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Heavy Metal Contamination in Two Tilapia Species (*Oreochromis macrochir* and *Coptodon rendalli*) From the Kabompo River, Zambia: A Food Safety and Human Health Risk Assessment

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ABSTRACT

Aquaculture sustainability is threatened by heavy metal contamination, particularly in regions where rivers are the primary water sources. The present study assessed the concentrations of cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) in two key aquaculture species, *Oreochromis macrochir* and *Coptodon rendalli*, from the Kabompo River, Zambia. Using atomic absorption spectrophotometry (AAS), Ni (0.003–0.065 mg/kg ww), Zn (0.41–3.90 mg/kg ww), Cu (3.42–6.81 mg/kg ww) and Mn (0.04–0.24 mg/kg ww) were detected in muscle tissue, while Cd, Co and Pb were below detection limits. All detected metal concentrations were within permissible limits set by national and international standards for food safety, indicating a negligible immediate health risk to both fish and human consumers. A two-way ANOVA showed that concentrations of Cu, Zn and Mn were significantly influenced by both sampling site and fish species ($p < 0.05$), whereas Ni varied significantly only by site. The overall order of metal concentration was Cu > Zn > Mn > Ni. Furthermore, key physicochemical water parameters (dissolved oxygen, pH, temperature, conductivity, salinity and total dissolved solids) showed no significant spatial variation and remained within optimal ranges for tilapia aquaculture. This study provides baseline information on heavy metal concentration in edible tissues of *Oreochromis macrochir* and *Coptodon rendalli* from the Kabompo River, contributing to food safety assessment and aquaculture management in the region. However, continued monitoring is recommended to safeguard long-term fishery sustainability and food security.

1 | Introduction

Freshwater fish resources play a vital role in global food security and nutrition, particularly in developing countries where inland fisheries provide an important source of affordable animal protein. However, aquatic ecosystems are increasingly threatened by environmental contamination, especially heavy metals, which persist in water bodies, accumulate in sediments and bioaccumulate in fish tissues, posing risks to both ecosystem health and human consumers (Tchounwou et al. 2014; Rahman et al. 2012). However, this growth is accompanied by increasing environmental challenges, notably the risk of heavy metal contamination, which can accumulate in aquatic systems and threaten the ecosystem and human health (Obayemi et al. 2023; Obayemi and Komolafe 2022; Ouma et al. 2022; Hasimuna, Gweon, et al. 2025). Although metals like iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) are required in small amounts for vital biological functions, they can be toxic when their concentrations rise above safe limits. Non-essential metals, including cadmium (Cd), lead (Pb) and mercury (Hg), are toxic even at low concentrations, causing organ dysfunction, physiological damage and bioaccumulation in aquatic organisms (Hasimuna et al. 2024; Hasimuna, Jere, et al. 2025; Mitra et al. 2022; Gunatilake 2016).

Heavy metals enter aquatic environments through anthropogenic activities such as industrial effluents and agricultural runoff, as well as natural processes like geological weathering (Bashir et al. 2020; Olowojuni et al. 2025). Once introduced, metals persist in water and sediments, facilitating uptake and accumulation in fish tissues, which poses a direct public health concern. Chronic dietary exposure via fish consumption has been linked to renal failure, hepatic damage and cardiovascular diseases (Al-Busaidi et al. 2011; Rahman et al. 2012; Magna et al. 2021). In addition, histopathological studies have shown that metal bioaccumulation can induce organ-specific alterations in fish, including gill hyperplasia, muscle degeneration and liver congestion, highlighting sub-lethal physiological effects even when metal concentrations are moderate (Obayemi and Komolafe 2022).

Research in other tropical African reservoirs has provided baseline data on heavy metal accumulation and associated human health risks. For instance, Obayemi et al. (2023) reported that while concentrations of Cd, Cu, Pb, Mn, Ni and Zn in *Coptodon zillii* and *Parachanna obscura* were below FAO and WHO recommended limits, continuous consumption could pose cumulative health risks. In the Kabompo River, a recent study has reported metal concentrations in water and sediments, showing localised enrichment of Zn, Cu and Pb at specific sites (Hasimuna, Gweon, et al. 2025). However, information on bioaccumulation in edible fish tissues and the resulting dietary health risks remains limited, motivating the present study. These observations highlight the importance of site- and species-specific monitoring to safeguard food safety.

The Kabompo River supports capture fisheries and emerging aquaculture, particularly of tilapia species such as *Oreochromis macrochir* and *Coptodon rendalli*. Its passage through mineral-rich regions increases the potential for heavy metal contamination. Therefore, this study aimed to (1) quantify seven heavy metals (Cd, Co, Cu, Mn, Ni, Pb, Zn) in the muscle tissues of *Oreochromis macrochir* and *Coptodon rendalli* from selected sites along the Kabompo River and (2) assess the potential health risks associated with the consumption of these fish. The findings provide critical baseline data for environmental monitoring, public health guidance and sustainable aquaculture development in Zambia.

2 | Methodology

2.1 | Study Area and Sampling Sites

A total of 60 fish, comprising 30 *Oreochromis macrochir* and 30 *Coptodon rendalli* specimens, were collected from the Kabompo River in Mufumbwe District, Zambia (Figure 1). Sampling was conducted between April and August 2024, encompassing the late rainy season (April) and the dry season (May–August) in north-western Zambia. This period was selected to allow easy field access and relatively stable hydrological conditions, while providing representative data on metal accumulation in fish during typical fishing periods. Sampling sites were located approximately 5 km apart along the river and were primarily selected based on variations in human activities, including fishing intensity, agricultural land use and proximity to tributaries, rather than precise distance measurements. These characteristics represent functionally distinct pollution-risk zones within the river continuum (Hasimuna, Gweon, et al. 2025).

The Kabompo River traverses a heterogeneous landscape that includes protected sections within West Lunga National Park and its adjoining Game Management Area, as well as downstream stretches influenced by surrounding communities and their livelihood activities. Fisheries management in the river is overseen by the Department of Fisheries, while conservation responsibilities within the protected areas fall under the Department of National Parks, with support from partner organisations such as the World-Wide Fund for Nature. This combination of contrasting land use and management regimes creates spatial variability along the river, which is justified by treating the sampling sites as ecologically distinct locations for analysis. A detailed description of the study area and the characteristics of each sampling site is provided in our earlier work (Hasimuna, Gweon, et al. 2025).

2.2 | Fish Sample Collection and Preparation

Fish samples were collected using gillnets deployed at the designated sampling sites at approximately 6:00 P.M. to allow passive overnight capture. Nets were hauled the following morning after 08:00 A.M., and fish were retrieved upon recovery of the fishing gear, with mortality occurring naturally as a result of entanglement. The target species, *Oreochromis macrochir* and *Coptodon rendalli*, were identified in situ using standard taxonomic keys (Skelton 2001; Utsugi and Mazingaliwa 2002). No live fish was handled or experimentally manipulated during the study, and all specimens were processed only after capture for subsequent laboratory analyses. Morphometric measurements, including standard length and total length, were recorded immediately in the field using a graduated measuring board, while total body weight was measured to the nearest 0.01 g using a portable electronic balance. Following measurements, specimens were placed on ice in insulated cool boxes maintained at approximately 0°C–4°C and transported within 8 h to the Mufumbwe Veterinary Field Laboratory, where they were refrigerated. The samples were subsequently transported to the Zambia Bureau of Standards (ZABS) Laboratory in Lusaka and stored at −20°C until further processing. In the laboratory, each fish was dissected using a clean stainless-steel knife, and approximately 2–3 g of dorsal muscle tissue was excised for analysis. The tissue samples were oven-dried to constant weight, homogenised using an acid-washed mortar and pestle and sieved through a 1 mm mesh to obtain a uniform powder. For digestion, 1 g of the dried homogenised tissue was accurately weighed for each analytical replicate.

