Analysis of Heavy Metals and Human Health Risk Implications Associated with Fish Consumption from the lower Omo River (Lotic) and Omo Delta Lake(Lentic), Semiarid Region of southern Ethiopia

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Abstract

A study was done for the first time to investigate the level of heavy metals in commercially important fish species (*Lates niloticus and Oreochromis niloticus)* and the likely human health risk .120 fish samples (60 from each water ~~body)~~body) were collected by fishermen ~~from~~ from lower Omo River (Lotic) near Omorate town and Omo delta Lake (Lentic), adjacent to Lake Turkana. Flame Atomic Absorption Spectrometer ~~(**FAAS**, novAA400p)~~ was used for the analysis of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) from samples of fish tissues (liver and muscle). The risk assessment tools for human health were the target hazard quotient (THQ), the Hazard index (HI), and the target cancer risk (TCR). ~~Mean~~ The mean level of heavy metals detected in the liver and muscle of *L. niloticus* from lower Omo River generally occurred in the order of Fe > Zn > Pb > Cu > Mn > Cr > Co > Ni and Pb > Cu > Mn > Co > Ni respectively whereas~~.~~, the mean level of metals in muscle and liver tissues of *O. niloticus* were in an order of Fe > Pb > Zn > Mn > Cu > Cr > Co > Ni and Pb > Zn > Mn > Fe > Cu.> Co > Ni respectively. Similarly, the mean level of heavy metals detected in the liver and muscle of ~~*Lates niloticus* (~~*L. niloticus*~~)~~ from Omo delta Lake generally occurred in an order of Fe > Zn > Pb > Cu > Mn > Cr > Co > Ni and Fe > Pb > Zn > Mn > Cu > Co > Cr >Ni respectively ~~while~~ while the mean level in muscle and liver tissues of ~~*Oreochromis niloticus* (~~*O. niloticus*~~)~~ from the Omo

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delta were in an order of Fe > Pb > Zn > Mn > Cu > Cr > Co > Ni and Pb > Fe > Zn > Mn > Co > Cu > Ni respectively. The mean concentration of Pb in the tissues of *L. niloticus and O. niloticus* from the lower Omo River ~~were~~ was above the FAO/WHO permissible limits. Similarly, the mean level of Pb and Cr in the tissues of *L. niloticus and O. niloticus* from Omo Delta Lake were above the FAO/WHO permissible. The TCR result due to Ni exposure through consumption of muscle of *L. niloticus and O. niloticus* is an alarming value which might enhance the likelihood of developing cancer. These results are a warning signal for early intervention and call for regular monitoring of the freshwater ~~fish .Consequently~~fish. Consequently, investigating pollution level and human health risks of heavy metals in fish tissues from lower Omo River Omo delta is imperative both for environmental and public health concerns. It is time for policy makers and concerned bodies to take appropriate action at this alarming level.

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Introduction

Aquatic products are becoming more popular for human consumption since they are a great source of protein, omega-3 fatty acids, vitamins, selenium, and calcium (Kalantzi et al., 2016). As part of a balanced diet, the American Heart Association advises two servings of fish per week (Neff et al., 2014). Aquatic products have favorable health impacts, but they may also contain contaminants since they contain high amounts of fat and protein, which have negative effects on human health (Usydus et al., 2009).

Various natural and anthropogenic causes, such as the discharge of home or industrial sewage, storm runoff, leaching from landfills/dumpsites, and atmospheric deposits, can cause heavy metals to end up in aquatic environments (Rahman et al., 2016).One of the noticeable contaminants of the freshwater ecology and food supply is heavy metals (Manal et al., 2014) which can pose serious risks to both humans and aquatic life (Solomon et al., 2016).The danger of eating heavy metals from contaminated food is rising in developing nation such as Ethiopia (Berehanu et al., 2016, Samuel et al., 2020).Humans may consume fish muscles that have accumulated heavy metals (Lubna et al., 2015, Gure et al., 2019), which could pose health risks to various vital organs including the kidney (Manal et al., 2014), liver and brain (Safiur et al., 2012),lung and heart (Mir et al., 2021)and reproductive system at cell, tissue and organ level (Javed and Usmani,2011).The presence of excessive amounts of heavy metals in fish tissues has the potential to negatively impact early development, growth, behavior, and reproduction concerns, as well as damage to fish species' neurological systems (Taslima et al., 2021).As a result, the accumulation of heavy metals in human diets, including fish, has emerged as an urgent worldwide danger that demands attention, particularly in developing nations like Ethiopia.

In recent years, the lower Omo River (Lotic) and the Omo delta (Lentic) have seen anthropogenic activities such as agricultural, industrial, and economic development (USEPA, 2010, Wakjira and Getahun, 2017).These activities have threatened the quality of the Lotic and the Lentic water bodies (Ojwang et al., 2010, Avery, 2012),which may be related to the presence of heavy metals in fish tissues. In addition to urbanization along the shoreline, the lower Omo River (Lotic) and the Omo delta (Lentic) are subject to extensive agricultural processing, manufacturing, and agrochemical-based irrigation projects(Avery, 2012). Additionally, other studies showed that fish tissues from Lake Turkana, which is close to the Omo delta (the subject of the current study), had higher levels of heavy metals (Magu et al., 2016, Christof et al., 2017). These could attribute to the occurrences of heavy metals in the fish tissues being investigated in the present study.

To the best of our knowledge, yet no study has been reported on the level of heavy metals in fish tissues and associated human health risks in the lower Omo River (Lotic) and the Omo delta (Lentic). However, studies being done on the concentration of elements in fish tissue from Lake Turkana in Kenya (Magu et al., 2016, Christof et al., 2017), where the southernmost tip of the Omo River extends into it. Thus, information on the concentration of heavy metals in fish tissues from the lower Omo River (Lotic) and the Omo delta (Lentic) is crucial for both environmental and public health issues. Therefore, the current study aimed to assess human health risks of heavy metals in commonly consumed fish spices (*L. niloticus* and *O. niloticus*) collected from the lower Omo River (Lotic) and the Omo delta (Lentic). The concentrations of nine heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) were measured in the samples, and the non-carcinogenic and carcinogenic health risks to adults and children associated with consuming fish were calculated respectively.

