FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 9 April Special Issue, 2025, pp 311 - 316
DOI: https://doi.org/10.33003/fjs-2025-09(AHBSI)-3530

TOXIC METALS BIOACCUMULATION IN DIFFERENT ORGANS OF FISH HARVESTED FROM ZOBE DAM, KATSINA STATE, NIGERIA USING RELATIVE INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Instrumental neutron activation (INAA) technique has been applied to investigate the concentrations of possibly toxic elements in organs of *Oreochromis niloticus* and *Clarias gariepinus* from Zobe Dam, Katsina State. Consumption of fish offers unique nutritional and health benefits and is considered a key element in a healthy diet. However, despite its nutritional value, fish living in a polluted environment can accumulate the toxic metals at high concentration causing serious risk to human health when consumed. The main goal of this study is to investigate the possible toxic elements concentrations as well as assess the possible health implications they may pose to human via the fish consumption. Six (6) possibly toxic elements As, Br, Co, Cr, V, and Ba were detected by the INAA techniques. The dietary intake of some of the possibly toxic elements was estimated for tissue by considering WHO/FAO tolerable daily intake of adults (TDI/70 kg). Other organs were neglected according to the consumption pattern of the population in the study area. Our result of the estimated dietary intake showed that, the concentration of the detected toxic elements in tissue of both fish species were considerably below the estimated daily tolerable human consumption limits set by the WHO/FAO except for Cobalt which shows concentration higher than the recommended daily intake limit. Therefore, consumption of tissue of the studied fish species harvested from Zobe Dam is considered safe.

Keywords: Bioaccumulation, Concentration, Consumption, INAA, Zobe Dam

INTRODUCTION

Fish and fishery products contribute significantly in food and nutritional security around the globe (FAO, 2010). Consumption of fish as a source of protein provide a unique nutritional and health benefits and is considered to be major element in a healthy diet. Increased concern is given to fish as a source of essential nutrients in our diets, not only high value proteins, but more importantly a unique source of micronutrients and long chain omega 3 fatty acids (FAO, 2010). Fish consumption is also known to have health benefits among the adult population. Strong evidence highlighted how consumption of fish particularly oily fish reduces the risk of coronary heart diseases mortality (Muzaffarian and Rimm, 2006), (FAO, 2011). Foods from the aquatic environment are major sources of macro and micronutrients necessary for a healthy diet (FAO/WHO, 2011). In addition to the health benefits of these nutrients, fish is also a key provider of a range of micronutrients, not commonly available from other sources in the diets of the poor. Fish is found to contain more protein source compared to other animal protein source. FDF (2009) found that almost half of the total animal protein consumed in Nigeria is from fish and fish products and this makes it to occupy a unique position being the cheapest source of animal protein.

However, despite its nutritional benefit to human, fish living in a contaminated environment can easily accumulate the toxic metals in high concentrations causing serious health threat to human when consumed. Fish and in general seafood may pose risks to human health because they may be exposed to toxic substances, such as toxic elements and persistent organic pollutants (Guerin et al., 2011). Some potential toxic elements are also found to enter into the food chain through processing (Hurrel *et al.*, 1989). Their existence in the environment has been a source of concern to environmentalist and health practitioners due to their health implications (Awofolu *et al*., 2005; Borgmann, 1983). Fish are widely used in evaluating and assessing the health and safety of aquatic ecosystem because pollutants build up in the food chain and are responsible for adverse effects and death of animals in the aquatic ecosystems (Farkas et al., 2002; Baby et al., 2010). Many studies have been conducted on the accumulation of trace elements in fish and other organisms in aqueous environments (Medeiros et al., 2012). An investigation was carried to evaluate the level of some essential and toxic elements of interest in fish and oysters from western India using energy dispersive x-ray fluorescence EDXRF (Daniel et al., 2021). The result indicates that, the elemental levels in finfish are within the maximum tolerable limits set by various international standards and guidelines except for arsenic and copper which concentrations exceeded the maximum guidelines. The impact of water pollution with heavy metals on fish health was studied (Mustafa and El-Sayed, 2014). Their result indicates that there is a strong evidence of heavy metals concentrations in different fish tissues due to numerous factors, such as environmental concentrations, environmental conditions (i.e pH, water temperature and hardness etc.), industrial waste which are potential sources of heavy metals into the environment. Study was carried (Sadauki et al., 2023) on the comparative survey of parasites of African Catfish Clarias gariepinus in Ajiwa and Zobe reservoirs showed that the occurrence and mean intensity of parasitic infection were higher in samples from Zobe reservoir than those in the Ajiwa reservoir. The relative INAA technique has been effectively utilized and found efficient (Joseph et al., 2019, 2023, 2024). The aim of the present study is to investigate the composition, the extent of the concentration, as well as the health implications of the selected toxic elements in organs of some fish species harvested from Zobe Dam using Instrumental Neutron Activation Analysis (INAA) technique.