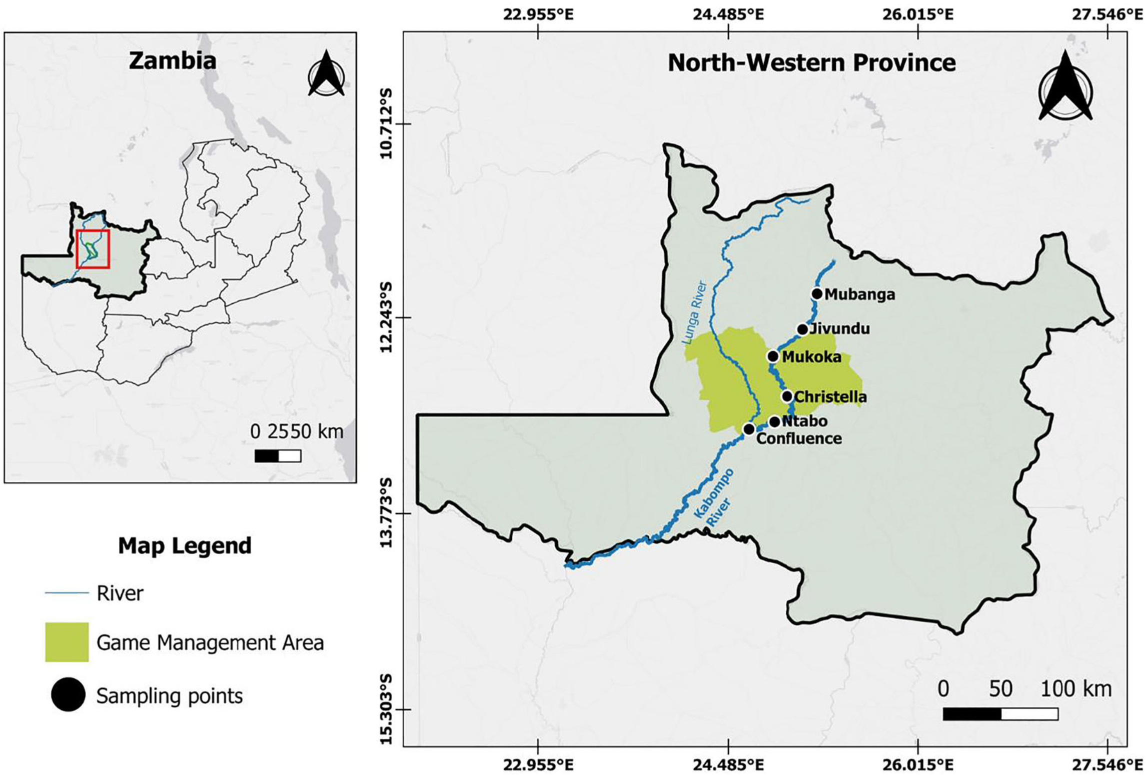

FIGURE 1 | Study area in the Kabompo River, North-Western Province, Zambia.

2.3 | Heavy Metal Analysis and Quality Assurance

Heavy metal analysis was carried out using established analytical procedures to ensure accuracy, precision and reproducibility of the results. Dried and homogenised fish muscle samples prepared as described in Section 2.3 were used for metal determination. A total of eight metals (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn) were initially analysed in fish muscle tissues. However, the present study reports only copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn), as these metals were consistently detectable across samples, exhibited clear site- and species-specific variation and have well-established reference doses (RfDs) suitable for subsequent human health risk assessment. For acid digestion, approximately 1 g of the powdered tissue sample was accurately weighed into a clean digestion flask, after which a mixed acid solution of concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) was added. The mixture was heated on a hot plate until complete digestion was achieved, as indicated by the formation of a clear solution. The digests were then allowed to cool to room temperature and filtered through Whatman No. 42 filter paper to remove any undissolved residues. The resulting filtrates were quantitatively transferred into volumetric flasks and diluted to a known volume using deionised water.

Metal concentrations were determined using flame Atomic Absorption Spectrophotometry (AAS; Shimadzu AA-7000, Japan). Instrument calibration was performed using certified standard solutions prepared across appropriate concentration ranges for each metal analysed. Procedural blanks and calibration standards were analysed alongside the samples to monitor potential contamination and instrument drift during analysis. Quality assurance and quality control measures included the analysis of replicate samples and spiked samples to assess analytical precision and recovery. Recoveries for the analysed metals fell within acceptable limits, confirming the reliability and robustness of the analytical procedure.

2.3.1 | Digestion Procedure

Ground tissue samples (1 g) were digested with 15 mL of aqua regia (3:1 hydrochloric acid [HCl]: nitric acid [HNO_3] in test tubes under a fume hood. The mixture was heated until the volume was reduced to approximately 4–5 mL. The resulting digest was cooled, filtered through Whatman No. 42 filter paper into a 50 mL volumetric flask, and made up to the mark with deionised water. A procedural blank was prepared alongside the samples using an identical process.

2.3.2 | Instrumental Analysis

The AAS was calibrated with multi-element standard solutions for quantitative analysis. The wavelengths (λ) and method detection limits for each element are summarised in Table 1 to ensure selective detection and clarity.

2.3.3 | Quality Control and Assurance

A rigorous quality assurance/quality control (QA/QC) protocol was implemented to ensure data accuracy and precision. All

TABLE 1 | Wavelengths (λ) and method detection limits for the heavy metals analysed in fish muscle samples by flame atomic absorption spectrophotometry.

Metal	Wavelength (nm)	Method detection limit (mg/L)
Cd	227.3	0.005
Co	238.7	0.01
Cu	323.7	0.01
Mn	279.9	0.05
Ni	230.0	0.02
Pb	217.6	0.01
Zn	213.2	0.05

analyses were performed in triplicate, and results are reported on a dry weight basis. To prevent cross-contamination, all glassware and plasticware were soaked in 30% HNO_3 overnight and thoroughly rinsed with deionised water before use. The accuracy of the analytical method was verified through the analysis of standard reference materials and spiked samples, which yielded percentage recoveries ranging from 90% to 95% for all target metals. Furthermore, a procedural blank was analysed after every five samples to monitor for potential background contamination, and instrument calibration was verified at regular intervals throughout the analysis sequence. This QA/QC protocol aligns with established methodologies described in our previous work (Hasimuna, Gweon, et al. 2025).

2.4 | Human Health Risk Assessment of Fish Contaminated With the Four Heavy Metals

To assess the possible human health risks from the consumption of the two fish species from the Kabompo River, equations were used in establishing safe limits. These indices were compared with the maximum permissible limits set for food used for human consumption.