Materials & Methods

Description of the study area

The Omo River (Lotic) basin (**Figure 1**) is one of Ethiopia's most important river systems having a drainage area about 79,000 km^2 (CSA, 2019). It starts off in the southwestern Ethiopian highlands at 2,200 m above sea level (a.s.l) and flows in its lower portion (Omo delta), finally ending up in Lake Turkana at an altitude of 365 m (a.s.l) (Wakjira and Getahun, 2017).

The Omo delta (Lentic) is situated in the Eastern Arm of the Great Rift Valley of East Africa (Avery, 2012),which is about 50 kilometers from Omorate Town towards downstream direction. The Omo delta is situated in the Eastern Arm of the Great Rift Valley of East Africa(Avery, 2012) which lies across the Ethiopia–Kenya border in Southern Ethiopia lowlands forming Bird's foot delta with an area of 98 km^2(Wakjira and Getahun, 2017). It starts off in the southwestern Ethiopian highlands at 2,200 meters above sea level (a.s.l) and flows in its lower portion (Omo Delta)at an altitude of (Wakjira and Getahun, 2017). It is nearly 820 km South of Addis Ababa (Capital city) and 508 km from the regional city (Hawassa).

Fish sample collection and storage

Thirty fish samples from each species (*L. niloticus* and *O. niloticus*) were taken from the Omo Delta Lake and the River water, respectively. Sample collection and storage were carried out in accordance with APHA and EMERGE procedures (Rosseland et al., 2001, APHA, 2017). Fish samples were taken from fishermen who collected fresh traps of *L. niloticus* and *O. niloticus* from the sampling sites [4°48'13.67"N36°2'0.37"E, 4°48'10.80"N36°2'9.16"E, 4°48'4.20"N 36° 2'8.98"E, 4°47'52.59"N 36° 2'4.80"E, 4°47'38.38"N 36° 2'16.96"E] of the River water using plastic nets. The fish samples were washed with deionized water just before dissecting the tissues. Fish were then dissected at the field using plastic blades to get liver and muscle tissue. Each liver and muscle tissue samples were carefully covered with aluminum foil and sealed separately in polyethylene bags instantaneously after removal. The tissues were then separately labeled based on species and tissue type. The wrap samples were cautiously put in an icebox and then transported to Arbaminch Minch University of Chemistry Laboratory immediately after dissection and wrapping in an icebox and then reserved in a freezer at -20 °C until analyses.

Sample preparation and digestion

The Samples were prepared following USEPA guideline (USEPA, 2010). Accordingly, fish tissues (muscle and liver) were oven dried separately at 60° until constant weight was obtained. To make powder, the dried tissues were then crushed using mortar and pestle. The Powdered tissue samples (0.5g) were separately made ready for digestion. Ash digestion was carried out by taking 0.5 grams of muscle and liver tissue and put into a dish which was then transferred into a furnace at a temperature of 550 °C for 4 hours. After each sample was turned completely in to ash, it was removed and cooled in desiccators. The ash samples with 10 ml of 20% HNO3 in 50 ml beakers was placed on a hot plate and then heated slowly at 120 °C for 30 minutes. After digestion and cooling, dilution and filtration process were carried out using distilled water and filter paper (Whatman No. 42) respectively. Digestion procedures were done using the protocol in the analytical methods for Atomic Absorption Spectrometry (Perkin, 1996).

Sample analysis

Analysis was carried out in accordance with APHA guideline (APHA, 2017).The heavy metal content of the fish tissues were analyzed via Flame Atomic Absorption Spectrometer **(FAAS,** novAA400p).Analytical grade standards of each target heavy metal were used to sketch the calibration curves.

Human health risk assessment

Non- Carcinogenic risks

Using the Target Hazard Quotient (THQ) and Hazard Index, it was determined whether consuming heavy metals from fish muscles would likely pose non-carcinogenic health concerns to humans. The THQ result signifies health risk for a single heavy metal while HI result signifies cumulative risk in fish muscle. THQ and HI values < 1 show that the health effects will be unlikely to happen on exposed individual. However, when the THQ and HI values are >1.0, implies that the potential non-carcinogenic health hazards will be likely to experience on exposed individual. The larger the THQ value, the greater the possibility of risk to the exposed individuals (USEPA, 2011).The non-carcinogenic risk was assessed for both adults and children who ingest fish muscle from the lower Omo River and Omo Delta ,one to seven days per week. The THQ and HI were estimated using EPA guideline (USEPA, 2011, USEPA, 2019) using equations 1 and 2 below

$$\mathrm{THQ} = \frac{(EF\times \mathrm{ED}\times \mathrm{IR}\times \mathrm{Cm})}{(\mathrm{RfD}\times \mathrm{WAB}\times \mathrm{AT})}10^{-3} \quad \ldots\ldots\ldots\ldots \quad \text{Eq(1)}$$

$$HI = \sum \mathrm{THQ} \ldots\ldots\ldots\ldots\ldots \text{Eq(2)}$$

HI= THQPb+ THQCd + THQNi + THQMn + THQCr + THQCu + THQCo + THQZn + THQFe.

