

Research Article

Ecotoxicological and Bacteriological Assessment of Water and Fish from Hadejia River, Nigeria: Public Health and One Health Implications

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ABSTRACT

Hadejia River, Jigawa State, Nigeria, is under anthropogenic influence for fisheries and irrigation. Water quality parameters, total bacterial count, and the 5 metals (Pb, Cd, Hg, As, Cr) were estimated in 3 fish species: *Clarias gariepinus*, *Oreochromis niloticus*, and *Synodontis sp.* at 3 points over 3 seasons (n=35). The species are commercially important, occupy contrasting trophic niches, and were used as bioindicators of metal exposure. The atomic absorption spectrometry (AAS) method was used to analyze metals, the American Public Health Association (APHA) standards were used to analyze water quality, and the pour-plate method was used to analyze bacteria. The visualisation was done using proportional symbol maps and target hazard quotient (THQ) charts. For adults, As (THQ 3.80) and Pb (1.14) exceeded 1, and the hazard index (HI) was 6.40; for children, As (8.87), Pb (2.66), Hg (1.40), and Cd (1.21) exceeded 1, and the HI was 14.93. Total coliform and *E. coli* were present at all sites, with the highest counts downstream; the downstream site was higher than WHO limits for DO and BOD5. There was a significant difference ($p=0.002$) between the total plate counts for species. Exploratory PCA showed that there were two variance structures, chemical and microbiological, with arsenic and bacterial load as the most significant contributors. Results were interpreted based on the source-pathway-receptor framework (geogenic and anthropogenic sources, floodplain-shallow groundwater exchange, adult and child consumers) and a One Health perspective, as the co-occurrence of metals and faecal bacteria can favour the emergence of antimicrobial resistance. Child-focused fish-consumption advisories, monitoring of landing sites, sanitation, and improvement of cold-chain are the top actions. Future studies should focus on species-specific arsenic toxicity and carcinogenic risk assessment, monitoring of sediments and groundwater, and pathogen and antimicrobial-resistance characterization of fish and water isolates.

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1 Introduction

Population growth, agricultural intensification, informal industrialization, and poor environmental governance are driving a process of degradation of the sub-Saharan African rivers in a rapid and accelerated fashion (United Nations Environmental Protection Agency [UNEP], 2023). In Nigeria, small-scale industries, uncontrolled disposal of solid wastes, and enhanced irrigation agriculture have led to the surface water systems being loaded with heavy metals and pathogenic microorganisms (Akoto et al., 2008). The Hadejia-Nguru wetlands are one of the main sources of the Hadejia River, which flows eastwards through Jigawa State and is a wetland of global ecological importance that is a Ramsar-designated wetland (RCS, 2021). Floodplain farming of rice, sorghum, and vegetables is supported by the river, which maintains thick artisanal fishing populations. River fish is one of the primary sources of protein for the population of the area and is traded in the regional markets; thus, the chemical and microbiological safety of fish is a direct and immediate health problem for the population (FAO, 2022).

Among the various contaminants, heavy metals, particularly lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr), are the most bioaccumulative in fish muscle tissues and biomagnify through aquatic food chains, making them the top priority contaminants in river fisheries (Habu et al., 2025; Onokebhabge et al., 2022). Even dietary toxins, when exposed to sub-acute chronic toxicity, are reported to cause nephrotoxicity, hepatotoxicity, neurotoxicity, carcinogenesis, and endocrine disruption (Jaishankar et al., 2014). According to WHO (2021) and the United States Environmental Protection Agency (USEPA, 2023), the physiological vulnerability of children and women of reproductive age, and the excessive relative intake of dietary metals per unit body weight are the risk factors. Children are particularly susceptible to the effects of dietary and environmental toxicants, as they consume more food per kg of body weight, ingest some dietary metals at higher rates, and have developing organ systems, which means that exposure assessments based

on adult parameters can significantly underestimate risk (Landrigan & Goldman, 2011; USEPA, 2011).

Estimated daily intake (EDI) and target hazard quotient (THQ), hazard index (HI) (USEPA, 2023) have been adopted as the internationally standardized approach to the assessment of non-carcinogenic risks from fish consumption. More recent methodological development has integrated classical risk indices with multivariate and graphical tools, including spatial symbol maps, principal component analysis (PCA), and bar-chart THQ visualization to provide a simpler and communication-friendly description of multi-metal contamination structures and their risk connotations (Jolliffe & Cadima, 2016; Onokebhagbe et al., 2022). These authors again emphasized that graphical techniques could augment tabular data to show the heterogeneity of the specimen at the level of the graphical data and the relationship between the variables that is not provided by the tabular data, which is only in the form of means.

The microbiological safety of freshwater fish, in addition to the levels of chemical contamination, is a rather important aspect of food safety in Nigeria (FAO, 2023). Total plate count (TPC) is the most commonly used worldwide indicator of aerobic bacterial load and handling hygiene (International Commission on Microbiological Specifications for Foods [ICMSF], 2018). Species-specific bacteriological risk information for Hadejia River is not available in the literature, but some of the most commercial species found in inland waters of the Nigerian waters include *Clarias gariepinus*, *Oreochromis niloticus* and *Synodontis sp.* (FAO, 2022; WHO, 2020). Interest in the corridor has been gaining momentum in research on the quality of surface water and the impact of wastewater, but there is no published work so far on the combined assessment of heavy metals' bioaccumulation as well as the quantification of the risk from harvested fish (WHO, 2022; UNEP, 2023).

Most contamination studies of African surface waters and wetlands see the presence of metals as primarily a result of human activity and consider the water body separately from its geologic and hydrologic context. An informative approach would be a holistic source-pathway-receptor (SPR) approach, which asks what the geogenic and anthropogenic sources are that provide the source of contaminants, what ways geology, sediment chemistry, floodplain inundation and groundwater-surface water exchange control the quantity and chemical form of the contaminants along the pathway, and who is exposed at the receptor end (Goes, 1999; Smedley &

Kinniburgh, 2002). Bacteriological results are also connected to antimicrobial resistance (AMR) and the One Health approach, as the presence of metals and faecal bacteria is often closely linked in the aquatic environment (Seiler & Berendonk, 2012; UNEP, 2023; Mackenzie & Jeggo, 2019). The interpretation of chemical and bacteriological results in this study is therefore made from the perspective of the SPR approach and the One Health perspective.

The study aimed to: (1) assess the levels of contamination of the river water for the three species of fish sampled at Hadejia River; (2) evaluate the non-carcinogenic dietary risk for adults and children at Hadejia River with the use of Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI) as derived from USEPA (2023) method; (3) use proportional symbol map to determine the spatial pattern of the three fish species and TPC; (4) compare the TPC among the fish species, using one-way analysis of variance (ANOVA); (5) explore the contamination structure using PCA and THQ bar charts; and (6) interpret the result within the framework of One Health and source-pathway-receptor and implications for antimicrobial resistance.