MATERIALS AND METHODS

Instrumental Neutron Activation (INAA) Technique

Neutron activation analysis (NAA), particularly, instrumental neutron activation analysis (INAA) is a technique with a very notable precision mainly for the determination of trace concentrations of elements in samples and also used to obtain information on the spatial distribution of a neutron field via neutron activation detectors (Majerle, 2006). This technique is based upon the conversion of stable nuclei to other, mostly radioactive nuclei via nuclear reactions, and measurement of the reaction products. The use of the INAA (relative) method for calculating the concentration of every element in the sample irradiated with reactor thermal neutron reduces the NAA equation to the simplest form (IAEA, 1990; Joseph et al., 2011; Alhassan et al., 2024):

$$\frac{w}{w_{st}} = \frac{N_s D_{st}}{N_{st} D} = \frac{N_s e^{-\lambda td(st)}}{N_{st} e^{-\lambda td}} \qquad (1)$$

Where; N_s= net photo peak area of radionuclide of interest in sample, N_{st} = net photo peak area of radionuclide of interest in standard, W= weight of element in sample irradiated, W_{st}= weight of the element in standard irradiated, $D = e^{-\lambda td}$ =decay factor for sample, $D_{st} = e^{-\lambda td(st)}$ =decay factor for standard, t_d= decay time for sample, $td(st)$= decay time for standard, λ= decay constant for radionuclide of interest

The concentration of the unknown element in the sample denoted by Cs is given by

$$C_s = \frac{W}{M} \qquad (2)$$

Where; M= known weight of the irradiated sample containing the unknown weight of the element irradiated, W= unknown weight of the element irradiated.

Study Area

Zobe Dam was established in July, 1977. It is located in the southern part of Dutsin-Ma in Katsina State of Nigeria (Isah, 2009). Before Dam establishment, the settlers had a history of blacksmithing and agrarian farming activities. The Dam has an average storage capacity of 170 million m^3 and about 8137 hectares of land for irrigation. It covers an estimated surface area of 39.6 km^2. The annual rainfall is estimated to be about 817 mm per year. Dam irrigation area is located between 12º23'18''N of northern latitude and 7º28'29''E of eastern longitude. In the east, the area is delimited by Kuki and Sayaya, in the north by Dan Makubiri and Safana, and in the south by Danmusa and Makera. The area lies between 450 m and 490 m above Mean Sea Level (MSL). The majority of the population are Hausa Fulani, and their primary occupation is farming and animal rearing. The establishment of the new Federal University Dutsin-Ma a few years ago contributed majorly in the rise in population and activity in the local government.