Although heavy metal concentrations in *Oreochromis macrochir* and *Coptodon rendalli* were determined on a dry weight basis, human exposure occurs through the consumption of fresh (wet) fish muscle. Therefore, prior to estimating the estimated daily intake (EDI) and target hazard quotient (THQ), dry weight concentrations were converted to wet weight concentrations using the fish muscle moisture content according to the equation:

$$C_{ww} = C_{dw} \times (1 - MC) \quad (1)$$

where C_{ww} is the metal concentration on a wet weight basis (mg/kg), C_{dw} is the concentration on a dry weight basis (mg/kg) and MC is the fractional moisture content of fish muscle. An average moisture content of 75% (MC = 0.75) was assumed, consistent with reported values for freshwater tilapia species (USEPA 2008; Ahmed et al. 2019). The resulting wet weight concentrations were subsequently used in all EDI and THQ calculations to ensure that the human health risk assessment accurately reflects food safety implications associated with the consumption of tilapia from the Kabompo River, Zambia.

2.4.1 | EDI of the Four Heavy Metals

The EDI was used to quantify the potential daily intake of Cu, Ni, Zn and Mn through the consumption of *Oreochromis macrochir* and *Coptodon rendalli*. The EDI does not represent a safe limit but rather provides an estimate of dietary exposure, which was subsequently evaluated against toxicological RfDs in the risk characterisation stage. The EDI was calculated using the following equation (USEPA 2008; Ahmed et al. 2019; Hasimuna, Gweon, et al. 2025):

$$EDI = \frac{(Cn \times IGr)}{Bwt} \quad (2)$$

where Cn represents the metal concentration in fish muscle expressed on a wet weight basis (mg/kg), following conversion from dry weight concentrations using the fish muscle moisture content; IGr denotes the daily fish ingestion rate, assumed to be 55.5 g/day for adults and 52.5 g/day for children; and BW represents the average body weight, assumed to be 70 kg for adults, following standard exposure assessment assumptions widely applied in human health risk studies (USEPA 2011; Ahmed et al. 2019; Simukoko et al. 2022; Khalefa et al. 2022; Atwah et al. 2025; Hasimuna, Jere, et al. 2025).

2.4.2 | THQ

To evaluate the combined impact of these heavy metals on consumers, the THQ was computed to reflect the cumulative contamination from all metal elements recommended by the United States Environmental Protection Agency (USEPA 2008). This formula assesses the ratio of EDI to the oral RfD for Cd and Pb, with ratio values below 1 indicating a negligible risk according to USEPA guidelines.

The THQ formula, expressed as Equation (2) (USEPA 2008), integrates exposure duration (E_d), exposure frequency (E_p) and the average time for the non-carcinogenic element ($At = Ea \times Ep$).

$$THQ = \frac{Ea \times Ep \times EDI}{At \times RfD} \times 10^{-3} \quad (3)$$

TABLE 2 | Water quality parameters from different sampling sites.

Site	Temp	pH	DO	Cond	TDS	Salinity
Mubanga	22.33 ± 1.31	8.48 ± 0.22	5.70 ± 0.90	249.67 ± 10.50	120.33 ±4.04	0.12 ± 0.03
Jivundu	21.67 ± 1.25	8.52 ± 0.23	8.53 ± 1.46	247.67 ± 6.51	122.00 ±7.00	0.12 ± 0.03
Mukoka	22.49 ± 1.24	8.42 ± 0.20	5.63 ± 1.26	251.67 ± 5.51	124.67 ±9.52	0.12 ± 0.02
Christella	21.67 ± 1.21	8.76 ± 0.79	6.57 ± 1.40	234.00 ± 8.00	117.33 ±7.51	0.12 ± 0.04
Ntabo	22.50 ± 1.65	8.73 ± 0.18	5.63 ± 1.15	250.00 ± 10.00	123.33 ±9.50	0.13 ± 0.01
Confluence	21.70 ± 1.55	8.12 ± 0.11	9.37 ± 1.58	211.33 ± 7.02	134.33 ±7.51	0.11 ± 0.02

Note: Temp is temperature. DO is dissolved oxygen. Cond is conductivity. TDS is the total dissolved solids.

2.4.3 | Hazard Index (HI)

The HI was calculated by adding all the THQ values using Equation (3) (Mwakalapa et al. 2019; Simukoko et al. 2022).

$$HI = THQ\ (Cd) + THQ\ (Pb) \qquad (4)$$

where *HI* value < 1.0 suggests that adverse effects are unlikely to occur. Conversely, an *HI* value ≥ 1.0 indicates an increased probability of adverse effects. When the *HI* value ≥ 10, the risk is considered significant, with the potential for both chronic and acute health impacts (USEPA 2000; Lei et al. 2015; Ahmed et al. 2019).

2.4.4 | Water Sampling and Analysis

Water quality parameters were measured at each of the six sampling sites along the Kabompo River at the time of fish collection to characterise the environmental conditions influencing tilapia habitat and metal bioavailability. Water samples were collected at approximately 20–30 cm depth using pre-cleaned polyethylene bottles. In situ measurements of temperature (°C), pH, dissolved oxygen (DO; mg/L), electrical conductivity (µS/cm), total dissolved solids (TDS; mg/L) and salinity (‰) were conducted using a calibrated portable multiparameter water quality meter. The instrument was calibrated daily according to the manufacturer's instructions prior to field measurements. All measurements were performed in triplicate at each site, and mean values ± standard deviations were recorded (Table 2).

2.5 | Data Analysis

Descriptive statistics, including mean concentrations and standard deviations, were calculated to summarise heavy metal levels in the two fish species across all sampling sites. The Shapiro–Wilk test and Fligner–Killeen test were employed to assess normality and homogeneity of variances, respectively. As no significant deviations from normality ($p > 0.05$) or homogeneity of variances ($p > 0.05$) were detected, parametric statistical methods were applied. A two-way analysis of variance (ANOVA) was conducted to evaluate the effects of sampling sites, fish species and their interaction on heavy metal concentrations. Tukey's honestly significant difference (HSD) test was used for post hoc comparisons to identify pairwise differences among sites. To explore patterns in heavy metal distribution, principal component analysis (PCA) was performed, identifying the primary contributing metals and highlighting similarities across sites and species. Hierarchical cluster analysis (HCA) was applied to classify sampling sites and fish species based on their heavy metal profiles, with results visualised using a dendrogram. The similarity profile (SIMPROF) test was used to detect statistically distinct clusters. Additionally, a permutational multivariate ANOVA (PERMANOVA) was conducted to confirm the effects of sampling site and fish species, revealing significant differences ($p < 0.001$). Correlation analysis was performed to examine inter-metal relationships, with statistical significance set at $p < 0.05$. All analyses were conducted using R software (R Core Team 2023).

3 | Results

Muscle tissues of *Oreochromis macrochir* and *Coptodon rendalli* were analysed for eight heavy metals (Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn). These elements exhibited clear spatial and interspecific variability and have well-established RfDs, making them appropriate for subsequent statistical analysis and human health risk assessment. The remaining metals were either below analytical detection limits or occurred sporadically and were therefore excluded from further quantitative and risk-based evaluations.

3.1 | Water Quality Parameters

The water quality parameters observed across the six sampling sites (Table 2) were within the acceptable ranges for tilapia survival and culture. Temperature ranged from 21.67°C ± 1.05°C to 22.50°C ± 1.55°C in six sites, while pH values were between 8.12 ± 0.11 and 8.76 ± 0.79, indicating slightly alkaline conditions conducive for tilapia growth. DO levels varied from 5.63 ± 1.15 mg/L to 9.37 ± 1.58 mg/L, ensuring adequate oxygen availability for fish metabolism. Conductivity (211.33 ± 7.02 µS/cm to 251.67 ± 5.51 µS/cm), TDS (117.33 ± 7.51 mg/L to 134.33 ± 7.51 mg/L) and salinity (0.11 ± 0.02 ‰ to 0.13 ± 0.03 ‰) were all within optimal ranges for freshwater tilapia aquaculture. These results affirm the suitability of the studied sites for tilapia farming under the prevailing environmental conditions.

