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Toxic Metal Residues in Gills and Muscles of Freshwater Fishes: Implications for Chronic Noncarcinogenic Risk Assessment

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ABSTRACT: We determined the concentrations of metals in gills and muscles of fish species from Ezu River and associated noncarcinogenic risk from the exposure. The following fish species; *Clarias gariepinus, Trachurus trachurus, Parachana africana and Heterotis niloticus* were collected from potential contamination hotspots of the river and analysed for zinc (Zn), arsenic (As), mercury (Hg), copper (Cu), cadmium (Cd) and lead (Pb). Concentrations of the metals ranged between Cu: 0.01-0.03 mg kg^{-1}, Cd: 0.02-0.12 mg kg^{-1}, Zn: 0.13-0.22 mg kg^{-1}, Hg: 0.02-0.07 mg kg^{-1}, As: 0.02-0.25 mg kg^{-1}, and Pb: 0.01-0.03 mg kg^{-1}, and Cu: 0.01-0.02 mg kg^{-1}, Cd: 0.02-0.2 mg kg^{-1}, Zn: 0.10-0.27 mg kg^{-1}, Hg: 0.01-0.07 mg kg^{-1}, As: 0.08-0.45 mg kg^{-1} and Pb: 0.01-0.02 mg kg^{-1} in gills and muscles, respectively. Zn and As were higher in the muscles and gills of the sampled fish species. Concentrations of Cu, Hg Cd, and Pb appeared similar in both the gills and muscles. Metal concentration gradient across hotspots was evident in all the sections of the river. Ugbenu and Ugbene had high concentrations of Zn and As with the concentration of As being above limit by the Codex (FAO/WHO). The noncarcinogenic risk evaluation showed negligible effects from oral exposure to the metals examined. While the risk of health impact from oral exposure is negligible under the conditions of current study, the elevated levels of the heavy metals in the examined fish species are indicative of metal enrichment of the river and thus call for a regulatory control to safeguard the aquatic resources of the river.

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The presence of heavy metals in the environment has become a significant global concern due to their toxicity, persistence, non-biodegradability, and tendency to accumulate (Islam *et al.*, 2018). They remain inert in the environment and are considered conservative pollutants when undisturbed (Ahmed *et al.*, 2019). However, rapid industrialization, urbanization, population growth, and human activities, particularly in developing countries, have led to widespread pollution by heavy metals (Hossain *et al.*, 2018). These metals are discharged into rivers in substantial quantities, resulting in their strong accumulation and biomagnification along the water, sediment, and aquatic food chain, causing detrimental effects on aquatic populations (Xu and Tao, 2004). The presence of metals in the aquatic environment can be attributed to both natural processes and human activities (La Colla *et al.*, 2017). Anthropogenic activities, such as rapid industrialization and urbanization, can significantly increase metal concentrations (Rahman *et al.*, 2012). The contamination of aquatic ecosystems with metals has gained worldwide attention, leading to numerous studies on the accumulation of these elements in aquatic biota (Yilmaz *et al.*, 2007). The increasing global consumption of fish can be attributed to their nutritional benefits, including proteins, vitamins, omega-3 fatty acids, and essential minerals (Varol *et*

