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Human Health Risk Assessment of Heavy Metals (Lead, Cadmium and Copper) in Fresh Water Tilapia Fish (*Oreochromis niloticus*) from Eleyele River, Ibadan, Southwestern Nigeria

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Abstract Heavy metals concentrations such as lead (Pb), Cadmium (Cd) and Copper (Cu) were analyzed in muscles and gills of fresh water tilapia fish (*Oreochromis niloticus*) collected from Eleyele river, Ibadan, Nigeria using Atomic Absorption Spectrophotometer (AAS). The mean concentrations of Pb, Cd and Cu in the fish muscles were 6.69±3.89 mg/kg, 1.30±0.71 mg/kg and 1.30±0.45 mg/kg, respectively while the mean concentrations in gills were 5.16±2.40 mg/kg, 0.84±0.39 mg/kg and 2.12±1.27 mg/kg, respectively. Statistical analysis showed no significant difference ($p>0.5$) in the bioaccumulation of Pb and Cu contents in muscles and gills of the fish samples but showed significant difference ($p<0.5$) for Cd contents in muscles and gills. The mean concentrations of Pb and Cd exceed the Food and Agriculture Organization (FAO) permissible legal limits of fresh fish for human consumption in both muscles and gills. The presence of high contents of these heavy metals indicates that the river is highly contaminated. Thus consumption of fish or any aquatic animals from Eleyele river is dangerous to human health. Analysis of human health risks from consumption of fish also suggests that the general population is subjected to significant serious health hazard from ingestion of individual heavy metal and of course the sum of the metals together.

Keywords Human health risk; *Oreochromis niloticus* fish; heavy metals; muscles and gills; Eleyele river

1. Introduction

The pollution of the aquatic environment with heavy metals due to urbanization and industrialization has become a worldwide problem in the recent years. Heavy metals in aquatic environment and aquatic biota pose a risk to fish consumers and other wildlife. Heavy metals may enter aquatic ecosystem through different natural and anthropogenic sources including industrial or domestic sewage, agricultural activities, runoff, leaching from landfills/dumpsites and atmospheric deposits and cause water pollution which lead to fish contamination [1-3]. Although, essential metals must be taken up from food, water or sediment by fish for its normal metabolism or body function [4]. Heavy metals such as mercury, cadmium and lead have no biological or beneficial function in living body [5, 6] and can be highly toxic. Heavy metals can be accumulated by fish through both the food chain and water, and may pass it to human beings through food which result to acute and chronic diseases as fish are located at the end of aquatic food chain. Distribution of metals between the different tissues depends on the mode of exposure in term of dietary and/or aqueous exposure [7]. The release of industrial, agricultural and domestic wastes into aquatic ecosystems affect natural levels of surface water, sediment and biological organisms [8] such as fish.

Aquatic organisms accumulate metals to concentrate many times higher than present in water [9, 10] and can bioconcentrate metals at different levels in their body organs [11]. The process whereby an organism concentrates metals in its body from the surrounding media (air, water and soil/sediment) or food, either by absorption or ingestion is refers to as bioaccumulation [12, 13]. Fish can regulate metal concentrations to a certain limits after which bioaccumulation occurs [13, 14] and this varies from one organ to the other.

It has been shown that heavy metals accumulation in fish tissue is mainly depend on the concentration of metals in water and exposure period, with some environmental factors such as temperature, pH, salinity and hardness [15]. Also ecological needs, age, sex, size, feeding habits and biological conditions of fish affect the accumulation of heavy metals in their organ tissues [4, 15].

Fish, an aquatic animal are often at the top of the aquatic food chain and are known for their ability to bioconcentrate heavy metals in considerable amount in the tissues and recognized as good accumulators of organic and inorganic pollutants [6, 16]. It plays an important role in human nutrition and valuable and cheap food item and source of protein to man. Therefore, they need to be carefully screened to ensure that high levels of toxic heavy metals are not transferred to man through consumption [10, 17]. Eleyele river serves as a major source of potable water after purification in Ibadan and fishing is carried out daily on the river [10]. There is no formal control of effluent discharge from industries and homes into the river hence, important to monitor the levels of metals in the river using fish as biomonitor. Biomonitors give specific bioavailability of the pollutants level [15]. The aim of this study is to analyze the levels of heavy metals (Cu, Cd and Pb) in Tilapia fish (*Oreochromis niloticus*) of Eleyele river in Ibadan, Nigeria and compare the levels with the recommended limits. The human health risk assessment was also determined. The study is limited to edible organs (muscles and gills) as these are mostly consumed.