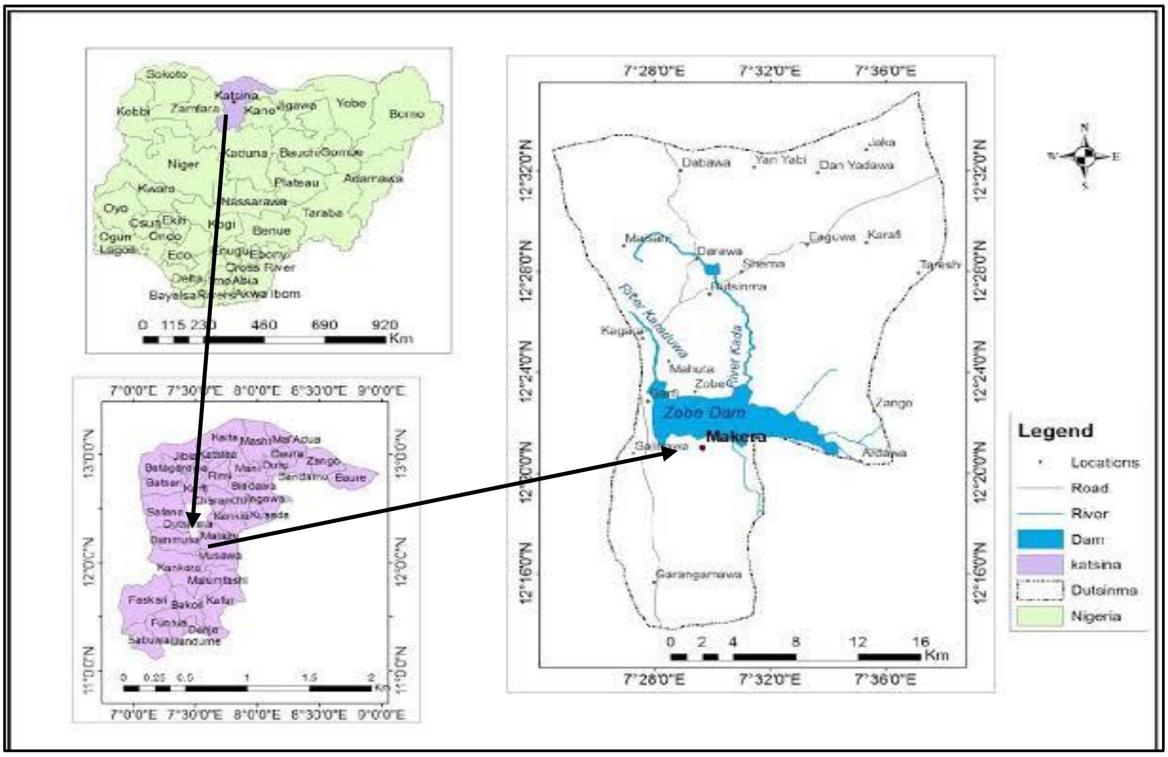

Figure 1: Dutsin-Ma Map Showing Zobe Dam (Insert: Nigeria and Katsina State)

Sample Collection and Preparation

Fish species (*Oreochromis niloticus* and *Clarias gariepinus*) were collected directly from fishermen at their landing site at Zobe Dam in Dutsin-Ma Local Government, Katsina State. The Fish Samples were prepared at Ummaru Musa Yaradua University Central Science Laboratory. The fish organs (Gill, Bone, tissue, and liver) were cut using dissecting kit, they were air dried for some minutes and later put in an Oven at 105°C. The samples were then put on a desiccator and grinded using motor and pestle. A total of 8 samples were prepared and taken to Center for Energy Research and Training (CERT) Zaria. Elemental analysis of the prepared samples was carried out using Instrumental Neutron Activation Analysis (INAA) Technique. For verification and quality control purpose, high grade Certified Reference Materials (CRMs) supplied by International Atomic Agency (IAEA)

452 SCALOP, (IAEA) 336 LINCHE were used as comparator standard monitors in this work NIST 1515 and NIST 1547, they were also prepared following the same procedure.

Irradiation and Measurements

Irradiation of the samples and standards was carried using NIRR-1 at Center for Energy Research and Training CERT Zaria. During the irradiation, neutrons of flux 5.0 x 10^{11} $ncm^{-2}s^{-1}$ were accessed. Using rabbit carriers, the samples and standards were sent into the reactor through a pneumatic pressure. NIRR-1 was used to irradiate the samples with a thermal neutron flux 5.0 x 10^{11} $ncm^{-2}s^{-1}$ for 6 hours for the long irradiation. The samples were then taken to a detecting set up consisting of a high purity germanium (HPGe) detector connected to a PC based multi-channel analyzer (MCA) in a fixed sample to detector geometry. For short lived elements, the first counting was done immediately and followed by a second one 2 hours later. The samples were then allowed to decay further for the analysis of long-lived elements. Using the procedure for all samples, the results in tables 1 & 2 were obtained.