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al., 2017; Adel *et al*., 2016; Varol and Sünbül, 2020). However, due to advancing industrialization, population growth, and increased anthropogenic activities, the contamination rate of aquatic environments has escalated (Matouke and Abdullahi, 2020). Consequently, aquatic environments worldwide are experiencing pollution from toxicants and heavy metals. Studies have revealed the accumulation of heavy metals in various aquatic species such as prawns, crayfish, crabs, bivalves, and fish (Hazrat *et al*., 2020; Anandkumar *et al*., 2019; Rawtani *et al*., 2016), leading to an increased risk to human health through the consumption of fish contaminated with heavy metals (Liang *et al*., 2018). Heavy metal toxicity poses a significant threat to freshwater organisms, leading to adverse effects on their physiological and metabolic processes (Emere and Dibal, 2013). Essential heavy metals like zinc, copper, iron, chromium, nickel, and manganese play crucial roles in biological systems. However, in excessive amounts, they become toxic, while non-essential heavy metals such as cadmium and mercury are toxic even in trace amounts (Shah, 2017). Assessing the presence of toxic heavy metals in freshwater organisms serves as a bio-indicator of their impact on these organisms and provides insights into the degree of water pollution (Farkas *et al*., 2000). Environmental pollutants like heavy metals can result in a reduction in population density and species diversity. Additionally, changes in aquatic ecosystems have significant implications from a human perspective, as human demands are placed on these systems (Obiakor *et al*., 2013). Heavy metal toxicity can lead to decreased energy levels and damage to vital organs such as the brain, lungs, kidneys, liver, and blood composition. Prolonged exposure to high concentrations of heavy metals can initiate gradual and progressive physical, muscular, and neurological degenerative processes, leading to diseases like multiple sclerosis, Parkinson's disease, Alzheimer's disease, and muscular dystrophy. Furthermore, repeated long-term exposure to certain metals and their compounds may increase the risk of cancer (Shah, 2017). Continuous monitoring of aquatic animals is crucial in understanding the potential risks associated with human consumption (Khaled, 2013). Fish have been widely recognized as bio-indicators of metal pollution in aquatic environments and suitability for human consumption from a toxicological viewpoint (Younis and Nafea, 2012). Fish serve as a link for the transfer of toxic heavy metals from water to humans, highlighting their importance (Khaled, 2013). Exceeding recommended limits of trace elements in fish consumption can have toxic effects, whether acute, chronic, or sub-chronic, and heavy metals can exhibit neurotoxic, carcinogenic, mutagenic, or teratogenic properties. As fish occupy higher trophic levels in the food chain, they are commonly used as bio-indicators for pollutants (Idriss and Ahmad, 2015; Authman *et al*., 2015). Moreover, fish have been a significant protein source for human consumption for many years. Therefore, the human body is highly susceptible to elevated concentrations of heavy metals in fish (Ali and Khan, 2018). Analyzing the levels of heavy metals in fish can help investigate the impacts of anthropogenic activities on ecosystems and human health. This study therefore, evaluated toxic metal residues in the gills and muscles of fish species collected from Ezu River in Anambra State and its implication for chronic noncarcinogenic risk assessment.

MATERIALS AND METHODS

Study Area: This study was conducted in Ezu River, Awka North L.G.A, in Anambra State. The river lies on latitude 6 º23.4'N and longitude 6º55.8'E (Figure 1). The area have typical semi-tropical rainforest vegetation, characterized by freshwater swamps. They have a humid climate with a temperature of about 30.6^{0}C (870F) and a rainfall between 152 and 203cm annually. The fish samples were collected from four sections of the river namely; Amansea, Ebenebe, Ugbenu and Ugbene. Ezu River is the largest tributary of Anambra River and traverses various communities that depends on the river for their domestic activities and the aquatic resources of the river. The river is exposed to waste disposal, sand mining, marketing activities and sewage disposal. Industries have also been located along the corridors of the river generating and dispersing effluents into the river.

Collection of fish samples: Samples of fish were bought directly from fishermen from the four sections of the river. The samples were placed in a cooler containing ice blocks and immediately transported to the laboratory where the samples were preserved and awaiting dissection and preparation of tissues for analysis.

Heavy metal analysis of the fish samples: The analysis was conducted using atomic absorption spectrophotometer (Varian Techtron Spectra B) with the recorder staged at 10mV and aspiration rate at 6ml min^{-}1. Quality assurance and control methods were carefully practised ensuring accurate procedures.