2 Materials and Methods

2.1 Study Area and Sampling

The Hadejia River originates from the Kano River system and runs to the east of Jigawa State, ending in the Hadejia-Nguru wetlands (Amoo et al., 2024). The research area was 40 km of the Hadejia Local Government Area (LGA), located between latitudes 12.430° N and 12.447° N, and longitudes 10.027° E to 10.037° E, where intensive rice farming, market gardening, livestock watering, artisanal fishing, and residential activities were found. (Figure 1). Three locations were used, namely Site 1 (upstream reference; Lat. 12.44°N, Long. 2.03°N; point B in Figure 1) in the middle of the main township, and Site 3 (near the coast; Lat. 12.45°N, Long. Site 3 (downstream; Lat. 10.05°E) is close to a rice milling cluster. 12.45°N, Long. 10.08°E; point C). The study reaches are located within the Quaternary Chad Formation, the sandstones, silts, and clays of which are interbedded, downstream from the basement-complex terrain of the Kano catchment where the river originates (Muhammad et al., 2023). On the Hadejia-Nguru floodplain, the annual flooding recharges a shallow unconfined aquifer tapped by shallow tube wells by farmers, thus creating a hydraulic link between surface water and shallow groundwater (Goes, 1999).

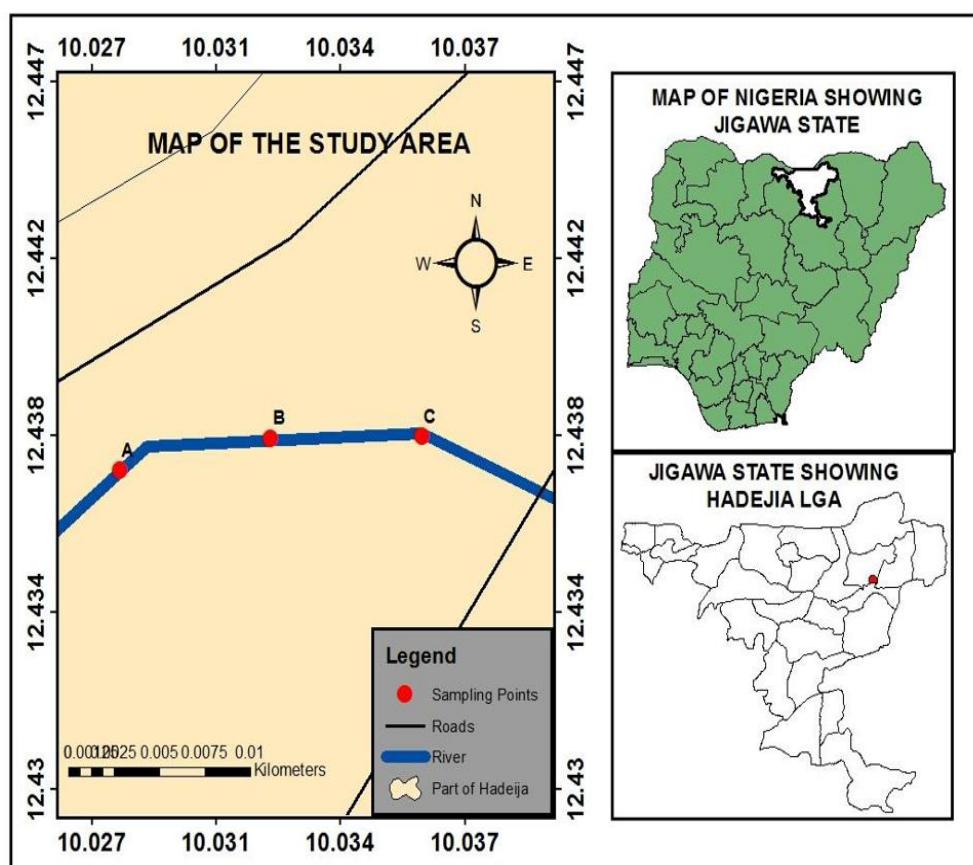


Figure 1: Map of the study area showing the three sampling points

2.2 Selection of Study Species

The selection of the three species was based on three criteria: (i) dietary and commercial importance, being the species that are widely landed and consumed as fresh water fish in the inland waters of Nigeria (FAO, 2022; Froese & Pauly, 2023); (ii) contrasting trophic position and habitat use which enables exposure through various routes to be compared and (iii) established use of fish as bioindicators of metal contamination, fish are known to integrate exposure from water, sediments and food and to accumulate metal in their edible parts which passed on to humans (Authman et al., 2015). The species thus act as bioaccumulation indicators (sentinels) of exposure to metals, as well as the medium of exposure for consumers. They have different roles in the ecology. *Clarias gariepinus* (African Catfish) is a benthopelagic predator and scavenger, omnivorous, tolerant of low DO and low-quality water, and persists where others do not. *Oreochromis niloticus* (Nile tilapia) is an omnivorous, largely herbivorous–detritivorous species that consumes phytoplankton, periphyton, and detritus, thus connecting primary production to higher trophic levels and to consumers such as humans. *Synodontis sp.* A bottom-feeding, omnivorous catfish (squeaker catfish), lives in close association with bed sediments where many metals are concentrated (Froese & Pauly, 2023). They do not have regulatory significance as indicator species, and

they do not indicate a particular source, but rather reflect exposure over time.

2.3 Sample Collection

Sampling was conducted in three seasons (dry season (January–March), early rains (May–June), and late rains (September–October) following the guidelines outlined in APHA (2017). Thirty-five fish samples (12 *Clarias gariepinus*, 12 *Oreochromis niloticus*, and 11 *Synodontis sp.*) were purchased in each site directly from the artisanal fishers. Measuring, weighing, and storage of fish were done under the conditions of ice and sterile polyethylene bag before it was promptly transported to African Biotechnology and Genomics Laboratory in Abuja for analyses. Species identification was carried out using the assistance of Froese and Pauly (2023). Water samples (1 L, n = 12 at each site for each season) were collected at a 20–30 cm depth in pre-cleaned High-Density Polyethylene (HDPE) bottles and analysed immediately.

2.4 Heavy Metal Assay

Each fish was cut open, and a piece of dorsal muscle tissue (around 5 g of fish) was homogenized and digested in wet acid (4:1 (v/v) $\text{HNO}_3\text{:HClO}_4$) until it was dissolved (APHA, 2017; Habu et al., 2025). The samples were subsequently diluted to 50 mL of ultrapure water and filtered through Whatman No. 42 filter paper before being analysed using flame atomic absorption

spectrophotometry (FAAS; Shimadzu AA-7000, Japan) technique, with the use of hydride generation for analysing As and Hg in the samples (Bate et al., 2024). QA/QC involved certified reference material (DORM-4), blank analyses, and duplicate analysis of every ten samples. Recoveries ranged from 92.1–106.4%. Detection limits: Pb = 0.005; Cd = 0.002; Hg = 0.001; As = 0.003; Cr = 0.004 mg/kg were taken into consideration during analysis. Total (inorganic plus organic) arsenic was measured, but no separation of arsenic species was carried out.

