0.006±0.13^{a}	0.274±0.11^{a}
SF2	0.660±0.10^{a}	0.179±0.10^{a}	0.123±0.10^{a}	0.025±0.10^{a}	0.184±0.10^{a}
SF3	6.189±0.30^{a}	3.230±0.40^{b}	0.463±0.20^{a}	0.020±0.10^{b}	0.403±0.10^{a}
IF1	0.820±0.10^{a}	0.247±0.11^{a}	0.045±0.10^{a}	0.019±0.02^{a}	0.300±0.20^{a}
IF2	0.890±0.10^{b}	0.300±0.20^{a}	0.247±0.30^{b}	0.009±0.10^{a}	0.257±0.10^{a}
IF3	6.830±0.10^{a}	0.043±0.12^{a}	0.305±0.10^{a}	0.068±0.01^{a}	0.165±0.14^{a}
FF1	0.959±0.10^{a}	0.201±0.10^{b}	0.123±0.10^{a}	0.002±0.10^{b}	0.189±0.10^{a}
FF2	2.530±0.50^{a}	1.493±0.20^{a}	0.238±0.10^{a}	0.044±0.10^{a}	0.112±0.10^{a}
FF3	3.606±0.70^{a}	3.316±0.80^{a}	0.403±0.30^{a}	0.065±0.10^{a}	0.097±0.10^{a}
FAO/WHO LIMIT (mg kg^{-1})	50	0.6	0.4	1.0	0.5

Columns sharing similar superscripts are not significantly different at p>0.05, Fish samples: Refer to Table 1

although there is evidence that lead accumulation in fish is most probably originated from contaminated water rather than diets[8]. It should be noted that lead accumulates in human tissues and hence are harmful to human health.

The results obtained for manganese analysis were below the WHO (0.6 mg kg^{-1}) recommended limit of manganese in fish except in smoked fish and fresh fish procured from Nkwo Amenyi market with concentration value of 3.230 and 3.316 mg kg^{-1}, respectively. The results from these findings are in agreement with the findings of other researchers[20,18]. Manganese in trace amounts is an essential element. However, excess accumulation of manganese result in poisoning or toxicity in fish[4]. This is because they combine with body bio molecules such as metal-binding proteins and enzymes to form stable biotic compounds, thereby mutilating their structures and hindering them from performing designated functions within the body system of fish[9]. Manganese interferes with iron metabolism, especially haemoglobin formation.

Mercury was the least accumulated metal in the studied fish samples with smoked fish procured from Nkwo Amenyi market showing the highest concentration value of 0.403 mg kg^{-1} and least concentration value of 0.097 mg kg^{-1} was observed in fresh fish sold in the same market. The results obtained were below the FAO/WHO (0.5 mg kg^{-1}) permissible limit of mercury in fish. Hence, the result revealed that mercury does not present any health risk to the consumers of these fish species at the time of this study. Mercury concentration in the analyzed samples compared favourably with the results obtained in some similar studies by Daniel *et al.*[7] and Ogundiran *et al.*[20]. Mercury has several adverse effects on human beings which include tiredness, loss of appetite and weight loss. Kidney failure, muscular weakness and paralysis can result from acute intake[16]. The primary route of exposure for fish is through ingestion of contaminated food[10].

A critical look at the analytical results showed the ranges of zinc in the fish samples to be 0.660-6.830 mg kg^{-1}. Zinc concentration was highest in imported frozen fish procured from Nkwo Amenyi market and least in fresh fish obtained from Eke Amawbia market as shown in Table 2. Dominance of zinc in the studied fish samples agrees with the finding of other researchers[13,19,21]. The zinc value obtained compared favorably with that obtained for catfish[21]. The concentration of zinc found in all the fish samples studied were below the FAO/WHO (0.5 mg kg^{-1}) recommended limit of zinc in fish. Therefore, the concentration of zinc in the fish sample does not pose any health risk to fish consumers in Awka metropolis. This is because zinc is an essential trace metal and micro nutrient in living organisms, found almost in all cells and being involved in nucleic acid synthesis and occurs in many enzymes. Zinc is being involved in more complicated functions such as immune system, neurotransmission and cell signaling[1]. However toxic effects of zinc in human beings include anaemia, diarrhoea, fever, renal failure, reproductive failure, nephritis and extensive nephropathy[5].

CONCLUSION

This study revealed that the fish samples, smoked fish, imported frozen fish and fresh fish consumed in Awka, Anambra State, Nigeria contain heavy metals; mercury, lead, cadmium, mercury and zinc in varying concentrations. However, this study revealed that cadmium, mercury and zinc in the fish samples were all below the limits recommended by FAO/WHO except lead and manganese whose levels exceeded the FAO/WHO standard in smoked fish and fresh fish obtained from Nkwo Amenyi market. The observed high levels of lead and manganese in this study could be attributed to anthropogenic sources such as fossils fuel combustion, indiscriminate disposal of domestic and industrial effluents on land and water bodies.

SIGNIFICANCE STATEMENT

This study discovered that imported frozen fish (*Scomber scombrus*) procured from the three studied markets in Awka did not contain significant quantities of the tested heavy metals. This implies that proper fish inspection was carried out at the port of origin in Chile and port of entry in Nigeria, thereby certifying the fish fit for human consumption. This study will help the researchers to recommend as well as participate in more intensive checks on the sources of heavy metal contamination of locally smoked and fresh fish containing excessive levels of heavy metals especially lead and manganese. This will therefore encourage further studies on sources of heavy metal contamination and possible solution(s) to the problem of heavy metal contamination of aquaculture products.

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