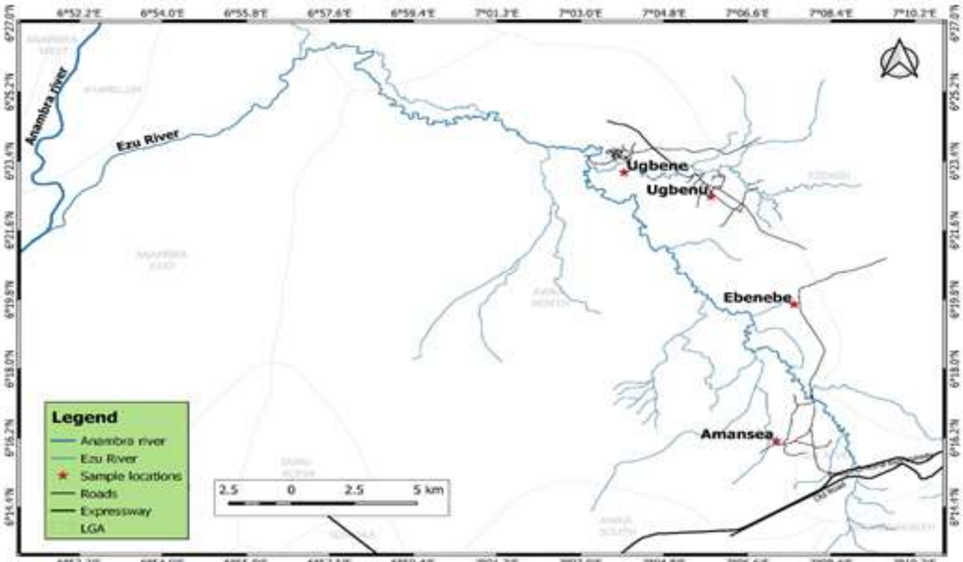

Fig. 1: Map showing the different sections of Ezu River

Distilled water was used to wash the fish samples to remove surface contaminants before dissection. During heavy metal analysis, precautions were followed to prevent cross-contamination. Each analytical analytical batch of ten runs was accompanied by an acid blank and three certified reference materials (CRM). Mean recoveries were in an acceptable range (90-99.5%) compared to the CRM theoretical or certified values for the metals (Ezeonyejiaku and Obiakor, 2017). Results were expresses in mg kg^{-1} dry weight and compared with FAO/WHO Codex Alimentarius (or Food Code). The Codex has worked since 1963 to create harmonised international food standards to protect the health of consumers and ensure fair trade practices (FAO/WHO, 2004, FAO/WHO, 2020).

Exposure and noncarcinogenic risk assessment: The concentration of heavy metals in the muscles and gills of the fish samples collected from the river were used to evaluate the human health risk from fish consumption by calculating the estimated daily intake of metals (EDIM) and the target hazard quotient (THQ). The calculations were based on the standard assumption of US environmental protection agency (EPA) risk analysis.

Estimated Daily Intake of Metals: The estimated daily intake of metals was calculated using the equation:

$$\text{EDIM} = \frac{C \times FIR}{BW}$$

Where C= average heavy metal concentration, FIR=food ingestion rate was considered to be 0.04kg/day^{-1} based on (Ezeonyejiaku *et al*, 2020). BW= average body weight which is 70kg (USEPA, 2005).

Target Hazard Quotient (THQ): The target hazard quotient used to characterize oral metal exposure and chronic noncarcinogenic risk was calculated according to USEPA (2011) guideline using the equation:

$$THQ = \frac{ADI}{RFD}$$

$$ADI = \frac{MC \times IR \times EF \times ED \times CF}{BW \times ATn}$$

Where MC= metal mean concentration(mg/kg), IR= daily ingestion rate(0.04kg), Ef= exposure frequency(365days/year), ED= exposure duration for non-carcinogen (30years), CF= conversion factor (0.21), BW= average adult body weight(70kg), ATn= average exposure time for non-carcinogen (10,950 days) and RFD= reference dose of individual metals (Zn=3.00e-1, Cu=4.00e-2, Hg=1.60e-4, Cd=1.00e-3, As=3.00e-4 and Pb= 3.50e-3).

The hazard index (HI) was further calculated from THQ values as follows:

HI = THQ (Zn) + THQ (Cu) + THQ (Hg) + THQ (Cd) + THQ (As) + THQ (Pb).

If THQ <1, the fish is considered safe and is unlikely to cause treat, and if THQ >1 the fish is exposed to toxic level of the heavy metals and can cause threat.

Data analysis: Data normality was checked using the Shapiro–Wilk test, while Levene's test was used to analyse homogeneity of variance. For normally distributed data, a parametric one sample Student's t test was used to compare metal concentrations with FAO/WHO standards. For data that could not be normalised, a nonparametric Mann–Whitney U test was applied for comparison.