2.5 Health Risk Assessment

The dietary exposure paradigm developed by the USEPA (2023) was used to calculate the non-carcinogenic health risk for two receptor groups, adults and children, with children being more susceptible due to their greater consumption of food per kg of body weight (Landrigan & Goldman, 2011; USEPA, 2011). Modelled exposure for the dietary (fish ingestion) pathway. The estimated daily intake (EDI) was estimated by:

$$EDI = \frac{(C \times IR \times EF \times ED)}{(BW \times AT)} \dots \dots \text{eqn. 1}$$

where C = concentration of metal in fish muscle (mean) (mg/kg); IR = ingestion rate (adults 0.2 kg/day; children 0.1 kg/day, assumed to be half of the adult ingestion rate); EF = frequency of exposure (365 days/year); ED = duration of chronic exposure (adults 30 years; children 6 years); BW = body weight (adults 70 kg; children 15 kg, representative of children aged 1–6 years; USEPA, 2011); AT = averaging time (ED × 365 days; 10,950 days for adults and 2,190 days for children).

Target hazard quotient (THQ) was calculated using:

THQ = EDI / RfD (Equation 2)

$$THQ = \frac{EDI}{RfD} \dots \dots \dots \text{eqn. 2}$$

where RfD (USEPA, 2023): Pb = 5.0×10^{-4} ; Cd = 5.0×10^{-4} ; Hg = 1.0×10^{-4} ; As = 3.0×10^{-4} ; Cr = 5.0×10^{-4} (mg/kg/day).

Cumulative HI = Total sum of all the THQs. A THQ > 1 or HI > 1 means that it is a health issue of concern under the additive toxicity assumption (Chary et al., 2008; USEPA, 2023). Adults and children were analyzed separately in terms of THQ and HI. Additionally, there are two other exposure pathways for children: ingestion of incidental soil and sediment and dermal contact, but concentrations of metals in sediment and soil were not measured in this study and could not be quantified.

2.6 Water Quality Assessment

Physicochemical parameters were measured by: nephelometric turbidity (HACH 2100P), iodometric Winkler titration for DO and BOD₅, and gravimetric measurement of TDS at 180 °C. Total coliform counts

were done according to the WHO (2022).

2.7 Bacteriological Analysis of Fish Tissue

Fish muscle (10 g) was homogenized in 90 mL of sterile buffered peptone water (60 s in stomacher) to create 10^{-1} dilution. The 10^{-6} serial dilution was poured onto Plate Count Agar (PCA, Oxoid) and incubated at 37 °C for 48 ± 2 h under aerobic conditions (APHA, 2017; Amoo et al., 2025). The colonies on plates were counted (25–250), and the results were recorded in the form of log₁₀ CFU/g fresh weight.

2.8 Statistical and Graphical Analysis

The normality test was conducted using the Shapiro-Wilk test, and the homogeneity of variance test was done using the Levene test. One-way ANOVA with Tukey HSD post-hoc ($\alpha = 0.05$) was used for comparison of the TPC at the species level. The Pearson correlation analysis was used to examine the relationships among variables. Python 3.11 was used to conduct multivariate analyses with the help of pandas, numpy, scipy, and matplotlib. Proportional symbol maps of the site-level water-quality and bacteriological parameters were created, with the area of the symbol proportional to the site mean and the symbols overlaid on the study-area base map, with the symbols coloured according to whether the WHO guideline was exceeded. Proportional symbol maps of the water-quality and bacteriological parameters for the site were created, with the area of the symbol proportional to the site mean and the symbols overlaid on the study-area base map, with the symbols coloured according to whether the water-quality parameter exceeded the WHO guideline. Data were analyzed using inferential statistics in IBM SPSS v26.0 (IBM Corporation, Armonk, NY, USA).

3 Results

3.1 Concentrations of Heavy Metals in the Tissue of Fish Samples

The average concentrations of heavy metals in the dorsal muscle tissue are shown in Table 1. As was the dominant metal (overall mean: 0.40 mg/kg), followed by Pb (0.20 mg/kg), Cd (0.09 mg/kg), Cr (0.06 mg/kg), and Hg (0.02 mg/kg). The concentration of all individual species was lower than that allowed by WHO (2021), but THQ values were higher than 1.0 for adults for As and Pb, and for children for As, Pb, Hg, and Cd (Table 3). *Synodontis sp.* had the highest As (0.42 mg/kg) and Pb (0.22 mg/kg) of the three species, which is in line with its feeding ecology being benthic.

Table 1: Mean Heavy Metal Concentrations (mg/kg) in Muscle Tissue of Fish from Hadejia River

Fish Species	Pb (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	As (mg/kg)	Cr (mg/kg)	N
<i>Clarias gariepinus</i>	0.18±0.03	0.08±0.01	0.02±0.00	0.37±0.05	0.05±0.01	12
<i>Oreochromis niloticus</i>	0.21±0.04	0.10±0.02	0.02±0.00	0.41±0.06	0.07±0.01	12
<i>Synodontis</i> sp.	0.22±0.03	0.09±0.01	0.02±0.01	0.42±0.04	0.06±0.01	11
Mean (All)	0.20	0.09	0.02	0.40	0.06	35
WHO Limit (mg/kg)	0.30	0.10	0.50	1.00	1.00	—

Values are mean $\pm$ SD. Pb = lead; Cd = cadmium; Hg = mercury; As = arsenic; Cr = chromium. n = number of specimens per species.

3.2 Physicochemical and Microbiological Water Quality: Spatial Patterns

The water quality parameters are summarized in Table 2; site means are plotted as proportional symbols in Figure 2 on the study-area base map. The pH values were found to be within the WHO (2022) limit at all the sites. Dissolved oxygen decreased from 6.8 mg/L (upstream) to 4.7 mg/L (downstream), which violated the WHO minimum of 5.0 mg/L at Site 3 (Figure 2b). BOD₅ was 6.2 mg/L (guiding value is below 5.0 mg/L) downstream (Figure 2a). At Site 3, the turbidity was the highest and had a value of 31.5 NTU (Figure 2c). The progressive faecal contamination was confirmed in the downstream site, as the total coliforms (143cfu/100mL) and *E. coli*

(51cfu/100mL) were well above WHO limits of zero. (Figure 2e and f). The turbidity and total coliform and *E. coli* values were found to be above the guideline at all three sites while DO and BOD₅ only exceeded the guideline at Site 3, and TDS didn't exceed the guideline at any site (Figure 2d). The biggest symbols for all parameters except DO are found at Site 3, where a uniform downstream trend in organic load, particulate load, and faecal contamination is observed. Pearson correlation revealed strong positive associations between downstream distance and BOD₅ ($r = 0.94$, $p < 0.01$), total coliforms ($r = 0.98$, $p < 0.01$), and turbidity ($r = 0.91$, $p < 0.01$), and a strong negative correlation with DO ($r = -0.97$, $p < 0.01$).

