

Research Paper

Health Risk Assessment and Levels of Toxic Heavy Metals in *Oreochromis niloticus* and *Clarias gariepinus* from Alwero and Abay River Basin

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Abstract

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This study investigated concentrations of toxic heavy metals (Cr, Pb, Hg, Sn, As, Co, Cd) in the muscle and gill tissues of *Oreochromis niloticus* and *Clarias gariepinus* from Ethiopia's Alwero and Abay Rivers, alongside the associated human health risks. Eighty fish samples (20 per species per river) were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). Metal accumulation was significantly higher in gills than in muscle across all samples ($p < 0.05$). In muscle tissue, species-specific metal accumulation patterns varied by site. Notably, while target hazard quotient (THQ) and hazard index (HI) values remained below 1, indicating no significant non-carcinogenic risk, the carcinogenic risk (CR) for inorganic arsenic in *C. gariepinus* from the Abay River (1.36×10^{-4}) exceeded the acceptable threshold of 10^{-4} . Furthermore, chromium, lead, and arsenic levels in the Abay River frequently exceeded global safety limits established by the European Commission and the FAO/WHO. Conversely, fish from the Alwero River generally fell within safe consumption limits, despite elevated tin levels in gills. These findings underscore an urgent need for pollution source control and regular biomonitoring to safeguard public health and ensure food security in these critical Ethiopian fisheries, as unchecked exposure to carcinogenic metals may pose serious long-term health risks.

1. Introduction

Pollution by heavy metals is a global problem because these elements are persistent, non-biodegradable, and harmful to human health as well as to the environment (Jadaa & Mohammed, 2023; Abdullah et al., 2024). Heavy metals are particularly harmful to aquatic ecosystems because they are often introduced into rivers and lakes through industrial discharges, agricultural runoff, and municipal wastewater (Singh et al., 2022). Fish possess a remarkable ability to

sequester metallic pollutants within their systems, absorbing these toxins through a combination of respiratory intake, food consumption, and direct contact with benthic deposits (Agbugui & Abe, 2022). Consequently, fish act as the primary vector for transferring metallic contaminants from aquatic environments to human populations through the food web (Habib et al., 2024).

Fish act as an economical source of good-quality, complete protein, along with crucial minerals and

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omega-3 fatty acids, thus posing metal contamination concerns in some commercially valuable fish species globally (Singh et al., 2022; Habib et al., 2024). Research has extensively reported fish metal exposure levels beyond the internationally accepted limits. The non-carcinogenic and carcinogenic risks associated with metal exposure in fish have been quantified using parameters like Estimated Daily Intake (EDI), Target Hazard Quotient (THQ/HQ), Hazard Index (HI), and Cancer Risk (CR) (Agbugui & Abe, 2022; Jadaa & Mohammed, 2023; Abdullah et al., 2024). Some reports have reported bioaccumulation patterns for different fish species and their tissues, along with the identification of vulnerable groups like children (Dippong et al., 2024; Phaenark et al., 2024; Oros, 2025).

Fish are widely used to assess whether an ecosystem is polluted by examining both local sources of pollution and the movement of pollutants through the food chain (Dippong et al., 2024; Phaenark et al., 2024; Younis et al., 2024). In Ethiopia, the Abay and Alwero Rivers are socio-economically important freshwater systems that support artisanal and commercial fisheries (Anteneh et al., 2023; Bor, 2025). However, ongoing developments in agriculture and urban areas, along with inadequate waste management practices, may have increased the amount of metals entering these systems and affected the safety of popular fish species, such as *Oreochromis niloticus* and *Clarias gariepinus* (Delilo, 2020; Asmamaw et al., 2021). Despite this, comparative information on metal burdens in edible versus non-edible tissues of these species, and the consequent health risks for local consumers, remains scarce.

This study addresses the gaps by (i) determining the concentrations of specific metals (Cr, Pb Hg, Sn, As and Cd) present in edible and non-edible tissues of *O. niloticus*, and *C. gariepinus* from the Abay and Alwero Rivers; (ii) determining differences between species and rivers of metal

accumulation; and (iii) assessing potential human health risks by estimating EDI and HQ (and related indices) for comparison to international guidelines such as those developed by the FAO/WHO and EU (Agbugui & Abe, 2022; Jadaa & Mohammed, 2023; Abdullah et al., 2024).

2. Method and Materials

2.1. Study Area Description

The study was conducted in the Abay River and the Alwero River, located in basins with potential for productive inland fisheries and notable susceptibility to metal contamination in water, sediment, and edible fish tissues (Melake et al., 2023). The Abay River, Ethiopia's longest river, serves as the sole natural outlet of Lake Tana and originates from the northwestern highlands (11°36'93.6"N, 37°24'53.85"E) (Figure 1). Its headwater rises at approximately 1,830 m a.s.l. in the southwest portion of Lake Tana. It has a watershed of about 15,054 km² and has high-energy flow, deep volcanic canyons, and a controlled flow at the Chara Chara weir (Mulatu et al., 2024). Rapid urban development in Bahir Dar and adjacent towns, combined with existing hydropower and irrigation projects, has already changed the natural flow patterns and caused moderate to severe ecological impacts downstream of these projects (Roth et al., 2018; Mulatu et al., 2024).

The Alwero River (07°86'N, 34°50'E) is in Abobo Woreda (Figure 1), within Ethiopia's Gambella Region in the lowland Baro–Akobo basin; hence, it is one of Ethiopia's most species-rich drainage basins and contains large areas of wetland floodplains (Melake et al., 2023). The basin also provides support for the people who inhabit the vicinity of the River basin, who rely on fishing, pastoralism, and shifting cultivation for subsistence. Human actions that have affected the basin include large-scale agribusinesses and irrigated rice and oil-palm farms, as well as small-scale artisanal gold mining activities in the Alwero River, where human agricultural

activities and use of agrochemicals have increased the trace metals in aquatic food web systems (Melake et al., 2023). Both Abay and Alwero Rivers support rich and diverse aquatic communities, and they contain many commercially important species such as *O. niloticus*, *C. gariepinus*, *Labeobarbus*

intermedius, *Lates niloticus*, and *Bagrus docmak*, which account for a significant proportion of the total fish harvested in inland lakes and provide local people with a readily available, affordable source of animal protein (Melake et al., 2023; Bor, 2025).

