Helminthic Fish Parasites, Fish Tissues, and Environmental Samples as Indicators of Heavy Metal Contamination in Koka Reservoir, Ethiopia

By:

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ABBREVIATIONS/ACRONYMS

AAU = Addis Ababa University

AT = Average lifetime

AO = Awash Open waters

AOAC = Association of Official Analytical Chemists

AWR = Awash River

APHA = American Public Health Agency

BCF = Bio-concentration factor

BW = Body weight

CF = Average heavy metal Concentration in Fish

EDI = Estimated Daily Intake

EP = Exposure Period

EPA = Environmental Protection Agency

ER = Exposure Rate

FAAS = Flame Atomic Absorption Spectrophotometry

FAO = Food and Agriculture Organization of the United Nations

HI = Hazard Index

ICP-OES = Inductively Coupled Plasma Optical Emission Spectroscopy

IR = Daily Intake of fish consumed

KW = Koka Waste

LEL = Least Effect Level

MM1 = Modjo Mouth 1

MM2 = Modjo Mouth 2

MR = Modjo River

MTs = Metallothionein

MPI = Metal Pollution Index

NFALRC = National Fishery and Aquatic Life Research Center

ORD = Oral Reference Dose

PCB = Polychlorinated biphenyls

ISQG = Interim Sediment Quality Guideline

PSQG = Provincial Sediment Quality Guideline

SEL = Severe Effect Level

THQ =Target Hazard Quotients

USEPA = United States Environmental Protection Agency

WHO = World Health Organization

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Yaada qofaan

Sibiilonni ulfaatoo ta’an itti fufiinsa, summii fi baayoo kuufamuu isaanii irraa kan ka’e sirna ikoo bishaanii irratti balaa guddaa fidu. Baayoomonitoriin, orgaanizimoota baayooindicator fayyadamuun qulqullina bishaanii fi fayyaa sirna ikoo madaala. Dhiheenya kana paraasitoonni qurxummii sadarkaa kuufama isaanii keessummeessitoota isaanii caalaa ol’aanaa ta’uu isaaniitiin akka agarsiiftuu baayoo-agarsiiftuu filannoo faalama sibiilota ulfaatootti yaadameera. Qorannoon kun sadarkaa kuufama sibiilota ulfaatoo gosoota qurxummii daldalaaf barbaachisoo ta’an sadii, paraasitoota helminthic isaanii akkasumas saamuda biyyee fi bishaanii kuufama Kokaa keessatti madaaluuf kan akeeke ture. Qorannoon qaxxaamuraa saamuda qurxummii 337 yeroo rooba duraa (Caamsaa) bitame irratti gaggeeffameera. Qurxummiin kun qurxummii Ambaazaa, Korsoo, fi Kaarpii kan lakkoofsa saayimenii 105, 197 fi 35 ta’e kan hammatedha. Saamuda biyyee fi bishaanii iddoowwan ja’a irraa walitti qabameera. Ambaazaa keessatti paraasitoonni nematode sadii adda baafamaniiru isaanis; Kontracecum, Porocecum, and Camallanus. yoo ta’u Kontracecum kan argame Korsoo keessatti. Tishuu Ambaazaa keessatti sibiilonni tartiiba Fe>Zn>Cu>Mn>Cr. Faallaa kanaatiin Korsoo fi Kaarpii keessatti tartiiba kuufama sibiilaa Fe>Zn>Mn>Cu>Cr ture. Xiyyeeffannaan sibiilaa paraasitoota Ambaazaa fi Korsoo keessatti tartiiba Fe>Zn>Cu>Mn>Cr fi Fe>Mn>Zn>Cr>Cu hordofeera. Xiyyeeffannaan sibiilaa seedimentii keessatti tartiiba Fe>Mn>Zn>Cr>Cu hordofeera. Qorannoon kun abdii Ambaazaa, Korsoo fi Kontracecum akka agarsiistota faalama sibiilota ulfaatoo ta’uu danda’an ibseera. Dandeettii kuufama isaaniitti fayyadamuun hordoffii naannoo guddisuu fi faalama sibiilota ulfaatoo qaamolee bishaanii keessatti mul’atan hir’isuuf tarkaanfiiwwan gidduu seensaa yaada kennuu dandeenya.

አህፅሮት

ከባድ ብረቶች በውሃ ውስጥ ባሉ ስነ-ምህዳሮች ላይ በፅናት፣ በመርዛማነታቸው እና በሰውነት ውስጥ መከማቸታቸው ምክንያት ከፍተኛ ስጋት ይፈጥራሉ። አመላካች ፍጥረታትን በመጠቀም የአካባቢን ጤና መከታተል፣ የውሃ ጥራት እና የስነ-ምህዳር ጤናን ይገመግማል። የዓሣ ጥገኛ ተውሳኮች ከአስተናጋጆቻቸው የበለጠ ከፍተኛ የማከማቸት ችሎታ ስላላቸው የከባድ ብረቶች ብክለትን እንደ አማራጭ አመላካች በቅርቡ ቀርበዋል። ይህ ጥናት በቆቃ ማጠራቀሚያ ውስጥ የሚገኙትን የከባድ ብረቶችን ክምችት መጠን በሦስት ለንግድ አስፈላጊ የሆኑ የዓሣ ዝርያዎች፣ የሄልሚንቲክ ጥገኛ ተውሳኮች፣ እንዲሁም ደለል እና የውሃ ናሙናዎችን ለመገምገም ያለመ ነው። በቅድመ-ዝናብ ወቅት (ግንቦት) ከተገዙ 337 የዓሣ ናሙናዎች ጋር ተሻጋሪ ጥናት ተካሂዷል። ዓሣዎቹ አምባዛ፣ ቆሮሶ እና ካርፕ በናሙና ቁጥር 105፣ 197 እና 35 በቅደም ተከተል ይገኙበታል። የደለል እና የውሃ ናሙናዎች ከስድስት ቦታዎች ተሰብስበዋል። በአምባዛ ውስጥ ሶስት የኔማቶድ ጥገኛ ተውሳኮች (ኮንትራሴከም፣ ፖሮሴከም እና ካማላነስ) ተለይተዋል። በቆሮሶ ውስጥ አንድ ኔማቶድ (ኮንትራሴከም) ብቻ ተገኝቷል። በአምባዛ አካል ውስጥ ብረቶች በ Fe>Zn>Cu>Mn>Cr ቅደም ተከተል ተከማችተዋል። በተቃራኒው በቆሮሶና ካርፕ የብረት ክምችት ቅደም ተከተል Fe>Zn>Mn>Cu>Cr ነበር። በአምባዛ እና ቆሮሶ ጥገኛ ተውሳኮች ውስጥ ያለው የብረት ክምችት Fe>Zn>Cu>Mn>Cr እና Fe>Mn>Zn>Cr>Cu በቅደም ተከተል ተገኝቷል። በደለል ውስጥ ያለው የብረት ክምችት Fe>Mn>Zn>Cr>Cu ቅደም ተከተል ተገኝቷል። ይህ ጥናት የአምባዛ፣ ቆሮሶ እና ኮንትራሴከም ከባድ ብረት ብክለትን እንደ እምቅ ጠቋሚዎች አጉልቶ አሳይቷል። የማከማቸት አቅማቸውን በመጠቀም የአካባቢ ቁጥጥርን ማሳደግ እና በውሃ አካላት ላይ ያለውን የከባድ ብረት ብክለትን ለመቀነስ የጣልቃ ገብነት እርምጃዎችን ልንጠቁም እንችላለን።

ABSTRACT

Heavy metals pose a significant threat to aquatic ecosystems due to their persistence, toxicity, and bioaccumulation. Biomonitoring, using bioindicator organisms, assesses water quality and ecosystem health. Fish parasites have recently been proposed as alternative bioindicators of heavy metal pollution due to their higher accumulation levels than their hosts. This study aimed to assess the level of heavy metal accumulation in three commercially important fish species, their helminthic parasites as well as sediment and water samples in Koka Reservoir. A cross-sectional study was conducted with 337 fish samples bought during the pre-rainy season (May). The fish included African catfish (Clarias gariepinus), Nile tilapia (Oreochromis niloticus), and Common carp (Cyprinus carpio) with specimen number 105, 197, and 35, respectively. Sediment and water samples were collected from six sites. Three nematode parasites were identified in C. gariepinus (Contracaecum, Porrocaecum, and Camallanus sp.), while only one nematode (Contracaecum sp.) was found in O. niloticus. In the tissue of C. gariepinus, metals accumulated in the order Fe>Zn>Cu>Mn>Cr. Conversely, in O. niloticus and C. carpio, the order of metal accumulation was Fe>Zn>Mn>Cu>Cr. The metal concentration in parasites of C. gariepinus and O. niloticus followed the orders Fe>Zn>Cu>Mn>Cr and Fe>Mn>Zn>Cr>Cu, respectively. The metal concentration in sediments followed the order Fe>Mn>Zn>Cr>Cu. This study highlighted the prospect of C. gariepinus, O. niloticus and Contracaecum sp. as potential indicators of heavy metal pollution. By taking advantage of their accumulation abilities, we can enhance environmental monitoring and suggest intervention measures to reduce heavy metal pollution in water bodies.

Keywords: Clarias gariepinus, Cyprinus carpio, Bioconcentration, Biomonitoring, Health risk, Heavy metals, Lake Koka, Nematodes, Oreochromis niloticus, Sediment

1. CHAPTER ONE: INTRODUCTION

1.1. Background and Justification

Heavy metals pose a significant threat to aquatic ecosystems due to their persistence, toxicity and bioaccumulation (Hassan et al., 2018). While naturally occurring in low concentrations from weathering processes, modern industrial and agricultural activities alongside water bodies significantly increase their levels, pushing them beyond safe limits (Ali et al., 2019). Due to their non-biodegrade feature, heavy metals remain in the environment for extended periods, accumulating in sediments and aquatic organisms, particularly fish (Sevcikova et al., 2011). This bioaccumulation has detrimental effects on fish. Effects include absorption and accumulation in organs like gills and liver (Zeitoun and Mehana, 2014); oxidative stress leading to lipids and DNA damage; histopathological changes leading to disruptions in tissue structure and function (Zeitoun and Mehana, 2014); and developmental impairments leading to malformations, reduced survival rates and altered enzymatic activity during embryonic and early life stages (Jezierska et al., 2009). Furthermore, contaminated fish pose a threat to human health as they enter the food chain, causing biomagnification (Jezierska et al., 2009). This highlights the urgent need for monitoring and mitigation strategies to control and reduce heavy metal pollution in aquatic ecosystems.

Environmental pollution related ecological changes can be demonstrated through different strategies, amongst them being biomonitoring. Biomonitoring is a technique that uses living organisms to indicate or evaluate the state of their environment, especially in relation to environmental pollution. It can be done either in a passive or active way. Passive biomonitoring can reveal the accumulation of toxic substances in the organs and tissues of organisms, reflecting the pollution level in their environment. It also shows the negative impacts of pollution on the ecosystem, such as loss of sensitive species, and reduced biodiversity. Active biomonitoring examines the behavioral, physiological, and cellular responses of organisms exposed to toxic substances in controlled or altered conditions (Fränzle, 2003). Biomonitoring, bioindicators, assesses water quality and ecosystem health. It can measure the pollution level and its consequences using the organisms that live in the water.

Not all organisms can be used as bioindicators. Bioindicators are organisms that can reflect the condition of their environment by their presence, absence, or characteristics. They should have some desirable traits, such as sensitivity to pollution, wide distribution, easy identification, and ecological relevance. These traits can help better evaluate the condition of the environment they are residing in. However, finding a bioindicator that meets all these criteria is difficult, so the choice of bioindicator may depend on the purpose of monitoring (Zhou et al., 2008). Fishes are among the aquatic organisms that can be used as bioindicators of heavy metal pollution. The sensitivity of fish to a variety of toxicants enables them to indicate the state of the ecosystem, because they are sensitive to various toxicants, they can show the natural and altered features of their environment, and they can accumulate heavy metals in their tissues as the top predators of the aquatic food web (Authman et al., 2015). However, fish are not ideal bioindicators, because their bioaccumulation capacity can vary depending on several factors, such as diet, storage, excretion, water quality, season, size, feeding habit, and behavior. These variations can occur even within the same area (Dural et al., 2007; Abdallah and Morsy, 2013). Recently, fish parasites have been proposed as alternative or complementary bioindicators of heavy metal pollution, because they can accumulate heavy metals in their tissues at higher levels than their hosts, they can interact with environmental stressors and affect the biomarker responses of their hosts, and they can provide information on the biodiversity, life cycle, and migration patterns of fish species (Sures, 2003, 2004; Vidal-Martínez et al., 2010; Biswal and Chatterjee, 2020; Mehana et al., 2020; Jerônimo et al., 2022). Moreover, fish ectoparasites with direct lifecycle can also indicate water quality changes, due to their rapid reproduction rates (Martins et al., 2015). These parasites are more ideal bioindicators of heavy metal pollution than free-living fishes, because they reflect the effects of environmental parameters across various trophic levels. Several studies have demonstrated their usefulness in different aquatic ecosystems around the world (Erasmus et al., 2020; Mostafa et al., 2023). However, there is still a lack of information on their role in Ethiopia, where many water bodies are affected by anthropogenic activities. Therefore, this study aimed to assess the level of heavy metal accumulation in three commercially important fish species, their helminthic parasites as well as sediment and water samples in Koka Reservoir.