RESULTS AND DISCUSSION

Quality Assurance and Quality Control

A proactive action to reduce the likelihood of errors in an analytical technique is quality assurance. It also involves quality control, which is the procedure used to check for problems after the analysis is complete. The preparation of the test portion, choice of the analytical protocol, calibration, instrument performance checks, irradiation, decay, measurement, spectrum analysis and interpretation, internal and external quality control, and procedures for ensuring the technical proficiency of the personnel involved are all covered in detail for each step in the neutron activation analysis process. In this investigation, two CRMs—NIST 1515 for apple leaves and NIST 1547 for peach leaves—were employed for quality control in INAA determinations (Tables 3 and 4).

We calculated the U-score, Z-score, and relative bias (RB) in order to assess the lab performance. The following formulae were used to calculate these measurements:

$$\text{Uscore} = \frac{|X_{Lab} - X_{Ref}|}{\sqrt{\mu_{Lab}^2 + \sigma_{Ref}^2}} \quad (3)$$

$$\text{Zscore} = \frac{|X_{Lab} - X_{Ref}|}{\mu_{Lab}} \quad (4)$$

$$\text{R-bias} = \frac{X_{Lab} - X_{Ref}}{X_{Ref}} \times 100 \quad (5)$$

Where; X_{LAB} represents the laboratory results, μ_{LAB} is the standard deviation, $X_R$ef represents recommended uncertainty, and $\sigma_R$ef is the standard uncertainty.

The laboratory performance is evaluated as follows; satisfactory if U-score ≤ 1, and satisfactory if Z-score is ≤ 2, questionable for 2< Z-score < 3, and unsatisfactory if Z-score is ≥ 3.

Table 1: Comparison of recorded values with the certified values in the certified reference materials of NIST 1515 (apple leaves)

Elements mg/kg	NIST 1515 Certified Values	NIST 1515 Recorded Values	U-score	Z-score	R-bias
Ba	49±2	53.9±7.3	0.65	2.45	9.6
Br	1.8	1.49±1.2	0.26	-	-17.2

Values represent mean ± standard deviation

Table 2: Comparison of recorded values with the certified values in the certified reference materials of NIST 1547 (peach leaves)

Elements mg/kg	NIST 1547 Certified Values	NIST 1547 This work measured values	U-score	Z-score	R-bias
Ba	124±4	136.4±11.8	1	3.1	10
Br	11	10.45±3.2	0.17	-	-5

Values represent mean ± standard deviation

The illustrated results in tables (1 & 2) show that, all element concentrations are in good agreement with the certified values after using two CRMs; NIST 1515 and NIST 1547. This calculation exposed a great quality result found in this practice which could be achieved through the statistical assessment and evaluation. The statistical parameters U-score and Relative bias calculated for all elements are acceptable only Z-score shows no satisfaction in concentrations of some elements.

Concentrations of Elements in Organs of *Oreochromis niloticus* and *Clarias gariepinus*

Four different organs (Tissue, liver, gill, bone) were analyzed for possibly toxic elements that may be detected. The results obtained were presented in tables (3 & 4) of this work. Six (6) potential toxic elements were detected from the analyzed organs of the studied fishes from Zobe Dam using INAA technique which includes As, Co, Br, Cr, V and Ba.

Table 3: INAA concentrations of element in various organs of *Oreochromis niloticus*.

Toxic Elements	Bone	Gill	Liver	Tissue
Co	0.39±0.07	0.87±0.09	2.93±0.14	0.29±0.07
Cr	BDL	2.66±0.66	BDL	BDL
As	0.25±0.07	BDL	BDL	BDL
Br	20.1±0.2	28.1±0.2	27.0±0.2	20.0±0.2
V	2.22±0.11	1.34±0.16	1.25±0.13	0.30±0.08

BDL means; Below Detection Limit

Table 4: INAA concentration of elements in various organs *of Clarias gariepinus.* BDL means; Below Detection Limit