~~Where :THQ~~Where: THQ is a non-carcinogenic health risk; The average life expectancy in Ethiopia is 65 years for adults and 6 years for children. ED is the exposure duration (USEPA, 2005, WHO, 2015); EF is exposure frequency which is 365 days/year for people who eat fish muscle 7 times a week and 52 days/year for those who eat once a week (FAO, 2014);IR is average fish ingestion rate of an individual in a day (g/day/person) which is 30g for adult and 15g for children in Ethiopia (USDA, 2000, USEPA, 2005).The RfD is the oral reference dose which is the daily ingestion of a contaminant that is unlikely to cause health effects in a life time as defined by USEPA (2003) in mg kg^{-1}/day were 0.001for Cd, 0.003 (Cr) , 0.03 (Hubbe et al.); 0.040 (Cu), 0.7 (Neff et al.), 0.020 (Ni), 0.14 (Mn), 0.0035 (Pb) and 0.30 (Zn);BW is the average body weight equivalent to 60 kg for adults and 21kg for children in Ethiopians (WHO, 2012);AT is the average exposure time for non-carcinogens which is 365 days/year x ED;Cm is the average concentration of heavy metals in fish muscles (mg/kg dry weight);WAB is the average body weight to be 60 kg for adults and 21kg for children in Ethiopians (WHO, 2012). Ethiopians fish consumption habits have recently been increasing in areas with adequate supply of fish (Yared et al., 2013). Over 10 kg/person of fish can be consumed annually per person in such a community (FAO, 2014). Accordingly, the average daily intake of fish by adults in Ethiopia was calculated to be 30 g/d per person (Yared et al., 2013, Samuel et al., 2020). According to USDA (2000) and USEPA (2005), child consumption is about one-half that of adults. Therefore; the daily average fish ingestion rate for children (Gure et al.) in Ethiopia was estimated to be 15g/d/person (USDA, 2000, USEPA, 2005)

Carcinogenic Risk (TCR)

Target Carcinogenic risks (TCR) estimates an individual's possibility of developing 'cancer over a lifetime while exposed to a potential carcinogen and the acceptable risk levels for carcinogens range from 10^{-4} to 10^{-6} '(USEPA, 1989).The TCR was estimated using equation 3 below.

$$\text{TCR} = \frac{(EF\times \text{ED}\times \text{Cm}\times \text{IR}\times \text{CSFO})\times 10-3}{(\text{WB}\times \text{AT})} \ldots\ldots \text{Eq(3)}$$

Where: TCR is the target cancer risk; CSFO is an oral carcinogenic slope factor in mg/kg/day and the values include1.7 mg kg^{-1}/day for Ni, 0.5 (Cr), 0.001(Cd) and 0.0085 for Pb (USEPA, 2010). The other parameters are presented in Equations 1 and 2.

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Precision of the method and validation of the results were checked by the recovery test (APHA, 2017).The fish sample was spiked with known concentration of heavy metals and spiked samples were digested in triplicate using the same method used for the original samples. The percent recovery was then calculated using equation 4.

$$\text{Recovery} = \frac{(Spiked\ result - Unspiked\ result)}{(\text{Amount added})} \times 100\% \quad \text{.....Eq(4)}$$

All recovery values were within the acceptable range (80%-120%) for heavy metal analysis (Harvey, 2000)and are summarized in **Fig 2 below**

Data Analysis

The data were analyzed using IBM SPSS 21 statistical software. Variations in the levels of heavy metals between fish tissues and species were evaluated using t-test. Results were compared with literature values of the previous findings and FAO/WHO standard limits.

Results

Levels of Heavy metals in O. niloticus in Omo River and Omo Delta

Table 1 lists the average levels of metals found in O. niloticus muscle and liver tissues from the Omo River and Omo Delta. Manganese (Mn) was found in Omo River at mean concentrations of 0.356 mg kg-1 in the liver and 0.379 mg kg-1 in the muscle of O. niloticus. Whereas ,in Omo delta Mn was 0.387 mg kg-1 and 0.384 mg kg-1 in liver and muscle of O. niloticus respectively. The mean level of elements in the liver and muscle tissues of *O. niloticus* from Omo river were in the array Fe >Pb> Zn >Mn> Cu > Cr > Co > Ni and Pb> Zn >Mn> Fe > Cu.> Co > Ni respectively. Whereas in Omo delta, the mean concentrations in the liver and muscle tissues of O. niloticus were in the following order: Fe >Pb> Zn >Mn> Cu > Cr > Co > Ni and Pb> Fe > Zn >Mn> Co > Cu > Ni.

The concentration of Zinc in muscle tissue of O. niloticus was 0.424 mg kg-1 and 0.394 mg/kg in Omo River and Omo Delta respectively (**Table 1**). The level of liver tissues in *O. niloticus* in the current examination was (0.556 mg kg-1) in the Omo Delta and (0.477 mg kg-1) in the Omo River. Copper (Cu) levels in O. niloticus muscle tissues ranged from 0.129 mg kg-1 to 0.071 mg kg-1, with Omo River having greater levels. The mean concentration of Cu in the liver tissues of *O. niloticus was* 0.189 mg kg-1 and 0.291 mg kg-1 in Omo River and Omo delta respectively.

The average Cr content in *O.niloticus* tissue is displayed in Table 1.The average Cr concentration in *O. niloticus* liver from the Omo River was 0.145 mg kg-1, which was less than the average Cr concentration from the Omo delta, which was 0.154 mg kg-1. Cr was not detected in the muscle tissues of O.niloticus in the Omo River and Omo delta.

O. niloticus muscle and liver samples from the Omo River and the Omo delta both contained no detected amount of cadmium. The Lead (Pb) level of muscle tissues ranged from 0.790mg kg^{-1} and 0.597 mg kg^{-1} with higher in the sample from Omo River. The analysis showed that there was no significant difference (p.value>0.39) in the mean concentration of lead in the muscle tissue of O. niloticus between the Omo river and Omo delta (**Table 2**).