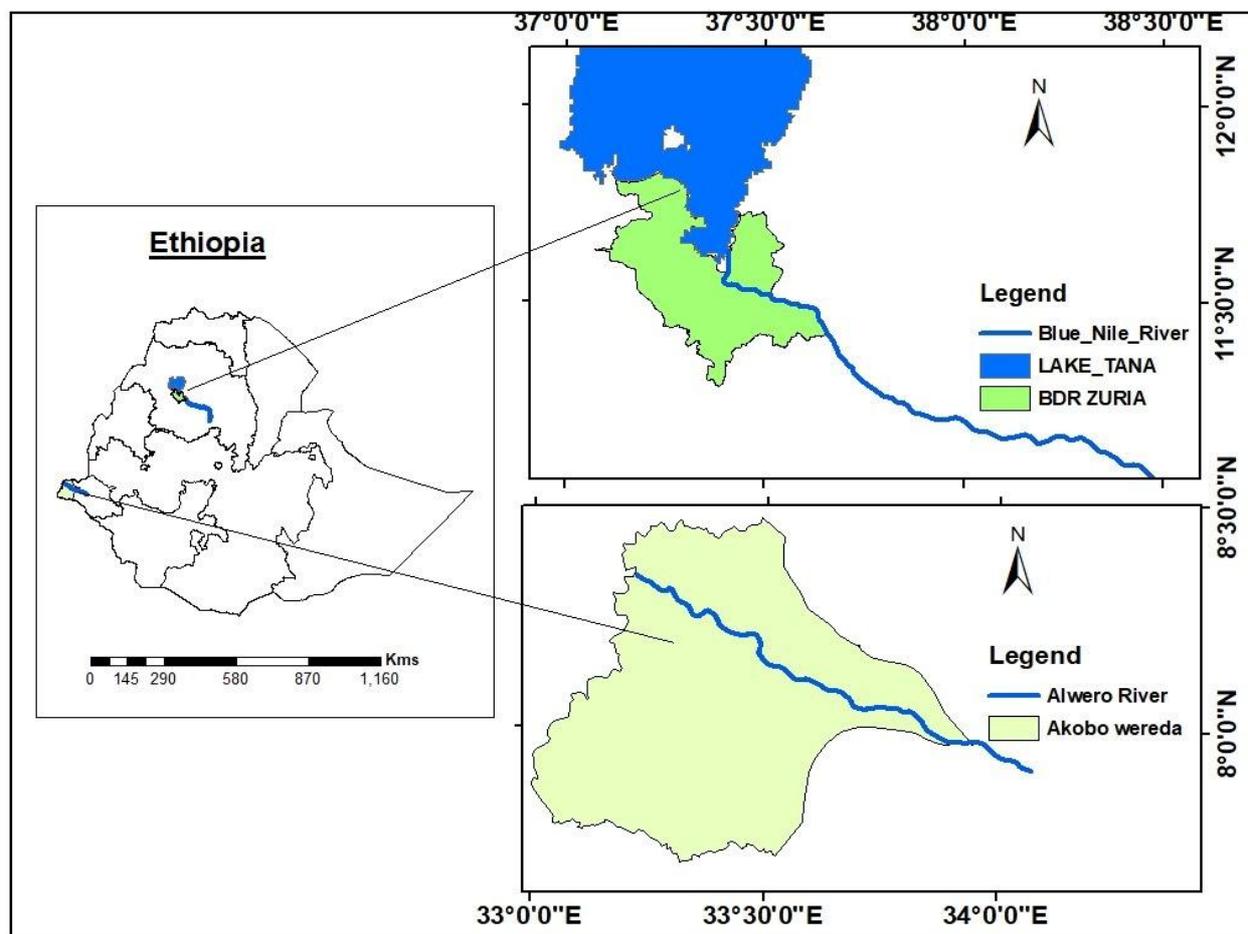


Figure 1: Map of Ethiopia illustrating the locations of the Abay and Alwero River study sites

2.2. Collection of Fish Sample

Fish sampling was conducted in both Alwero and Abay Rivers, with 20 fish sampled from each site for both species, i.e., *O. niloticus* and *C. gariepinus*. Prior to sampling, the fish were rinsed with deionized water to remove any metallic residue on the scales or the surface of the fish. Using the EMERGE protocol, which is described in Rosseland et al. (2001), the fish muscle and gill tissue were sampled using a set of specialized surgical blades and scissors made of stainless steel. Prior to the sampling procedure, the surgical equipment was treated

with a 70% nitric acid solution. After collecting the fish tissue, it was immediately encased in aluminum foil and stored in a deep freezer at -20°C for 72 hours. For the final analytical procedure, the fish tissue was transported through air transport in ice boxes to Horticoop Ethiopia PLC for analysis of heavy metal content using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES).

2.3. Sample Preparation and Digestion Protocol

To maintain the composition of the sample, the fish samples were crushed into powder using a

mortar and pestle. Microwave-assisted digestion was performed as per the methods proposed by Junior et al. (2024), as it helps in the preservation of volatile constituents of the sample and reduces the amount of reagents consumed during the digestion of the sample. About 0.5 g of the sample was weighed, and the digestion was performed by mixing it with 14 ml of 65 % HNO₃, 2 ml of 30 % H₂O₂, 2 ml of concentrated H₂SO₄, and 1 ml of concentrated HCl in Teflon vessels as per the methods proposed by Mohammed et al. (2017). The digested sample was filtered into 50 ml conical flasks, and the sample was diluted up to the desired volume by using 3 % HNO₃. The final analysis was performed by making 1:10 dilutions of the sample using 3 % HNO₃ and reagent blanks to check for the presence of any contaminant in the sample.

2.4. Instrumental Analysis and Quality Control

Elemental analysis of chromium (Cr), lead (Pb), mercury (Hg), arsenic (As), copper (Co), cadmium (Cd), and tin (Sn) was carried out using inductively coupled plasma optical emission spectrometry (ICP OES, AMETEK), a device operated by Horticoop Ethiopia PLC. Instrument calibration was carried out using a multi-element standard solution. Metal concentrations were reported as mg/kg dry weight (DW). Instrument precision was ensured through the use of a homogeneous sample mixture and microwave digestion conditions for the complete degradation of the sample.

2.5. Human Health Risk Assessment

2.5.1. Non-carcinogenic Risk Assessment

To evaluate the non-carcinogenic health risks associated with the ingestion of metallic contaminants via fish consumption, both the Target Hazard Quotient (THQ) and the Hazard Index (HI) were calculated (USEPA, 2019). THQ or HI values < 1 imply a low probability of adverse effects on an individual's health, whereas THQ or HI values > 1.0 imply a high probability

of adverse health effects from consumption of fish muscle. The THQ for each metal was calculated as:

$$THQ = \frac{EF \cdot ED \cdot FIR \cdot C}{RfD \cdot WAB \cdot ATn} \times 10^{-3}$$

where EF equals the number of days that people in a year are exposed to fish, ED is the length of time over which adults are exposed to fish, FIR is the weight of fish that adults eat each day and C is how much metal is in muscle from fish (mg kg⁻¹ DW). RfD is the number of fish that is safe to eat each day (mg kg⁻¹ day⁻¹). WAB is the average weight of adult humans (kg), i.e., 70 kg; the number of days that non-cancer-causing agents can cause cancer is equal to the average length of time from exposure to death (365 days year⁻¹ x ED); for carcinogenic agents, 70 years x 365 days.