1.2. Statement of the problem

Koka Reservoir, which serves a variety of socioeconomic functions, is gravely endangered by point and non-point sources of pollutants from industry and intensive farming activities in their catchment. Heavy metals are among the main pollutants that are constantly discharged into the lake due to intensive human activities such as industrialization, urbanization, and agricultural activities (Ermias Deribe et al., 2011; Kassahun Tessema et al., 2020). A study by Kassahun Tessema et al. (2020) investigated the presence of heavy metals, including Mn, Cr, Pb, Zn, Cu, Ni, and Cd, in both the physical and biological systems of Koka Reservoir. The findings revealed varying levels of bioaccumulation and biomagnification of these heavy metals. While the weekly intake estimates of most metals remained below tolerable values, the biomagnification of Chromium (Cr) posed a significant carcinogenic risk. Specifically, concentrations of Cr in the muscle tissue of *Clarias gariepinus, Oreochromis niloticus*, and *Cyprinus carpio* exceeded the acceptable limits set by the World Health Organization (WHO), indicating a potential health hazard for consumers (Bealemlay Abebe Melake et al., 2023).

Assessing the human health risks associated with consuming fish containing accumulated heavy metals necessitates continuous monitoring of these metals in the aquatic ecosystem, utilizing reliable indicators of heavy metal pollution capable of reflecting their bioavailability and biological impact (Zhou et al., 2008). While fish are commonly used as bioindicators due to their ability to accumulate metals and exhibit physiological and biochemical alterations in response to pollution (Rashed, 2001; Vinodhini and Narayanan, 2008), alternative indicators such as parasites, particularly helminths, have been proposed. Helminths can accumulate and reflect the effects of heavy metals in both their hosts and the environment, offering a complementary approach to assessing heavy metal pollution (Sures, 2003, 2004; Vidal-Martínez et al., 2010). However, as useful as fishes are in heavy metal bioaccumulation studies, the usefulness of their parasites as indicators of heavy metal accumulation in any water bodies in Ethiopia have not been explored so far. Some publications demonstrated the prevalence of different parasites in commercially important fish species. For example, Yewubdar Gulelat et al. (2013) found the prevalence of helminth parasite in fishes harboring Koka Reservoir to be 66.3%. Higher prevalence was exhibited in *C. gariepinus* than any fish in the lake.

In recent years researchers have tried to use fish and fish parasite prevalence as indicators of ecosystem health (Haileleul Micho et al., 2020; Habtamu Tadesse, 2022). Considering the mounting evidence of parasites as excellent indicators of environmental change, measuring them for heavy metal accumulation has proven to be useful in quantifying environmental pollution. Among the different groups of helminths, cestodes have been shown to have the highest potential for metal accumulation and biomonitoring, as they have a high surface area to volume ratio, a permeable tegument, and a lack of digestive tract (Sures et al., 1999). However, nematodes, which are the second most abundant group of helminths in fish, have also been studied for their ability to accumulate and indicate heavy metal pollution. Nematodes have some advantages over cestodes, such as a wider distribution, a higher diversity, and a shorter life cycle (Paperna, 1996). Several studies have reported the metal accumulation patterns and profiles of different nematode species in fish and compared them with those of their hosts and other parasites (Wilson and Kakouli□Duarte, 2009; Olivero-Verbel and Caballero-Gallardo, 2013; Mazhar et al., 2014). These studies have shown that nematodes can accumulate metals in different degrees and proportions, depending on the metal type, the nematode species, the host species, and the environmental conditions. Some nematodes have been found to accumulate metals at higher levels than their hosts, while others have shown lower or similar levels. The divergence in metal accumulation profiles of nematodes is linked with the specificity of microhabitats, cuticle morphology, and interspecific competition (Mazhar et al., 2014). This research work, therefore, attempted to identify whether the helminth parasites (nematodes specifically) of the three commercially important fish species (*C. gariepinus*, *O. niloticus* and *C. carpio*) can be used as indicators of heavy metal contamination in Koka Reservoir.

1.3. Research Questions

- Is there a difference in heavy metal accumulation between helminthic parasites and tissues/organs of the three commercially important fish species in Koka Reservoir?
- Is there a difference in heavy metal accumulation between sediment and water samples taken from sites with different levels of anthropogenic impacts in Koka Reservoir?

1.4. Objectives

1.4.1. General objective

To evaluate the effectiveness of helminth fish parasites as bioindicators of heavy metals contamination in Koka Reservoir for monitoring and sustainable aquatic ecosystem management in Ethiopia.

1.4.2. Specific objectives

- To determine the concentration of heavy metals in fish tissues, nematode parasites, sediments, and water samples collected from different sites with different levels of anthropogenic impacts in Koka Reservoir.
- To compare metal accumulation patterns and profiles of three commercially important fish species in their tissues and their helminth parasites in Koka Reservoir.

1.5. Hypothesis

There is no significant difference between the metal accumulation of the fish and their nematode parasites, and between the sediment and water samples collected from different sites in the Koka Reservoir.

1.6. Limitations of the study

This study aimed to provide information about the heavy metal accumulation capacity of helminth parasites and the fish species they harbored. Since, a cross-sectional study was carried out; the effect of seasonality was not taken into consideration. The study was conducted at a time where pollutants/contaminants were expected to be concentrated as it was during the dry season. While this might provide detailed information, acknowledging the pattern of accumulation in the parasite would have provided a great deal of insight. In this study, it was possible to analyze the presence of only five essential metals based on the history of the site. However, assessing the presence of other metals (non-essential) would help in understanding the state of heavy metal contamination taking place in the site and the ability of the parasites in accumulating them.

2. CHAPTER TWO: LITERATURE REVIEW

2.1. Heavy metal pollution and its effect on living things

Contamination of soil and water as a result of human activity-generated industrial and urban wastes is a major environmental issue. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, and sewage sludge application to agricultural soils are responsible for transferring of contaminants into non-contaminated sites as dust or leachate also contribute towards contamination of our ecosystem (Gupta and Singh, 2011). Contamination can be caused by a variety of inorganic and organic substances, such as heavy metals, combustible and putrescible materials, hazardous refuse, explosives, petroleum products, phenol, and textile dyes. As heavy metals are key components of natural contaminants, they cause a distinct challenge compared to other chemical pollutants (Jadhav et al., 2010).

All metals are toxic at greater concentrations because they lead to oxidative stress by producing free radicals. Metals may also be toxic because they can substitute for critical metals in pigments or enzymes, which would interfere with the operation of those substances. Heavy metals, therefore, make the land unfit for plant development and eliminate biodiversity (Gupta and Singh, 2011).

When mined ores are deposited on the earth surface for manual dressing, heavy metal pollution of surface and underground water sources leads to significant soil pollution. It can increase when the extracted ores are discarded on the ground. The pollution caused by agricultural activities can also affect the tissues of animals and aquatic life. These animals consume and drink from areas where heavy metals are present. Humans are also exposed to heavy metals when they consume food and other living things that are contaminated. This can lead to various health conditions. The effects can be in the form of neurotoxicity, mutagenicity, carcinogenicity, or teratogenicity (Duruibe et al., 2007).

Most contaminants in aquatic environments tend to build up in organisms, where they can accumulate to levels that are hundreds or thousands of times higher than the pertinent water levels (Bervoets and Blust, 2003). Bioaccumulation is the term used for the process by which some contaminants in the sediment are absorbed by benthic organisms. In a process known as biomagnification, the toxins are ingested by bigger animals that eat these contaminated organisms, moving up the food chain with increasing concentrations (Begum et al., 2009).

Fish and other aquatic life forms pick up pollutants both directly from contaminated water and indirectly through the food chain. Fish become contaminated with heavy metals when chemical additives that contain traces of these metals are applied. In heavy metal contaminated reservoirs, a significant decrease in fish populations or even extinction of the entire fish population was observed (Khayatzadeh and Abbasi, 2010). Toxicants generally shorten the lives of parasites, possibly as a result of their binding to the active sites of enzymatic molecules, which ultimately causes impairment or death by deactivating any of a wide range of enzyme systems (Pietrock and Marcogliese, 2003).

The presence of toxicants, the temperature, and other environmental variables all have an impact on growth. Between fish species, depending on age, developmental stage, and other physiological variables, metals may or may not be present. The fish gain mass and length when the right circumstances are present, including the right temperature and an adequate supply of food. On the other hand, fish development may be hampered in water that has been contaminated with toxins, such as heavy metals. One of the most obvious signs of toxic metal activity on fish larvae is growth inhibition (Khayatzadeh and Abbasi, 2010). Long-lived fish at the top of the aquatic food chain concentrate a lot of contaminants, including metals, and are therefore, frequently used as biological indicators (Rashed, 2001).

2.2. Indicators of heavy metal pollution

The use of biological indicator organisms to identify areas of trace metal pollution seems to be the most appealing method because these organisms not only concentrate metals from water, making analysis relatively cheap and simple, but they may also provide a moving time-averaged value for the relative biological availability of metals at each site under investigation (Phillips, 1977). Animals' limited capacity to eliminate heavy metals right afteringestion, combined with their ability to inactivate the metal by binding to metallothionein (MTs), favors metal buildup (Zhou et al., 2008). Due to these reasons, different organisms are employed as indicators of heavy metal pollution. These include plankton (phyto- and zooplankton), insects, mollusks, fish, plants, birds (Zhou et al., 2008), mammals (Stankovic and Stankovic, 2013) and parasites

(Mehana et al., 2020) amongst others. For example, fishes are able to show the natural features and the changes that happen in them. The different stages in growth, the fecundity rate, and their metabolism can all be used in monitoring aquatic pollution (Zhou et al., 2008). Moreover, their location within the food web allows them to be the end receivers of the accumulated heavy metal as they are the top predators within the aquatic ecosystem (Authman et al., 2015). This makes them good indicators of heavy metal pollution. However, different scenarios should not be overlooked when considering fish as an indicator of heavy metal pollution. Authman et al. (2015) reported that the bioaccumulation capacity of fishes depends on the food ingested, the way it is stored and the mechanism of expulsion from the body. Another factor that plays a role in the varaiation in the accumulation capacity of fish is the status of the water, the season and the length and weight (Dural et al., 2007). The feeding habit, the environment and behaviour of the fish were also reported to have an effect in the accumulation capacity of heavy metals. These variations were reported to occur even within the same area (Abdallah and Morsy, 2013). In hindsight, while fishes can be used as indicators of heavy metal pollution, these variations does not make them the most ideal organisms.

2.3. Parasites as indicators of ecosystem health

Poulin (2007) defined parasitism as a close relationship between species in which one organism, the parasite, inhabits or lives on another organism, the host, harming it in some manner and becoming structurally adapted to its mode of existence. Parasites are divided into two based on their site of occurrence; endo- and ectoparasites. Endoparasites are the parasites that are usually found within the body of the host. Some of their examples include acanthocephalans, cestodes, nematodes, protozoans and digeneans. Parasites that are found on the exterior part of the host are referred to as ectoparasites. Arthropods and monogeneans are some of the examples (Bush et al., 2001). Endo- and ectoparasites are typically found to be the best accumulators and effective indicators of pollution in aquatic environments (Sures, 2004).

Parasites are successful (Poulin and Morand, 2000) because they have evolved on numerous separate instances across a wide range of phyla by going through many transitions (Weinstein and Kuris, 2016). They are part of biological systems and make up a sizeable percentage of the global biodiversity (Dobson et al., 2008). In some ecosystems, they also achieve significant biomass, abundance, and productivity (Kuris et al., 2008).

Parasites are valuable indicators of the health of an ecosystem (Marcogliese and Cone, 1997). This is because they have different life cycles amongst different species in which they infect an organism that is usually used as a marker as they go through progressive stages (Williams and MacKenzie, 2003). Ecosystem health can't be directly quantified therefore research on parasites and the host-parasite relationship would be a more significant and useful method of keeping track of ecosystem health (Steedman, 1994). Since fish parasites are a significant component of aquatic biodiversity, they exhibit strong correlations with changes in the hosts' internal and external environments. Changes in the hosts may directly or indirectly result in changes in the parasites (Palm, 2011).

Toxins in contaminated water can cause immediate harm to parasites, and some toxins may even preferentially concentrate in parasite tissues (Sures et al., 1997). Toxins that weaken the immune system of the host may be advantageous for other pathogens like fish monogeneans and parasitic ciliates on the gills. It is likely that toxins reduce mucous formation, a fish's primary line of defense against gill parasites, which explains why ciliate intensity and prevalence rises in response to a variety of pollutants. However, parasites appear to be susceptible to harmful chemicals (Lafferty, 1997). The possibility of using fish parasites as environmental markers was suggested due to the connection found between pollution and fish parasites (Sures, 2004).

As fish parasites and fish food, parasites unquestionably have an impact on food webs. Their diversity and abundance are both high, and their combined biomass indicates that they play an often-rivaling role in ecosystem energetics (Marcogliese, 2004). A parasite-infected species may cause an effect on the remainder of the food chain either through a bottom-up or top-down cascade, depending on where it is found within the food web. Therefore, all organisms found throughout the food web are therefore subjected to the impacts of parasitism. These effects strongly imply that it is essential to take parasites into account when managing and conserving their hosts (Marcogliese, 2004). Fishes are impacted by parasites on a group and ecosystem level. Additionally, parasites rely on the host communities in which they reside, and elements of parasite communities are influenced by fish communities. Parasites may become less abundant over time because they are susceptible to extensive fishing and pollution. For these reasons, parasites in fish may serve as helpful markers of the health of both fish and aquatic life in general (Biswal and Chatterjee, 2020).