Table 2: Physicochemical and Microbiological Parameters of Hadejia River Water at Three Sampling Sites

Parameter	Site 1 (Upstream)	Site 2 (Midstream)	Site 3 (Downstream)	WHO/FEPA Limit
pH	7.2±0.3	7.5±0.2	7.8±0.4	6.5–8.5
Temperature (°C)	27.4±1.2	28.1±0.9	29.3±1.5	< 30
Dissolved Oxygen (mg/L)	6.8±0.5	5.9±0.7	4.7±0.8*	≥ 5.0
BOD ₅ (mg/L)	2.1±0.4	3.8±0.6	6.2±0.9*	< 5.0
Turbidity (NTU)	12.3±2.1*	18.7±3.4*	31.5±4.8*	< 5.0
TDS (mg/L)	142±18	198±24	267±31	< 500
Total Coliforms (CFU/100 mL)	28±6*	74±12*	143±21*	0
<i>E. coli</i> (CFU/100 mL)	6±2*	22±4*	51±9*	0

Note: Values are mean $\pm$ SD ($n = 12$ per site per season). BOD₅ = five-day biochemical oxygen demand; TDS = total dissolved solids; NTU = nephelometric turbidity units. * = exceeds WHO guideline.

WHO limits sourced from WHO (2022).

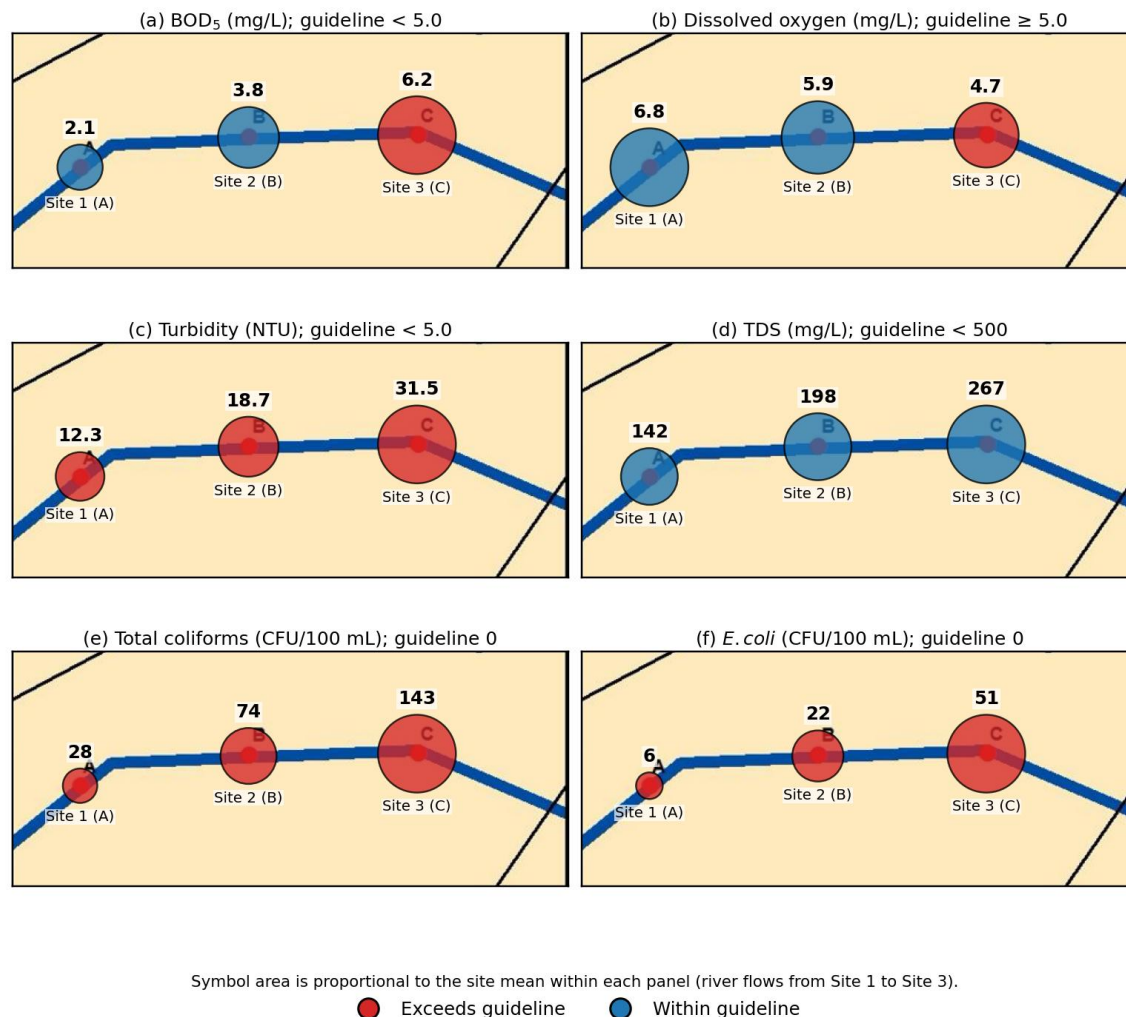


Figure 2: Proportional symbol maps of water-quality and bacteriological parameters at the three sampling sites on Hadejia River: (a) BOD₅, (b) dissolved oxygen, (c) turbidity, (d) total dissolved solids, (e) total coliforms and (f) *E. coli*. Symbol area is proportional to the site mean within each panel; red symbols exceed the WHO guideline, and blue symbols are within it.

3.3 Heavy Metals and Microbial Load PCA

Figure 3 presents a bar graph of THQ for each metal for adults and children with a horizontal reference line at THQ = 1.0, the USEPA non-carcinogenic risk level. The first visual representation makes it immediately evident that the role that arsenic is playing in fueling the risk profile is the largest, at 3.80, which is nearly 3.3 times greater than the highest of the remaining metals (Pb, THQ = 1.14). The non-cancer exposure risk to the average fish consumer is large because the HI is increasing and is now greater than the USEPA safety limit of 6.40. For children, EDIs were 2.3 times higher than for adults, with the higher intake per unit body weight (0.0067 kg fish per kg body weight per day). THQ exceeded 1 for As (8.87), Pb (2.66), Hg (1.40), and Cd (1.21), and only Cr (0.79) remained below 1. The child HI was 14.93, almost 15 times the safety limit and 2.3 times the adult HI. The proportion of the HI was the same for both groups and each metal, so children were assumed to eat the same diet composition as adults. The risk relationships are

explicitly displayed on the THQ bar chart (Figure 3).