3.2 | Correlation of Water Quality Parameters

Pearson correlation analysis revealed clear patterns of association among the measured water quality variables as summarised

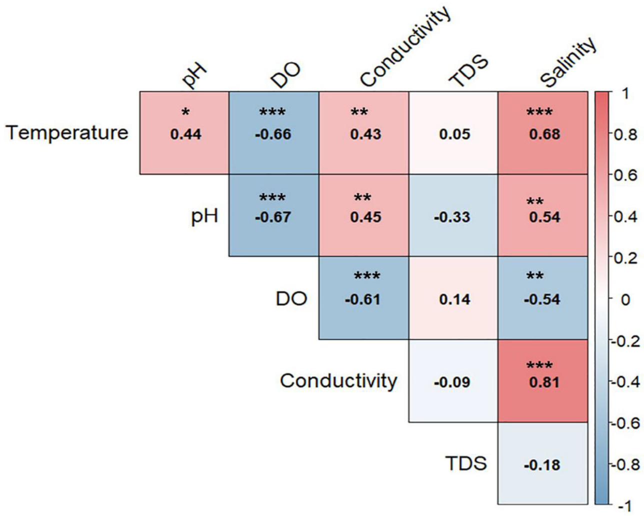

FIGURE 2 | Pearson correlation matrix showing the relationships among water quality parameters measured across sampling sites. Correlation coefficients (r) are indicated within cells, with colour intensity representing the direction and strength of associations. Asterisks denote levels of statistical significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

in Figure 2. Temperature exhibited positive associations with parameters linked to ionic content and salinity, while showing an inverse relationship with DO. Similarly, pH covaried positively with conductivity and salinity but was negatively associated with DO. Conductivity and salinity displayed a strong concordant relationship, indicating their shared control by dissolved ions, whereas TDS showed comparatively weak or negligible associations with the other variables. Overall, DO consistently demonstrated inverse relationships with several physicochemical parameters, highlighting its sensitivity to thermal and ionic conditions within the system. These correlation structures are visually represented in Figure 2, which illustrates the direction and relative strength of associations among variables.

3.3 | Heavy Metal Assessment in Two Aquaculture Fish Species

3.3.1 | Concentration of Heavy Metals in *Oreochromis macrochir* and *Coptodon rendalli*

Two-way ANOVA revealed that sampling site had a significant main effect on the concentrations of Ni, Zn, Cu and Mn in both *Oreochromis macrochir* and *Coptodon rendalli* ($p < 0.05$). Similarly, fish species also showed a significant main effect on metal accumulation ($p < 0.05$). No significant interaction between site and species was observed for any of the metals analysed ($p > 0.05$), indicating that the spatial patterns in metal concentrations were consistent across both species. The mean concentrations of metals at each site and for each species are presented in Table 3.

Across sampling sites, the Confluence site recorded significantly higher concentrations of Ni, Cu and Mn, compared to the other locations ($p < 0.05$), whereas Mubanga, Jivundu and Christella generally exhibited lower concentrations of Ni and Cu.

TABLE 3 | Concentrations of Ni, Zn, Cu and Mn (mg/kg dry wt) in two fish tilapia species across six sampling sites.

Heavy metals	Fish species	Mubanga	Jivundu	Mukoka	Christella	Ntabo	Confluence
Ni	*O. macrochir*	0.01 ± 0.01^{a}	0.03 ± 0.01^{ab}	0.03 ± 0.01^{b}	0.02 ± 0.01^{a}	0.01 ± 0.01^{ab}	0.26 ± 0.02^{c}
	C. rendalli	0.03 ± 0.01^{a}	0.02 ± 0.01^{ab}	0.06 ± 0.02^{b}	0.01 ± 0.01^{a}	0.06 ± 0.02^{ab}	0.16 ± 0.02^{c}
Zn	*O. macrochir*	11.64 ± 1.51^{b}	3.67 ± 0.61^{a}	15.58 ± 2.44^{c}	5.18 ± 0.08^{a}	10.37 ± 2.21^{b}	12.37 ± 1.46^{b}
	C. rendalli	9.06 ± 1.98^{b}	1.64 ± 0.64^{a}	14.55 ± 1.50^{c}	4.20 ± 1.00^{a}	9.49 ± 2.62^{b}	11.11 ± 1.09^{b}
Cu	*O. macrochir*	18.38 ± 2.34^{ab}	16.13 ± 2.99^{a}	22.77 ± 0.84^{cd}	22.24 ± 1.98^{c}	21.63 ± 2.16^{bc}	27.25 ± 1.68^{d}
	C. rendalli	15.29 ± 2.30^{ab}	13.67 ± 2.06^{a}	20.58 ± 2.39^{cd}	19.69 ± 2.78^{c}	16.57 ± 1.48^{bc}	23.08 ± 1.99^{d}
Mn	*O. macrochir*	0.22 ± 0.12^{a}	0.48 ± 0.14^{bc}	0.37 ± 0.13^{abc}	0.55 ± 0.21^{c}	0.28 ± 0.08^{ab}	0.96 ± 0.15^{d}
	C. rendalli	0.16 ± 0.10^{a}	0.38 ± 0.13^{bc}	0.23 ± 0.04^{abc}	0.40 ± 0.10^{c}	0.21 ± 0.10^{ab}	0.74 ± 0.12^{d}

Note: Values are mean ± standard deviation. Different superscript letters within the same row indicate significant differences among sampling sites for a given metal and species based on post hoc multiple comparison tests ($p < 0.05$). Main effects of sampling site and fish species were significant, while their interaction was not significant (two-way ANOVA).

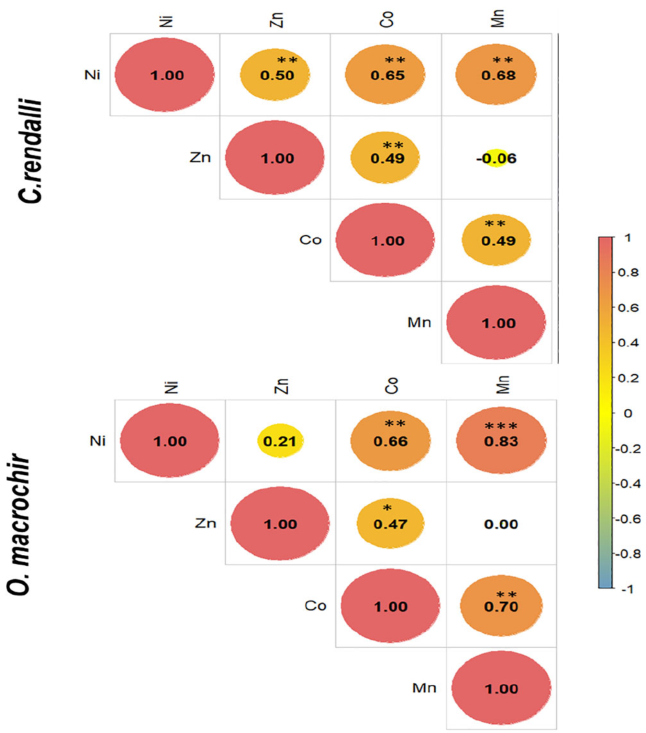

FIGURE 3 | Pearson correlation coefficients among Ni, Zn, Co and Mn in the muscle tissues of *Oreochromis macrochir* (upper panel) and *Coptodon rendalli* (lower panel) collected from the Kabompo River. Circle size and colour intensity are proportional to the strength of the correlation, with warmer colours indicating stronger positive relationships. Asterisks denote levels of statistical significance ($p < 0.05$; *$p < 0.01$; **$p < 0.001$).