In the past, parasites were just perceived as organisms that needed to be treated or removed as their only role was to cause harm (Marcogliese, 2005). However, present-day understanding of the host-parasite relationships showed that they are the result of antagonistic interactions that coevolved into precise reciprocal adaptations. Therefore, parasites take advantage of particular biological traits of their hosts to guarantee their transmission, survival, and maintenance of viable populations. As a consequence, parasites may have varying degrees of impact on the biology of their host. Individual hosts are subjected to energetic demands from parasites, which may have an impact on their morphology, fecundity, reproduction, behavior, and longevity (Marcogliese, 2004). Their effects reach host groups, communities, and even ecosystems in addition to individual hosts. Amongst the many things that parasites can control, they have the power to control host populations (Hudson et al., 1998), and modify ecosystem processes (Frainer et al., 2018). However, they are not able to establish free-living groups (Dunne et al., 2013).

In addition to changing host parameters typically used as indicators of physiological or reproductive state, parasites are also capable of having antagonistic or synergistic effects on variables commonly used in fish ecology and fisheries. Sures and Siddall (2003) conducted a study on an acanthocephalan, *Pomphorhynchus laevis*, parasitising a fish, *Leuciscus cephalus*, and found that the level of heavy metal acummulated inside the parasite was much greater than the level found in the fish. Thus, reducing the effect the fish would have encountered if not for the presence of the parasite. Sures and Knopf (2004) demonstrated that the fish, *Anguilla anguilla*, exposed to polychlorinated biphenyl (PCB) 126 would be immunosuppressed when getting infected by the parasite *Anguillicola crassus.* Without the presence of the pollutant, PCB 126, the fish would have been able to develop an antibody against the parasite.

The ecology of fish, their predators, and their prey may be affected by changes in host behavior brought on by parasites. These changes may affect host distribution trends, habitat selection, diet makeup, sexual behavior, and other factors (Timi and Poulin, 2020). Nevertheless, little is known about the crucial functions that parasites play in the environment (Marcogliese, 2004). There are different methods in which parasites and environmental pollution can interact. As they pose an impact on the physiology and behavior of the host, parasites can hinder established bioindication processes. This might result in misleading results. However, as these entities react in different ways to anthropogenic pollution, they can be used as both effect and accumulation indicators (Sures, 2004).

2.4. Fish parasites as accumulation indicators of heavy metal pollution

Accumulation indicators can reveal important details about the chemical state of their habitats and behavior (and the changes in prevalence, mean intensity, or mean abundance of the parasite in the case of fish parasites that are used as bioindicators) by monitoring changes in their physiology. Sentinels that accumulate chemicals should do so effectively and come to a state of equilibrium where their consumption and excretion are equal. There shouldn't be any toxic effects on the body as a result of this uptake and bioconcentration of chemicals (Sures, 2004).

The existence or absence of some organisms can reveal information about the chemical composition of their surroundings. Others exhibit the ability to concentrate environmental pollutants inside their tissues despite being less impacted by toxic substances (Rosenberg and Resh, 1993). Up to this point, studies have primarily concentrated on the use of parasites as accumulation indicators of heavy metals. This is connected to the various types of lipophilic and hydrophilic substance accumulation. Hydrophilic substances are dispersed more evenly among tissues, whereas lipophilic chemicals primarily accumulate in fat and are subsequently biomagnified along food webs. Low-fat-content parasites are unable to bioconcentrate lipophilic substances above the amounts found in host tissues (Heinonen et al., 2000; Ruus et al., 2001).

It is possible to initiate complex physiological cascades by altering very fundamental processes (Sures, 2004). Numerous studies on the uptake and accumulation of metals by parasites have been published, in addition to the impacts of infection on the uptake and accumulation of organic pollutants in host tissues (Sures, 2003). The majority of these investigations used fish helminths, which accumulate metals to levels several thousand times higher than those of their hosts and above ambient environmental concentrations. Only metazoic endoparasites have had the ability to accumulate metal among fish parasites studied. This was because protozoan parasites are too small to provide sufficient material for a legitimate chemical investigation. Ectoparasites like monogeneans, crustaceans, and leeches are typically primarily affected by the water in the area and are most likely comparable to related free-living organisms in their pattern of accumulation. These organisms include turbellarians, non-parasitic crustaceans, and annelids (Bergey and Weis, 2002).

Due to these restrictions, endohelminths are the focus of the overwhelming majority of papers on metal accumulation in parasites. Except for *Anguillicola crassus* and *Philometra ovata*, a general pattern shows that among the most prevalent groups of fish parasites, nematodes do not appear to be suitable as sentinels, whereas cestodes and particularly acanthocephalans are characterized as having a greater metal accumulation capacity (Sures, 2003). According to Sures and Siddall, (2003), the parasites *Pomphorhynchus laevis* and *Paratenuisentis ambiguous* were able to accumulate higher concentrations of cadmium (Cd) and lead (Pb) than their hosts. Likewise, Sures and Reimann (2003) reported that the parasite *Aspersentis megarhynchus* was able to accumulate higher levels of silver (Ag), cobalt (Co), and nickel (Ni) than their corresponding hosts. These reports amongst others further solidify the claim that acanthocephalans are the best indicators of heavy metal pollution.

It has also been reported that cestodes can serve as reliable heavy metal markers. Sures et al. (1997) reported that *Bothriocephalus scorpii,* a marine cestode, contained higher levels of Pb and Cd concentrations than their hosts. *Carryophyllaeus laticeps,* a tapeworm, was found to hoard more Cd, Pb, and zinc (Zn) in their tissue compared to their host (Jirsa et al., 2008). Baruš et al. (2007) have also reported that *Anguillicola crassus* and *Philometra ovata,* different species of nematodes, could indicate heavy metal pollution as they accumulate greater heavy metal concentrations than their host.

Al-Hasawi (2019) conducted a study in which three parasite groups (Trematodes, Nematodes, and Acanthocephalans) were tested for their heavy metal bioaccumulation capacity within the same host. His results indicated that the acanthocephalan (*Sclerocollum rubrimaris*) served as the best indicator. Therefore, the parasite species' bioconcentration factors decide their capacity to serve as reliable bioindicators of metal pollution.

The extraordinary ability of some intestinal parasites, such as the acanthocephalans, to bioaccumulate heavy metals in their tissues at concentrations up to 1,000 times greater than those of the host tissues (Mehana et al., 2020) and up to greater than 2,700 times greater than in the tissues of their final host (Sures, 2004) is one of their most notable traits. However, the capacity for bioaccumulation varies among different endoparasites. Baudrimont and De Montaudouin, (2007) reported that cockles, a marine mollusk, subjected to Cd pollution with digenean parasites have lower concentrations of the protein metallothionein than cockles without the parasites. This shows that while parasites are usually regarded as hazardous, they can accumulate heavy metals and reduce the impact on the host.

2.5. Fish parasites as effect indicators of heavy metal pollution

When it comes to parasites, effect indication typically concentrates on the level of individual organisms, populations, and communities. The direct toxicity of substances on free-living parasitic stages may allow for the impact indication of specific organisms. Even though many parasites have free-living larval stages, the majority of toxicological investigations have been carried out using trematode miracidia and cercariae (Pietrock and Marcogliese, 2003). This was because of their relatively large size and the fact that thousands can be obtained from a single infected snail.

These investigations involved the chemical treatment of a known number of larval stages, and analysis of the resulting longevity, viability, and infectiousness of these stages. It is clear that large concentrations of environmental pollutants, such as metals, generally decrease the survival of the test organisms because toxicants bind and cause a number of enzymes to become inactive. Cercarial test systems seem less promising than traditional impact indication methods (Borcherding and Wolf, 2001). The relatively brief lifespan of cercariae makes it impossible to regularly use a monitoring system based on these organisms.

However, the research conducted by Pietrock and Marcogliese (2003) can be used to assess how pollution affects parasite transmission and the resulting makeup of parasite assemblages. Analyses of changes in parasite populations and communities about environmental pollution are also common (Kennedy, 1997). Both directly and indirectly through impacts on intermediate, paratenic, and final hosts, environmental pollution has an impact on parasite populations and societies (Overstreet, 1997). However, rather than being correlated with environmental changes, population or community shifts could also be unpredictable (Kennedy, 1990).

The interpretation of parasitological evidence in the setting of pollution is therefore highly complex and uncertain. As parasites show effects over different trophic levels, the analysis of their community and population can be rather informative to other methods using other animals.

2.6. Impacts of chromium, copper, iron, manganese and zinc

Heavy metals are widely distributed within the earth's crust and are persistent in the natural environment. They can enter the human body through either ingestion (water and food) or inhalation (air). They can further be introduced to the environment by both natural and anthropogenic sources. Some of the heavy metals like chromium, copper, iron, manganese, and zinc have an essential role as they play a role in the metabolism in animals and humans. However, even essential heavy metals can result in toxicity in both humans and the environment if they are found at elevated levels. This is attributed to their ability to accumulate in natural systems (Gautam et al., 2014).

2.6.1. Chromium

Chromium's (Cr) role in the metabolism of carbohydrates makes it an essential nutritional metal (Farag et al., 2006). It can be found in the form of trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) oxidation states depending on the physicochemical characteristics of the environment. While they are said to be essential, it was reported that the hexavalent form is more toxic to both human and animal health. The reason behind its toxicity is because of its powerful oxidative potential and ability to cross cell membranes (Lushchak et al., 2009). Both natural and anthropogenic sources can be the reason for the existence of chromium in the aquatic environment (Gautam et al., 2014). The natural sources can be due to the eruption of volcanoes, the weathering of rocks, soils and sediments (Mohan et al., 2006). On the other hand, the anthropogenic sources come from effluents discharged from leather tanneries, textiles, electroplating, metal finishing, mining, dyeing and printing industries, ceramic, photographic and pharmaceutical industries amongst others (Arunkumar et al., 2000). The widespread use of these industrial applications has resulted in enormous amounts of discharged effluents that contain chromium into the environment. The presence of the toxic form of chromium is usually the result of the poor treatment given to wastewaters prior to discharging. Hence, its accumulation over time eventually causes harm to the environment and the organisms residing in it (Sfakianakis et al., 2015).

2.6.1.1. Environmental risk

Chromium is assimilated by fish through either ingestion of food/water that is polluted or through gill uptake. The up taken Chromium is usually accumulated within the liver of the fish compared to the other organs (Lushchak et al., 2009). However, the toxicity affects all organs.

When different organs get affected, the overall behavior and growth of the fish gets affected (Vinodhini and Narayanan, 2008). Additionally, alterations in hematology, histology, and morphology take place (Vera-Candioti et al., 2011). One of the serious effects includes the impairment of the fish immune system in fresh water which will make the fish susceptible to many more diseases. Reduction in the rate of photosynthesis was reported in the case of plants (Gautam et al., 2014).

2.6.1.2. Health risk

Damage to the liver and kidney is one of the most well-known effects of exposure to high levels of chromium. Skin ulceration and damage to the central nervous system were also reported (Gautam et al., 2014). The carcinogenicity of the hexavalent chromium was also conveyed. The most common type reported being lung cancer. The reduction of the trivalent chromium to the hexavalent chromium can result in cancer development when prolonged exposure is faced. While lung cancer itself is a serious health issue, chromium was additionally reported as the cause of other respiratory diseases as well. The uptake of huge amounts of this metal can ultimately lead to death (Saha et al., 2011).

2.6.2. Copper

Copper's (Cu) role as a main constituent of metabolic enzymes and micronutrient makes it an essential element (Monteiro et al., 2009). It is required by organisms for the activation of their enzymes which makes it essential for both humans and animals. This element was used since primitive times in the production of utensils, pipes, and electrical wires. Additionally, they were used in the manufacture of brass and bronze. Nowadays, the environment is polluted with copper due to activities like mining, metallurgy and industrial applications (Gautam et al., 2014). However, the main source of copper pollution is attributed to its' use as a biocide. Its extensive use in fungicides, algaecides, molluscicides, insecticides and discharge of wastes results in its accumulation in the aquatic environment. This is because these products get washed into the aquatic environment from agricultural and urban application sites (Kiaune and Singhasemanon, 2011). The environment and the organisms residing in it are faced with copper toxicity when copper concentrations reach elevated levels (Abdel-Tawwab et al., 2007).

2.6.2.1. Environmental risk

In the aquatic environment, copper displays an intricate biogeochemical cycle and speciation pattern. The interaction between the metal and the physicochemical properties are the ones that govern the destiny of copper within the aquatic environment (Kiaune and Singhasemanon, 2011). Copper was reported to be toxic even at levels that do not exceed high limits for different aquatic organisms (Gautam et al., 2014). For example, fish is said to accumulate copper within its organ, mainly liver, even when it is found at low concentration. It might be because fish can collect copper through diet or directly from the environment (Sfakianakis et al., 2015). Some of the symptoms to copper exposure by fish include mucus around the body surface, under and between the gills. Different tissues like the kidney hematopoietic tissue, mechanoreceptors, chemoreceptors and other tissues get altered as well (Authman et al., 2015). Furthermore, poor growth, reduced immune response, changes in appearance and behavior, low fertility, reproductive problems, and shortened life span are some of the effects to the chronic exposure of copper in fish (Yacoub and Gad, 2012).