3.4 Health Risk Assessment: EDI, THQ and Hazard Index for Adults and Children

Table 3 shows the EDI, THQ, percent HI contribution, and the risk classification for adults and children. Adult EDI (1.14×10^{-3} mg/kg/day) and THQ (3.80) for Arsenic were the highest and accounted for 59.4% of the total HI. Lead produced a THQ of 1.14 (17.8% of HI). Combined, the THQ for cadmium (0.52), mercury (0.60), and chromium (0.34) were not significantly above 1.0, but they did combine for 22.8% of the HI. The growing HI of 6.40 and more than 6 times the USEPA safety limit shows that there is a significant non-carcinogenic risk to the general fish consumer. Children had a higher intake per unit body weight (0.0067 kg fish per kg body weight per day), and thus the EDIs were 2.3 times higher than those of adults. THQ exceeded 1 for As (8.87), Pb (2.66), Hg (1.40), and Cd (1.21), and only Cr (0.79) remained below 1. The child HI was 14.93, almost 15 times the safety limit and 2.3 times

the adult HI. The same diet composition was assumed for both groups, which led to the same proportion of the HI for both children and adults for each metal. These risk

relationships are directly shown on the THQ bar chart (Figure 3).

Table 3: Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Non-Carcinogenic Health Risk for Adults and Children

Metal	Mean Conc. (mg/kg)	RfD (mg/kg/day)	Adult EDI (mg/kg/day)	Adult THQ	Child EDI (mg/kg/day)	Child THQ	HI Contribution (%)
Pb	0.20	5.0×10^{-4}	5.70×10^{-4}	1.14	1.33×10^{-3}	2.66	17.8
Cd	0.09	5.0×10^{-4}	2.57×10^{-4}	0.52	6.00×10^{-4}	1.21	8.1
Hg	0.02	1.0×10^{-4}	5.71×10^{-5}	0.60	1.33×10^{-4}	1.40	9.4
As	0.40	3.0×10^{-4}	1.14×10^{-3}	3.80	2.66×10^{-3}	8.87	59.4
Cr	0.06	5.0×10^{-4}	1.71×10^{-4}	0.34	3.99×10^{-4}	0.79	5.3
HI				6.40		14.93	100

Note: Adults: IR = 0.2 kg/day, ED = 30 years, BW = 70 kg, AT = 10,950 days. Children: IR = 0.1 kg/day (assumed), ED = 6 years, BW = 15 kg, AT = 2,190 days; EF = 365 days/year for both groups. THQ > 1 indicates potential non-carcinogenic health concern.

3.5 Target Hazard Quotient Bar Chart

A bar chart of THQ for the 5 metals for adults and children with a horizontal reference line for the USEPA non-carcinogenic risk level (THQ = 1.0) is presented in Figure 3. The first visual representation makes it clear at a glance the role that arsenic is playing in fueling the risk profile: its adult bar is 3.80, which is almost 3.3 times higher than the highest other metal (Pb, THQ = 1.14). The size of the arsenic bar in comparison to the threshold line highlights the fact that arsenic, alone at its average concentration in fish tissue, puts chronic consumers at risk of dietary exposure of about 3.8 times the safe reference level (8.9 times for children). With a lead level

slightly above that for adults, and 2.7 times the lead level for children, it shows its worth as a secondary risk factor. The bars of Cd, Hg, and Cr are below the threshold line for adults, while in children, the bars of Cd and Hg are above the threshold line. Combined with the HI from As and Pb in the HI framework, their sum results in the lamentably high HI for adults of 6.40 and the HI for children of 14.93. The bar chart is thus an easy-to-communicate tool for stakeholders in regulation and public health to represent the technical THQ/HI framework graphically as cumulative risk burden and metal-specific risk burden.

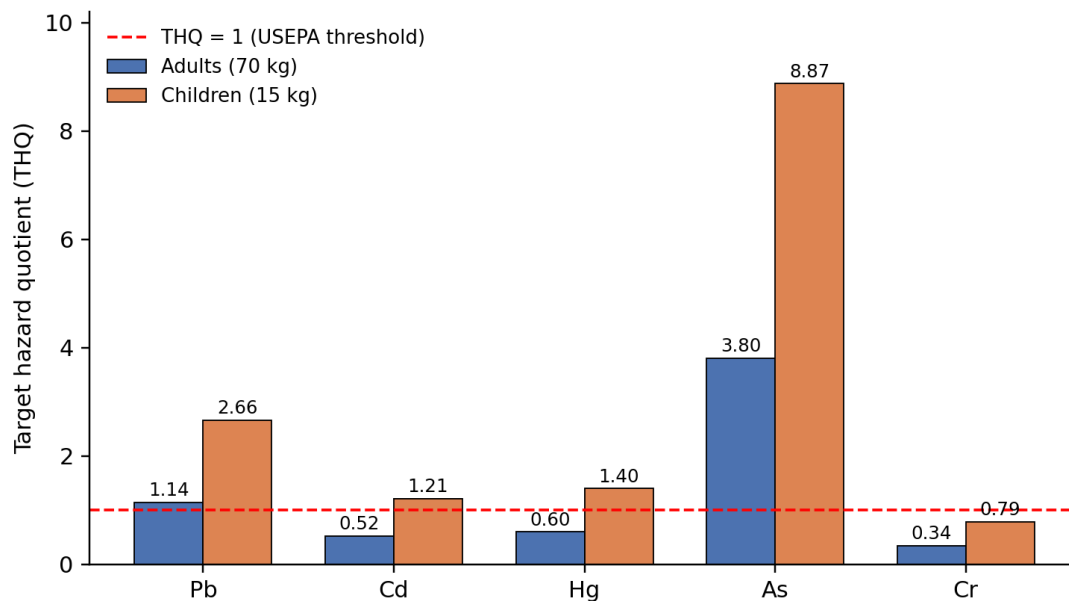


Figure 3: Target Hazard Quotient (THQ) for Five Heavy Metals in Fish from Hadejia River for Adults and Children.

3.6 Total Plate Count per Fish Species

Table 4 shows the ANOVA results, and the descriptive statistics of TPC are shown in Table 5. From the results of one-way ANOVA, $F(2, 32) = 12.45$, $p = 0.002$ (F-critical =

3.29), it is concluded that there are significant differences between the species in terms of TPC. *O. niloticus* and *Synodontis* sp. had the highest mean TPC (5.14 log CFU/g, Tukey group b), respectively. (4.87 log CFU/g, group b)

and *C. gariepinus* (4.21 log CFU/g, group a). Post hoc tests were performed as pairs comparing *C. gariepinus* with both *O. niloticus* ($p = 0.018$) and *Synodontis sp.*, to ensure that there was a significant difference between the two pairs. ($p > 0.05$); no significant difference was found

between *O. niloticus* and *Synodontis sp.* ($p > 0.05$). ($p = 0.621$). It was found that the highest TPC of *O. niloticus* specimens was 6.50 log CFU/g and the lowest was found in the *C. gariepinus* specimens with a TPC of 3.10 log CFU/g (Table 5).