The Omo River and Omo Delta had mean iron concentrations of 0.268 mg kg-1 and 0.411 mg kg-1, respectively, in the muscle tissues of O. niloticus. The current study's findings showed that the Fe concentration of liver in O. niloticus of was 1.100 mg kg-1 in the Omo River and 1.74 mg kg-1 in the Omo delta. Statistics showed that the average Iron concentration in O. niloticus muscle from the Omo River and Omo Delta was significant (p value < 0.01).

The concentration of Nickel in muscle of O. niloticus is shown in table 1. Omo River and Omo Delta had mean level of nickel in muscle of O. niloticus was 0.010 mg kg-1 and 0.013 mg kg-1, respectively. However, the current findings indicate that the mean Ni content of the liver in O. niloticus is 0.014 mg kg-1 and 0.018 mg kg-1 in the Omo River and Omo Delta respectively. Cobalt content in muscle tissues *O. niloticus* varied 0.054mg kg^{-1} to 0.080 mg kg^{-1} in Omo River and Omo delta. There was statistically significant site (Omo river and Omo delta) dependent (p value < 0.01) variation in the mean Co concentration in the muscle tissues *O. niloticus.*

Levels of Heavy metals in *L. niloticus* in Omo River and Omo Delta

The level of Manganese in muscle tissue of *L. niloticus was* 0.383mg $kg\text{-}^{1}$ and 0.385mg/kg in Omo River and Omo Delta respectively (**Table 3**).The maximum mean level of heavy metals detected in liver of *L. niloticus* was Fe (2.918 ± 1.47 mg kg^{-1})) and the minimum mean level was Ni (0.011 ± ±0.003). Mean levels of the metals in liver and muscle of *L. niloticus* generally occurred in an order of Fe > Zn >Pb> Cu >Mn> Cr > Co > Ni and Pb> Cu >Mn> Cr > Co >Ni respectively.

The concentration of Zinc in muscle tissue of *L. niloticus* was 0.642 mg kg-1 and 0.428 mg/kg in Omo River and Omo Delta respectively (**Table 3**). Copper (Cu) level in *L. niloticus* muscle tissues varied between 0.157 mg kg-1 and 0.13 mg kg-1 in the Omo River and Omo delta respectively. However, the mean copper concentration in liver tissues of *L. niloticus* ranged from 0.481 mg kg-1 to 0.407 mg kg-1 in the Omo River and Omo delta, respectively.

Chromium (Cr) level ranged from being below detection limit to 0.154 mg kg^{-1}. Higher level of Cr was observed in the liver tissues of *L. niloticus*. L. niloticus muscle tissue had a statistically significant site (Omo River and Omo delta) dependent variation in mean Co concentration (p value< 0.02). Lead (Pb) level in L. niloticus muscle tissues varied between 0.793 mg kg-1 and 0.890 mg kg-1 in the Omo River and Omo delta respectively. As presented in table 4, the mean Pb concentration in L. niloticus muscle tissue varied statistically significantly by site (p value < 0.01) (Omo river and Omo delta).

Mean concentration of Fe in muscle tissue of L. niloticus was 0.509 mg kg-1 and 0.94 mg/kg in Omo River and Omo Delta respectively. Omo river and Omo delta, the mean Fe levels in L. niloticus muscle tissue differed statistically (p value <0.01) substantially (**Table 4**). Mean Nickel (Ni) concentration ranged from 0.011mg kg-1 to 0.019 kg-1 in Omo River and Omo delta respectively. Similarly, the mean Nickel levels in L. niloticus muscle tissue varied statistically significantly (p value <0.01) depending on the location (Omo river and Omo delta).

The statistical significant differences in the mean content of the detected heavy metals between the fish tissues of both fish species are presented in table 5 using T-test. The t-test (P<0.05) revealed that there were significant differences in the mean level of all heavy metals except for Mn, Cu, and Ni in muscle and liver tissues of L. niloticus (**Table 5**). Similarly, significant differences were observed in the mean levels of heavy metals between muscle and liver tissue of O. niloticus, with the exception of Zn, and Co. There were also species-dependent statistically significant differences in mean content of Pb, Ni, and Co in liver tissues of L. niloticus and O. niloticus. Likewise, the mean contents of Pb, Fe, and Ni in muscle tissues of both species were significantly different (P<0.05).

Human Health Risk of Heavy metals through consumption of fish from Omo River

The non-carcinogenic health risks associated with the nine heavy metals in children and adults who consumed muscle of *L. niloticus and O. niloticus* from the Lower Omo River were assessed using THQ and HI indices. The index results (THQ and HI) through eating muscle of fish within ‘one to seven times a week are presented’ in Table 6.The THQ values in the muscle of *L. niloticus*and O. *niloticus* were in the order of Pb> Cu >Mn> Co > Zn > Fe > Ni and ‘Pb> Cu

>Mn> Co > Ni >Zn > Fe' respectively. The index (HI) values due to consumption of *L. niloticus* muscle were 0.15 (for adults) and 0.21(for children). Similarly, the HI values in *O. niloticus* were 0.10 (for adults) and 0.18 (for children). .Maximum THQ and HI values were observed for Pb whereas the minimum was observed in Ni (**Table 6**).

In all the evaluated samples, the THQ for heavy metals in fish muscle ingested by adults and children were less than one. Similarly, the Hazard Index (HI) of the detected heavy metals was less than one. The mean contribution of THQ value to HI showed that Pb, Cu, and Mn contributed about 97 % to HI through muscle of fish tissues. Pb while single-handedly contributed about 90% to HI via the muscle tissues of both fish species.

The likely target cancer risk due to ingestion of Pb and Ni through muscle of *L. niloticus* and *O. niloticus* for 1 to 7 days a week are presented in Table 6. The target cancer risk (TCR) values in the muscle of both *L. niloticus* and *O. niloticus* were in an order of Ni>Pb (**Table 7**).