When averaged across all sites, *Oreochromis macrochir* tended to accumulate slightly higher concentrations of Cu and Mn than *C. rendalli*, while Zn and Ni concentrations were comparable between the two species. Overall, the mean metal concentrations in both species followed the order: Cu > Zn > Mn > Ni.

3.3.2 | Correlation Between Heavy Metals in the Two Fish

The Pearson correlation analysis indicated positive associations among several heavy metals measured in the muscle tissues of the two tilapia fish species (Figure 3). Notably, strong correlations were observed between Ni and Mn ($r = 0.76$, $p < 0.001$), while Cu showed moderate correlations with both Ni ($r = 0.60$, $p < 0.001$) and Mn ($r = 0.64$, $p < 0.001$). Zinc exhibited comparatively weaker correlations with Ni ($r = 0.31$) and Cu ($r = 0.50$) and a near-zero association with Mn ($r = 0.01$) as summarised in the figure below.

3.3.3 | Multivariate Analysis

The HCA classified heavy metal concentrations in the two fish species from different sampling sites into five primary clusters based on similarities (Figure 4). The SIMPROF test indicated that black lines represented statistically similar groups of fish species from different site clusters in terms of their heavy metal profiles, whereas red lines denoted groups exhibiting greater variability or dissimilarity. Notably, heavy metal concentrations from the Confluence site formed a distinct and homogeneous group, clearly separated from other sampling sites. Similarly, Ntabo, Mubanga and Mukako clustered into another homogeneous group, while Jivundu and Christella formed a separate, distinct cluster.

The PCA biplot (Figure 5) illustrates the distribution of heavy metals (Ni, Zn, Cu and Mn) across six sampling sites. The first two principal components, Dim1 and Dim2, explain 62.1% and 25.8% of the total variance, respectively, contributing to a cumulative variance of 87.9%. The PCA confirms that the heavy metal concentrations at Confluence were distinct from those at other sites, with Cu, Ni and Mn being the key differentiating metals. Mukoka, on the other hand, was associated with Zn, making it dissimilar to the other sites. Significant differences in heavy metal concentrations among sites were confirmed by the analysis of similarities (ANOSIM) for abundance ($R = 0.83$; $p < 0.01$). Pairwise comparisons revealed clear dissimilarity in heavy metal concentrations between Mubanga versus Jivundu ($R = 0.98$) and Mubanga versus Confluence ($R = 1$), while the concentration between Mubanga versus Ntabo was similar ($R = 0.07$; $p \geq 0.29$). The heavy metals concentrations between two species were not different ($R = 0.32$; $p>0.05$).

3.4 | Human Health Risk Assessment of Consuming Fish From Kabompo River

3.4.1 | EDI

The EDI for the two targeted age groups, adults and children, was calculated and summarised in Tables 4 and 5. In both species, the present study revealed that consumers were exposed to low doses of heavy metals through the consumption of contaminated fish as a dietary source. The EDIs for both age groups followed the descending order: Cu > Zn > Mn > Ni.

3.4.2 | THQs for Both Adults and Children

The estimated THQs for Cu, Mn, Ni and Zn are presented in Tables 6 and 7 for adults and children. Generally, the estimated THQ values were lower for adults, compared to children across all sampling sites, indicating a higher potential non-carcinogenic risk in children.

3.4.3 | Hazard Indices in Adults and Children

The calculated hazard indices (HIs) for both adults and children are presented in Table 8. The HI values for adults ranged from 0.017 at Jivundu to 0.03 at the Confluence, while for children, the values ranged from 0.07 at Jivundu to 0.14 at the Confluence. Across all study sites, the HIs for both age groups remained well below the threshold value of 1, indicating no significant non-carcinogenic health risks associated with consuming fish from these locations. Children were observed to be more susceptible, with HI values approximately four to five times higher than those of adults.

TABLE 4 | Calculated estimated daily intake (EDI) for adults (mg/kg dry wt.).

Metal	Fish species	Mubanga	Jivundu	Mukoka	Christella	Ntabo	Confluence
Nickel	*O. macrochir*	0.01	0.03	0.02	0.02	0.01	0.20
Nickel	*C. rendalli*	0.02	0.01	0.04	0.01	0.05	0.12
Zinc	*O. macrochir*	9.18	2.89	12.29	4.09	8.18	9.76
Zinc	*C. rendalli*	7.15	1.30	11.47	3.31	7.49	8.76
Copper	*O. macrochir*	14.49	12.72	17.96	17.54	17.06	21.49
Copper	*C. rendalli*	12.06	10.78	16.23	15.53	13.06	18.20
Manganese	*O. macrochir*	0.17	0.38	0.29	0.43	0.22	0.76
Manganese	*C. rendalli*	0.12	0.30	0.18	0.32	0.17	0.58

TABLE 5 | Calculated EDI for children (mg/kg dry wt.).

Metal	Fish Species	Mubanga	Jivundu	Mukoka	Christella	Ntabo	Confluence
Nickel	*O. macrochir*	0.04	0.12	0.10	0.08	0.04	0.90
Nickel	*C. rendalli*	0.10	0.05	0.20	0.05	0.21	0.55
Zinc	*O. macrochir*	40.73	12.84	54.54	18.13	36.30	43.30
Zinc	*C. rendalli*	31.72	5.75	50.93	14.70	33.23	38.90
Copper	*O. macrochir*	64.32	56.46	79.70	77.85	75.72	95.39
Copper	*C. rendalli*	53.52	47.86	72.02	68.93	57.99	80.77
Manganese	*O. macrochir*	0.76	1.68	1.31	1.93	0.99	3.35
Manganese	*C. rendalli*	0.55	1.34	0.81	1.41	0.74	2.58

TABLE 6 | Calculated target hazard quotients (THQs) for adults.

Metal	Fish species	Mubanga	Jivundu	Mukoka	Christella	Ntabo	Confluence
Nickel	*O. macrochir*	$8.00\ e^{-07}$	$2.70\ e^{-05}$	$2.30\ e^{-05}$	$1.70\ e^{-05}$	$9.00\ e^{-06}$	$2.03\ e^{-04}$
Nickel	*C. rendalli*	$2.10\ e^{-05}$	$1.20\ e^{-05}$	$4.40\ e^{-05}$	$1.10\ e^{-05}$	$4.80\ e^{-05}$	$1.23\ e^{-04}$
Zinc	*O. macrochir*	$9.17\ e^{-07}$	$2.89\ e^{-03}$	$1.23\ e^{-02}$	$4.09\ e^{-03}$	$8.18\ e^{-03}$	$9.76\ e^{-03}$
Zinc	*C. rendalli*	$7.15\ e^{-03}$	$1.29\ e^{-03}$	$1.15\ e^{-02}$	$3.31\ e^{-03}$	$7.49\ e^{-03}$	$8.76\ e^{-03}$
Copper	*O. macrochir*	$1.45\ e^{-02}$	$1.27\ e^{-02}$	$1.80\ e^{-02}$	$1.75\ e^{-02}$	$1.71\ e^{-02}$	$2.15\ e^{-05}$
Copper	*C. rendalli*	$1.21\ e^{-02}$	$1.08\ e^{-02}$	$1.62\ e^{-02}$	$1.55\ e^{-02}$	$1.31\ e^{-02}$	$1.82\ e^{-02}$
Manganese	*O. macrochir*	$1.70\ e^{-04}$	$3.78\ e^{-04}$	$2.94\ e^{-04}$	$4.34\ e^{-04}$	$2.23\ e^{-04}$	$7.55\ e^{-04}$
Manganese	*C. rendalli*	$1.23\ e^{-07}$	$3.02\ e^{-04}$	$1.81\ e^{-04}$	$3.18\ e^{-04}$	$1.67\ e^{-04}$	$5.81\ e^{-04}$

TABLE 7 | Calculated THQs for children.