2.5.2. Index of Hazard (HI)

The cumulative health risk, expressed as the Hazard Index (HI), was estimated by the following equation:

$$HI = \sum_{i=1}^n THQ$$

Values of HI and THQ < 1 indicate a low likelihood of adverse, non-cancer effects occurring. Values > 1 could indicate a possible hazard to the exposed population (USEPA, 2012).

2.5.3. Carcinogenic Risk Assessment

Chronic Daily Intake (CDI, mg kg⁻¹ day⁻¹) via ingestion was calculated for each metal as:

$$CDI_{fish} = \frac{C_{fish} \cdot FIR \cdot ED \cdot EF}{BW \cdot AT} \times 10^{-3}$$

RfD refers to the oral reference dose (mg kg⁻¹ day⁻¹); SF refers to the slope factor for carcinogenicity (mg kg⁻¹ day⁻¹); and CDI refers to the chronic daily intake of carcinogenic substances (mg kg⁻¹ day⁻¹). Cancer risk is measured as the probability without units indicating that a person will develop a cancerous tumour. The SF for lead (Pb) is derived from the

average chronic daily intake over a lifetime of exposure to lead (Pb). The slope factor (SF) for Pb is $(8.5 \times 10^{-3} \text{ mg kg}^{-1} \text{ day}^{-1})$ (USEPA, 2005), while the slope factor (SF) for inorganic arsenic (As) due to ingestion is $(1.5 \text{ mg kg}^{-1} \text{ day}^{-1})$; Ferreira-Baptista & De Miguel, 2005; Lim et al., 2008).

The cumulative lifetime cancer risk (CR) from lifetime exposure to particular carcinogenic chemicals (e.g., arsenic and lead) was estimated. The formula used for the assessment of CR is as follows:

$$\text{Cancer risk} = (\text{CDI-RfD}) * \text{SF}$$

According to USEPA (2015) and IARC classifications, CR values below 10^{-6} are considered negligible, values between 10^{-6} and 10^{-4} are regarded as acceptable or tolerable, and values exceeding 10^{-4} indicate an unacceptable carcinogenic risk.

2.6. Statistical Analysis

Descriptive statistics for the mean and standard deviation for metal concentration and risk index data were computed. Normality tests, such as the Shapiro-Wilk test, were conducted. Tests for assumptions, normality, and homogeneity showed that there were differences between the species or sites. This was confirmed by one-way ANOVA tests. In cases where normality and homogeneity could not be assumed, non-parametric tests such as the Kruskal-Wallis test and the Dunn post-test were conducted. Statistical significance was assumed to be $p < 0.05$.

3. Results

3.1. Heavy Metal Concentration in *O. niloticus* and *C. gariepinus*

Heavy metal concentrations (Co, Cr, Cd, Hg, Sn, Pb, and As) in the muscle and gill tissues of *O. niloticus* and *C. gariepinus* from the Alwero and Abay Rivers are presented in Table 1. Statistical analysis showed significant differences in spatial and tissue-specific variation ($p < 0.05$) between

the Alwero and Abay Rivers. The average concentration of detected elements present in *O. niloticus* from the Alwero River was as follows: $\text{Sn} > \text{Cr} > \text{Hg} > \text{Pb} > \text{Co} = \text{Cd}$ for muscles, and $\text{Sn} > \text{Pb} > \text{As} > \text{Cr} > \text{Hg} > \text{Co} > \text{Cd}$ for gills; however, concentrations found in *O. niloticus* from the Abay River had the following order: $\text{As} > \text{Sn} > \text{Cr} > \text{Pb} > \text{Hg} > \text{Cd} > \text{Co}$ for muscle, and $\text{Sn} > \text{As} > \text{Pb} > \text{Cr} > \text{Hg} > \text{Co} > \text{Cd}$ for gills (Table 1).

Heavy metals accumulated in *O. niloticus* in a manner that gill tissues typically contained considerably higher levels than muscle tissues, across both Rivers ($p < 0.05$) (Table 1). *Oreochromis niloticus* from the Abay River had the greatest concentration of metals overall, particularly for Pb ($4.56 \pm 0.02 \text{ mg kg}^{-1}$) and Hg ($2.40 \pm 0.02 \text{ mg kg}^{-1}$) in gill tissues, while As was most abundant at $17.65 \pm 0.01 \text{ mg kg}^{-1}$ in muscle tissues. Conversely, in the Alwero River, *O. niloticus* displayed a remarkably high concentration of Tin (Sn), reaching $126.74 \pm 0.01 \text{ mg kg}^{-1}$ in the gills and $51.50 \pm 0.00 \text{ mg kg}^{-1}$ in the muscle. However, Arsenic (As) was not detected (ND) in the muscle of samples from the Alwero River, contrasting sharply with the Abay River, where Arsenic reached its highest muscle concentration ($17.65 \text{ mg kg}^{-1} \text{ DW}$) (Table 1).

Clarias gariepinus exhibited distinct tissue-specific bioaccumulation and significant spatial variation ($p < 0.05$) (Table 1). In the Alwero River, metal concentrations in the muscle followed the order $\text{Sn} > \text{Cr} > \text{Hg} > \text{Cd} = \text{As} > \text{Co} = \text{Pb}$, whereas the gills showed an order of $\text{As} > \text{Sn} > \text{Cr} > \text{Pb} > \text{Hg} > \text{Co} > \text{Cd}$. In the Abay River, the hierarchy shifted; the muscle concentrations ranked as $\text{As} > \text{Sn} > \text{Cr} > \text{Hg} > \text{Pb} > \text{Cd} > \text{Co}$, and the gills followed the sequence $\text{As} > \text{Sn} > \text{Hg} > \text{Cr} > \text{Pb} > \text{Cd} > \text{Co}$ (Table 1). The gills of *C. gariepinus* caught in Alwero River contained significant levels of both Arsenic (As) ($12.83 \pm 0.01 \text{ mg kg}^{-1}$) and Pb ($3.02 \pm 0.00 \text{ mg kg}^{-1}$), while the Cr levels for gills ($3.11 \pm 0.00 \text{ mg kg}^{-1}$) were also significantly higher than muscle ($1.73 \pm 0.01 \text{ mg kg}^{-1}$) (Table 1). Additionally, the

gills of the fish collected from Abay River have been reported to have significant levels of As (30.92±0.01 mg kg⁻¹), while the gills of the same fish from this site had only 0.58±0.03 mg kg⁻¹ of As.