2.6.2.2. Health risk

Irritation to the nose, eyes and mouth are some of the effects of exposure to elevated levels of copper. If the exposure continues for a long time, damage to the kidney and stomach, which will result in diarrhea, vomiting and loss of strength and in the worst cases result in death (Gautam et al., 2014). Furthermore, it can cause the inactivation of enzymes resulting in the imbalance of an organism's vital processes (Katranitsas et al., 2003).

2.6.3. Iron

Iron (Fe) is the fourth most abundant element on the earth's crust. Its inner and outer core composition however suggests that it's the most abundant element on Earth (Frey and Reed, 2012). Iron is an essential element due to its role as a macronutrient in organisms (Rabajczyk and Namieśnik, 2014). Locomotion, replication, cellular processes, biosynthesis, regulation of gene expression and energy production are some of its roles (Frey and Reed, 2012). As this metal is incorporated in many applications, there are different forms of iron that can be found in the natural environment. In addition, different minerals of iron can be formed by replacing magnesium and aluminum in aluminosilicates. There are two oxidation states in which iron occur at; (+2) and (+3). Their existence depends on the rotation between carbon, sulfur and oxygen. Even though the changes are affected by different factors, the origin of this element comes from various minerals (Rabajczyk and Namieśnik, 2014). Iron is usually an element that is easily accumulated by organisms. This is caused by its presence in all links as it goes in the food chain. Even though this element occurs naturally and can contribute to its existence in the aquatic environment, human activities can also result in its significant accumulation in the aquatic environment. Discharge of industrial wastes, landfills, corrosion of pipes, containers and other equipment made of iron are some of the anthropogenic contributions of iron (Rabajczyk and Namieśnik, 2014).

2.6.3.1. Environmental risk

The main effect of iron toxicity in fish is related to respiratory problems even though liver is the target organ (Omar et al., 2014). This is due to the alkaline nature of the gill surface which enables the oxidation of ferrous iron leading to the loss of normal respiration (Abbas et al., 2002). The effect can go as far as killing the fish as the respiration organ collapses due to reduction in the area and damage to the respiratory epithelium. The collapse of the respiratory organ, like gill, results in the reduction of the distance for the diffusion between water and blood.

2.6.3.2. Health risk

Humans are usually exposed to iron toxicity through dietary intake. The toxicity gets more severe especially in the case of newborn babies and infants as it becomes acute. This is caused due to the rapid absorption by the gastrointestinal tract. The organs that are affected are the liver, kidneys and the cardiovascular system (Gautam et al., 2014). Furthermore, it was reported that excessive intake of iron can lead to chronic intoxication (Zoni et al., 2007). Myocardial infarction, lung cancer, and bad cholesterol were also some of the mentioned effects to the exposure to high iron levels (Rabajczyk and Namieśnik, 2014).

2.6.4. Manganese

Manganese (Mn) is an abundantly distributed essential metal. It occurs in a compound form mixed with elements like sulfur, oxygen, silicone, chlorine, and carbon. Even though manganese can exist in 11 oxidation states ((-3) to (+7)), the most common ones are the oxidation states (+2) and (+4). However, the former is the reactive soluble one while the latter is the insoluble one in the aquatic environment. Circulation between these two oxidation states can be caused due to oxidation and reduction reactions. Oxygen concentration, redox potential, and pH are some of the factors that control the existence of manganese in the environment. Normal development, body function, and regulation of metabolic and non-metabolic functions are some of the roles of manganese. It can be found in the environment due to both natural and anthropogenic sources. While it holds importance, its presence in high concentrations can be rather toxic (Pinsino et al., 2012).

2.6.4.1. Environmental risk

Manganese can be introduced into the environment due to both natural and anthropogenic sources. The natural sources come from decomposed plants, eroded rocks, and soils. The anthropogenic sources are due to the use of pesticides, fungicides, water purification agents, and imaging instruments in the medical field (O'Neal and Zheng, 2015). Both sources eventually end up in water bodies. This is due to the reduction of manganese to the more soluble form making its end point effortlessly to water bodies (Nádaská et al., 2010). Even though the two sources are responsible for the release of manganese into the environment, occupational circumstances were also reported as the main reason for human exposure to manganese (Howe et al., 2004).

2.6.4.2. Health risk

When it comes to human beings, occupational exposure was the most important reason for manganese toxicity in humans. Even if the exposure was at or below occupational standards, it was reported that the effect remained severe (Barrioni et al., 2019). The undue ingestion of manganese can result in bronchitis, nerve dent, lung embolism (Zoni et al., 2007), and in worst cases showing symptoms that are similar to Parkinson's disease. When exposure to high levels of manganese takes place, harm to the central nervous system was reported to happen (Madhav et al., 2020).

2.6.5. Zinc

Zinc (Zn) is the second most abundant element on the earth's crust. It is an essential element as it is required for nucleic acid synthesis and is part of many enzymes (Sfakianakis et al., 2015). Furthermore, it is considered a micronutrient and is involved in cell signaling, immune system, and neurotransmission (Celik and Oehlenschläger, 2004). The main source of zinc comes from the mining and metallurgical processing of its ores and its use in the different industrial applications like the production of galvanized ironwork, plumbing and paints, and even burning of coal (Gautam et al., 2014). These are the sources that contribute to the accumulation of zinc water, soil and air. Especially in the case of the aquatic environment, it may occur in the form of soluble zinc complexes, or it can be attached to suspended matter (Linnik and Zhezherya, 2018). Even though Zinc is said to be an important element of human diet, excess amount of it can be harmful (Gautam et al., 2014).

2.6.5.1. Environmental risk

Even though fish can be affected by zinc itself, usually the effect is amplified with the presence of other metals (Ezeonyejiaku and Obiakor, 2011). The outcome happens due to the uptake of calcium cation which interrupts the usual water uptake activities leading eventually to death. Toxicity of zinc can cause problems regarding growth, hatching, reproduction and survival in fish (Niyogi and Wood, 2006). Furthermore, respiratory and cardiac changes are some of the other effects. While the target organ for the accumulation of zinc is kidney (Omar et al., 2014), the damage of different organs like the gill and liver were also reported (Ezeonyejiaku and Obiakor, 2011).

2.6.5.2. Health risk:

Even though zinc is said to be an important element of human diet, excess amount of it can be harmful. This is because it might end up causing cholesterol problems and anemia (Gautam et al., 2014). Furthermore, it was reported that an elevated concentration of zinc may cause loss of appetite, muscle stiffness, and nausea (Jain et al., 2010).

3. CHAPTER THREE: MATERIALS AND METHODS

3.1. Description of the Study Area

Koka Reservoir is located between latitudes 8°18′– 8°28′N and longitudes 38°59′–39°09′ E. It is situated 90 kilometers southeast of Addis Ababa, the capital of Ethiopia, at an elevation of 1590 meters above sea level. Its maximum and mean depths are 14 m and 9 m, respectively, and it covers a surface area of approximately 255 km^2 (Reyntjens and Tesfaye Wudneh, 1998). The Awash River is primarily responsible for the inflow and outflow of water. Additionally, during the wet season, the Modjo River produces inflow. Along the western shore, a substantial floodplain borders the lake. According to Tenalem Ayenew (2004), annual rainfall in the Rift Valley region ranges from 600 to 800 mm, and the mean annual temperatures ranged from 20-26°C (average 23 °C). The conductivity of lake water ranged from 200 to 393 µS/cm, and the pH varied between 8 and 9. Due to suspended silt entering from the Awash River, the Secchi depth of Koka Reservoir is low and ranges from 12-28 cm (Fasil Degefu et al., 2011). The reservoir's sediments consist of very fine particles easily captured by a 200 µ mesh net (Melaku Mesfin et al., 1988). Some of the commercially important fish species inhabiting Koka Reservoir are the African catfish (*C. gariepinus*), Nile tilapia (*O. niloticus*), common carp (*C. carpio*), and Large Barbs (*Labeobarbus intermedius*) (Gashaw Tesfaye and Wolff, 2018).

Table 1. Geographical coordinates of sediment and water sampling sites in Koka Reservoir.

Sampling Site	**Latitude**	**Longitude**
Koka Waste (KW)	08°21.583' N	039°00.209' E
Mojo Mouth 1 (MM1)	08°25.769' N	039°05.118' E
Mojo Mouth 2 (MM2)	08°25.610' N	039°05.0615' E
Awash Open water (AO)	08° 22.592' N	039°03.504' E
Mojo River inlet (MR)	08° 25.956' N	039°13.708' E
Awash River inlet (AWR)	08° 24.213' N	039°0.684' E

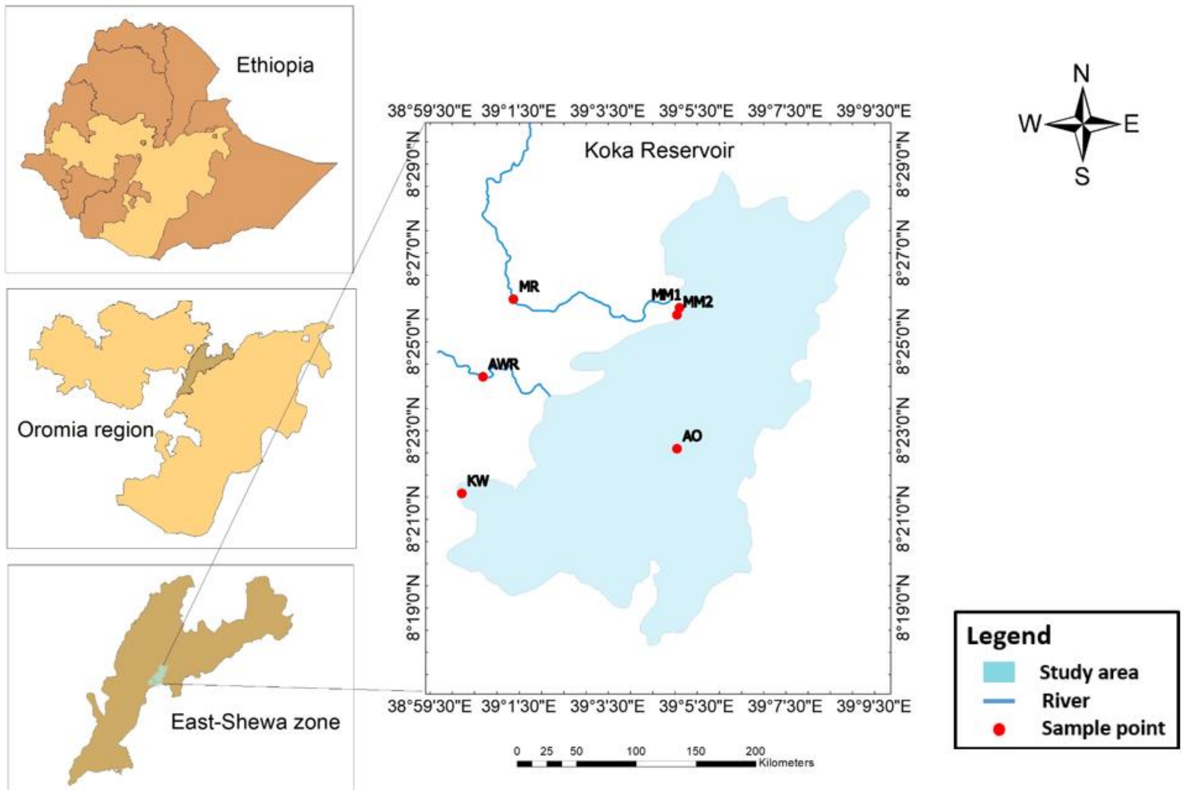

Figure 1. Map of Koka Reservoir indicating locations of sampling sites

Six sampling stations were selected strategically so that potential human interference whether in the form of agricultural or industrial activities can be investigated. The geographical coordinates of sampling sites were recorded using a GPS device (Garmin 72H). Sampling Site 1 (KW) is located where wastewater from the nearby village (Kanteri) is discharged into the reservoir. Sampling sites 2 and 3 (MM1 and MM2) are positioned at the confluence points of Modjo and Awash Rivers. Sampling site 4 (AO) is situated within the open water area of the Awash River. Sampling site 5 (MR) is located at the mouth of Modjo River's entry point into the reservoir, while Sampling site 6 (AWR) is positioned at the mouth of the Awash River. All these sites were selected to evaluate the extent of the contamination resulting from both the river inflows and local activities surrounding the reservoir, providing valuable insights into environmental impacts.

3.2. Methodology

3.2.1. Sampling and Sample collection

A cross-sectional study was conducted in which 337 fish samples were bought during the pre-rainy season (May) in Koka Reservoir. The fish specimens were bought from the fish landing sites along the reservoir and they comprised of African catfish (*C. gariepinus*)*,* Nile tilapia (*O. niloticus*)*, and* common carp (*C. carpio*) with specimen number 105, 197, and 35 respectively. Additionally, sediment and water samples were collected from 6 sites to assess the level of heavy metals and determine any significant difference between them. Furthermore, samples were taken from the muscle tissue and organs (gill, liver, and accessory organ (in case of *C. gariepinus*)) of the fish to analyse their heavy metal bioaccumulation capacity in comparison to that of their parasites. These fish species were chosen because they were reported for their high parasite prevalence in the rift valley lakes (Eshetu Yimer, 2000; Yewubdar Gulelat et al., 2013; Daniel Hussien and Jossy Bekele, 2015; Hailekiros Gebreegziabher Gebremedhn and Assefa Kebede Tsegay, 2017) and due to their commercial importance, which will help in predicting some of the health issues that might ensue in case of consumption. However, it's worth noting that the species *C. carpio* lacked parasites and was consequently excluded from the nematode parasite level estimation. As a result, the comparison for heavy metal accumulation between fish organs and parasites was not conducted for this species. Additionally, upon further identification of parasites in *C. gariepinus* and *O. niloticus*, three nematode parasites were identified in *C. gariepinus (Contracaecum, Porrocaecum, and Camallanus sp.),* while only one nematode (*Contracaecum sp.*) was found in *O. niloticus.* Therefore, only the nematode *Contracaecum* sp. was chosen as the ideal helminth species in this study as it was common to both fish species and had sufficient weight. It is worth highlighting that this study was the first to identify the presence of the parasite, *Porrocaecum* sp. in Ethiopia.