Metal	Fish species	Mubanga	Jivundu	Mukoka	Christella	Ntabo	Confluence
Nickel	*O. macrochir*	$3.50\ e^{-05}$	$1.19\ e^{-04}$	$1.02\ e^{-04}$	$7.70\ e^{-05}$	$3.90\ e^{-05}$	$9.00\ e^{-04}$
Nickel	*C. rendalli*	$9.40\ e^{-05}$	$5.30\ e^{-05}$	$1.96\ e^{-04}$	$4.90\ e^{-05}$	$2.13\ e^{-04}$	$5.46\ e^{-04}$
Zinc	*O. macrochir*	$4.07\ e^{-02}$	$1.28\ e^{-02}$	$5.45\ e^{-02}$	$1.80\ e^{-02}$	$3.63\ e^{-02}$	$4.33\ e^{-02}$
Zinc	*C. rendalli*	$3.17\ e^{-02}$	$5.75\ e^{-03}$	$5.09\ e^{-02}$	$1.47\ e^{-02}$	$3.32\ e^{-02}$	$3.89\ e^{-02}$
Copper	*O. macrochir*	$6.43\ e^{-02}$	$5.64\ e^{-02}$	$7.97\ e^{-02}$	$7.78\ e^{-02}$	$7.57\ e^{-02}$	$9.54\ e^{-02}$
Copper	*C. rendalli*	$5.35\ e^{-02}$	$4.78\ e^{-02}$	$7.20\ e^{-02}$	$6.89\ e^{-02}$	$5.80\ e^{-02}$	$8.08\ e^{-02}$
Manganese	*O. macrochir*	$7.56\ e^{-04}$	$1.68\ e^{-03}$	$1.30e^{-03}$	$1.92\ e^{-03}$	$9.91\ e^{-04}$	$3.35\ e^{-03}$
Manganese	*C. rendalli*	$5.46\ e^{-04}$	$1.34\ e^{-03}$	$8.05\ e^{-04}$	$1.41\ e^{-03}$	$7.42\ e^{-04}$	$2.58\ e^{-03}$

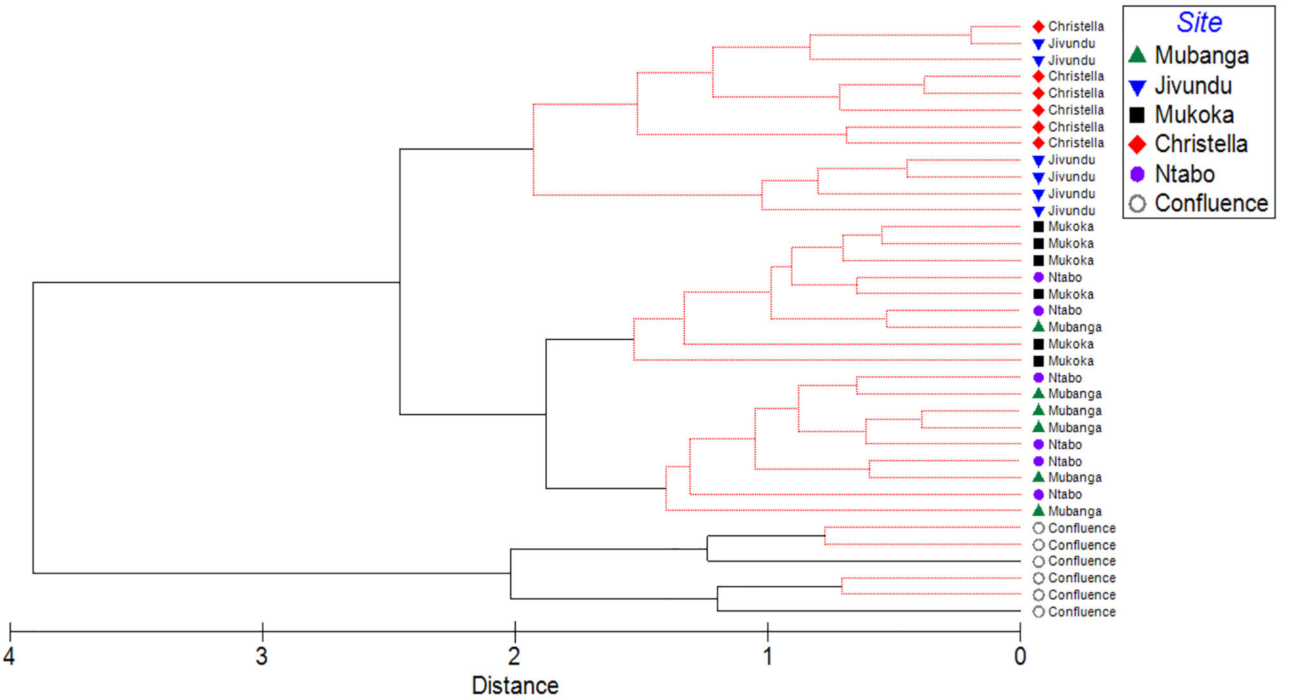

FIGURE 4 | Hierarchical cluster plot of heavy metal levels from *O. macrochir* and *C. rendalli* at six sampling sites.

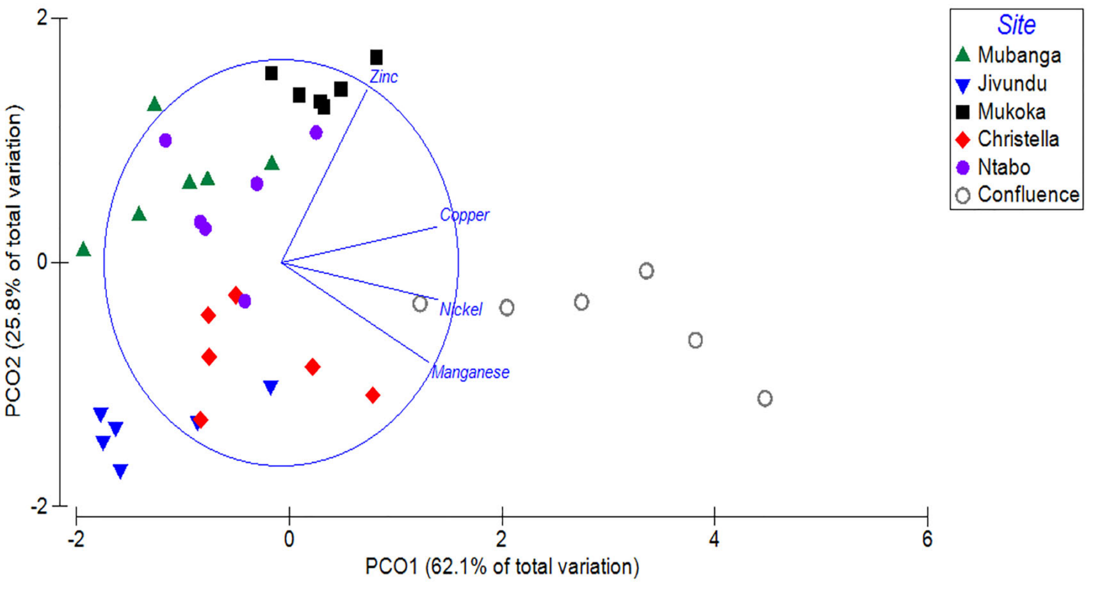

FIGURE 5 | Principal component analysis (PCA) showing the distribution of heavy metals among different sites.