Human Health Risk of Heavy metals through consumption of fish from Omo Delta

Using the THQ and HI indices, the non-carcinogenic risks of the heavy metals identified in the muscle of L. niloticus and O. niloticus from the Omo delta were evaluated in adults (**Table 8**) and children (**Table 9**). Tables 8 and 9 for adults and children, respectively, show the index findings (THQ and HI) from eating fish muscle one to seven times a week. The THQ values in the muscle of *L. niloticus* and O. *niloticus* were in the order of Pb> Cr > Cu >Mn> Co > Zn > Fe> Ni and Pb>Mn> Co > Cu > Zn > Ni> Fe respectively. The index (HI) values due to consumption of *L. niloticus* muscle were 0.139 (for adults) and 0.199 (for children). Similarly, the HI values in O. *niloticus* were 0.09 (for adults) and 0.136 (for children).Maximum THQ values were observed for Pb both in L. niloticus and O. niloticus whereas the as the minimum was observed for in muscle of Fe in O.niloticus and Ni in L. niloticus.

The probable target cancer risk due to ingestion of Cr, Pb, and Ni through muscle of *L. niloticus* and *O. niloticus* for 1, 3, 5, and 7 days a week are presented in Table 10. The target cancer risk (TCR) values in the muscle of both *L. niloticus*and *O. niloticus* were in an order of Ni>Cr>Pb (**Table 10**).The THQ for heavy metals in fish muscle consumed by adults and children was less than one in every sample that was examined, and the Hazard Index (HI) of the identified heavy metals was also less than one.

Discussion

The Lead (Pb) level of muscle tissues ranged from 0.597mg kg-1 and 0.890 mg kg-1 with higher

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Formatted: Complex Script Font: Italic in the sample from Omo Delta, this may be due to the water nature in the Omo River and the Omo delta. The results of the present study are different from those of Gure et al. (2019), Felly et al. (2020), and Fredrick et al. (2021) who reported the mean concentration of lead at 8.39 mg kg1, 9.99 mg kg1, and 5.8 mg kg1, respectively. The Pb content of muscle tissues in both sites which is Omo river and Omo delta of this finding was higher than earlier report by (Magu et al., 2014; Samuel et al., 2020). Heavy agricultural runoff comprising pesticides, agrochemicals, and fertilizers, as well as petrol from fishing boats that contains lead, may be the source of the highest Pb value. However, the Pb content of liver tissue of O. niloticus of the current finding was lower than reported by Dugasa and Endale (2018) Pb (1.63 mg kg-1)- . and Pb (6.02 mg kg-1) by Gure et al. (2019).The mean Pb levels in the muscle tissue of O. niloticus between the Omo river and Omo delta were above the FAO/WHO recommended limits in the human diet (FAO/WHO, 1989).This shows that muscle tissue from Omo River and Omo delta are not safe for human consumptions with respect to Pb toxicity.

Lead (Pb) level in *L. niloticus* muscle tissues varied between 0.793 mg kg-1 and 0.89 mg kg-1 in the Omo River and Omo delta respectively. The results of the current study were higher than those of the prior studies on the Pb concentrations of 0.131 ± 0.048mg kg-1 and 0.181 ± 0.664 mg kg-1, respectively, in the River Nile (Aswan) and Nasser Lake, as reported by (Al-Hossainy et al., 2017). Similarly, our finding was higher than the previous study by (Machiwa, 2005) who reported Pb concentration in the range < 0.01–0.08 in Lake Victoria, Tanzania. Additionally, the Pb content of L. niloticus muscle tissues of this finding was higher than earlier report by (Magu et al., 2014; Lubna et al., 2015; Samuel et al., 2020) but lower than those reports by (Felly et al., 2020; Fredrick et al., 2021). Additionally, the Pb content of muscle in L. niloticus of the present study was higher than the previous report by (Magu et al., 2014), but lower than those reports by (Felly et al., 2020; Fredrick et al., 2021). It's possible that heavy rains carried the wastes down, contributing to the greater metal level in *L. niloticus* (Dural et al., 2007; Saei-Dehkordi and Fallah, 2011). The mean Pb levels in *L. niloticus* muscle tissues were above the FAO/WHO recommended limits in the human diet (FAO/WHO, 1989).

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In the muscle of O. niloticus, manganese (Mn) was discovered in Omo River at mean amounts of 0.379 mg kg-1. In contrast, the muscle of O. niloticus contained 0.384 mg kg-1 of Mn in Omo delta. Rather than the water naturally becoming more enriched with Mn, anthropogenic activities are typically to blame when Mn concentrations in natural waters above 0.2 mg/l (Nagpal, 2001). Salinity, water hardness, pH, and the presence of other contaminants are some of the variables that affect manganese toxicity in aquatic environments.

The results of this study showed that the concentration of Mn in O. niloticus muscle (0.379 mg kg-1) was lower than Mn (1.972 mg kg-1) content of muscle tissues (O. niloticus) reported by Ermias et al. (2015) in Lake Hawassa, Ethiopia, and Mn (0.77 mg kg-1) reported by Emmanuel et al. (2021) in the Volta River Basin of Ghana. However, comparable to those found in the muscle of O. niloticus species from Lake Hawassa as reported by Abayneh et al. (2003) Mn (0.55mg kg-1).The observed value is below the permitted human food intake level established by FAO/WHO in 1989.

Zinc levels in O. niloticus muscle tissue were 0.424 mg kg-1 in the Omo River and 0.394 mg/kg in the Omo Delta, respectively. The Zn level in muscle tissues of O. niloticus —from Omo river(0.424 mg/kg) of the current findings was comparable with those reported by Zenebe (2011) in Ethiopia (0.26 mg kg-1) but, less than Magu et al. (2014) from freshwater fish in Kenya (0.647 mg kg-1) and 19.36 mg kg-1 reported by Lubna et al. (2015) from the Langat River in Malaysia. The present investigation found that the concentration of Zn in O. niloticus muscle from the Omo delta was lower than that in the muscle of the same species as reported by Abayne et al. (2003), Ermias et al. (2015), and Samuel et al. 2020 (4.76 mg kg-1, 21.11 mg kg-1, and 19.36 mg kg-1, respectively). According to FAO/WHO (1989), the mean zinc concentration in the muscle of *O. niloticus* from the Omo River and Omo Delta was lower than the MPL in the human diet. This suggests that, in terms of zinc toxicity, *O. niloticus* from the Omo River and Omo Delta may be safe for ingestion by humans.