2. Materials and Methods

Eleyele river is a freshwater that is located at the north-eastern part of Ibadan, Nigeria. Ibadan is within latitudes E03°51′00″ and E03°52′30″ and longitudes N07°25′00″ and N07°26′30″ (Fig. 1). The river receives effluents discharged from the cassava processing site, waste water from domestic activities from the neighboring homes. The river is also dam for treatment to supply potable water to the people in Ibadan [18]. The river is also surrounded by small scales industries such as metals scrap dealers and mechanic workshops. Ibadan fall under tropical hinterland climate zone with 1000 to 1500 mm annual rainfall, temperature range of 21–25°C and relative humidity range of 50–80%. The dry season range between November and March, with December and January characterized by northeastern-southwestern dry, cold and dusty harmattan trade wind from the Sahara Desert while the rainy season range between April and October [19].

2.1. Sample Collection and Preparation

The fishes analysed were economic specie and procured from the fishermen at the shore of the river in July, 2016 (rainy season) and transported to the laboratory in an ice chest and stored inside freezer below 0°C before analysis. The average weight and length of the fishes were 67.5 g and 14.7 cm, respectively. All reagents used were of analytical reagent grade. All plastic and glass ware were thoroughly washed with detergent and cleaned by soaking overnight in 10% nitric acid solution and then rinsed thoroughly with distilled water before use. The stock solutions of metals (1000 mg/l) were prepared by dissolving appropriate salts of the corresponding metals and working standards were obtained from the stock by dilution of measured aliquots from the stock solutions.

2.2. Sample Analysis

The wet digestion method described by [20] involving 1:1 (v/v) concentrated nitric acid, HNO_3 and 30% hydrogen peroxide, H_2O_2 (30%) mixture was used. The fish samples were washed with distilled water and allowed to thaw or defrost at room temperature. Fishes were dissected with clean stainless steel instruments in the laboratory. Exactly 1.0 g fresh fish muscle and gill were weighed into separate beakers and 10 ml of freshly prepared nitric acid-hydrogen peroxide mixture added and covered with watch glass for initial reaction to subside. The beakers were then placed in a water bath on hot plate and the content heated for 2 hours at temperature of 160°C until the solution were clear and reduced to 2-5 ml. the digest were allowed to cool and then filtered through Whatman no. 42 filter paper into 50 ml standard flask and diluted to mark with distilled water. The procedures were carried out in duplicate. Procedural blank was also carried out. These digests were transferred into plastic sample bottles for heavy metals (Cu, Cd and Pb) determination using atomic absorption spectrophotometer (AAS) Perkin Elmer A Analyst 200 model and the results expressed as mg/kg wet weight of fish sample.

The statistical analysis of data was carried out using one way analysis of variance (ANOVA) to compare results which were presented as mean ± standard deviation and $p<0.5$ was considered to indicate statistically significant difference while significantly different means were separated using Duncan's Multiple Range Test.

Source: Google Earth Pro

Figure 1: Map of the study area showing Eleyele river

2.3 Human Health Risk Assessment

2.3.1. Estimated Daily Intake of heavy metals from fish

Heavy metals can enter human body through several pathways which includes food chain, dermal or skin contact and breathing or inhalation but all these are negligible relative to oral intake [21].

Estimated Daily Intake (EDI) (mg/kg body weight (bw)/day) of heavy metals from consumption of fish was obtained by equation below [22, 23]:

$$EDI = \frac{C \times F_{IR}}{B_{WA}}$$

where, C, F_{IR} and B_{WA} are the concentration of heavy metals in fish (mg/kg fresh weight, fw), average daily fish consumption/ingestion rate which is 40 g/person/day obtained from a dietary surveys in Nigeria as reported in literature [24-27] and body weight average of 70 kg, respectively [25].

2.3.2. Target hazard quotients (THQ) determination

Generally, there are two methods of estimating health risks. One is based on carcinogenic effects while the other is based on non-carcinogenic effects [28]. The THQ, which is the ratio between the exposure and the reference doses (RfDo) is used to express the risk of non-carcinogenic effects [28-30]. In other word, the THQ index can also be defined as the ratio determined dose of a pollutant to the reference dose [23].