TABLE 8 | Hazard indices (HIs) for adults and children across study sites.

Site	HI (adults)	HI (children)
Mubanga	0.02	0.12
Jivundu	0.02	0.07
Mukoka	0.03	0.13
Christella	0.02	0.09
Ntabo	0.03	0.11
Confluence	0.03	0.14

4 | Discussion

4.1 | Contribution of Mining to Aquatic Contamination

Mining remains the backbone of Zambia's economy followed by agriculture. The country exploits minerals such as copper, cobalt, nickel, gold and manganese, with ore extraction and refining occurring domestically. However, these activities contribute significantly to aquatic contamination, necessitating regular assessments to safeguard both ecosystem and human health. Mining discharges metals into water bodies through acid mine drainage, tailings and runoff, increasing concentrations of toxic substances in sediments and biota over time. Artisanal and small-scale mining (ASM), which often lacks adequate waste management, may exacerbate contamination (Hasimuna et al. 2023; Sihoka et al. 2024). Consequently, fish from rivers within or near mining zones require continuous monitoring. This is supported by Obayemi et al. (2023), whose study of tropical African reservoirs documented metal accumulation in *Coptodon zillii* and *Parachanna obscura*. While the immediate THQ and HI risks were not elevated, the authors warned that continuous exposure poses potential long-term health concerns.

4.2 | Detected and Undetected Heavy Metals in Fish Tissues

In the present study, Ni, Zn, Cu and Mn were detected in fish muscle, whereas Pb, Cd and Hg were below detection limits. Similar patterns have been reported in other less industrialised regions of sub-Saharan Africa, including Zambia, where non-essential heavy metals are often undetectable due to relatively low industrial activity (Hasimuna et al. 2022, 2023; Kamzati et al. 2020; Mwambene et al. 2023). Previous studies have shown that metal bioaccumulation in fish tissues is influenced by environmental availability and species-specific uptake mechanisms (Jomova et al. 2022; Girgis et al. 2019; Castro-González et al. 2008), and the observed detection patterns in the present study are consistent with these findings. In addition, histopathological evidence from tilapia indicates that even moderate metal exposure can induce tissue alterations, emphasising the need for ongoing surveillance (Hasanein et al. 2022; Obayemi and Komolafe 2022).

4.3 | Metal Concentrations and Potential Health Implications

Among the detected heavy metals, Cu concentrations were highest across all locations, exceeding the United States Food and Drug Administration (FDA) recommended limit of 20 mg/kg at Mukoka, Christella, Ntabo and Confluence (Table 4). However, all concentrations remained below the FAO/WHO Codex Alimentarius Commission (2012) and WHO (1989) recommended maximum of 30 mg/kg wet weight (ww). At the Confluence site, Cu levels were approximately 91% of the 30 mg/kg ww threshold, indicating that while elevated, they are still within the safe limit. The elevated Cu levels could be attributed to extensive copper mining activities in Zambia, particularly in the study area, where rivers receive effluents from nearby copper mines (Hasimuna et al. 2023, 2024; Mbewe et al. 2016; Sihoka et al. 2024). Although copper is an essential trace element involved in red blood cell production, immune support and antioxidant functions (Cheng et al. 2022; Rock et al. 2000; Hasan et al. 2023), excessive intake can result in toxicity, leading to kidney and liver failure, gastrointestinal distress (nausea, vomiting and diarrhoea), and, in severe cases, contact dermatitis (Gaetke and Chow 2003; Izydorczyk et al. 2021).

The FAO guidelines permit Zn concentrations of up to 30 mg/kg. In this study, the highest Zn concentration was recorded at Mukoka (15.58 mg/kg), while the lowest was observed at Jivundu (1.64 mg/kg), and both were well below the recommended maximum permissible limit (Table 4). As all recorded Zn values were below half of the permissible threshold, the analysed fish species do not pose a significant risk of Zn contamination. Rock weathering and agricultural activities, including pesticide, fertiliser and herbicide applications, are likely primary sources of Zn contamination (Hasimuna et al. 2023; Sihoka et al. 2024; Shen et al. 2019). As an essential metal, Zn plays a vital role in biological cellular functions, particularly in red blood cell production, immune function and growth. However, Zn concentrations exceeding 40 mg/kg ww are considered toxic and have been associated with kidney and liver impairment, lethargy and anaemia (Sihoka et al. 2024; Simfukwe et al. 2024). Higher Zn concentrations, exceeding 1.6 mg/kg in *Oreochromis* species, have also been documented by Sihoka et al. (2024), Sani et al. (2022) and Hossain et al. (2021).

Similarly, Mn and Ni levels remained below the recommended consumption limits of 1 and 67.9 mg/kg, respectively, as set by WHO and FAO. Manganese is essential for enzyme function, wound healing and antioxidant defence; however, excessive exposure may adversely affect neuronal function (Hasan et al. 2023; Hasimuna, Jere, et al. 2025). Nickel plays a role in enzyme activity and iron metabolism but has also been linked to carcinogenic effects when present in excessive concentrations (Grimsrud et al. 2002; Seilkop and Oller 2003). Both Mn and Ni naturally occur at low environmental concentrations, yet rock weathering and agricultural runoff can contribute significantly to their presence in aquatic ecosystems (Tchounwou et al. 2012; Rai et al. 2019; Hasimuna et al. 2021; Hasimuna, Jere, et al. 2025). The Mn and Ni concentrations observed in this study align with findings by Sihoka et al. (2024), Hasimuna et al. (2022, 2023, 2024), Mbewe et al. (2016), Mwambene et al. (2023) and Sani et al. (2022) in Zambian natural waters.

4.4 | Spatial Distribution and Human Health Risk Assessment

Spatial variations in metal concentrations were evident, with the Confluence exhibiting higher levels of Ni, Cu and Mn, whereas Christella, Jivundu and Mubanga displayed lower concentrations of Ni and Cu (Figure 3). The elevated metal levels at the Confluence likely reflect increased deposition from upstream sources, driven by mining activities and natural geological processes. As a convergence point for effluents transported by tributaries, this site experiences significant metal accumulation in both sediments and water. Conversely, the lower concentrations observed at Christella, Jivundu and Mubanga may indicate reduced anthropogenic influence or more effective natural dilution. Similar patterns of elevated heavy metal concentrations at confluence points have been reported by Wang et al. (2025) and Qi et al. (2022). Notably, the elevated Cu and Zn concentrations in fish muscle at the Confluence are consistent with localised enrichment in sediments and water in the same area reported in our previous study (Hasimuna, Gweon, et al. 2025; Magna et al. 2021). This suggests that environmental metal availability strongly influences bioaccumulation patterns. Additionally, Dendievel et al. (2022) observed that metal concentrations tend to be higher in downstream river sections due to cumulative deposition.