Ethical clearance was granted by the Institutional Review Board of the College of Natural and Computational Sciences, Addis Ababa University with reference number CNCSDO/158/15/2023.

3.2.2. Sample preparation

3.2.2.1. Fish and fish helminthic parasites samples

The length and weight of each fish species was recorded prior to dissection using a measuring tape to the nearest 0.01 cm and a weighing balance to the nearest 0.01 g, respectively. Categorization based on length (L), weight (W) and condition factors (K) of fish were carried out for *C. gariepinus*, and *O. niloticus.* The categories were large (1), medium (2), and small (3).

The dissected fish were macroscopically inspected for endoparasites. Most of the parasites of *C. gariepinus* were found in the mesentery whereas they were found in the pericardial cavity in *O.*

niloticus. The helminthic fish parasites identified in the infected fish were then assigned to their respective taxonomic groups based on their morphological characteristics and were identified to the lowest genus level following the guidelines by Scholz et al. (2018). After being mixed into a composite, the helminthic fish parasites were stored at -20°C until further analysis for heavy metals. Prior to conducting heavy metals analysis, helminthic parasitic pools were created for each of the fish species in order to get sufficient weight for parasite heavy metal analysis. Similarly, representative fish tissue and organ samples were collected and stored at -20°C until they were processed for heavy metal analysis. The procedure outlined by Bruno et al. (2006) was used to examine samples from parasites and fish tissues (muscle tissue and organs) with some modifications.

3.2.2.2. Water samples

The water samples were taken from 6 sites with varying levels of anthropogenic disturbance. Volume of water sample collected from each site was 500 ml. The water sample was then filtered through a Whatman filter paper No. 41 (0.45 µm pore size) and acidified with suprapure HNO_3 to a pH of < 2 to prevent precipitation of metals (APHA, 1998).

3.2.2.3. Sediment samples

The sediment samples were taken from 6 sites with varying levels of anthropogenic disturbance. Sediment samples were dried in an oven (SP305520) at 100 ◦C for about 6 hours and grounded using mortar and pestle. One gram of dried samples was sieved through a 200 µm sieve to normalize for particle size. The sieved samples were then placed in a 50 ml Erlenmeyer flask and heated with the gradual addition of 1M HNO_3. Heating continued until all particles were dissolved and then the digestate produced was filtered using Whatman (0.45 µm) filter papers to remove silicate residue. At last, the filtrate was diluted to 25 ml using 1M HNO_3 for heavy metal analysis. The analysis was conducted following the procedure described in EPA (1996) with some modifications.

3.2.2.4. Ash content

0.7 g of fish parasite, 3 g of muscle, gill and liver organs, 1.5 g of accessory organ and 1 g of sediment samples were weighed into a previously ignited, cooled and weighed silica dish. The dish and its content were ignited first gently and then at 500◦C for 4 h in a muffle furnace (Carbolite). The dish and its content were cooled in a desiccator and reweighed. It was then returned into the muffle furnace for 30 min, desiccated and reweighed until constant weight. The experiment was carried out in duplicate. Ash content was determined by calculating the difference in weight and converting to percentage following the procedure described in AOAC, (2000).

3.3. Heavy metal analysis

Fish parasite, muscle tissue, organ and sediment samples were ashed in a furnace at 500 ◦C for 4 hours. Then the ash was transferred into a 50 mL volumetric flask. 1M HNO_3 was added gradually while heating for digestion. After digestion, the resulting solutions were analysed for heavy metals (Cr, Cu, Fe, Mn and Zn). The water samples were directly analyzed for heavy metals. Heavy metals analysis was conducted using both Flame Atomic Absorption Spectroscopy (FAAS) (AA500) at National Fishery and Aquatic Life Research Center (NFALRC), Sebeta for the elements Cu, Fe, Mn, Zn and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) at Addis Ababa University (AAU), Addis Ababa for the element Cr. The flame wavelength, sample aspiration rate and backgrounds were optimized in agreement to the manufacturers' guideline. Standards with analytical concentrations equivalent to the instrument's linear response range were prepared and used for calibration.

Table 2. Working condition of FAAS

Element	**Wavelength (nm)**	**Bandwidth (nm)**	**Sensitivity (mg/l)**	**Detection limit (mg/l)**	**Working range (mg/l)**
Copper	324.7	0.4	0.03	0.004	0.018 – 4.0
Iron	248.3	0.2	0.05	0.0046	0.03 – 8.0
Manganese	279.5	0.4	0.02	0.002	0.01 – 3.5
Zinc	213.9	0.4	0.01	0.003	0.01 – 3.0

Table 3. Working condition of ICP-OES

Element	**Wavelength (nm)**	**RF Power (KW)**	**Nebulizer Flow (L/min)**	**Plasma Flow (L/min)**	**Aux Flow (L/min)**
Chromium	267.716	1.2	0.7	12	1

3.3.1. Heavy Metal Pollution Indexes

3.3.1.1. Metal Pollution Index

Metal Pollution Index (MPI) is an index used to evaluate the degree of metal accumulation in the environment (water, sediment), and living organisms (fish). It helps to assess the risk in humans in case of consumption. Furthermore, it provides the potential of heavy metal accumulation capacity of different fish species (Yin et al., 2024). Elevated MPI values indicate more contamination levels (Marengo et al., 2018). The equation for calculating Metal Pollution Index (MPI) according to Usero et al. (1996) is;

$$MPI = (CF_1 \times CF_2 \times CF_3 \times \ldots CF_n)^{1/n}$$

Where, CF_1; Heavy metal concentration of Element 1, CF_2; Heavy metal concentration of Element 2...Element n.

3.3.1.2. Bioconcentration Factor of Fish Parasites

Bioconcentration refers to the uptake and retention of a substance that takes place through respiratory surfaces (such as gill or skin). This means it doesn't consider the uptake that takes place through ingestion. Organisms can bioconcentrate different substances from their immediate environment like from water or sediments making them ideal for monitoring purposes. Thus, they can be used to calculate the bioconcentration factor by comparing the concentration of substances in the organism with the concentration in the environment (Alexander, 1999). A value of bioconcentration factor (BCF) greater than 1 indicates that the metal is concentrating within the organism at a level higher than its immediate environment. Similarly, a value less than 1 can indicate that the metal is either metabolized or excreted by the organism (Arnot and Gobas, 2006). That means BCF can be calculated using the formula:

$$BCF = \left(C \left[\frac{host\ tissue}{environment} \right] \right)$$

Where the host tissue refers to different organisms, for example fish and the environment refer to sediment or water. Modifying the formula to fit the purpose of the study brings us to another formula. According to the procedure by Sures et al. (1999), the bioconcentration factor (BCF) was computed for each species of fish that has parasites as:

$$BCF = \left(C \left[\frac{parasite}{host\ tissue} \right] \right)$$

Though host tissue describes different fish species in this scenario, specific sites in the fish like the muscle, gill, liver and accessory organ (in case of *C.gariepinus*) were used to evaluate the extent of their bioaccumulation capacity when compared to parasites. Furthermore, to understand the bioaccumulation capacity of parasites to the environment, this equation was used:

$$BCF = \left(C \left[\frac{parasite}{environment} \right] \right)$$

Where: the environment could mean either sediment or water.

3.4. Human Health Risk Assessment

Human health risk assessment is computed to estimate the health associated problems that could take place when the food (such as fish) that is contaminated (like heavy metals) is consumed. Different risk assessment tools are employed to calculate the risk. Some of them are Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI). There is the Oral Reference Dose (ORD) as well, but this value is usually given and helps in calculating THQ. The EDI estimates the level of exposure caused by a person consuming specific heavy metals present in fish tissues orally (Radwan et al., 2022). Fish tissues refer to muscle tissues because they constitute higher edible sections of the fish even though they usually accumulate less contaminants compared to the other organs like gill and liver (Akhbarizadeh et al., 2018). The equation for obtaining EDI is;

$$EDI\ (mg^{-1}\ kg^{-1}\ day^{-1}) = (CF \times IR \times ER \times EP/BW \times AT) \times 10^{-3}$$

Where CF; Average heavy metal concentration in fish muscle (mg/kg), IR; Daily Intake of fish consumed (kg/day), ER; Exposure Rate (365 days/year), EP; Exposure Period over a lifetime (years), BW; Body Weight (kg), AT; Average Lifetime (years x 365 days/year).

Ethiopia's overall fish consumption was estimated at less than 0.5 kg/year (Demelash Kefale and Addisu Genetu, 2023). However, fish consumption at Koka Reservoir was estimated at 10 kg/year, making the daily intake (IR) of fish consumed as 0.0274 kg/day as the area is considered as a fish producing and consuming area (Abebe Cheffo et al., 2016). The life expectancy of female and male Ethiopians were reported to be 70.5 and 66.9 years, respectively making the mean exposure period (EP) of the population at 68.7 years (WHO, 2024). In Africa, the average body weight (BW) was reported as 60.7 kg (Walpole et al., 2012). The IR, EP, and BW values were rounded up to 0.03 kg/day, 69 years, and 61 kg, respectively for ease of calculation.

Target Hazard Quotient (THQ) is a risk assessment metric utilized to quantify the potential adverse effects resulting from exposure to specific hazardous substances, particularly heavy metals. It serves as a valuable tool in evaluating the non-cancer health risks associated with environmental contaminants and guiding regulatory measures to safeguard public health (Pongpiachan et al., 2018; Radwan et al., 2022). If the value of THQ is 1 or less than 1, health complications are unlikely to occur. However, if the value exceeds 1 then potential health impacts are to be expected. The equation for calculating THQ is given as:

THQ = EDI/ORD

Where; EDI; Estimated Daily Intake (mg^{-1} kg^{-1} day), and ORD; Oral Reference Dose (mg^{-1} kg^{-1} day). The highest allowable oral dosage of a hazardous substance is known as the oral reference dose (ORD). It stands for the threshold below which a lifetime of exposure should not have any deleterious consequences on non-cancerous health. It assesses the risk related to chemical exposure (USEPA, 1993). The oral reference dose (ORD) for the elements Cr, Cu, Fe, Mn, and Zn were 1.5, 0.04, 0.7, 0.14, and 0.3 $mg^{-1}kg^{-1}$day, respectively (USEPA, 1993).

The Hazard Index (HI) is an index that is determined by aggregating the individual Hazard Quotients (HQs) to evaluate the cumulative health hazards associated with all designated target metals. A value of HI <10 means the person has less non-carcinogenic risk. The equation for calculating HI is;

ΣTHQ

3.5. Prevalence and intensity of fish parasites

There are different metrics used to quantify the severity of infection by parasites. Some of them are mean prevalence and intensity. Mean prevalence describes the part of the population infected with a parasite group (Shaw et al., 2018). The equation for calculating mean prevalence is indicated below (Bush et al., 1997):

$$\text{Mean Prevalence (\%)} = \left[\frac{Number\ of\ \inf ected\ fish}{Number\ of\ exa\min ed\ fish}\right]*100$$

Intensity, which is referred to as intensity of infection describes the number of a specific group of a parasite in an infected host (Shaw et al., 2018). The equation for calculating Intensity is (Bush et al., 1997):

Intensity = $\frac{Total\ number\ of\ fish\ parasites}{Number\ of\ infected\ fish}$

3.6. Fulton's Condition Factor

Fulton's condition factor (K) is a tool implemented in fishery science to understand the overall well-being of an individual fish by considering its length and weight (Nash et al., 2006). A value where K equals 1 indicates a good condition while a value less than 1 indicates poor condition (Ravikumar et al., 2023). The equation used to calculate Fulton's condition factor is:

$$K = \left[\frac{W}{L^3}\right]*100$$

Where K; Fulton's condition factor, W; Weight (g), L; Length (cm). The length considered is the total length.

3.7. Data analysis

Data were organized using Microsoft Excel (2013). Statistical analysis was performed using SPSS (version 23). The data were checked for normality using Shapiro-Wilk test. The means of the heavy metal concentrations between the different organs of different fish species, environmental samples and their bioconcentration factors were compared using one-way analysis of variance (ANOVA) followed by Tukey's HSD Post Hoc test when significant difference was observed for normally distributed data and Kruskal-Wallis test for non-normally distributed data. Independent sample t-test was performed to compare between the BCF values for normally distributed data and Mann-Whitney U test was applied for data that doesn't follow normal distribution. Association between exposed variables and the prevalence of infection was determined using chi-square. Exposed variables indicate sex, length, weight, and condition factor. The strengths of these associations were measured by means of odds ratio at 95% confidence interval (CI). Statistical significance was established at 95% confidence interval with P value of less than 0.05.

4. CHAPTER FOUR: RESULTS

4.1. Heavy metal concentration of different fish species, their parasites, and environmental samples

The tables in this section show the concentrations of heavy metals (Cr, Cu, Fe, Mn, and Zn) in different tissues of three fish species; *C. gariepinus*, *O. niloticus*, and *C. carpio,* their parasites*; Contracaecum* sp. as well as sediment and water samples from Koka Reservoir, Ethiopia.