Table 1: Oral reference doses (RfDo) of heavy metals [31]

Heavy metal	RfDo (mg/kg.day)
Pb	3.6×10^{-3}
Cd	5.0×10^{-4}
Cu	3.7×10^{-2}

Table 2: Standard assumptions for health risk calculation

Assumption	Reference
The ingested dose is equal to the absorbed contaminants or pollutants dose	[32]
Cooking has no effect on the pollutants toxicity in seafood	[28, 33]
The average adult body weights of a Nigerian is 70 kg	[25]
The average lifetime of a Nigerian is 70 years	

The methodology for the determination of THQ was described in the United States Environmental Protection Agency (USEPA) Region III risk-based concentration table [31]. The dose calculations were carried out using standard assumptions for health risk from an integrated USEPA risk analysis (Table 2). A THQ value below 1 indicates the level of exposure is smaller than the reference dose; there is likely no obvious risk or any adverse effects from the toxicant over a lifetime of exposure at this level of daily exposure [28, 29]. Conversely, the THQ value higher than 1, the toxicant may produce an adverse effect or health risk to exposed population. This means the dose is equal to or greater than the reference dose [29, 30] and the higher the THQ value, the higher the probability of exposed population experiencing long term carcinogenic effects [23].

The models for estimating THQs are [28, 34]:

$$THQ = \frac{E_{Fr} \times E_{DA} \times F_{IR} \times C}{RfD_o \times B_{WA} \times A_{TN}} \times 10^{-3}$$

where E_{Fr} is exposed frequency (365 days/year); E_{DA} is the exposure duration (70 years, average lifetime); F_{IR} is the food ingestion rate (g/person/day); C is the mean heavy metal concentration in fish (mg/kg); $RfDo$ is the oral reference dose (mg/kg/day, Table 1); B_{WA} is the average adult body weight (70 kg) and A_{TN} is the averaging exposure time for non-carcinogens (365 days/year x number of exposure years, assuming 70 years in this study). It has also been reported that exposure to two or more toxicants may result in additive and/or interactive effects [28, 29, 35, 36]. Therefore, in this study, the total THQ is treated as the arithmetic sum of the individual metal THQ values.

Total THQ = THQ (toxicant 1) + THQ (toxicant 2) + THQ (toxicant 3)

where 1, 2 and 3 denote Pb, Cd and Cu concentrations.

3. Results and Discussion

The mean concentrations of Pb, Cd and Cu in muscles and gills in fresh water tilapia fish (*Oreochromis niloticus*) are presented in Table 1. The mean Pb contents in muscles and gills of the fish samples were 6.69±3.89 mg/kg and 5.16±2.40 mg/kg, respectively. Statistically, there is no significant difference ($p>0.05$) between the values obtained in muscle and gill. The minimum concentration of 0.5 mg/kg Pb was recorded in gill while the maximum concentration of 11.00 mg/kg Pb was recorded in muscle. The Pb contents in both muscle and gill exceed the acceptable limit of 0.5 mg/kg [37]. These values recorded in this study were higher than the 0.7 – 2.4 µg/g recorded from seven fish species collected from some lakes in Tokat, Turkey [38], 0.0145 µg/g wet weight mean Pb in fish tissue from selected river in Kuantan, Pahang, Malaysia [30], 1.49 µg/g dry weight mean Pb value obtained in *O. niloticus* muscle from Al-Khadoud Spring, Al-Hassa, Saudi Arabia [13] and 0.393±0.04 mg/kg wet weight mean Pb content in Tilapia fish *Oreochromis niloticus* muscle from Eleyele reservoir, Ibadan, Nigeria [39] but far lower than the Pb value of 31.95 µg/g dry weight obtained in muscle of *O. niloticus* from Sabal dranage Al-Menoufiya Province, Egypt [15]. The value was however in agreement with the recorded Pb concentration range of 0.009 – 10.1 mg/kg (wet weight) in Yangtze river, China [29] and 6.82 ± 2.28 mg/kg obtained from the Fosu Lagoon in Cape Coast, Ghana [36].