Table 4: One-Way ANOVA Summary for Total Plate Count (log CFU/g) by Fish Species from Hadejia River

Source of Variation	Sum of Squares	Df	Mean Square	F-value	p-value
Between Species (Groups)	18.34	2	9.17	12.45	0.002*
Within Groups (Error)	23.55	32	0.74	—	—
Total	41.89	34	—	—	—

Note: * $p < 0.05$. TPC = Total Plate Count (log CFU/g). F-critical (2, 32, $\alpha = 0.05$) = 3.29. Post-hoc Tukey HSD: *C. gariepinus* vs *O. niloticus*, $p = 0.018$ *; *C. gariepinus* vs *Synodontis sp.*, $p = 0.031$ *; *O. niloticus* vs *Synodontis sp.*, $p = 0.621$.

Table 5: Descriptive Statistics and Tukey HSD Post-Hoc Groupings for TPC by Fish Species

Fish Species	N	Mean TPC (log CFU/g)	SD	Min	Max	Tukey Group
<i>Clarias gariepinus</i>	12	4.21	0.84	3.10	5.60	A
<i>Oreochromis niloticus</i>	12	5.14	0.91	3.80	6.50	B
<i>Synodontis sp.</i>	11	4.87	0.78	3.50	6.10	B
Overall	35	4.74	0.90	3.10	6.50	—

Note: TPC = Total Plate Count. Tukey groups: species sharing the same letter are not significantly different ($\alpha = 0.05$). One-way ANOVA: $F(2, 32) = 12.45$, $p = 0.002$.

4 Discussion

4.1 Spatial Structure of Contamination and Multi-Endpoint Patterns

The proportional symbol maps show that the river is not uniformly affected: BOD₅, turbidity, TDS, total coliforms, and *E. coli* all increase from Site 1 to Site 3, while DO falls below the guideline at Site 3 (Figure 2). The greatest change occurs between the midstream site, which is near a rice milling cluster, and the downstream site, which fits with the cumulative input of domestic wastewater, agricultural runoff, and processing effluents along the reach, and its associated oxygen demand (Sures et al., 2017; Dippong et al., 2024). The discrete site, in this way, avoids the assumption of a continuous surface between the three sampling points, which would not be justified by these few discrete sites. The variability of TPC among the fish ranged by about 2 logarithmic units (3.10–6.50 log CFU/g), while the variability of metals around their respective means was comparatively small. Thus, the variability of TPC among the fish is the widest among the measured parameters, and bacterial contamination of fish tissue is the most variable. The highest variation was noticed for arsenic and this was correlated with the species differences in particular the high As concentration in *Synodontis sp.* that is in close contact with sediments (WHO, 2021). These graphics verify and express what numerical data in the ANOVA and concentration Tables indicate, but in a way that can be understood by non-technical groups such as regulatory inspectors and policy stakeholders (FAO, 2021).

The PCA indicated that the data on the specimens were grouped in two clusters, and that chemical and bacteriological contamination axes were autonomous.

The downstream location ($PC1 < -2$) has higher concentrations of metals than TPC, and is dominated by specimens of *Synodontis sp.* that have higher concentrations of As and Pb (Table 1), and is more exposed to metals via the benthic pathway (Bate et al., 2024). The right cluster ($PC1 > 2$) is a cluster of specimens with a high TPC and relatively low concentrations of metals, a broad representation of *O. niloticus* that have been exposed to intensive post-harvest handling. The fact that $PC1$ (metal-driven) and $PC2$ (TPC-associated) are partly orthogonal, and chemical/microbiological hazards in Hadejia River fish are the result of a combination of partly independent exposure paths, is confirmed, meaning that regulation or monitoring based on either dimension would fail to capture a significant portion of the overall load of health risks. This methodological observation can be used practically to design the multi-endpoint fish safety surveillance programmes in the area (ICMSF, 2011).

4.2 THQ Bar Chart Analysis and Significance of Health Risk for Adults and Children

Quantitative data on risk is represented graphically using the THQ bar chart, and in the process, arsenic and lead are graphically highlighted as the two metals of primary health concern in the Hadejia River fish. The hegemony of the arsenic bar at 3.80 in comparison to a threshold reference line of 1.0 is especially dramatic, and serves to make a point that tabular THQ values alone might not convey as fully to policy audiences as to present the fact that arsenic is not only slightly above the threshold but actually is approximately four times the acceptable level of single metal risk. It is highly significant for

regulatory purposes because inorganic arsenic is a Group 1 human carcinogen (IARC, 2022), and its modeled EDI would lead to a cancer risk much higher than the cancer risk limit set by the USEPA for lifetime exposure of 10^{-6} . The bar chart is, therefore, presented with a double communicative function: that the bar chart is symbolic of non-carcinogenic findings of THQ, and that at the same time the bar chart suggests the need for immediate carcinogenic risk analysis of arsenic.

Children are the ones who suffer the most. The four metals had THQs greater than 1, and the values of Cd and Hg (1.21 and 1.40) used for adults are higher than the values for children, indicating that the risk would have been underestimated with an adult-only evaluation (Landrigan & Goldman, 2011). This child HI of 14.93 is derived directly from the higher intake/body-weight ratio; it is dependent on the assumed child portion size (0.1 kg/day), which was not measured and is subject to further refinement based on consumption surveys. Lead is particularly important because early developmental exposure has been linked to neurodevelopmental effects and there is no known threshold for safety (Landrigan & Goldman, 2011; Jaishankar et al., 2014).

The proximity of the lead bar to the threshold (1.14) sends a clear but relevant message: For adults, the lead bar is a marginal exceedance and may not be a concern for the risk characterization limited to means, but for the risk characterization extended to account for the heterogeneity in the specimen, it is a toxicologically relevant exceedance. The aggregate THQ value of 1.14 is calculated from the mean value of the fish. Some proportion of the fish in the market has a higher THQ value than the mean value in the aggregate analysis, as illustrated by the following example: An individual *Synodontis sp.* collected has a Pb level of 0.22 mg/kg. The individual fish THQ is approximately 1.25, which is higher than the aggregate mean THQ.

One of the relevant contaminants is arsenic. Total arsenic was measured, and the inorganic arsenic reference dose was applied to this. However, the inorganic fraction is generally low, and arsenic is mainly present in organic forms in fish muscle with much lower toxicity (EFSA, 2009). This arsenic THQ should therefore be used as a conservative upper bound until arsenic speciation is performed, and the carcinogenic risk should be based on the inorganic fraction.