Table 1. Heavy metal concentrations (mean ± SD, mg kg⁻¹ DW) in the muscle and gill tissues of *O. niloticus* and *C. gariepinus* from the Alwero and Abay Rivers

	Water bodies	Tissue	Co	Cr	Cd	Hg	Sn	Pb	As
<i>O. niloticus</i>	Alwero River	Muscle	0.08±0.00 ^a	1.55±0.01 ^a	0.08±0.00 ^a	0.81±0.00 ^a	51.50±0.00 ^a	0.49±0.00 ^a	ND
		Gill	0.65±0.01 ^b	3.32±0.01 ^{bc}	0.41±0.01 ^b	1.46±0.01 ^b	126.74±0.01 ^b	3.73±0.00 ^b	3.35±0.00 ^a
	Abay River	Muscle	0.41±0.01 ^c	2.57±0.01 ^d	0.33±0.00 ^c	1.57±0.01 ^b	6.55±0.01 ^c	1.91±0.01 ^c	17.65±0.01 ^b
		Gill	1.16±0.01 ^d	3.40±0.02 ^b	0.58±0.01 ^b	2.40±0.02 ^c	12.18±0.01 ^d	4.56±0.02 ^d	11.18±0.01 ^c
<i>C. gariepinus</i>	Alwero River	Muscle	0.08±0.01 ^a	1.73±0.01 ^e	0.16 ±0.01 ^d	1.32±0.01 ^d	5.03±0.01 ^e	0.08±0.00 ^e	0.16±0.01 ^d
		Gill	0.49±0.00 ^b	3.11±0.00 ^f	0.41 ±0.00 ^b	1.72±0.00 ^b	7.77±0.00 ^f	3.02±0.00 ^f	12.83±0.01 ^e
	Abay River	Muscle	0.16±0.01 ^a	2.13±0.01 ^d	0.25 ±0.01 ^{cd}	1.56±0.01 ^b	4.59±0.01 ^g	0.82±0.01 ^g	30.92±0.01 ^f
		Gill	0.08±0.01 ^a	1.19±0.03 ^g	0.17 ±0.03 ^d	1.25±0.03 ^{bd}	4.66±0.03 ^g	0.42±0.03 ^a	0.58 ±0.03 ^g

Values followed by different superscript letters within the same column are significantly different ($p < 0.05$). Comparisons were performed between the mean values of muscle and gill tissues for each species and location. ND: Not Detected (below the limit of detection), DW: Dry Weight

3.2. Heavy Metal Concentrations Comparison with International Safety Guidelines

Muscle tissue samples of *O. niloticus* and *C. gariepinus* were examined for heavy metal concentrations to determine whether fish consumption is safe from the Alwero and Abay Rivers (Table 2) using recommended levels from both WHO (1989) and EC (2006). Metal concentrations in the Alwero River were within safe levels, while many of the samples from the Abay River exceeded allowable concentrations. Specifically, Chromium (Cr) levels in *O. niloticus* from the Abay River reached 0.55 mg kg⁻¹, surpassing the 0.5 mg kg⁻¹ limit recommended by both WHO (1989) and EC (2006). Furthermore, Lead (Pb) concentrations in *O. niloticus* from the Abay River (0.41 mg kg⁻¹) surpassed the limits established by FAO/WHO (2011) and EC (2006) (0.3 mg kg⁻¹).

Total Arsenic (TAs) levels in the Abay River were significantly elevated in both *C. gariepinus* (5.73 mg kg⁻¹) and *O. niloticus* (3.76 mg kg⁻¹), far exceeding the thresholds of WHO/FAO (1995) (0.5 mg kg⁻¹), FAO (2004) (1.5 mg kg⁻¹), and SADOH (2004) (3.0 mg kg⁻¹). Furthermore, Inorganic Arsenic (In As) concentrations in the

Abay River (0.573 mg kg⁻¹ for *C. gariepinus* and 0.376 mg kg⁻¹ for *O. niloticus*) were more than seven times the FSANZ (2000) safety limit of 0.05 mg kg⁻¹. In contrast, concentrations of Cadmium (Cd) and Tin (Sn) across all sites and species remain within the safe limits prescribed by EC and FAO guidelines.

Table 2. Comparison of the levels of heavy metals in muscles of selected fish species (mg kg⁻¹ wet weight) with international criteria guidelines

Sampled fish species and location	Pb	Hg	Co	Cr	Cd	Sn	TAs	In As
<i>O. Niloticus</i> , Alwero	0.10	0.16	0.02	0.30	0.02	10.10	0.00	0.000
<i>O. Niloticus</i> , Abay	0.41	0.33	0.09	0.55	0.07	1.39	3.76	0.376
<i>C. Gariepinus</i> , Alwero	0.02	0.29	0.02	0.38	0.03	1.09	0.03	0.003
<i>C. Gariepinus</i> , Abay	0.15	0.29	0.03	0.39	0.05	0.85	5.73	0.573
Organization guidelines	Pb	Hg	Co	Cr	Cd	Sn	TAs	In As
EC 2013			0.05			50		
WHO 1989				0.5				
FAO/WHO 2011	0.3				0.1			
WHO/FAO 1995						50	0.5	
FAO 2004							1.5	
WHO 1985/ 2004							2.0	
SADOH 2004		1.0				50	3.0	
FAO 1983	0.4	0.5			0.05			
EC,2006	0.3	0.5		0.5	0.03			
FSANZ 2000							0.01	0.05

^a FAO, 1983/ 2004 & WHO, 1985. 2004; Ahmad and Al-Mahaqeri, 2015

3.3. Estimated Daily Intake and Public Health Risk Assessment

To assess dietary exposure to elemental contaminants, estimated daily intake (EDI) was calculated and compared to the respective international safety limits. The EDI for all contaminants evaluated in both River basins was below the acceptable daily intake (ADI) and provisional tolerable weekly intake (PTWI) limits (Table 3). However, spatial analysis indicated that people consuming fish from the Abay River had a significantly higher risk of being exposed to contaminants than individuals consuming fish from the Alwero River. Notably, the highest EDI for inorganic arsenic (As) in *C. gariepinus* from the Abay River was 224×10^{-06} mg kg⁻¹ body weight day⁻¹ and for chromium (Cr) it occurred in *O. niloticus* from the Abay River (2.15×10^{-04} mg kg⁻¹ body weight day⁻¹). The highest EDI for tin (Sn) in *O. niloticus* from the Alwero River was 3.9×10^{-04} mg kg⁻¹ body weight day⁻¹, while the EDI for As in *O. niloticus* from the Alwero River was below the limit of detection (Table 3).