The mean concentration of Cd in muscles of the fish was 1.3±0.71 mg/kg and 0.84±0.39 mg/kg in gills. The values show significant difference ($p<0.05$). The lowest and highest Cd concentrations of 1.00 mg/kg and 5.25 mg/kg respectively were recorded in gill of the fish. The mean concentration of Cd in both muscle and gill exceed the maximum permissible limit 0.5 mg/kg [37]. The values obtained in this study was also higher than 0.1 – 1.2 µg/g reported from lakes in Turkey [38], 0.0004 µg/g wet weight mean Cd in fish tissue from selected river in Kuantan, Pahang, Malaysia [30], 0.275±0.47 mg/kg reported from the Fosu Lagoon in Cape coast, Ghana [36], 0.073±0.02 mg/kg in fresh tissue of Tilapia fish *Oreochromis niloticus* from Eleyele reservoir, Ibadan, Nigeria [39], 0.28 µg/g dry weight mean Cd value obtained in *O. niloticus* muscle from Al-Khadoud Spring, Al-Hassa, Saudi Arabia [13] and Cd concentration range of 0.00 – 2.00 mg/kg wet weight obtained in Yangtze river [29] but lower than 3.40 µg/g dry weight mean Cd content reported in muscle of *O. niloticus* from Sabal dranage Al-Menoufiya Province, Egypt [15]. Higher Cd and Pb mean concentrations of 0.96±0.15 and 9.69±1.54 mg/kg, respectively were also reported in fish muscles from Buriganga river, Bangladesh [40].

Pb and Cd are classified among the most toxic heavy metals which have no known biochemical benefits to animals and humans. Effects of Pb on fish can lead to formation of veil-like film on the body, anemia, vomiting, loss of appetite, convulsions, irritability, headache, blood pressure, lung and stomach ulcer, irreversible damage to brain, renal failure, liver damage in humans and even death [36, 41-44]. Cadmium is known to be an endocrine disturbing substance and may lead to the, kidney damage, development of prostate cancer and breast cancer in humans [36, 44, 45].

The mean content of Cu was 1.30±0.45 mg/kg in the fish muscles and 2.12±1.27 mg/kg in gills. The statistical analysis also show no significant difference ($p>0.05$) between the values. However, mean Cu content is within the permissible level of 30 mg/kg [37]. The Cu maximum concentration of 2.12±1.27 mg/kg was recorded in the fish gill. These values were in agreement with 1.0 – 4.1 µg/g recorded by [38] from some lakes in Turkey and 0.22±0.08 mg/kg dry weight reported by [36] from the Fosu Lagoon in Cape Coast, Ghana but higher than 0.0205 µg/g wet weight mean Cu in fish tissue from selected river in Kuantan, Pahang, Malaysia [30] and 0.236±0.11 mg/kg wet weight in Tilapia fish *Oreochromis niloticus* tissue from Eleyele reservoir, Ibadan, Nigeria by [39]. The range of Cu values was lower than the 0.361 – 18.76 mg/kg wet weight values recorded in Yangtzer river, China [29] while the mean content of Cu in this study (Table 3) was lower than 2.46 µg/g dry weight mean Cu value obtained in *O. niloticus* muscle from Al-Khadoud Spring, Al-Hassa, Saudi Arabia [13] and 4.60 µg/g dry weight mean Cu value recorded in muscle of *O. niloticus* from Sabal dranage Al-Menoufiya Province, Egypt [15]. Cu is necessary for normal body functions at require amount but its excess may leads to nervous system disorder and damage to brain, anemia, liver and kidney damage [44, 46].

The mean concentrations of heavy metals in gill of the fish decrease in order of Pb > Cu > Cd while that of muscle is Pb > Cd/Cu.

Table 3: Mean concentration ± SD (mg/kg wet weight) of heavy metals in fish of Eleyele river

Fish Tissue	N	Pb	Cd	Cu
Muscle	7	6.69±3.89	1.30±0.71	1.30±0.45
(range)		(1.25-11.00)	(0.50-1.50)	(0.25-2.25)
Gill	7	5.16±2.40	0.84±0.39	2.12±1.27
(range)		(0.50-10.50)	(1.00-5.25)	(ND-1.50)

ND = not detected; SD = standard deviation; N = number of fish sample

The mean EDI values for the consumption of fish muscle for the studied area are 3.82, 0.74 and 0.74 mg/kg.day for Pb, Cd and Cu, respectively while that of fish gill are 2.95, 0.48 and 1.21 mg/kg.day for Pb, Cd and Cu, respectively (Table 4). The mean EDI through fish consumption were generally high for the studied area. The highest mean EDI from fish consumption in both muscle and gill is from Pb while the lowest is from Cd. The EDI values is generally in order of Pb > Cu > Cd.