Table 4. Heavy metal concentration (mean±SD) of different tissues/organs (μg/g) of fish from Koka Reservoir, Ethiopia.

Fish species	Tissues	Cr	Cu	Fe	Mn	Zn	MPI
Clarias gariepinus	Muscle	*0.99±0*[a]	2.59±1.49[a]	15.23±1.93[a]	n.d	17.28±0.05[a]	3.68
	Gill	2.14±0.03[b]	5.15±1.16[a]	120.66±10.17[b]	32.36±0.87[a]	48.67±0.26[b]	18.37
	Liver	0.21±0.12[c]	22.78±5.67[b]	60.46±4.10[c]	1.60±0.69[b]	21.63±1.9[ac]	6.31
	Accessory organ	0.48±0[d]	0.07±0.1[c]	89.39±2.01[d]	1.52±0.01[bc]	30.73±1.05[d]	2.69
p-value		<0.001	0.001	<0.001	<0.001	<0.001	
Oreochromis niloticus	Muscle	*0.54±0.1*[a]	2.27±1.56	16.50±1.4[a]	n.d	16.23±1.41[a]	3.19
	Gill	3.66±1.1[b]	n.d	126±5.36[b]	34.21±3.62[a]	39.21±9.45[b]	14.4
	Liver	0.78±0.30[ac]	41.36±34.48	85.22±28.84[ab]	24.87±6.49[a]	32.25±0.99[c]	18.56
p-value		0.007	0.152	0.043	0.007	0.003	
Cyprinus carpio	Muscle	*0.66±0.01*	n.d	38.34±23.68	n.d	25.06±0.31	3.63
	Gill	1.05±0.01	0.66±0.14	68.83±0.45	21.97±8.77	23.33±0.1	7.54
	Liver	0.39±0	n.d	56.06±0.76	4.08±0.45	16.57±0.1	4.3
p-value		0.102	0.123	0.102	0.095	0.102	
WHO/FAO	Muscle	0.05[2]	30[1,2]	100[1]	0.01[1]	40[1]	

where n.d: not detected, Cr; Chromium (FAO, 2003), Cu; Copper (WHO,1989), Zn; Zinc (WHO,1989), Fe; Iron (WHO,1993), Mn; Manganese (WHO, 1993), MPI; Metal Pollution Index, WHO; World Health Organization, FAO; Food and Agriculture Organization of the

United Nations. The superscripts indicate 1: WHO; 2=FAO. Italicized results indicate values that exceeded WHO/FAO standards. Superscripts indicate the results of ANOVA and post-hoc tests: similar superscripts denote no significant difference (P>0.05), while different superscripts denote significant differences.

The results indicated significant variations in metal accumulation across species and tissue types. In *C. gariepinus*, gill tissues exhibited the highest concentrations of Fe (120.66±10.17 µg/g) and Zn (48.67±0.26 µg/g), with corresponding high Metal Pollution Index (MPI) values of 18.37. Liver tissues of this species showed elevated Cu (22.78±5.67µg/g) and Fe (60.46±4.10 µg/g) levels. In *O. niloticus*, the gills had the highest concentrations of Fe (126±5.36 µg/g) and Zn (39.21±9.45 µg/g), and the liver also showed significant Cu (41.36±34.48 µg/g) and Fe (85.22±28.84 µg/g) content, with an MPI of 18.56. Similarly, *C. carpio* contained high Fe in gill (68.83±0.45 µg/g) and liver tissues (56.06±0.76 µg/g), with gills also accumulating high Zn (23.33±0.1 µg/g). Notably, some measured values exceeded the WHO and FAO standards for muscle tissues, indicating potential health risks.

Table 5. Heavy metal concentration (mean±SD) of parasites (µg/g) collected from different fish species sampled from Koka Reservoir, Ethiopia

Fish species	**Parasite**	**Cr**	**Cu**	**Fe**	**Mn**	**Zn**	**MPI**
Clarias gariepinus	*Contracaecum* sp.	1.06±0	8.47±0	96.93±0	7.62±0	17.44±0	10.3
Oreochromis niloticus	*Contracaecum* sp.	0.44±0.38	0	55.94±48.45	32.03±27.74	22.64±19.60	7.08

Parasites collected from *C. gariepinus* and *O. niloticus* also exhibited varying heavy metal concentrations. Parasites from *C. gariepinus* showed significant levels of Fe (96.93±0 µg/g) and Zn (17.44±0 µg/g), resulting in an MPI of 10.3. In contrast, parasites from *O niloticus* had lower metal concentrations of Fe (55.94±48.45 µg/g) and Mn (32.03±27.74 µg/g), with an MPI of 7.08.

Table 6. Heavy metal concentration (mean±SD) of sediment (μg/g) and water (mg/l) samples collected from different sites in Koka Reservoir, Ethiopia and WHO standards for drinking water

Sample type	Site	Cr	Cu	Fe	Mn	Zn	MPI
Sediment	KW	3.88 ± 0.12^{a}	n.d	191.47±6.4	60.37 ± 1.42^{acdef}	n.d	8.52
	MM1	*33.40±0.4*^{b}	n.d	189.14±2.03	54.53 ± 2.48^{acde}	29.50±1.13	25.2
	MM2	21.88 ± 0.91^{c}	1.40±0.85	166.58±31.33	65.68 ± 6.17^{acedf}	39.38±3.83	26.55
	AO	13.03 ± 0.53^{d}	2.91±0.14	148.84±13.33	65.22 ± 2.16^{acdef}	46.19±1.24	27.93
	MR	*55.63±1.68*^{e}	n.d	150.48±21.59	89.30 ± 10.52^{b}	29.96±7.47	29.51
	AWR	4.83 ± 0.02^{a}	1.15±0.17	128.07±75.8	60.98 ± 1.45^{acdef}	19.39±16.08	15.31
p-value		<0.001	0.162	0.542	0.004	0.008	
ISQG		37.3	35.7	-	460	123	
PSQG	**LEL**	26	16	21,000	460	120	
	SEL	110	110	43,766	1100	820	
USEPA		43.4	31.6	-	-	121	
Water	KW	0.01	n.d	n.d	n.d	n.d	0.4
	MM1	0.02	n.d	n.d	n.d	n.d	0.46
	MM2	0.01	n.d	n.d	n.d	n.d	0.4
	AO	0.01	n.d	n.d	n.d	n.d	0.4
	MR	0.02	n.d	n.d	n.d	n.d	0.46
	AWR	0.01	n.d	n.d	n.d	n.d	0.4
p-value		0.416					
WHO		0.05	2	0.3	0.4	3	

Where n.d: not detected, MPI; Metal Pollution Index, ISQG; Interim Sediment Quality Guideline (ISQG, 2002), PSQG; Provincial Sediment Quality Guideline (Jaagumagi, 1992), LEL; Lowest Effect Level, SEL; Severe Effect Level, USEPA; United States Environmental Protection Agency (USEPA, 2010), WHO; World Health Organization (WHO, 2011). Italicized results indicate values that exceeded the guidelines for sediments. Superscripts indicate the results of ANOVA and post-hoc tests: similar superscripts denote no significant difference (P>0.05), while different superscripts denote significant differences.

Environmental samples, including sediments and water collected from various sites of Koka Reservoir, revealed substantial heavy metal contamination. Sediment samples from MM1 and MR sites had the highest Cr concentrations (33.40±0.40 µg/g) and (55.63±1.68 µg/g), respectively. Fe concentrations were significantly high in all sediment samples, particularly at KW (191.47±6.4 µg/g). Water samples generally showed low metal concentrations, all being within WHO drinking water standards, and Cr ranging from 0.01 to 0.02 mg/l.

Table 7. Bioconcentration factor (BCF) as [(parasite)/(host tissue)] of *Clarias gariepinus* and *Oreochromis niloticus* from Koka Reservoir, Ethiopia

Fish species	**Tissue/ organ**	**Cr**	**Cu**	**Fe**	**Mn**	**Zn**
African catfish (Clarias gariepinus)	Muscle	1.07±0^{a}	3.92±2.26	6.42±0.82^{a}	-	1.01±0^{a}
	Gill	**0.5±0.01**^{a}	1.69±0.38	**0.81±0.07**^{b}	0.24±0.01^{a}	0.36±0^{b}
	Liver	5.82±3.14^{b}	0.38±0.1	1.61±0.12^{c}	5.27±2.29^{b}	0.81±0.07^{c}
	Accessory organ	2.2± 0.01^{bc}	31±39.53	1.08±0.02^{d}	5±0.03^{bc}	0.57±0.02^{d}
Nile tilapia (Oreochromis niloticus)	Muscle	1.24±0.20^{a}	-	5.11±0.42^{a}	-	**2.10±0.18**^{a}
	Gill	0.19±0.05^{b}	-	0.67±0.03^{b}	**1.42±0.16**	0.91±0.25^{b}
	Liver	0.98±0.48^{ac}	-	1.08±0.41^{bc}	2.02±0.54	**1.05±0.03**^{bc}

The table shows the mean and standard deviation of the BCF for *C. gariepinus* and *O. niloticus*. Bolded results indicate higher values and significant differences (t-test, P<0.05) between *O. niloticus* and *C. gariepinus* for a given element in similar organs. Superscripts indicate the results of ANOVA and post-hoc tests: similar superscripts denote no significant difference (P >0.05), while different superscripts denote significant differences.

Table 8. Bioconcentration factor (BCF) as [(parasite)/(sediment)] (µg/g) of *C. gariepinus* and *O. niloticus* from Koka Reservoir, Ethiopia

Parasite and fish species	Cr	Cu	Fe	Mn	Zn
***Contracaecum sp* (*C. gariepinus*)**	0.11±0.10	**5.63±3.16**	0.58±0.08	0.12±0.02	0.68±0.54
***Contracaecum sp* (*O. niloticus*)**	0.07±0.06	0	0.50±0.07	0.75±0.11	0.99±0.21

Bolded results indicate significant difference (t-test).

The bioconcentration factors (BCF) of heavy metals in parasites relative to fish tissue and sediment concentrations were also assessed. *C. gariepinus* displayed high BCFs for Cu in the accessory organ (31±39.52 µg/g) and Fe in the muscle (6.42±0.82 µg/g), indicating significant bioaccumulation. *O. niloticus* showed moderate BCFs across tissues, with notable values for Fe (5.11±0.42 µg/g) and Zn in muscle. For sediments, significant bioaccumulation patterns were observed in Cu (5.63±3.16 µg/g) in *C. gariepinus* and Zn (0.99±0.21 µg/g) for *O. niloticus*.

Table 9. Estimated Daily Intake (mg/kg/day) and Target Hazard Quotients (THQ) in fish muscles of *C. gariepinus*, *O. niloticus*, and *C. carpio* in Koka Reservoir, Ethiopia

Fish species	Heavy metals	EDI	THQ
C. gariepinus	Cr	4.87×10^{-7}	3.25×10^{-7}
	Cu	$1.27 \text{ X } 10^{-6}$	3.18×10^{-5}
	Fe	$7.49 \text{ X } 10^{-6}$	1.07×10^{-6}
	Mn	0	0
	Zn	$8.5 \text{ X } 10^{-6}$	2.83×10^{-5}
			$\mathbf{7.12 \times 10^{-5}}$
O. niloticus	Cr	2.66×10^{-7}	1.77×10^{-7}
	Cu	1.12×10^{-6}	2.79×10^{-5}
	Fe	8.11×10^{-6}	1.16×10^{-5}
	Mn	0	0
	Zn	7.98×10^{-6}	2.66×10^{-5}
			$\mathbf{6.63 \times 10^{-5}}$
C. carpio	Cr	3.25×10^{-7}	2.16×10^{-7}
	Cu	5.16×10^{-7}	1.29×10^{-5}
	Fe	1.89×10^{-5}	2.69×10^{-5}
	Mn	0	0
	Zn	1.23×10^{-5}	4.12×10^{-5}
			$\mathbf{8.11 \times 10^{-5}}$

where EDI; Estimated Daily Intake, IR; Daily intake, BW; Body weight, EP; Exposure period, Exposure rate (ER), Exposure period (EP). The bolded results are the Hazard Indexes (HI).

The estimated daily intake (EDI) and target hazard quotients (THQ) for heavy metals in the muscle tissues of the studied fish species showed potential health risks. *C. gariepinus* showed the highest EDI for Zn (8.5×10^{-6} mg/kg/day) with the corresponding THQ of 2.83×10^{-5}. Similarly, *O. niloticus* had high EDI for Fe (8.11×10^{-6} mg/kg/day) and THQ (1.16×10^{-5}). *C. carpio* exhibited significant EDI and THQ values for Fe and Zn, with THQ values indicating cumulative potential health risks.

4.2. Biometric Parameters of Fish Species and the Level of Helminthic Parasite Infestation in Koka Reservoir

The tables and figures in this section show the results of the overall morphometric data (length and weight) of three fish species; *C. gariepinus*, *O. niloticus,* and *C. carpio,* the mean prevalence and intensity of their parasites; *Contracaecum, Porrocaecum, and Camallanus* sp. with respect to different exposed variables from Koka Reservoir, Ethiopia.