The noncarcinogenic risks of heavy metals in the muscle tissues of *O. niloticus* and *C. gariepinus* from River Alwero and Abay were evaluated using Target Hazard Quotient (THQ) and Hazard

Index (HI) (Table 4). The result showed that THQs for heavy metals found in fish muscle were less than one (below the critical threshold of 1.0). The Target Hazard Quotient (THQ) for heavy metals via consumption of *O. niloticus* from the Alwero River followed the order Hg > Cr > Pb > Cd > Co > Sn. A similar trend was observed for *C. gariepinus*, with the sequence Hg > Cr > Cd > In-As > Pb > Co > Sn. In the Abay River, THQ values for *O. niloticus* were highest for Hg (2.83×10^{-02}), followed by In-As (1.7×10^{-02}), Cr (2.51×10^{-03}), Pb (1.40×10^{-03}), Cd (9.59×10^{-04}), Co (1.23×10^{-05}) and Sn (3.5×10^{-07}). Similarly, for *C. gariepinus* from the same site, THQ values followed the order: In-As (2.6×10^{-02}), Hg (2.48×10^{-02}), Cr (1.78×10^{-03}), Cd (6.85×10^{-04}), Pb (5.14×10^{-04}), Co (4.11×10^{-06}) and Sn (2.1×10^{-07}). Notably, THQ values recorded for both species in the Abay were consistently higher than those recorded in River Alwero (Table 4).

Results of the spatial analysis indicated a higher hazard profile in the Abay River for mercury (Hg) and inorganic arsenic (In-As), with Hg and In-As as the two main contributors to risk in this watershed. The highest target hazard quotient (THQ) of Hg (2.83×10^{-02}) occurring in *O. niloticus* from the Abay River was also significantly higher than that of In-As

(THQ=2.6x10⁻⁰²) occurring in *C. gariepinus* from this sample site, which was significantly greater than the level recorded from the Alwero population (THQ=1.4x10⁻⁰⁴). There was a significant difference between the HI values for *C. gariepinus* and *O. niloticus* at the two sites, where *C. gariepinus* had an HI value of 5.40x10⁻⁰² and *O. niloticus* had an HI value of 5.03x10⁻⁰²;

the abundance of Hg and In-As was contributing to the > 2-fold greater HI values for the two species collected from the Abay River than those collected from the Alwero basin (*C. gariepinus* = 2.72x10⁻⁰²).

Table 3. EDI and hazard quotient of heavy metals in muscles of fish species from Ethiopia

Area	Metals	Co	Cr	Cd	Hg	Sn	Pb	Tot-As	In-As
Alwero	ADI(mg kg-1bw/w)1	0.0217 ^a	0.233 ^b	0.07 ^c	0.0033 ^d	0.029 ^e	0.25 ^c	0.009 ^e	3 E ^{-03f}
	PTWI(mg kg-1bw/w)1	0.1519 ^a	1.631 ^b	0.49 ^c	0.0231 ^d	0.203 ^e	1.75 ^c	0.063 ^e	0.021 ^f
	EDI (mg kg-1bw/d)2 (<i>O. niloticus</i>)	7.83x10 ⁻⁰⁶	1.17x10 ⁻⁰⁴	7.83x10 ⁻⁰⁶	6.26x10 ⁻⁰⁵	3.9x10 ⁻⁰⁴	3.91x10 ⁻⁰⁵	ND	ND
Abay	EDI(mgkg-1bw/d)2 (<i>C. gariepinus</i>)	7.83x10 ⁻⁰⁶	1.49x10 ⁻⁰⁴	11.7x10 ⁻⁰⁶	11.4x10 ⁻⁰⁵	42.7x10 ⁻⁰⁵	0.78x10 ⁻⁰⁵	1.17x10 ⁻⁰⁵	1.17x10 ⁻⁰⁶
	EDI (mg kg-1bw/d)2 (<i>O. niloticus</i>)	35.2x10 ⁻⁰⁶	2.15x10 ⁻⁰⁴	27.4x10 ⁻⁰⁶	12.9x10 ⁻⁰⁵	54.4x10 ⁻⁰⁵	16x10 ⁻⁰⁵	147x10 ⁻⁰⁵	147x10 ⁻⁰⁶
	EDI(mgkg-1bw/d)2 (<i>C. gariepinus</i>)	11.7x10 ⁻⁰⁶	1.53x10 ⁻⁰⁴	19.6x10 ⁻⁰⁶	11.4x10 ⁻⁰⁵	33.3x10 ⁻⁰⁵	5.87x10 ⁻⁰⁵	224x10 ⁻⁰⁵	224x10 ⁻⁰⁶

¹ADI: Acceptable daily intake, calculated from provisional tolerance weekly intake; PTWI (ADI= PTWI/7); ²EDI: Estimated Daily Intake; TAs= Total arsenic; IAs = Inorganic arsenic; ^a Nordic Council of Ministers (2015); ^b Cheung et al., 2008; ^c FAO /WHO, 2010; ^dJECFA (2003).; ^e FSANZ 2003; ^fANZFA 1999.

Table 4. THQ and HI of heavy metals due to consumption of species from Ethiopia

Area	Metals	Co	Cr	Cd	Hg	Sn	Pb	In-As	HI
Alwero	RfD (mg kg-1 day-1)1	0.1 ^g	0.003 ^g	0.001 ^h	1.6E ^{-04h}	54.98 ⁱ	0.004 ^h	3E ⁻⁰⁴ⁱ	
	THQ (mg kg-1bw/d) (<i>O. niloticus</i>)	2.74x10 ⁻⁰⁶	1.37x10 ⁻⁰³	2.74x10 ⁻⁰⁴	1.37x10 ⁻⁰²	2.5x10 ⁻⁰⁶	3.42x10 ⁻⁰⁴	ND	1.57x10 ⁻⁰²
	THQ (mg kg-1bw/d) (<i>C. gariepinus</i>)	2.74x10 ⁻⁰⁶	1.74x10 ⁻⁰³	4.11x10 ⁻⁰⁴	2.48x10 ⁻⁰²	2.7x10 ⁻⁰⁷	6.85x10 ⁻⁰⁵	1.4x10 ⁻⁰⁴	2.72x10 ⁻⁰²
Abay	THQ (mg kg-1bw/d) (<i>O. niloticus</i>)	1.23x10 ⁻⁰⁵	2.51x10 ⁻⁰³	9.59x10 ⁻⁰⁴	2.83x10 ⁻⁰²	3.5x10 ⁻⁰⁷	1.40x10 ⁻⁰³	1.7x10 ⁻⁰²	5.03x10 ⁻⁰²
	THQ (mg kg-1bw/d) (<i>C. gariepinus</i>)	4.11x10 ⁻⁰⁶	1.78x10 ⁻⁰³	6.85x10 ⁻⁰⁴	2.48x10 ⁻⁰²	2.1x10 ⁻⁰⁷	5.14x10 ⁻⁰⁴	2.6x10 ⁻⁰²	5.40x10 ⁻⁰²