4.3 Source–Pathway–Receptor Framework and Geological Controls

Results interpretation of a source–pathway–receptor chain (Table 6) reveals that contamination of fish in the Hadejia River is not solely anthropogenic. The high concentration of the heavy metals measured in this study

is consistent with the multi-source anthropogenic contamination dominating an underlying geogenic arsenic background found in the catchment sedimentary and weathered terrain (Smedley & Kinniburgh, 2002). All three species have a prevailing As profile that is consistent with the geologic contribution of As in the region and the levels of anthropogenic contribution from the phosphate fertilizers and agricultural pesticides used in the Hadejia irrigation belt. Habu et al. (2025) also obtained similar results, with As (0.31–0.58 mg/kg) enriched in vegetables in the Kano wastewater irrigation systems, thereby confirming the enrichment of Arsenic in the region. The concentrations are comparable to those reported by Amoo et al. (2022) for lead in fish in River Salanta, Kano (0.15–0.28 mg/kg), owing to the presence of cars, battery wastes, and paint wastes. The slightly elevated metal levels in the fish *Synodontis sp.* seem biologically plausible, as it lives in the sediment and is exposed to 5–50 times higher concentrations of metals attached to sediment than to the water above it (Förstner & Wittmann, 2012).

The amount and presence of metals reaching the biota are both under geologic control. The study reach is located in the Quaternary Chad Formation, which has silts and clays and an Fe/Mn coating that absorb and immobilize As, Pb, and Cd in bed sediments (Muhammad et al., 2023; Förstner & Wittmann, 2012). Redox conditions and pH vary with seasonal flooding and wetting–drying cycles in these sediments, with the potential for release of arsenic under reductive conditions as a result of reductive dissolution of iron oxides (Smedley & Kinniburgh, 2002). The fraction that is mobile and its speciation, inorganic and organic arsenic, depend on sediment mineralogy and the redox state during flood seasons and not solely on anthropogenic input.

A second pathway is the groundwater–surface water interaction, which has rarely been considered in contamination studies of the wetlands. Annual flood events in the Hadejia–Nguru floodplain also recharge a shallow unconfined aquifer which hosts wetland vegetation and shallow tube wells of the farmers (Goes, 1999). The contaminated water, which includes the metals and faecal contamination, can therefore enter the shallow aquifer, and the shallow aquifer can supply water to river water and wetland water during the dry season. This exchange would be linked to the floodplain exposure of drinking water and irrigation use by households. This pathway is included in this study as a hypothesis because there was no sampling of groundwater, and it is consistent with the relatively high arsenic concentrations observed in this pathway and the faecal indicator counts, and needs to be tested directly by sampling groundwater and sediments concurrently with surface water.

Table 6. Source–pathway–receptor summary for contamination of fish and water in the Hadejia River reach

Component	Elements in the Hadejia reach	Evidence from this study	Knowledge gap
Source: geogenic	Chad Formation sediments (sandstone, silt, clay); weathered basement material carried from the Kano catchment	Arsenic dominant in all species (mean 0.40 mg/kg)	Sediment and soil baseline for As and other metals
Source: anthropogenic	Phosphate fertilisers and pesticides; rice-milling effluent; domestic waste and open defecation; vehicle, battery and paint residues (Pb)	Downstream rise in BOD ₅ , turbidity, TDS and faecal indicators	Source apportionment
Pathway: surface	Runoff, irrigation return flow, effluent discharge; sediment–water partitioning; seasonal flooding	Seasonal sampling; spatial gradient (Figure 2)	Metals in river water and sediment not measured
Pathway: groundwater–surface water	Flood-plain recharge of the shallow unconfined aquifer and shallow tube wells; redox-driven arsenic mobility	Not measured	Groundwater and hydrochemical sampling
Pathway: food web	Uptake from water, sediment and prey; benthic feeding	<i>Synodontis</i> highest As and Pb; <i>O. niloticus</i> highest TPC	Paired tissue–sediment data
Receptor	Fish (sentinels); adult and child consumers; fish handlers and vendors; households using river water and shallow wells	THQ > 1 for adults (As, Pb) and children (As, Pb, Hg, Cd); HI 6.40 and 14.93	Soil-to-mouth and dermal routes; As speciation; pathogen and AMR profile

4.4 Water Quality Cases of Gradient Deterioration

The progressive downstream decrease in DO (6.8 to 4.7 mg/L) is below the WHO limit of 5.0 mg/L and is associated with high levels of BOD₅ (6.2 mg/L), turbidity (31.5 NTU), total coliforms (143 CFU/100 mL) and *E. coli* (51 CFU/100 mL). These gradients are measured by the high Pearson correlations between distance from upstream to downstream and the poor quality indicators (0.94–0.98 correlation for BOD₅, coliforms, turbidity). The merging of metal pollution and faecal pathogens at the downstream site where most of the artisanal fishing and fish washing is done creates a compounding chemical-microbiological risk which will greatly amplify the overall health risk compared to each hazard alone (Sures et al., 2017; Dippong et al., 2024).

4.5 Bacteriological Risk and Food Safety Implications on Species

There is a significant difference in the species-level TPC ($F(2, 32) = 12.45$, $p = 0.002$) and the highest TPC for *O. niloticus*, which is consistent with the biological and process-related explanations known. *O. niloticus* has a mucus-covered vascular skin, which is an excellent source of nutritional elements that would allow bacteria to colonize; it is the most commercially traded species, and transit times in the market chain are the longest for this species in comparison to the other 2 species, which are not as processed as the other species. The reduced TPC of *C. gariepinus* (4.21 log CFU/g) is in line with its mucus antimicrobial properties such as lysozyme-like factors proved by Wamalwa & Mwaura (2024). The overall mean TPC of 4.74 log CFU/g is slightly lower than the ICMSF (2011) acceptability of 5.0 log CFU/g, while the

mean TPC of *O. niloticus* is 5.14 log CFU/g, with a maximum value of 6.50 log CFU/g, which is higher than the ICMSF (2011) limit. The results confirm the food-safety-related concerns of Amoo et al. (2025) on the street-vended and ready-to-eat foods in the northwestern region of Nigeria, and reinforce the need for cold-chain infrastructure, hygiene education, and species-specific alerts on food-safety hazards that were highlighted by previous assessments of the cold-chain gaps in the study area (Gulma, 2025; SNCCRAFS, 2026).

4.6 One Health Implications: Antimicrobial Resistance and Shared Exposure

The presence of faecal indicator bacteria (*E. coli* up to 51 CFU/100 mL in water, TPC up to 6.50 log CFU/g in fish) and the presence of Pb, Cd, Hg and As in the same river–fish–human continuum is relevant to antimicrobial resistance. Co-resistance and cross-resistance are ways in which metals are co-selected for antibiotic resistance; metals of particular concern include Hg and Cd (Seiler & Berendonk, 2012). Resistant bacteria and resistance genes (RB & RBG) have been identified in aquatic environments that are exposed to human and animal excrement, as well as in multiple antibiotic-resistant *Escherichia coli* (MAR-EC) in northwestern Nigeria (Shiaka et al., 2020; Larsson & Flach, 2022; UNEP, 2023). The river serves as a source of food, irrigation water, and domestic water and fish are caught, handled, washed and traded in the same place, the system connects environmental, animal and human health, which is the essence of the One Health approach (Mackenzie & Jeggo, 2019).