RESULTS AND DISCUSSION

Table 1 and 2 presents the mean concentrations of heavy metals in the muscle and gills of fish samples collected from the different sections Ezu River. The results indicates higher concentrations of zinc (0.27±0.066) and arsenic (0.45±0.630) in the muscle tissue of the fish. Arsenic had the highest concentration in the gills and muscles of all the metals studied. Also, the concentration of arsenic in the muscle tissue of the fish was above FAO/WHO limit. Heavy metal contamination in aquatic ecosystems poses a significant threat to both the environment and human health (FAO, 2020). Monitoring the concentrations of heavy metals in fish tissues is crucial for assessing potential risks associated with their consumption. The study evaluated heavy metal levels in the muscles and gills of fish species collected from Ezu River and its noncarcinogenice risk assessment. Table 1 presents the mean concentrations of heavy metals in the muscle tissues of fish from the Ezu River. Among the metals studied, zinc and arsenic exhibited elevated concentrations. The mean concentration of zinc ranges from 0.10±0.013 mg/kg to 0.27±0.06 mg/kg in different locations along the river. Similarly, arsenic showed concentrations ranging from 0.07±0.040 mg/kg to 0.45±0.630 mg/kg. Also, zinc and arsenic had the highest concentration in the gills of the fish species sampled (table 2). These values are consistent with the study conducted by (Ezeonyejiaku *et al*., 2019), who reported elevated levels of zinc in fish species collected from a freshwater ecosystem. The similarity in zinc concentrations suggests that this metal is widely distributed in aquatic ecosystems affected by pollution. A study by Adegbola *et al*. (2021) on the heavy metal accumulation in fish species (*Clarias gariepinus* and *Sarotherodon melanotheron*) from industrially polluted Ogun and Eleyele Rivers, Nigeria, showed comparable or higher zinc levels in fish gills, suggesting that gills are efficient accumulators of zinc in polluted environments. This further supports the role of fish gills as indicators of heavy metal pollution.

Table 1: Mean concentration of heavy metals in muscles of fish from the Ezu River (mg/kg

Heavy Metals	**Ugbenu (*Clarias gariepinus*)**	**Ugbene *(Trachurus trachurus*)**	**Amansea *Parachana Africana*)**	**Ebenebe (*Heterotis niloticus*)**	**WHO/FAO**
Zinc	0.27±0.066	0.10±0.013	0.15±0.075	0.12±0.033	40
Copper	0.02±0.011	0.01±0.001	0.02±0.010	0.02±0.003	30
Mercury	0.01±0.009	0.01±0.012	0.07±0.003	0.05±0.004	0.5
Cadmium	0.12±0.153	0.03±0.014	0.02±0.003	0.02±0.003	0.5
Lead	0.01±0.001	0.00±0.003	0.02±0.006	0.02±0.001	0.2
Arsenic	0.45±0.630	0.21±0.18	0.08±0.069	0.07±0.040	0.3

Table 2: Mean concentration of heavy metals in gills of fish from the Ezu River (mg/kg)

Heavy metals	**Ugbenu (*Clarias gariepinus)***	**Ugbene *(Trachurus trachurus*)**	**Amansea *(Parachana Africana*)**	**Ebenebe (*Heterotis niloticus*)**	**WHO/FAO**
Zn	0.22±0.194	0.21±0.066	0.13±0.050	0.16±0.081	40
Cu	0.02±0.023	0.03±0.005	0.02±0.008	0.01±0.004	30
Hg	0.02±0.025	0.02±0.002	0.06±0.001	0.07±0.003	0.5
Cd	0.12±0.016	0.03±0.006	0.02±0.001	0.02±0.003	0.5
Pb	0.01±0.016	0.01±0.004	0.02±0.007	0.03±0.017	0.2
As	0.06±0.067	0.25±0.069	0.06±0.022	0.02±0.010	0.3