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The concentration of Zinc in muscle tissue of *L. niloticus* was 0.642 mg kg-1 and 0.428 mg/kg in Omo River and Omo Delta respectively. The level of Zn in muscle of L. niloticus in this finding was also higher than the earlier study by Jhon (2007). Similarly, our finding was higher than the previous study by (Machiwa, 2005) who reported Pb concentration in the range < 0.01–0.0189 mg/kg in Lake Victoria, Tanzania.

Copper (Cu) levels in O. niloticus muscle tissues ranged from 0.129 mg kg-1 to 0.071 mg kg-1, with Omo River having greater levels. The levels are below the maximum permitted limit in the human diet established by FAO/WHO (1989). The mean copper level of O. niloticus muscle tissues in this investigation was higher than the copper amount reported in prior studies by Zenebe (2011) Cu 0.03 mg kg-1. This may be attributed to agricultural runoff, which may carry higher values of these metals and arise from anthropogenic activities such as the use of chemical fertilizers and pesticides in agriculture land. However, the finding of the our study was lower than that of the previous studies, which included Cu (0.419 mg kg-1) (Magu et al., 2014), Cu(13.833 mg kg-1) (Ermias et al., 2015), and Cu (4.64 mg kg-1) (Gure et al., 2019).According to FAO/WHO(1989), O. niloticus from the Omo River and Omo Delta had mean Cu concentrations in their muscles that were below the MPL for humans' diets. This demonstrates that O. niloticus from the Omo River and Omo Delta may be safe for eating by humans in terms of Cu toxicity.

Copper (Cu) level in *L. niloticus muscle* tissues varied between 0.157 mg kg-1 and 0.130 mg kg-1 in the Omo River and Omo delta respectively. The levels are below the FAO/WHO (1989) maximum permissible limit in the human diet. The mean Cu content of muscle tissues in O. niloticus of this study was higher than the earlier report by Zenebe (2011).Similarly, the present finding were higher than those of the prior studies on the Cu concentrations in the range 0.001–0.097 (Machiwa, 2005) in Lake Victoria, Tanzania. The mean muscle content of Cu in L. niloticus of the current study was lower than the previous study reports by (Jhon, 2007; Felly et al., 2020) but lower than the study report by Magu et al (2014).

The current finding showed that the mean Cu concentration in O. niloticus liver tissues was 0.407 mg kg-1 in the Omo Delta and (0.481 mg kg-1) in the Omo River, which was higher than the finding reported by Dugasa and Endale (2018) Cu(0.029 mg kg-1). Nevertheless, the result was lower than that of the prior study Cu (8.28mg kg-1) (Gure et al., 2019).

According to the findings of the current investigation, *O. niloticus* tissue had a lower Cr concentration than that reported by Lubna et al. (2015); Cr = 1.48 mg kg-1; and Gure et al. (2019); Cr = 10.3 mg kg-1.However, the content of Cr in the liver tissue of O. niloticus was

0.126 mg kg-1 in the Omo River and 0.151 mg kg-1 in the Omo river, which was lower than the Cr (8.28 mg kg-1) content reported in a report by Gure et al. (2019) from the Gibe River in Ethiopia.

Chromium (Cr) level ranged from being below detection limit to 0.154 mg kg-1. Higher level of Cr was observed in the liver tissues of *L. niloticus.* The muscle content of Cr in L. niloticus of this result (0.039 mg kg-1) was lower than those study report by (Samuel et al., 2020; Emmanuel et al., 2021).However, the content of Cr in the liver tissue of L. niloticus of the current study was higher than the study reported by Dugasa and Endale (2018) and lower than the finding report of Gure et al. (2019) from Ethiopia.

The Omo River and Omo Delta had mean iron concentrations of 0.268 mg kg-1 and 0.411 mg kg-1, respectively, in the muscle tissues of *O. niloticus*. The results of the present investigation revealed that the muscle tissue of *O. niloticus* had less Fe than that reported by Abayneh et al. (2003) and Samuel et al. (2020), who reported 5.49 mg kg-1 and 11.34 mg kg-1, respectively. Similarly, the current findings show that O. niloticus have lower Fe contents in their muscle tissues than those found in research by (Abayneh et al., 2003; Dugasa and Endale, 2018; Emmanuel et al., 2021).The tissue levels of Fe are below the FAO/ WHO (2011) allowable limit. This suggests that, in terms of Fe toxicity, the muscle tissues of O. niloticus from the Omo River and Omo Delta may be safe for ingestion by humans. The current study's findings showed that the Fe concentration of liver in *O. niloticus* of was 1.100 mg kg-1 in the Omo River and 1.74 mg kg-1 in the Omo delta, which was greater than the earlier Fe (0.809mg kg-1) report by Dugasa and Endale (2018).

Mean concentration of Fe in muscle tissue of *L. niloticus* was 0.509 mg kg-1 and 0.94 mg/kg in Omo River and Omo Delta respectively. The tissue levels of Fe in this study are below the FAO/ WHO (2011) allowable limit. These concentrations are within the FAO/WHO (1989) recommended permissible human diet intake levels. The Ni contents of muscle tissues in *L. niloticus* of the present finding were comparable with the reports by (Zenebe, 2011; Magu et al. (2014; Samuel et al., 2020) and lower than the studies recorded by (Emmanuel et al., 2021).