Table 4 shows the THQ for individual metal through fish consumption by population in the studied area of Ibadan, Nigeria. The THQ of Pb and Cd through the fish muscle consumption is greater than 1 and fish gill also very close to 1. These suggest that the population may experience significant health risks from the intake of individual heavy metal due to fish consumption. The THQ of Cd is relatively higher compare to Pb while that of Cu is the lowest and less than 1, indicating no health hazard to the public from Cu through fish consumption. The values of Pb and Cd are higher than the values obtained from other studies (Table 5). The low THQ value of Cu may be attributed to its relatively high oral reference dose value. The THQ for individual metal follow the sequence in decreasing order: Cd > Pb > Cu. The THQ value for Cu is lower than values obtained by other researcher but higher than value recorded by [30] (Table 5).

Table 4: The EDI and THQ of heavy metals through fish consumption

	EDI	THQ
Pb Muscle	3.82	1.06
Gill	2.95	0.82
Cd Muscle	0.74	1.49
Gill	0.48	0.96
Cu Muscle	0.74	0.02
Gill	1.21	0.03

Table 5: Comparison of estimated targeted hazard quotients (THQs) of heavy metals through fish consumption in different areas

Location	THQ of Heavy Metals			Reference
	Pb	Cd	Cu	
Ibadan, Nigeria	1.06	1.49	0.02	This study
Yangtze river, China	0.34	0.29	0.11	[29]
Tianjin, China	0.02	0.005	0.03	[34]
Pahang, Malaysia	0.0068	0.0008	0.0010	[30]

The total THQ (Fig. 2) for the population concern is far greater than 1 (2.57) with total THQ for fish muscle about twice that of fish gill (1.81). This also suggests adverse health effects due to additive effects of two or more pollutants [23, 25, 29, 35]. Though, a THQ greater than 1 may not reveal people actually experiencing adverse health effects since there is no evidence of an unacceptable non-cancer risk for the population eating fish from studied Eleyele river [29].

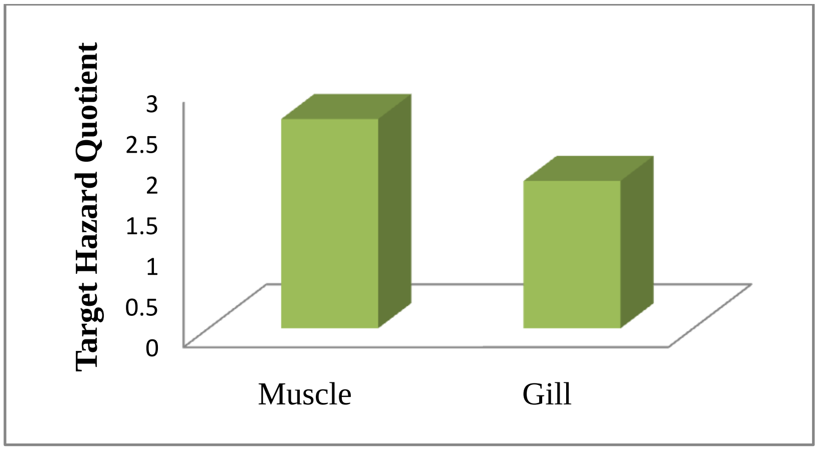

Figure 2: Total THQ of heavy metals through fish consumption

4. Conclusion

This study showed the clear indication of high pollution of Eleyele river. The consumption of fish from this river could pose serious health risks to the population because of high concentrations of studied heavy metals. Also, analysis of human health risks from consumption of fish suggests that the population is subjected to significant serious health hazard from ingestion of individual heavy metal (THQ > 1) and of course the sum of the metals together (Total THQ > 1). As fish is a valuable and nutritious food, which should be included in a balanced diet, human health risks associated with fish consumption were important, and the sources of heavy metal pollution in fish clearly should be controlled by the agents concerned. Anthropogenic input of pollutants into water bodies through various ways such as urban run-offs, industrial effluents and domestic sewage discharge should be discouraged as a measure to improve the aquatic environment.

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