Table 10. Descriptive statistics of the morphometric data for the fish species in Koka Reservoir, Ethiopia

Fish species	Variables	Mean	Standard deviation	Standard error	Variance
C.gariepinus	**Weight**	465.533	231.514	22.593	53598.732
N =105	**Length**	40.148	5.406	0.528	29.221
O.niloticus	**Weight**	344.662	157.772	19.877	7960.338
N = 197	**Length**	55.631	6.749	0.85	14.569
C.carpio	**Weight**	325.2	168.082	28.411	28251.4
N= 35	**Length**	27.874	4.645	0.786	21.580

Table 11. Length, weight, and condition factor categories for *Clarais gariepinus* and *Oreochromis niloticus*

Fish species	***Clarias gariepinus***			***Oreochromis niloticus***		
Category	**L (cm)**	**W (g)**	**K**	**L (cm)**	**W (g)**	**K**
1	> 45	> 800	> 0.8	> 19	> 160	> 2
2	40 - 45	400 - 800	0.7 - 0.8	17 - 19	80 -160	1.85 – 2
3	< 40	< 400	< 0.7	< 17	< 80	< 1.85

In *C. gariepinus*, both the length and weight categories correspond to condition factors less than 1. Conversely, both the length and weight category respond to condition factor values greater than 1 in *O. niloticus*.

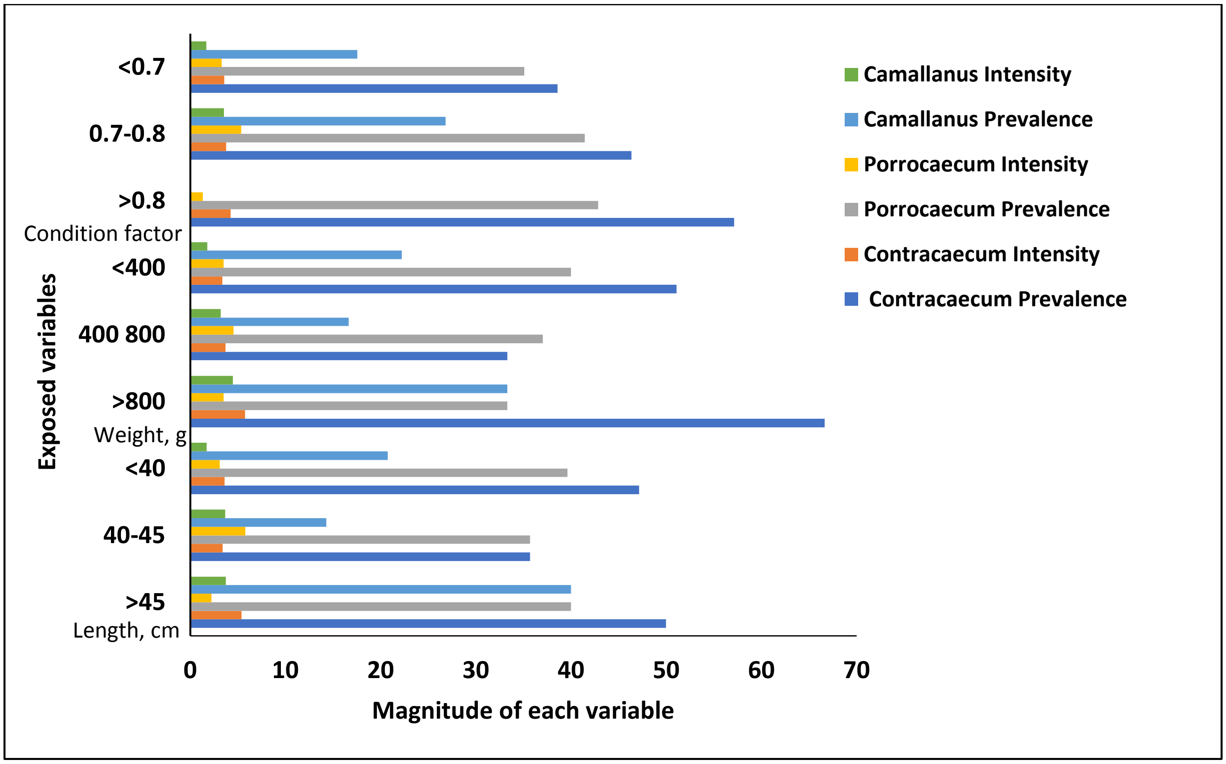

Figure 2. Prevalence and intensity of *Contracaecum*, *Porrocaecum*, and *Camallanus* sp. infection in *Clarias gariepinus* with respect to significant exposed variables (length (cm), weight (g) and condition factor)

The parasite *Contracaecum* sp. showed the highest mean prevalence and intensity in one exposed variables; weight (66.67% and 5.75), respectively. Both values were found in the large weighted group (>800 g). In the parasite *Porrocaecum* sp. the highest mean prevalence and intensity were found in the variables condition factor (42.86%) and weight (5.80), respectively. However, while the highest mean prevalence was found in the large condition factor (>8) variable, the highest intensity was found in the intermediate length (40-45 cm) variable. As for *Camallanus* sp. the highest mean prevalence and intensity were found in the exposed variables length (40%) and weight (4.50), respectively. Both were found in the large group category of their variables.

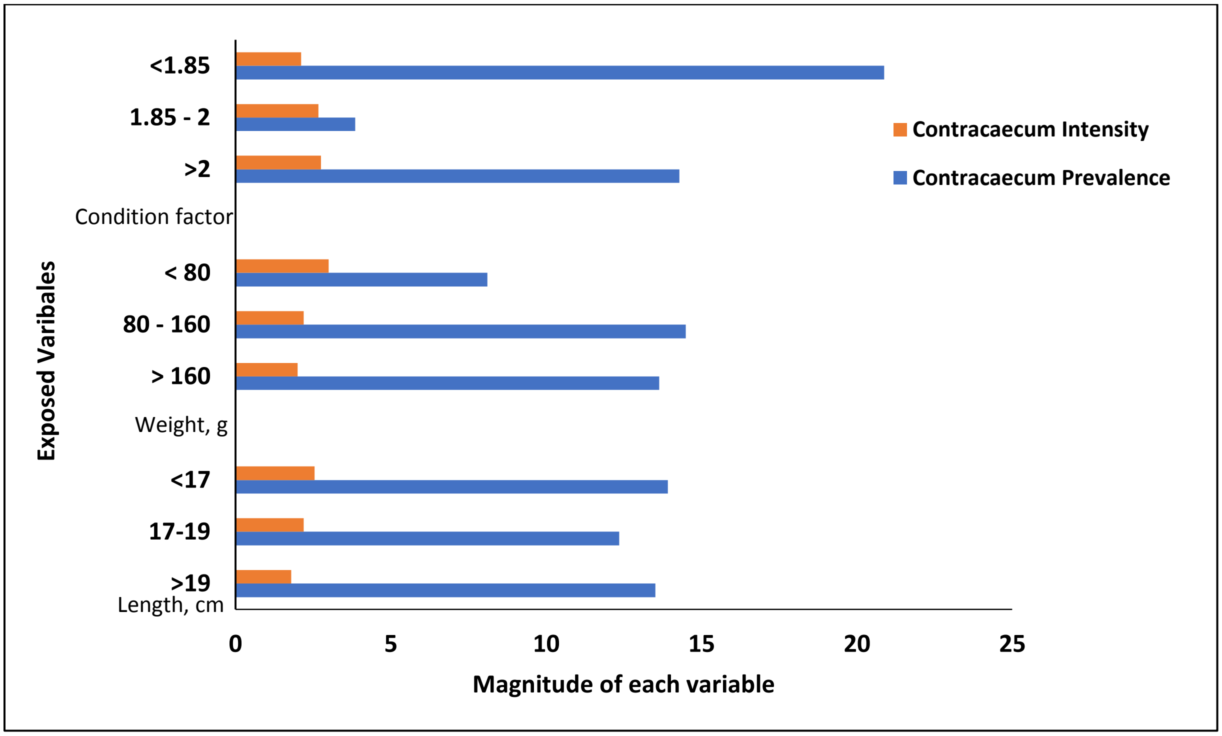

Figure 3. Prevalence and intensity of *Contracaecum* sp. infection in *Oreochromis niloticus* with respect to significant exposed variables (length (cm), weight (g) and condition factor)

The highest mean prevalence (20.88%) and intensity (2.75) of *Contracaecum* sp. was found in the variable condition factor. This was followed by the weight (14.49%) and the length (13.92%) variables, and the condition factor (2.67) and length (2.55) variables, for mean prevalence and intensity, respectively.

The chi-square tests conducted in this study revealed significant and non-significant associations between the prevalence of three nematode species (*Contracaecum*, *Porrocaecum*, and *Camallanus* sp.) in African catfish (*C. gariepinus*) and various exposed variables (sex, weight, length, and condition factor).

Table 12. Prevalence and intensity of *Contracaecum* sp. infection by sex, weight, length, and condition factor in *C. gariepinus*

Variables	No. examined fish	No. infected	No. of parasites recovered	Mean intensity of infection	Prevalence (%)	Odds ratio (95% CI)
Sex						
Males	53	19	72	3.79(3.11,4.4)	35.85	1
Females	52	26	96	3.69(2.96,4.4)	50	0.72(0.34,1.5)
X^2					2.33	
p-value					0.13	
Total	105	45				
Weight (g)						
< 400	45	23	78	3.39(2.65,4.1)	51.12	1
400-800	54	18	67	3.72(3.09,4.3)	33.34	0.48(0.21,1.0)
> 800	6	4	23	5.75(2.16,9.3)	66.67	1.91(0.37,9.8)
X^2					11.05	
p-value					0.004	
Total	105	45				
Length (cm)						
<40	53	25	90	3.6(2.91,4.3)	47.17	1
40-45	42	15	51	3.4(2.68,4.12)	35.71	0.62(0.29,1.3)
>45	10	5	27	5.4(3.03,7.77)	50	1.12(0.40,3.1)
X^2					2.58	
p-value					0.27	
Total	105	45				
Condition factor						
<0.7	57	22	79	3.59(2.95,4.2)	38.6	1
0.7-0.8	41	19	72	3.79(2.95,4.6)	46.34	1.37(0.71,2.64)
>0.8	7	4	17	4.25(1.65,6.8)	57.14	2.12(0.58,9.92)
X^2					3.66	
p-value					0.16	
Total	105	45				

For *Contracaecum* sp., a significant association was observed with the weight of the fish ($\chi 2$=11.05, p = 0.004). Fish weighing less than 400g and more than 800g exhibited higher prevalence rates compared to those within the 400-800g range. The association between sex ($\chi 2$=2.33, p = 0.13), length ($\chi 2$=2.58, p = 0.27), and condition factor ($\chi 2$=3.66, p = 0.16) with the prevalence of *Contracaecum* sp. was not significant. The odds ratio indicates that female fish had (28%) lower odds of being infected with *Contracaecum* sp. compared to male fish. However, this difference is not statistically significant, as evidenced by the wide confidence interval and the p-value greater than 0.05. Fish in the 400-800g range had (52%) lower odds of infection compared to fish weighing less than 400g. Conversely, fish weighing more than 800g exceeded by 1.91 times to be infected than those weighing less than 400g, although this finding is not statistically significant, given the wide confidence interval. The odds ratios suggest that fish of intermediate length (40-45 cm) showed 38% lower odds of infection compared to smaller fish (< 40 cm), while larger fish (> 45 cm) had slightly higher odds of infection (12% more likely). However, these associations are not statistically significant as indicated by the confidence intervals crossing 1. The odds ratios indicated that fish with a condition factor between 0.7 and 0.8 had 37% higher odds of infection compared to those with a condition factor less than 0.7. Fish with a condition factor greater than 0.8, showed more than twice higher than the odds of infection (2.12 times more likely) obtained for the reference group. Nonetheless, these differences are not statistically significant, given the confidence intervals.

Table 13. Prevalence and intensity of *Porrocaecum* sp. infection by sex, weight, length, and condition factor in *C. gariepinus.*

Variables	No. examined fish	No. infected	No. of parasites recovered	Mean intensity of infection	Prevalence (%)	Odds ratio (95% CI)
Sex						
M	53	16	68	4.25(3.35,5.15)	30.19	1
F	52	24	93	3.88(2.65,5.11)	46.15	0.51(0.24,1.09)
X^2					3.34	
p-value					0.07	
Total	105	40				
Weight (g)						
<400	45	18	63	3.5(2.77,4.23)	40	1
400-800	54	20	91	4.55(3.21,5.89)	37.04	0.88(0.42,1.84)
						0.75(0.14,4.00)
>800	6	2	7	3.5(2.04,4.96)	33.33	
X^2					0.61	
p-value					0.74	
Total	105	40				
Length (cm)						
<40	53	21	65	3.1(2.47,3.73)	39.62	1
40-45	42	15	87	5.8(4.09,7.51)	35.71	0.66(0.30,1.46)
						1.02(0.32,3.22)
>45	10	4	9	2.25(1.31,3.19)	40	
X^2					0.29	
p-value					0.86	
Total	105	40				
Condition factor						
<0.7	57	20	66	3.3(2.77,3.83)	35.09	1
0.7-0.8	41	17	91	5.35(3.62,7.08)	41.46	1.31(0.64,2.70)
>0.8	7	3	4	1.33(0.75,1.92)	42.86	1.39(0.32,5.97)
X^2					0.86	
p-value					0.65	
Total	105	40				

In the case of *Porrocaecum* sp., none of the variables (sex, weight, length, and condition factor) showed a significant association with the prevalence of infection. The chi-square values and p-values were as follows: sex (χ^2=3.34, p = 0.07), weight (χ^2=0.61, p = 0.74), length (χ^2=0.29, p = 0.86), and condition factor (χ^2=0.86, p = 0.65) (

Table *13*). While some trends were observed, such as lower odds of infection in females and varying odds based on weight and length, these were not statistically significant, indicating that other factors not measured in this study may influence the prevalence and intensity of *Porrocaecum* sp. infection in African catfish (*C. gariepinus*) in Koka Reservoir, Ethiopia.