Toxic Elements	Bone	Gill	Liver	Tissue
Co	0.23	0.70±0.12	0.21±0.02	0.42±0.06
Cr	BDL	BDL	0.40±0.09	1.89±0.48
As	0.09	BDL	0.21	BDL
Br	18.6±0.1	51.2±0.5	47.4±0.4	22.8±0.2
Ba	BDL	BDL	BDL	BDL
V	0.61±0.08	0.37±0.08	1.03±0.14	0.075

Chromium concentration was only observed in gill of *Oreochromis niloticus* while the remaining organs were at BDL. In *Clarias gariepinus*, the concentrations of Cr were observed in tissue and liver whereas Cr concentration was not detected in gill and bone. Cr is an essential nutrient metal necessary for metabolism of carbohydrate (Farag et al., 2014). Fish accumulate Cr by ingestion or by the gill uptake track and accumulation in fish tissues mainly liver occurs at higher concentrations than those found in the environment (Pacheco et al., 2013) and (Ahmed et al., 2013). Cr often accumulates in aquatic life adding the danger of eating fish that may have been exposed to high level of Cr. The maximum guideline of Cr intake set by EFSA is 0.3 mg/kg (EFSA, 2014). Cr concentrations in organs of *Oreochromis niloticus* are lower than the maximum guideline of EFSA except for gill which shows concentration higher than the EFSA's maximum guideline. In *Clarias gariepinus*, tissue and liver show concentrations higher than the maximum guideline of EFSA while gill and bone concentrations fall below the stipulated guideline and agrees with previous reports (Abubakar and Joseph, 2023, Abubakar et al., 2023).

Cobalt shows concentration of the range (0.23 to 2.93±0.14 mg/kg). The highest concentration was observed in liver of *Oreochromis niloticus* while the lowest was noticed in bone of *Clarias gariepinus*. Exposure to cobalt may cause cancer (NIOSH, 2019). Co concentrations in organs of the sampled fishes corroborates with the concentrations reported from previous studies (Anim et al., 2011; Abubakar and Joseph, 2023) in *Channa Obscura, Hepsetus odoe, Tillapia zilli inhabiting Densu* River, Niger. According to FEEDAP Panel (2009a) the potential cobalt intake of consumers from food of animal origin would not exceed 14 µg/day. This is equivalent to 0.0002 mg/kgbw. Thus, the concentrations of Cobalt in organs of the studied fishes are not within the tolerable limit. Generally, cobalt compounds that dissolve easily in water are more harmful than those that are hard to dissolve in water. Once cobalt enters the body, it is distributed into all tissues, but mainly into the liver, kidney and bones (ATSDR, 2004).

Arsenic (As) was the least accumulated heavy metal in organs of *Oreochromis niloticus* and *Clarias gariepinus* in the present study. Its concentration was observed only in bone of *Oreochromis niloticus* while in tissue, liver, and gill, it was untraceable. Arsenic concentration was also noticed in liver and bone of *Clarias gariepinus* while it was not detected in tissue and gill. The detected concentrations were of the range (0.09 to 0.25±0.07 mg/kg). Highest concentration was recorded in bone of *Oreochromis niloticus* while the lowest was recorded in bone of *Clarias gariepinus*. Arsenic is toxic to humans and can affect people of any age or health status. These results are consistent with earlier results (Abubakar and Joseph, 2023, Abubakar et al., 2023). Under MFR (1985) guideline, the maximum permissible limit for Arsenic intake was set at 1.0 mg/kg. Thus, the concentrations of As in all organs of the studied fishes are lower than the MFR guidelines.

Bromine has sometimes been considered to be possibly essential in humans with support of only limited circumstantial evidences and no clear biological role. The concentrations of Br detected by INAA were of the range (18.6±0.1 to 51.2±0.5 mg/kg). The highest concentration was noticed in gill of *Clarias gariepinus* while the lowest in bone of *Clarias gariepinus*. According to JMPR (1989), the maximum daily intake limit of Br is 1.0 mg/kg. Hence, concentrations of Br in all organs of the sampled fishes exceeded the maximum guideline.