TAs= Total arsenic, ¹RfD: Reference doses for metals; Hosseini et al., 2015; ^g Asefa (2015) Reference doses for Cr & Co set by FAO ; ^hUSEPA (2009) Reference doses (RfD) for Cd, Hg & Pb set by U.S. EPA; ⁱUSEPA (2010) Reference doses (RfD) for As ; ^jUSEPA (2000) Reference doses (RfD) for Sn set by WHO/FAO

3.4. Carcinogenic Risk Assessment of Lead and Arsenic

The potential for developing carcinogenic health issues from the ingestion of lead (Pb) and inorganic arsenic (In-As) via the muscle tissues of *O. niloticus* and *C. gariepinus* is detailed in Table 5. Accordingly, the lifetime cancer risk (CR) assessment associated with the consumption of *O. niloticus* and *C. gariepinus* reveals variations in the toxicological safety of the Alwero and Abay River basins (Table 5). The carcinogenic risk for freshwater *O. niloticus* and *C. gariepinus* from lead (Pb) ingestion was in the negligible (10^{-7} to 10^{-8}) range on both Rivers,

while Inorganic Arsenic (In-As) presents a significant public health concern for the Abay River basin. *C. gariepinus* in the Abay River had an In-As lifetime cancer risk (CR) of 1.36×10^{-4} and is above the lifetime human safety reliance CR value 1×10^{-4} to 1×10^{-6} (Table 5). This corresponds with a Chronic Daily Intake (CDI) of 9.08×10^{-5} mg kg⁻¹day⁻¹ for the same species (Table 5). In contrast, the Alwero River exhibited much lower risk profiles, with In-As concentrations in *O. niloticus* falling below detection limits and *C. gariepinus* showing a marginal CR of 7.14×10^{-7} .

Table 5. Cancer risk for lifetime contaminated heavy metal fish intake from Ethiopia

Metals	Fish species	Alwero River		Abay River	
		CDI	CR	CDI	CR
Pb	<i>O. niloticus</i>	1.59×10^{-05}	1.35×10^{-07}	6.50×10^{-05}	5.53×10^{-07}
	<i>C. gariepinus</i>	3.17×10^{-06}	2.69×10^{-08}	2.38×10^{-05}	2.02×10^{-07}
In-As	<i>O. niloticus</i>	ND	ND	5.96×10^{-05}	8.94×10^{-05}
	<i>C. gariepinus</i>	4.76×10^{-07}	7.14×10^{-07}	9.08×10^{-05}	1.36×10^{-04}

CDI= chronic daily intake dose (CDI, mg kg⁻¹day⁻¹); CR=cancer risk (unitless)

4. Discussion

4.1. Interspecific Variation of Heavy Metal Concentrations in *O. niloticus* and *C. gariepinus*

In both *O. niloticus* and *C. gariepinus* specimens collected from the Abay and Alwero Rivers, metallic accumulation was significant in the gill than in the muscle tissue. For example, Pb and Sn were found in the gills of *O. niloticus*, and Pb and Cr in *C. gariepinus*. This finding is aligned with several studies that have reported greater metallic accumulation in the gills than the muscle tissue of both *O. niloticus* and *C. gariepinus* (Chan et al., 2021; Pan et al., 2021; Blankson et al., 2023). Also, studies on *O. niloticus* and *C. gariepinus* in Lake Chamo (Reda & Ayu, 2016), Lake Ziway (Masresha et al., 2021), Koka and Aba Samueal Reservoirs (Dessie et al., 2026), Lake Hawassa and Bochasa stream (Samuel et al., 2020), and Omo River and Omo delta Lake (Kotacho et al., 2024) confirm that metabolically active organs (gills and livers) have significant concentrations

of heavy metals compared to muscle tissue. This significant accumulation of metals in gills might be due to their direct exposure to contaminated water and their primary function as sites of ion exchange and uptake of metals (Varol et al., 2025).

The Alwero River exhibited exceptionally high concentrations of tin (Sn) and arsenic (As) in gill tissues (126.74 and 17.65 mg kg⁻¹ DW, respectively), far exceeding values typically reported for tropical freshwater systems and mine-impacted sites (Chan et al., 2021; Albuquerque et al., 2021; Yang et al., 2022). Such outlier concentrations in Alwero fish might suggest a strong localized point source or distinctive geochemical setting of the basin. Potential contributors include industrial discharges (e.g., tanneries, textile and metal processing), agricultural inputs (fertilizers, pesticides, biocides), and small-scale mining, all of which are known to elevate As and Sn in

sediments and biota in other River systems (Yang et al., 2022; Macklin et al., 2023; Rojas-Conejo et al., 2025). In contrast to Cd, Pb, Cr, Hg, Cu, and Zn, which dominate most African and Asian biomonitoring studies and typically show only low to moderate As in fish tissues (Yang et al., 2022; Tolkou et al., 2023; Almafrachi et al., 2024), the Sn and As levels measured in *O. niloticus* and especially *C. gariepinus* from Alwero are among the highest reported. Moreover, as levels in Abay River fish muscle exceeded those reported for Ethiopian Rift Valley lakes (Dsikowitzky et al. 2013), where As was mainly concentrated in liver and gill and did not constitute a clear consumer health risk (Fernández-Trujillo et al., 2021). The very high Sn concentrations in Alwero fish, therefore, point to major local anthropogenic inputs; however, such high levels in any river-sourced fish are rarely recorded and may be attributed to industrial sources or agricultural chemicals (Parente et al. 2020; Almafrachi et al., 2024).

Chromium (Cr) concentrations in muscle tissue of *C. gariepinus* from Alwero (1.73 mg kg^{-1}) and Abay (2.13 mg kg^{-1}) Rivers were notably higher than those reported for Lake Hawassa and the Boicha stream ($0.08\text{--}0.29 \text{ mg kg}^{-1}$; Larissa et al., 2013; Samuel et al., 2020). Conversely, our values for *O. niloticus* ($1.55\text{--}2.57 \text{ mg kg}^{-1}$) were significantly lower than the 10.31 mg kg^{-1} reported in the Gilgel Gibe Reservoir (Gure et al., 2019). These differences may arise from variations in analytical methodologies, seasonal sampling fluctuations, and differing levels of anthropogenic pressure. Crucially, Chromium is known for its high depuration rate in teleost fish, as it is rapidly excreted and does not typically sequester in muscle tissue (Mahboob et al., 2014).