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The results of this investigation showed that the mean concentrations of nickel in *O. niloticus* muscle in Omo River and Omo Delta were 0.010 mg kg-1 and 0.013 mg kg-1, respectively. These concentrations are within the FAO/WHO (1998) recommended permissible human diet intake levels which could not impose an immediate adverse health effects. The Ni contents of muscle tissues in O. niloticus and L. niloticus of the present finding were comparable with the reports by (Zenebe, 2011; Samuel et al., 2020) and lower than the studies recorded by (Magu et al., 2014; Emmanuel et al., 2021). Cobalt content in muscle tissues O. niloticus varied 0.054mg kg-1 to 0.082 mg kg-1 in Omo River and Omo delta. The mean muscle level Co in O. niloticus was comparable with the previous report by Samuel et al. (2020) .However; the mean Co level of the current finding was lower than the study recorded in the muscle and liver tissue of O. niloticus by Gureet al. (2019).Cd concentrations in muscle and liver tissues of both fish species were below the detection limit in the Omo River and Omo Delta Lake.

The t-test ($P<0.05$) revealed that there were significant differences in the mean level of all heavy metals except for Mn, Cu, and Ni in muscle and liver tissues of *L. niloticus*. Similarly, significant differences were observed in the mean levels of heavy metals between muscle and liver tissue of O. niloticus, with the exception of Zn, and Co. There were also species-dependent statistically significant differences in mean content of Pb, Ni, and Co in liver tissues of *L. niloticus* and *O. niloticus*. Likewise, the mean contents of Pb, Fe, and Ni in muscle tissues of both species were significantly different ($P<0.05$).It was addressed by many researchers that fish species-dependent differences in heavy metal accumulation might be associated with feeding habits such as being carnivores, herbivores, or omnivores and habitat of fish species (Yılmaz et al., 2005; Kamal et al., 2023).Variations in the mean concentrations of heavy metals between L. niloticus and O. niloticus in the current study may be attributed to Variations in feeding habits and habitat use (Elias et al., 2014; Samuel et al., 2020). Biological factors including age and growing rates of fish species could also attribute to differences in heavy metal concentrations between L. niloticus and O. niloticus (Yılmaz et al., 2005; Ahmad et al., 2015) of the present study.

Differences in mean level of heavy metals between the fish tissues (liver and muscle) in this finding could be due to the ability of various metals to induce binding with carboxylate oxygen, the amino functional group, and nitrogen in the metal-binding proteins (Uysal et al., 2009; Gure

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Formatted: Font: Italic, Complex Script Font: Italic et al., 2019; Pramita et al., 2021).The variations between tissue contents of metals could also be ascribed due to differences in the physiological role of each tissue in which muscle generally accumulates lower levels of heavy metals (Ahmad et al., 2015;Olawusi et al., 2019).Many studies also confirmed that there was variation in heavy metal levels among fish tissues and species (Samuel et al., 2020; Gure et al., 2019), which was also observed in the current finding. In general, L. niloticus showed higher burden of heavy metals than *O. niloticus*. This could be due to differences of the two species in their behavior and feeding habits. Thus, the relative high level of metals in L. niloticus tissues of the present study could be accredited to their feeding habits as they are bottom-dwelling carnivores which feed on zooplankton, shrimp, clams, snails, insects and other fish species as compared to O. niloticus which feeds on algae and other vegetables (Magu et al., 2016; Elias et al., 2005). Carnivores are likely to accumulate high metal content of heavy metals than other fish (Ahmad et al., 2015).

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The THQ for heavy metals in fish muscle consumed by adults and children in all of the samples from the Omo River that were analyzed were all less than one, indicating that people are unlikely to face significant health concerns as a result of ingesting a single heavy metal through consumption of the fish muscles. The Hazard Index (HI) of the discovered heavy metals was also less than one, indicating that, at the time of the study, there was no significant risk to human health from consuming L. niloticus and O. niloticus muscle tissues from the Lower Omo River source. The mean contribution of THQ value to HI showed that Pb, Cu, and Mn contributed about 97 % to HI through muscle of fish tissues. Pb alone was responsible for 90% of the HI through the two fish species' muscular tissues. Therefore, when it comes to the non-carcinogenic dangers, more attention should be paid to the Pb level in the muscle of both fish species. Regarding the non-carcinogenic risks, Emanuel et al. (2021) found that the THQ value for Mn (0.00325) was greater in their study conducted in the Volta Basin River, Ghana, than it is in the current findings for Mn (0.0021). However, they reported lower THQ values for Ni (0.000108), Zn (9.2×10-5), and Fe (2.14×10-8) than the present study via intake of O. niloticus muscle by children. Similarly, Samuel et al (2020) from their study in Ethiopia from Boicha stream (Hawassa) reported higher THQ values for Fe (0.01), Co (0.001), Ni (0.002), Cu (0.02), and Zn (0.039) than the present findings. However, they reported lower THQ value for Pb (0.026) than the present finding (0.11) in muscle O. niloticus by an adult while consuming one to seven days a week.

Fish sampled from Omo river, the target cancer risk (TCR) values in the muscle of both *L. niloticus* and *O. niloticus* were in an order of Ni>Pb. The TCR values for Pb and Ni in this study were within the tolerable range of (10-6 to 10- 4) (USEPA, 2012) for all levels of exposure. The highest TCR value observed due to consumption of *L. niloticus* and *O. niloticus* muscle by children for Ni was 1.4×10^{-5} and 1.3×10^{-5} respectively. Similarly, the highest TCR value due to *L. niloticus* and *O. niloticus* by adults for Ni was 9.4 ×10-6 and 8.5×10-6 respectively. This demonstrated that, for all exposure levels, there was no risk to the health of *L. niloticus* and *O. niloticus* from ingesting Pb and Ni through muscle. It was also observed that children had a higher probability of developing risk when exposed to heavy metal pollution. Emmanuel et al.(2021) from their study in Volta Basin River, Ghana, reported lower target Cancer Risk (TCR) for Ni in children (5.5×10-8)than the present study (1.1×10-5).Similarly Samuel et al (2020) from their study in Ethiopia from Boicha stream (Hawassa) reported higher target Cancer Risk (TCR) for Ni in adult (5.51×10-5) than the present study (8.5×10-6). However, they reported lower TCR for Pb (7.65 ×10-8) than the present study (3.3×10-6) via intake of *O. niloticus* muscle by adults.