The presence of high levels of zinc and arsenic in the muscle tissues suggests potential bioaccumulation and biomagnification processes occurring in the fish species analyzed. Bioaccumulation refers to the accumulation of heavy metals in the tissues of organisms, while biomagnification describes the phenomenon of increasing metal concentrations at higher trophic levels in the food chain (Hazrat *et al*., 2020). This finding indicates that fish species from the Ezu River may be exposed to elevated levels of zinc and arsenic, which could pose risks to human health upon consumption. The most striking is the elevated concentration of arsenic in the muscle tissues of fish exceeding the WHO/FAO limit of 0.3 mg/kg^{-1}. Arsenic is a highly toxic heavy metal that can have detrimental effects on human health, including carcinogenic and mutagenic effects. Prolonged exposure to elevated levels of arsenic through the consumption of contaminated fish can lead to severe health issues, such as skin lesions, respiratory problems, and an increased risk of developing various types of cancer (La Colla *et al*., 2017). This finding is consistent with the research by Bawuro *et al*. (2018), which reported bioaccumulation of heavy metals in some tissues of fish in Lake Geriyo, Adamawa State, Nigeria. The presence of significant arsenic concentrations in fish tissues is an indication of widespread contamination in the Ezu River ecosystem.

The presence of other heavy metals, such as copper, mercury, cadmium, and lead, though at lower concentrations, also raises concerns. These metals can have toxic effects on various organ systems in humans, including the nervous, cardiovascular, and renal systems. Even low levels of exposure to these metals over time can lead to adverse health effects, including neurological disorders, kidney damage, and developmental abnormalities, especially in vulnerable populations such as children and pregnant women (Ezeonyejiaku *et al*., 2019).

Target hazard quotient (THQ) and hazard index (HI): Public health risk assessment was conducted based on target hazard quotient (THQ) following the US EPA acceptable value of 1 (USEPA, 2011). The THQs for As, Hg, Cu, Cr, Zn and Pb examined for the fish species in this study are shown in Table 3. The THQ calculations were below 1 for all metals. Hazard index (HI), which provides a cumulative chronic noncarcinogenic risk, was below 1 for all metals analysed indicative that oral exposure by ingestion to metal contaminated freshwater fish species-based food was not associated with any noncarcinogenic risk to public health.

Table 3: Target hazard quotient (THQ) and hazard index (HI) of heavy metal in muscle and gills of fish sample

Heavy Metals	Ugbenu		Ugbene		Amansea		Ebenebe	
	Muscle	Gills	Muscle	Gills	Muscle	Gills	Muscle	Gills
Zinc	1.08E-04	8.98E-05	3.86E-05	8.22E-05	6.28E-05	5.40E-05	6.80E-05	9.49E-05
Copper	6.15E-06	5.10E-06	4.35E-06	7.65E-06	5.70E-05	7.50E-05	9.14E-06	8.00E-06
Mercury	4.88E-03	1.65E-02	1.01E-02	1.31E-02	5.00E-04	5.00E-04	2.86E-06	4.00E-06
Cadmium	1.49E-02	1.38E-02	3.96E-03	3.42E-03	3.00E-04	3.00E-04	1.09E-05	1.14E-05
Lead	4.63E-04	4.80E-04	1.03E-04	4.63E-04	3.00E-04	2.00E-04	4.34E-05	9.14E-06
Arsenic	1.79E-01	2.42E-02	8.22E-02	1.01E-01	1.00E-04	1.00E-04	9.71E-06	1.71E-05
HI	1.99E-01	5.51E-02	9.64E-02	1.18E-01	7.19E-03	4.02E-01	2.40E-05	2.41E-05

The rising consumption of freshwater fish in Nigeria, driven by its nutritional benefits and affordability, raises concerns regarding the potential accumulation of heavy metals such as zinc, copper, mercury, cadmium, arsenic, and lead, in these fish species and its implications for human health. However, the estimated daily intakes of these metals were below the reference doses provided by JECFA (2000) and USEPA (2011).

Furthermore, the target hazard quotient values for the metals were below 1, indicating no immediate risk to the consumers. Regular monitoring of heavy metal concentrations in fish and implementing effective pollution control measures are essential to mitigate the contamination of aquatic ecosystems. Additionally, public awareness campaigns and educational programs should be implemented to inform local communities about the potential risks associated with consuming heavy metal contaminated fish and promote sustainable practices to reduce heavy metal pollution in Ezu River.

Conclusion: All the metals analysed except for arsenic, were found to be below the permissible limits set by the WHO/FAO. Consequently, fish species from the river were considered safe for consumption based on the human health risk assessment.

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