This study only measured TPC and indicator organisms and did not isolate or test for bacteria, therefore

no conclusions can be drawn regarding the prevalence of resistance. The public health implications are therefore restated as: (i) dietary metal exposure is a child-health problem, hence advisories need to focus on children and women of reproductive age first; (ii) joint surveillance of chemical and microbiological burdens is required at the downstream landing sites, as opposed to having two separate chemical and food-safety programmes; (iii) sanitation, wastewater control and cold-chain improvement can reduce both the faecal burden and the resistance burden; and (iv) the fish and water samples provide an entry point for pathogen identification, antimicrobial susceptibility testing and resistance-gene screening in a One Health surveillance system.

4.7 Study Limitations

There are some restrictions on this present study. The health risk model is now expanded to include children and the ingestion rate for children was assumed as 0.1 kg/day, which needs to be replaced with data from consumption surveys of riverine households. Dietary pathway was the only pathway assessed and incidental soil and sediment ingestion and dermal absorption (especially important for children who play in and around the river) were not quantified because sediment, soil and river-water metals were not measured. There was no separate assessment for pregnant women. As risk assessment of carcinogenicity by utilizing USEPA cancer slope factors was also out of scope and there is an urgent need to do so. Total arsenic was measured and assumed as inorganic, and the risk due to arsenic may be overestimated until the arsenic has been speciated. The groundwater and sediment were not sampled and therefore the geological and groundwater-surface water pathways were not measured, but inferred from the literature. The proportional symbol maps are for the water-quality and bacteriological parameters only, since site-resolved concentrations of these parameters are not presented. TPC was the only bacteriological test performed; it is necessary to identify specific pathogens (*Salmonella*, *Vibrio*, *Listeria*) and AMR profiling, which is based on Shiaka et al. (2020). The PCA was descriptive and exploratory; confirmatory multivariate analysis with more intensive sampling of other stretches of the river would have validated the spatial-ecological interpretation the more. Stratification of metal data would have been helpful at the higher order, and would have facilitated the characterization of risks over time. HI additive model may underestimate the risk of combinations of metal that are synergistically toxic (USEPA, 2023).

5 Conclusion

This marks the first multi-endpoint, graphically enhanced evaluation of bioaccumulation of heavy metals

in fish of the Hadejia River in Jigawa State, Nigeria, and associated dietary health risk (according to USEPA method), physicochemical water quality parameters, and species-specific bacterial contamination in fish. All exposures were to fish muscle, with As exceeding the USEPA oral RfD for chronic exposures in adults (59.4% of total non-carcinogenic risk) and with Pb exceeding the USEPA oral RfD for chronic exposures in adults (1.14% of total non-carcinogenic risk). The total HI (6.40), which is over six times the USEPA benchmark for safety, shows extreme multi-metal dietary risk to consumers. In children, the THQs of As (8.87), Pb (2.66), Hg (1.40), and Cd (1.21) exceeded 1, and the HI was 14.93, so children are the most exposed group. Progressive deterioration of water quality was also indicated in a series of progressive downstream water quality on proportional symbol maps, with the presence of total coliforms and *E. coli* concentrations at all locations exceeding the zero tolerance guideline. PCA revealed no correlation or dependence between the chemical and bacteriological contamination, thus indicating the need for combined multi-endpoint monitoring of chemical and microbial contamination of fish muscle. The visualization of THQ by bar chart clearly shows that As is the leading risk factor, followed by Pb; Cd, Hg, and Cr individually are below THQ in adults. TPC differed amongst the species [$F(2,32) = 12.45$; $p = 0.002$], with *O. niloticus* with the highest bacterial concentration (5.14 log CFU/g) which is higher than the acceptability limit (5.0 log CFU/g). The findings, in a source-pathway-receptor context, suggest that the sources are a mixture of geogenic and anthropogenic, pathways are geologically and hydrologically determined, and receptors are children and adults consuming the water. Co-occurrence of metals and faecal bacteria also raises One Health concerns regarding antimicrobial resistance.

It is recommended that the National Agency for Food and Drug Administration and Control (NAFDAC) consider conducting a follow-up study on each specimen for contamination. The State Ministry of Agriculture should implement the THQ/HI monitoring in fish landing sites following the USEPA monitoring approach, which includes age-specific (adult and child) exposure factors and proportional symbol maps of site-level results. Cancer risk assessment of inorganic arsenic, by age and including the food-water pathway, should be undertaken. Construction of community sanitation facilities should be given priority in rural communities along the riverine section to eradicate the problem of open defecation in the communities, particularly at Site 3 where the faecal contamination was very high. Agricultural extension should be used to promote pesticides and phosphate fertilizers which are approved by the WHO, through agricultural extension, with a view to conducting a

catchment wide audit of the arseniferous pesticides and phosphate fertilizers by the Hadejia-Jama'are River Basin Development Authority. Consumer education should be targeted towards fish vendors and consumers, educating on the high bacteriopathogenic risk from *O. niloticus*, the need for rapid processing and cooking methods, and not washing fish in the river. Fish consumption advice should prioritize children and women of reproductive age in the issuance of fish consumption advisories by health authorities.

Future research directions. To test metal–antibiotic co-selection, (i) arsenic and a carcinogenic risk assessment for adults and children; (ii) child-specific consumption surveys and inclusion of the soil-to-mouth and dermal pathways; (iii) paired sampling of surface water, sediment, groundwater, and fish across seasons, with site-resolved tissue data and geochemical or isotopic source apportionment; and (v) longitudinal multivariate monitoring with a visualization approach.

Implementation agenda. NAFDAC and the Jigawa State Ministry of Agriculture should initiate sampling of the sites of Nsibidi waste in the Basin within 1 year, publish a guide for children's consumption, prioritise sanitation at the downstream communities by RUWASA and local governments, and initiate a fertiliser and pesticide audit by the Basin Development Authority; and create a joint human-health, veterinary and environment working group to coordinate chemical and AMR surveillance in a One Health perspective within 1 year.

Declarations

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Author Contributions

Afeez Oladeji Amoo: Sampling, data collection, and preparation of the original draft. Adeniyi Olarewaju Adeleye: Conceptualization, supervision of the study, formal analysis, multivariate data analysis (principal component analysis), and editing of the manuscript. Mohammed Garba Daya: Project administration, study of artisanal fisheries, and technical support for the health risk assessment. Olukemi Adeleye and Catheine Iyabode Asaju: Methodology validation, statistical analysis (IBM SPSS), and interpretation of the biological aspect of the fish species. Nureni Babatunde Amoo: Designed the map of the study area and the proportional symbol maps. Mohammed Bello Yerima: Supervision and editing of the final draft.

Conflict of Interest

The authors declare no financial or personal relationships with other people or organizations that could have inappropriately influenced them in writing this manuscript.

Data Availability

The data that support the findings of this study will be available from the authors upon reasonable request.

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