The THQ for heavy metals in fish muscle consumed by adults and children was less than one in all of the Omo Delta samples that were analyzed, indicating that it is unlikely that people will face significant health hazards as a result of consuming fish muscles that contain heavy metals. The Hazard Index (HI) of the discovered heavy metals was also less than one, indicating that, at the time of the study, there was no significant risk to human health from consuming *L. niloticus* and *O. niloticus* muscle tissues from the Lower Omo River source. As can be seen from the risk assessment data, more emphasis should be given to the Pb level in the muscles of both fish species regarding the non-carcinogenic risks. Emanuel et al. (2021), from their study in the Volta Basin River, Ghana, recorded a lower THQ value for Mn (0.00325) than the present findings for Mn (0.009143) from *O. niloticus*. They also reported lower THQ values for Ni (0.000108), Zn (9.2×10-5), and Fe (2.14×10-8) than the present study via intake of O. niloticus muscle by adults and children. However, compared to the current results, Samuel et al. (2020) found that the THQ values for Fe (0.01), Co (0.001), Ni (0.002), Cu (0.02), and Zn (0.039) were greater in their study conducted in Ethiopia near Lake Hawassa. They did, however, disclose a lower THQ value for Pb (0.026) in muscle *O. niloticus* by an adult while consuming one to seven days a week than the current finding (0.08).

The target cancer risk (TCR) values in the muscle of both L. niloticus and O. niloticus were in an order of Ni>Cr>Pb in Omo Delta. The TCR values for Pb and Ni in this study were within the tolerable range of (10-6 to 10- 4) (USEPA, 2012) for all levels of exposure. The highest TCR value observed due to consumption of *L. niloticus and O. niloticus* muscle was by children for Ni 2.307×10-5 and 1.579×10-5 respectively. Similarly, the highest TCR value due to L. niloticus and O. niloticus by adults for Ni was 1.615 ×10-5 and1.105×10-5 respectively. This showed that, at all exposure levels, there was no risk of cancer from consuming Cr, Pb, or Ni through the muscle of *L. niloticus and O. niloticus*. It was also observed that children had a higher probability of developing risk when exposed to heavy metal pollution. Emmanuel et al.(2021) from their study in Volta Basin River ,Ghana, reported lower target Cancer Risk (TCR) for Ni in children (5.5×10-8) than the present study (2.307×10-5).Samuel et al (2020) from their study in Ethiopia from Lake Hawassa reported higher target Cancer Risk (TCR) for Ni in adult (5.51×10-5) than the present study (1.105×10-5). However, they reported lower TCR for Pb (7.65 ×10-8) than the present study (2.537×10-6) via intake of *O. niloticus* muscle by adults

Conclusions

The present study determined the heavy metal content in liver and muscle tissues of the widely distributed and economically most important fish species (*L. niloticus and O. niloticus*) as well as the possible risks to human health from Omo River Basin and Omo Deta, Southern Ethiopia. This study was the first of its kind done and the finding of the study has provided the first baseline information on the level of nine heavy metals in *L. niloticus and O. niloticus* from Ethiopian low land freshwater. All levels of heavy metals under evaluation, with the exception of Pb, were within the permissible limits established by FAO/WHO (1998) in the Omo River. The levels of all heavy metals under investigation, with the exception of Pb and Cr, were within the permissible limits established by (FAO/WHO, 1989) in the Omo delta. Higher accumulations of heavy metals were generally observed in the liver and muscle tissue of *L. niloticus* as compared to *O. niloticus* and generally, liver accumulated more heavy metals than muscle tissues.

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The level of heavy metal pollution found in fish tissue serves as a signal for quick action. The results of health risk assessments indicated that there was no potential damage to human health from exposure to the investigated heavy metals. However, the mean Pb levels discovered in the liver and muscle tissue of both fish species were higher than the FAO/WHO (1989) allowed level, suggesting that freshwater fish from the Omo river and Omo delta should be regularly monitored. Similar to this, the TCR result from Ni exposure from eating *L. niloticus and O. niloticus* muscle is frightening and may increase the risk of cancer in the future, particularly in youngsters due to numerous vigorous and prolonged development activities being carried out.Therefore, monitoring of heavy metal level in tissues of *L. niloticus and O.* ~~Ore~~*niloticus* is a vital concern and it is time for the policy makers to take appropriate action at this alarming level to protect freshwater fish and people from the threat of heavy metal pollution from the lower of River and Omo Delta.

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Authors' contributions

AA: The primary investigator of the study was responsible for the study's conception, design, writing of the proposal, data-gathering process, final analysis, and article production. Participating in the study design, GTY, SSS and YSB reviewed and critiqued the entire paper, paying particular attention to the procedure and analysis sections. The final manuscript was read and approved by all authors.

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Availability of Data and Materials

"The dataset are made available on the manuscript."

Ethical Approval:

Letters of support from the Department of Biology and Debub-Omo Agriculture and Fishery Bureau of Hawassa University were also obtained. Ethical clearance and approval were obtained from the Hawassa University Institutional Review Board (IRB) and approval number Bio/499/13. "All methods were performed in accordance with the relevant guidelines and regulations".

Consent for publication

"Not applicable "

Conflicts of Interests

The authors declare that we have no financial and non-financial competing interests

Declaration

"All authors have read understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors"

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