Vanadium (V) concentration in organs of *Oreochromis niloticus* ranged between 0.30±0.08 to 2.22±0.11 mg/kg. The highest concentration was observed in bone while the lowest was noticed in tissue. Other concentrations observed in liver and gills were 1.25±0.13 and 1.34±0.16 mg/kg respectively. The range of V concentrations in *Clarias gariepinus* was 0.075 to 1.03±0.14 mg/kg. The highest concentration was detected in liver while the lowest in tissue. Concentrations of 0.37±0.08 and 0.61±0.08 mg/kg were recorded in gill and bone respectively. Vanadium as micronutrient plays a role in carbohydrate and lipid metabolism, but can be toxic at high concentrations (Xenobiot, 2022). The maximum guideline for V is 0.5 mg/kg WHO/FAO (1976). This shows that, in *Oreochromis niloticus*, only tissue accumulates V concentration lower than the WHO/FAO guideline. In *Clarias gariepinus*, V accumulation in tissue and gill also falls below WHO/FAO guideline while liver and bone concentrations exceeded the stipulated limit.

Barium is not considered to be an essential element for human nutrition (Schroeder, 1972). At high concentration, barium causes vasoconstriction by its direct stimulation of arterial muscle, peristalsis as a result of the violent stimulation of smooth muscles and convulsions and paralysis following stimulation of the central nervous system (Stockinger, 1976). From this study, the concentrations of Ba in *Oreochromis nilticus* detected by INAA technique were of the range (10.7 to 83.6±16.4 mg/kg). The highest concentration was observed in bone of *Oreochromis niloticus* while the lowest concentration was noticed in tissue of the *Oreochromis niloticus*. Ba Concentration was not detected in all organs of *Clarias gariepinus.* According to WHO (2016), the maximum guideline of Ba intake is 0.21 mg/kg. Hence, concentrations of Ba in all organs of *Oreochromis niloticus* except for liver exceeded the maximum guideline.

Estimation of the Dietary intake of Trace Elements

The present study attempts to provide an estimation of the dietary consumption of essential and potential toxic elements contained in various organs of both *Oreochromis niloticus* and *Clarias gariepinus*. This will provide reliable information and facts to health practitioners and scientific literatures. The detected concentrations of some toxic elements in the studied samples are presented in tables (5 & 6) with the stipulated daily tolerable limits. The concentration data of the studied fish organs were compared with those provided by the WHO/FAO. The normal consumption values per day and per person of potential toxic elements contained in organs of the studied fishes were determined assuming an intake of 10 g (dry weight) of the studied fishes ration per person. The estimation of the dietary intake was conducted only for tissue. Other organs were neglected according to the consumption practice of the population in the study area. The assumed intake estimation of the fish tissue revealed that, the concentration of the potentially toxic elements in tissue of both studied fish species (As and Br) are well below the toxicological reference values provided by the WHO/FAO and were found within nutritional threshold. Also, Cr is within the recommended value, while Co exceeded the provided reference value. Therefore, consumption of tissue of the studied fishes is considered safe.

Table 5: Intake values (in mg/day) of some essential and toxic elements and tolerable daily intake of adult (TDI/70 kg) of WHO/FAO. INAA

Elements	Co	As	Br	Cr
Oreochromis niloticus	0.003	BDL	0.2	BDL
Clarias gariepinus	0.004	BDL	0.23	0.03
Males 19- 50	0.0024	150	70	0.035
Females 19- 50	0.0024	150	70	0.025
Pregnancy 19 - 50	0.0026	150	70	0.03
Lactation 19-50	0.0028	150	70	0.045

CONCLUSION

The present study tried to determine and evaluate the composition, the extent of the concentrations, and the possible health implications of some toxic elements in organs of two fish species (*Oreochromis niloticus* and *Clarias gariepinus*) from Zobe Dam, Katsina state which are proved to be the most consumed fishes by the people in the study area, by using Instrumental Neutron Activation (INAA) technique. Six (6) possibly toxic elements are detected which includes; As, Cr, Co, V, Ba, and Br. Moreover, it was found that, the concentration of the detected toxic elements in tissue of both fish species were considerably below the estimated daily tolerable human consumption limits set by the WHO/FAO except for Cobalt which shows concentration higher than the recommended daily intake limit. Finally, the outcomes of the present investigation might be used for nutritional data base.

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