For all sampled locations, the consumption of *Oreochromis* species posed no significant non-carcinogenic health risk (HI < 1), consistent with the findings of Obayemi et al. (2023). Nonetheless, cumulative exposure from multiple contaminants may still need attention in both adults and children (Khalefa et al. 2022; Li et al. 2013). Children remain more vulnerable due to lower body weight and developing organs, which may increase their relative exposure even at low metal concentrations (Bair 2022; Capitão et al. 2022). These findings underscore the importance of continued monitoring, particularly for vulnerable populations, despite the generally low health risk indicated by the current results.

4.5 | Linkages Between Water Chemistry and Metal Bioaccumulation in Fish

The observed correlations among water quality parameters and heavy metals in fish tissues provide insight into the physicochemical controls governing metal availability and uptake in the Kabompo River system. Strong inverse relationships between DO and temperature, alongside positive associations among conductivity, salinity and selected metals, are consistent with established limnological theory indicating that warmer, more mineralised waters enhance metal solubility and mobility, thereby increasing bioavailability. In parallel, the significant positive correlations among Ni, Mn, Co and Zn in fish muscle suggest shared environmental sources and similar uptake pathways, likely driven by dissolved and particulate metal pools influenced by upstream mining inputs and geochemical weathering (Shen et al. 2019; Miri et al. 2017; El-Moselhy et al. 2014). Such co-accumulation patterns are well documented in riverine systems where metals with comparable ionic behaviour and binding affinities respond similarly to changes in redox conditions, ionic strength and sediment–water exchange processes (Akpan et al. 2024; Panga et al. 2023; Liew et al. 2020; Membere and Abdulwasiu 2020; Shen et al. 2019; Mataba et al. 2016). From an ecotoxicological perspective, these coupled water–biota relationships indicate that even when individual metal concentrations remain below regulatory thresholds, changes in water chemistry can modulate cumulative exposure in fish, with implications for both ecosystem health and long-term human consumption risk (Atwah et al. 2025; Hasimuna, Gweon, et al. 2025; Hasimuna, Jere, et al. 2025; Khalefa et al. 2022; Shen et al. 2019; Niyogi et al. 2016). This reinforces the importance of integrated monitoring approaches that consider water quality dynamics alongside tissue burdens, particularly in mining-influenced catchments where subtle shifts in physicochemical conditions may precede measurable increases in biological accumulation.

4.6 | Ecological and Regulatory Implications

Beyond human health, elevated metal concentrations in aquatic ecosystems can impair fish growth, reproduction and survival, potentially disrupting aquatic food webs (Nagajyoti et al. 2010; Duruibe et al. 2007). For example, tilapia species that exhibit greater interaction with bottom sediments or consume benthic-associated food resources may experience increased exposure to particle-bound metals, as sediments often act as major reservoirs for trace metal contamination in riverine systems (Usero et al. 2005; Yi et al. 2011). In addition, differences in growth rate, lifespan and bioenergetic demands may affect cumulative metal burdens over time, even among closely related species (Dallinger 1993; Canli and Atli 2003). Although interspecific differences were statistically significant in the present study, both species displayed similar overall spatial patterns of metal concentrations across sampling sites, suggesting that shared environmental exposure remains the dominant driver of bioaccumulation in the Kabompo River. The presence of essential metals such as Cu, Zn, Mn and Ni at elevated levels may be toxic to aquatic organisms, particularly in early life stages. This study emphasises the imperative to strengthen monitoring and enforcement of discharge regulations in Zambia's mining regions. Supporting this, Obayemi and Komolafe (2022) demonstrated, using histopathological analyses, that physiological stress in fish can serve as an early warning indicator of environmental contamination, preceding detectable chemical accumulation. These findings support the implementation of integrative, ecosystem-based management approaches for the monitoring and mitigation of aquatic pollution. While current levels remain largely within permissible limits, the proximity of some sites to regulatory thresholds stresses the importance of adopting a precautionary approach to protect both ecological and public health. Policymakers should prioritise investment in early warning systems, environmental education and ecosystem-based management strategies to mitigate the long-term consequences of heavy metal accumulation.

4.7 | Limitations and Future Research

While this study provides valuable insights into heavy metal contamination in aquaculture fish species from the Kabompo River, several limitations must be acknowledged to contextualise the findings and guide future investigations.

- First, the analysis focused exclusively on the muscle tissues of fish, which are most relevant to human consumption. However, other organs such as the liver, kidneys and gills are known to accumulate higher concentrations of metals and could provide a more complete picture of bioaccumulation patterns and physiological effects. Excluding these organs may therefore underestimate the overall metal burden in fish.
- Second, the study did not account for key biological variables such as the age, sex or size of the sampled fish. These factors can significantly influence metal uptake and bioaccumulation due to variations in metabolism, growth rate, reproductive status and feeding behaviour. Without stratifying the samples by these variables, it is difficult to determine whether observed metal concentrations are consistent across fish populations or are influenced by demographic characteristics.

- Third, the sampling was conducted at a single time point, which does not capture temporal variations in metal concentrations. Seasonal changes in water flow, temperature and sediment load can alter metal distribution and bioavailability in aquatic environments. As such, the lack of seasonal data limits the generalisability of the findings across different hydrological periods.
- Fourth, potential sources of metal contamination in the river catchment were not explicitly identified or quantified. While the results suggest elevated levels of certain metals in specific areas, the absence of source attribution prevents the formulation of targeted mitigation or regulatory measures.
- Last, the present study was geographically limited to selected sites along the Kabompo River and may not reflect the broader regional context. Spatial heterogeneity in pollution sources and land-use activities could lead to variable contamination levels across different segments of the river system.

To address these limitations, future research should include multi-tissue analysis, consider fish-specific biological traits and incorporate seasonal sampling to better understand temporal dynamics. Longitudinal studies would also be useful to monitor trends over time. Furthermore, identifying and quantifying specific pollution sources such as agricultural runoff, mining activities or wastewater discharge would enhance the ability to manage and reduce contamination risks. Expanding the geographic scope and sample size will also improve the robustness and applicability of future findings for food safety and environmental protection.

5. Conclusion

This study demonstrated that *Oreochromis macrochir* and *Coptodon rendalli* from the Kabompo River accumulate essential trace metals including nickel, zinc, copper and manganese, while cadmium, cobalt and lead were below detection limits. Spatial variation in concentrations was evident, with copper levels at some sites approaching permissible limits, suggesting possible influence of anthropogenic inputs and natural geochemical conditions. Although zinc, manganese and nickel remained within international safety thresholds, elevated levels at the Confluence site warrant closer monitoring. The health risk assessment indicated that consumption of these species does not currently pose significant non-carcinogenic risks, supporting their continued role in aquaculture and food security. Nevertheless, sustained environmental monitoring and site-specific risk assessments are recommended to prevent long-term ecological and public health challenges. Importantly, these findings provide essential baseline data for managing heavy metal risks in aquaculture and ensuring safe fish production in Zambia and similar freshwater ecosystems.

Author Contributions

All the authors contributed to the study conception and design of the methodology. Data collection and analysis as well as preparation of the first draft of the manuscript were prepared by Oliver Jolezya Hasimuna. Hong Yang and Hyun S. Gweon supervised the work and reviewed previous versions of the manuscript. All authors read and approved the final manuscript. Oliver J. Hasimuna and Hong Yang acquired the funding.

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Ethics Statement

Ethical approval was not required as the study exclusively examined fish specimens that were deceased prior to data collection.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used to support the findings of this study are available and can be requested from the corresponding author upon reasonable request.

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