The arsenic concentrations found in *C. gariepinus* and *O. niloticus* species harvested from the Abay River (30.92 and 17.6 mg kg^{-1} , respectively) are high compared with who reported by Samuel et al. (2020) in Lake

Hawassa (0.14 and 0.32 mg kg^{-1}). This is most likely caused by both geogenic and anthropogenic factors. The Abay River Basin has a tectonically and volcanically active background, where arsenic-rich volcanic rocks, arsenic-bearing hydrothermal veins, and hot springs are known to leach naturally into groundwater and sediments (Alamirew et al., 2025). This would naturally increase the levels of arsenic in the sediments and groundwater, which could bioaccumulate in aquatic food chains (Osuna-Martínez et al., 2021; Patel et al., 2023). Superimposed on this background are possible anthropogenic effects of long-term agricultural runoff from the Basin, where the use of arsenical pesticides, herbicides, and wood preservatives is a well-documented non-point source of As into soils and surface waters (Kumari et al., 2016; Sankhla et al., 2024). Additionally, the influence of industrial wastewater discharge into the Abay River from tanneries and textile mills (Hishe et al., 2020), which could be a local anthropogenic point source of As, has in other areas around the world been documented as increasing As levels in adjacent River waters, sediments, and fish populations to levels posing clear human health concerns (Paul et al., 2025; Tang et al., 2025).

Clarias gariepinus showed higher concentrations for several metals than *O. niloticus* (See Table 1), suggesting species-specific uptake and physiology. Similar species effects are reported in Ethiopia; in Lake Chamo, *C. gariepinus* generally showed higher Cu, Mn, and Ni than *O. niloticus* (Reda & Ayu, 2016). According to Dessie et al. (2021), the concentrations of Cd, Pb, As, Cr, and Zn were higher in *C. gariepinus* than in *O. niloticus* from the Koka and Aba Samueal Reservoirs. These variations may reflect the divergent ecological niches of these species. Because *C. gariepinus* feeds on benthic invertebrates and sediments, the fish is in regular contact with contaminated sediments that contain heavy metals and has the potential to bioaccumulate more toxic substances than *O.*

niloticus, which is a primary consumer of planktonic organisms (Jibrin et al., 2025).

4.2. Heavy Metal Concentrations Comparison with International Safety Guidelines

A comparison of metal concentrations between *O. niloticus* and *C. gariepinus* with respect to international standards indicates that Alwero's fish are compliant, while Abay's fish demonstrate exceedances of Cr, Pb, and particularly As, indicating site-specific contamination which poses a potential human health risk. Such exceedances in edible fish are common in Rivers impacted by anthropogenic inputs (Biswas et al., 2023; Ahmed et al., 2024). *Oreochromis niloticus* from the Abay River had a concentration of Cr (0.55 mg kg^{-1}) higher than the permissible limits of WHO (1989) and EC (2006) (0.5 mg kg^{-1}). This result agrees with other studies conducted on industrially impacted rivers where Cr in fish often exceeds FAO/WHO limits due to tannery discharges (Lipy et al., 2021; Biswas et al., 2023). Further, Pb concentrations in *O. niloticus* (0.41 mg kg^{-1}) were also above the FAO/WHO (2011) and EC (2006) limits (0.3 mg kg^{-1}). Recent studies have indicated that *O. niloticus* have a greater tendency to bioaccumulate Pb relative to other species due to their foraging behavior in contaminated sediments (Fentie et al., 2025). These results are consistent with many studies from both Asia and Africa, where Pb concentrations have exceeded regulatory limits in both freshwater and estuarine fish, with sources of Pb being urban runoff, vehicular emissions, mining, and informally produced goods (Biswas et al., 2023; Ahmed et al., 2024). That Pb in Alwero fish remains below these limits suggests lower direct anthropogenic pressure and supports the interpretation that metal loading in Abay is driven by local pollution sources rather than regional background.

The most concerning outcome is the very high total and inorganic arsenic in Abay fish muscle. Total As in *C. gariepinus* (5.73 mg kg^{-1}) and *O.*

niloticus (3.76 mg kg^{-1}) clearly exceeds the 0.5 mg kg^{-1} benchmark proposed by WHO/FAO (2011) and even the more permissive 3.0 mg kg^{-1} limit of SADOH (2004). Lower total As levels in freshwater fish have already been associated with hazard quotients, hazard indices, and lifetime cancer risks that surpass acceptable ranges, particularly for children and high-consumption groups (Kotacho et al., 2024). In contrast, recent investigations on freshwater fish repeatedly show that inorganic arsenic can account for a sufficient fraction of total As to yield unacceptable cancer risk at concentrations close to, or even below, those observed in Abay fish (Polak-Juszczak & Richert, 2021; Almafrachi et al., 2024). Speciation studies from mine-impacted and industrially affected Rivers demonstrate that, although organic forms may dominate at high total As, the inorganic share often remains large enough to drive carcinogenic risk estimates above 10^{-4} – 10^{-3} for regular consumers (Gao et al., 2017; Jia et al., 2018). The In As levels in Abay fish therefore strongly suggest that routine consumption, especially at high local intake rates, would confer substantial carcinogenic risk.

4.3. Dietary Exposure and Estimated Daily Intake (EDI)

The Estimated Daily Intake (EDI) results indicate that consumption of *O. niloticus* and *C. gariepinus* from both the Alwero and Abay Rivers does not currently exceed the Acceptable Daily Intake (ADI) or Provisional Tolerable Weekly Intake (PTWI) values for the metals investigated. Nevertheless, pronounced spatial differences in exposure are evident. Consumers relying on fish from the Abay River experience substantially higher toxicological burdens, particularly for inorganic arsenic (In-As) and chromium (Cr). For example, the EDI for In-As via *C. gariepinus* from Abay reached $2.24 \times 10^{-4} \text{ mg kg}^{-1} \text{ body weight day}^{-1}$, and Cr exposure from *O. niloticus* was $2.15 \times 10^{-4} \text{ mg kg}^{-1} \text{ body weight day}^{-1}$, whereas arsenic in *O. niloticus* from Alwero was below the detection limit. This

pattern is consistent with studies from other Riverine systems, where EDIs for toxic metals often remain formally below guideline thresholds yet are systematically elevated at more impacted sites, especially for arsenic, mercury, and chromium (Kindie et al., 2020; Melake et al., 2022; Zhang et al., 2024; Mahmoud et al., 2025).

4.4. Non-Carcinogenic Health Risk Assessment (THQ and HI)

Based on assessments using the Target Hazard Quotient (THQ) and Hazard Index (HI), the assessed non-carcinogenic health risks are all significantly below the threshold of 1, indicating no expected negative health effects due to non-carcinogenic methods of exposure to metals through fish from Alwero and Abay Rivers. This is consistent with numerous studies from throughout Ethiopia that have found fish consumers generally faced low non-carcinogenic health risk from consumed fish despite localized metal contamination (Reda & Ayu, 2016; Gure et al., 2019; Kindie et al., 2020; Melake et al., 2022). However, the THQ and HI values are consistently higher in fishes from the Abay River when compared to those from the Alwero River, particularly due to the influence of mercury (Hg) and inorganic arsenic (IAs); therefore, fishes from the Abay River have an overall higher hazard potential than fishes from the Alwero River. These results are also consistent with other studies conducted in contaminated aquatic environments where the major contributors to the carcinogenic health risk are Hg and IAs, even at environmental daily intake levels well below regulatory standards (Panda et al., 2023; Zhang et al., 2024; Mahmoud et al., 2025).

The individual THQ for Hg among *O. niloticus* was the highest measured (2.83×10^{-2}), and for inorganic As among *C. gariiepinus*, measured at 2.6×10^{-2} . The maximum cumulative HI values were 5.40×10^{-2} and 5.03×10^{-2} among *C. gariiepinus* and *O. niloticus*, respectively, more than twice the maximum value among fish from the Alwero River, but much less than the

threshold of risk for non-carcinogens (Kalıpcı et al., 2022; Mishra et al., 2023). Similar multi-metal risk profiles have been documented from the Gilgel Gibe Reservoir (Gure et al., 2019); Lake Tana (Kindie et al., 2020); Lake Hawassa (Melake et al., 2022); Omo River and Omo Delta Lake (Kotacho et al., 2024); and Koka and Aba Samuel Reservoirs (Dessie et al., 2026), where agricultural intensification has introduced neurotoxic and carcinogenic trace elements into fisheries (Masresha et al., 2021), which means current exposures via these two Ethiopian fisheries are unlikely to pose significant noncarcinogenic risks for the adult population, but Abay River fish clearly represent a higher-risk commodity in relative terms.

4.5. Carcinogenic Risk Assessment of Lead and Arsenic

The lifetime cancer risk (CR) assessment reveals a stark contrast between the relatively benign levels of Lead (Pb) and the hazardous concentrations of Inorganic Arsenic (In-As). For Pb, CR values for both species in both Rivers (10^{-7} – 10^{-8}) fall well below the level established by USEPA (2012) as considered to be negligible for a lifetime of exposure (10^{-6} to 10^{-4}). Thus, there is little risk for developing cancer due to Pb alone based on current consumption. This study is consistent with Ethiopian studies from Lake Hawassa, Lake Koka, Lake Tana, Lake Hayqe, Borkena River, and the Omo system, where Pb-related CR generally falls inside the internationally accepted 10^{-6} – 10^{-4} range despite occasional exceedance of guideline concentrations in fish muscle (Samuel et al., 2020; Temesgen & Geleta, 2023; Fentie et al., 2025; Kotacho et al., 2024). In contrast, the carcinogenic risk attributable to In-As exhibits a marked spatial and species-specific differentiation. *O. niloticus* did not have detectable levels of In-As in the Alwero basin, while *C. gariiepinus* had an exceptionally low level of In-As ($CR = 7.14 \times 10^{-7}$) that still fell below regulatory limits and was comparable to

low-risk scenarios from other River fisheries (Shorna et al., 2021; Dwiyoitno et al., 2024).

The carcinogenic profile of the Abay River based on In-As is significantly greater compared to other Rivers. For *O. niloticus* specimens from the Abay River, the calculated Cancer Risk (CR) for In-As (8.94×10^{-5}) is close to the maximum risk of common use (10^{-6} – 10^{-4}), whereas *C. gariepinus* shows a CR of 1.36×10^{-4} at the Chronic Daily Intake (CDI) of $9.08 \times 10^{-5} \text{ mg kg}^{-1} \text{ day}^{-1}$, exceeding the benchmark of 10^{-4} established by USEPA (2012) and others to define minimal lifetime risk for cancer (Adegbola et al., 2021; Fan et al., 2025). Similar exceedances in CR driven by As have been reported in Lake Hawassa and Boicha stream, and the CR from both species are also above 10^{-4} ; therefore, their regular consumption might pose a risk, particularly from catchments that have been impacted by industrial or urban discharges (Samuel et al., 2020). Additionally, systematic reviews of surface waters in Ethiopia have indicated that *O. niloticus* and *C. gariepinus* are effective bio-concentrators of As and Se. Several systems have reported concentrations of dissolved and sediment-bound metals that exceed international standards, suggesting the potential for increased lifetime cancer risk from As in watersheds with elevated levels of pollution (Melake et al., 2023).

The markedly lower In-As-related CR in Alwero (non-detectable in *O. niloticus* and 7.14×10^{-7} in *C. gariepinus*) places this basin among the relatively low-risk Ethiopian systems, comparable to Lake Hawassa and Lake Koka scenarios where aggregate cancer risk indices remained within 10^{-6} – 10^{-4} despite some metals exceeding FAO/WHO limits in fish muscle (Melake et al., 2022; Temesgen & Geleta, 2023). At the same time, the Abay profile aligns more closely with high-impact settings such as Lake Hawassa and Boicha stream and Borkena, where Cr, As, and Cd concentrations in fish tissues were associated with non-negligible carcinogenic risk

and call for source control and tighter effluent regulation (Samuel et al., 2020; Melake et al., 2023).

5. Conclusion

While fish consumption from the Abay and Alwero Rivers does not currently pose non-carcinogenic health risks, the contamination levels of Cr, Pb, and As in the Abay River frequently exceed international safety guidelines. Heavy metal accumulation was found in gills more than in muscle tissues and was greater in the benthic feeding *C. gariepinus* than in *O. niloticus*. Of great concern is that the risk associated with carcinogenic inorganic arsenic in the Abay River was greater than the 10^{-4} threshold for *C. gariepinus*, indicating a serious public health hazard due to the long-term consumption of this fish. The results of this study demonstrate an urgent need for targeted pollution source control and continued biomonitoring to protect public health. Continued exposure to carcinogenic metals from the consumption of fish without immediate action may result in an increased cancer risk among residents. Therefore, implementing effective environmental management practices will be critical to the sustainability and safety of the fisheries in Ethiopia.

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