Table 14. Prevalence and intensity of *Camallanus* sp. infection by sex, weight, length, and condition factor in *C. gariepinus*

Variables	No. examined fish	No. infected	No. of parasites recovered	Mean intensity of infection	Prevalence (%)	Odds ratio (95% CI)
Sex						
M	53	13	45	3.46(2.81,4.1)	24.53	1
F	52	8	11	1.38(1.23,1.5)	15.38	1.79(0.65,4.9)
X^2					2.09	
p-value					0.15	
Total	105	21				
Weight (g)						
< 400	45	10	18	1.8(1.55,2.05)	22.22	
400-800	54	9	29	3.22(2.65,3.7)	16.67	0.70(0.31,1.5)
> 800	6	2	9	4.5(1.93,7.07)	33.34	1.75(0.34,9.0)
X^2					5.98	
p-value					0.05	
Total	105	21				
Length (cm)						
<40	53	11	19	1.73(1.51,1.9)	20.75	1
40-45	42	6	22	3.67(2.96,4.3)	14.29	0.64(0.26,1.6)
>45	10	4	15	3.75(2.15,5.3)	40	2.55(0.77,8.4)
X^2					14.31	
p-value					0.001	
Total	105	21				
Condition factor						
<0.7	57	10	17	1.7(1.51,1.89)	17.54	1
0.7-0.8	41	11	39	3.55(2.72,4.3)	26.83	1.72(0.73,4.06)
>0.8	7	0	0	0	0	0
X^2					25.1	
p-value					< 0.001	
Total	105	21				

For *Camallanus* sp., a significant association was found with weight ($\chi2=5.98$, $p = 0.05$), length ($\chi2=14.31$, $p = 0.001$), and condition factor ($\chi2=25.1$, $p < 0.001$) (Table 14). Fish with a length of less than 40 cm and a condition factor between 0.7 and 0.8 showed higher prevalence rates. The odds ratio indicates that female fish had 1.79 times higher odds of being infected with *Camallanus* sp. compared to male fish. However, this difference is not statistically significant, as indicated by the wide confidence interval (0.65, 4.98). Fish weighing less than 400g and more than 800g exhibit higher prevalence rates compared to those within the 400-800g range. Fish weighing more than 800g are 1.75 times more likely to be infected, but not significantly different. Fish larger than 45 cm are more likely to be infected compared to those smaller than 40 cm, with a significant association indicating that longer fish have higher odds of infection. Fish with a condition factor between 0.7 and 0.8 are more likely to be infected compared to those with a condition factor less than 0.7. No infections were observed in fish with a condition factor greater than 0.8.

Table 15. Prevalence and intensity of *Contracaecum* sp. infection by sex, weight, length, and condition factor in *O. niloticus*

Variables	**No. examined fish**	**No. infected**	**No. of parasites recovered**	**Mean intensity of infection**	**Prevalence (%)**	**Odds ratio (95% CI)**
Sex						
M	70	9	21	2.34(1.92,2.7)	12.86	1
F	127	17	38	0.3(-0.04,0.64)	13.39	0.95(0.39,2.3)
X^2					0.01	
p-value					0.92	
Total	197	26				
Weight (g)						
< 80	37	3	9	3(2.14,3.86)	8.11	1
80 - 160	138	20	44	2.2(1.86,2.55)	14.49	0.52(0.13,2.0)
> 160	22	3	6	2(1,3)	13.64	0.93(0.23,3.7)
X^2					1.99	
p-value					0.37	
Total	197	26				
Length (cm)						
< 17	79	11	28	2.55(1.96,3.1)	13.92	1
17 - 19	81	10	22	2.2(1.76,2.64)	12.35	1.15(0.13,2.0)
> 19	37	5	9	1.8(1.37,2.24)	13.51	1.11(0.23,3.7)
X^2					0.10	
p-value					0.95	
Total	197	26				
Condition factor						
<1.85	91	19	40	2.11(1.55,2.6)	20.88	1
1.85-2	78	3	8	2.67(2.39,2.9)	3.85	6.6(1.55,28.18)
>2	28	4	11	2.75(1.94,3.5)	14.29	4.17(0.87,20.0)
X^2					11.35	
p-value					0.003	
Total	197	26				

Males showed lower mean prevalence of infection (12.86%) than females (13.39%). Fish weighing 80-160g showed the highest mean prevalence of infection (14.49%) compared to those weighing less than 80g (8.11%) and more than 160g (13.64%). Fish smaller than 17 cm had a mean prevalence of 13.92%, those between 17-19 cm had a prevalence of 12.35%, and fish bigger than 19 cm had a prevalence of 13.51%. Concerning the condition factor (K), fish with a Fultons'condition factor (K) of less than 1.85 had the highest mean prevalence of infection (20.88%), followed by those with K greater than 2 (14.29%) and those with a K between 1.85 and 2 (3.85%).

Female Nile tilapia had slightly lower odds of being infected with *Contracaecum* sp. compared to males (OR = 0.95, 95% CI: 0.39, 2.33) (Table 15). However, this difference is not statistically significant, as shown by the wide confidence interval and a high p-value (0.92). The chi-square test ($\chi^2 = 0.01$) also confirms no significant association between sex and prevalence of infection. Nile tilapia weighing 80-160g have 48% lower odds of infection compared to those weighing less than 80g (OR = 0.52, 95% CI: 0.13, 2.02), and fish weighing more than 160g have 7% lower odds of infection (OR = 0.93, 95% CI: 0.23, 3.79). Neither of these differences is statistically significant, as indicated by the wide confidence intervals and a high p-value (0.37). The chi-square test ($\chi^2 = 1.99$) also indicates no significant association between weight and prevalence of infection. Fish with lengths between 17-19 cm have 15% higher odds of infection compared to those smaller than 17 cm (OR = 1.15, 95% CI: 0.13, 2.02), and fish higher than 19 cm TL have 11% higher odds of infection (OR = 1.11, 95% CI: 0.23, 3.79). These differences are not statistically significant, as shown by the wide confidence intervals and a high p-value (0.95). The chi-square test ($\chi^2 = 0.1$) confirms no significant association between length and prevalence of infection. Fish with a condition factor between 1.85 and 2 had significantly higher odds (6.6 times) of infection compared to those with a condition factor less than 1.85 (OR = 6.6, 95% CI: 1.55, 28.18). This result is statistically significant, as indicated by the confidence interval not crossing 1 and the low p-value (0.003). Fish with a condition factor greater than 2 have 4.17 times higher odds of infection (OR = 4.17, 95% CI: 0.87, 20.00), although this is not statistically significant given the wide confidence interval. The chi-square test ($\chi^2 = 11.35$) confirms a significant association between condition factor and prevalence of infection.

5. CHAPTER FIVE: DISCUSSION

5.1. Heavy metal concentration in fish, parasites, and environmental samples

In the tissue of *C. gariepinus*, metals accumulated in the order: Fe>Zn>Cu>Mn>Cr. Conversely, in *O. niloticus* and *C. carpio*, the order of metal accumulation was Fe>Zn>Mn>Cu>Cr (Table 4). The liver and gill are commonly selected as key organs for assessing heavy metal accumulation. The gill provide insight about the immediate environment (water), while the liver focuses more on storage (Romeo et al., 1999) as it was observed in their MPI values. All heavy metals show elevated concentrations in the gill except for Cu (liver) in both *C. gariepinus* and *O. niloticus* and Zn (muscle) in the case of *C. carpio*. Similar results were reported by different authors about the high concentration of Cu in the livers of *C. gariepinus* (Adeyeye and Ayoola, 2013), *O. niloticus* (Badr et al., 2014; Wondimagegne Asefa and Tarekegn Beranu, 2015; Abdel-Khalek et al., 2016; Mesele Dagne, 2017; Radwan et al., 2022), and *C. carpio* (Yousafzai et al., 2012; Danabas et al., 2020; Mancera-Rodríguez et al., 2023). This could be attributed to the liver's function in accumulating Cu as it plays a vital role in copper homeostasis (Das and Gupta, 2013). However, elevated concentrations of Cu can sometimes be found in other organs, such as gills (Wondimagegne Asefa and Tarekegne Beranu, 2015; Mahboob et al., 2020; Kareem et al., 2022; Saiyadi et al., 2022; Blankson et al., 2024). These variations in accumulation pattern could be due to differences in heavy metal concentrations in the environment (Das and Gupta, 2013). Factors such as the physico-chemical parameters of the water, the age and sex of the fish, its habitat, and physiological condition also affect toxic metal concentration (Andreji et al., 2012). High concentrations of Cr were reported in the gills of *C. gariepinus* (Dsikowitzky et al., 2013; Wondimagegne Asefa and Tarekegne Beranu, 2015; Mesele Dagne, 2017; Saiyadi et al., 2022), *O. niloticus* (Mesele Dagne, 2017) and *C. carpio* (Yousafzai et al., 2012; Danabas et al., 2018). This pattern may result from the gills being the first organ exposed to heavy metals (Fawad et al., 2017) and the presence of mucous, which aids in retention and absorption of metals (Macirella et al., 2019). Significant difference ($p<0.05$) was observed in all elements except for Cu in *O. niloticus*. Comparing the heavy metal concentration found in the muscles of *C. gariepinus*, *O. niloticus*, and *C. carpio* with that of the WHO/FAO standards show that all three fishes contained higher levels of Cr that exceed the permissible limit (Table 4). Similarly, a previous study on Koka Reservoir reported that Cr concentrations in the muscles of the three commercially import fish species were found to be above the standard limit (Kassahun Tessema et al., 2020). Furthermore, when comparing the results obtained with the findings of this study shows that an increase and decrease in Cr concentration in the muscle of *C. gariepinus* (0.45 Vs 0.99) and *C. carpio* (0.87 Vs 0.66) were found, respectively. The Cr concentrations in the muscles of *O. niloticus* were the same (0.52 Vs 0.54).This could be attributed to the increase in the concentration of Cr in the environment which resulted in the elevated concentration in the muscles of *C. gariepinus*.

The metal concentration in parasites of *C. gariepinus* and *O. niloticus* followed the orders Fe>Zn>Cu>Mn>Cr and Fe>Mn>Zn>Cr>Cu, respectively (Table 5). Least and no concentration was reported by the element Cr and Cu, respectively in *O. niloticus*. These differences in the concentration of heavy metals in parasites were attributed to different factors like the parasite type, developmental stage of the parasite as well as its location in the host (Nachev et al., 2013). However, as the same parasite is discussed in both *C. gariepinus* and *O. niloticus*, this variation can mainly be attributed to their differences in diet and feeding habit which can influence metal accumulation (Shaaban et al., 2021). In a study conducted on a reservoir in Ghana, it was reported that the tissues of *C. gariepinus* accumulated more heavy metals than *O. niloticus* and this is due to the bottom feeding habitat of the fish which in contact with sediments which are deemed as better accumulators of heavy metals than water (Blankson et al., 2024). The results also show similar pattern for all metals with the exception of Zn. These results were further observed in their MPI values as the MPI value in *C. gariepinus* (10.3) was found to be higher than the MPI value for *O. niloticus* (7.08). As no internal parasite was identified in *C. carpio*, it was excluded from the heavy metal analysis. The absence of parasites in *C. carpio* could be attributed to the immune system that sheltered the fish from infection. Additionally another possible reasoning is *C. carpio*'s nature of being mainly attacked by external parasites, like monogeneans (Rohlenová et al., 2011).

The metal concentration in sediments followed the order Fe>Mn>Zn>Cr>Cu (Table 6). A previous study conducted on the sediment of Koka Reservoir revealed was comparable to our results (Assefa Wosnie Ysheber, 2020). Furthermore, a more recent study conducted by Alemayehu Esayas Masresha et al. (2023) reported similar results except Iron which is missing in the analysis. In a similar study conducted on the sediment reported some heavy metals in reservoirs found in Switzerland, similar decreasing orders of Zn, Cr, and Cu (Wildi et al., 2004).

High concentrations were recorded for Fe and Mn in this study. This could be due to the natural processes that involve both catchment erosion and redox related dissolution of Fe-Mn containing minerals near or at sediment-water interface. It was reported by Granina et al. (2004) that the movement of Fe and Mn where the sedimentation rate is very high is usually attributed to an allochthonous input. Furthermore, their increase in the environment can be attributed to the external inputs that increase because of higher catchment loading (Abesser and Robinson, 2010). Significant difference ($p<0.05$) was observed in all elements in different sites except for Cu and Fe. Chromium concentration exceeded the ISQG, PSQG (LEL), and USEPA's for the site MR. Furthermore, the site MM1 was found to have exceeded the PSQG (LEL). High chromium concentration is usually linked to industrial activities and untreated waste released to water bodies. Many industries use chromium for the processing of their products including tanneries (Murthy et al., 2023). Hence, the presence of a tannery along Modjo River could have been the reason for the high values recorded in these sites. All heavy metals analyzed in water samples were below detection level except for Cr. Thus, the results obtained in this study are within the limits set by WHO's for drinking water quality standard (Table 6).

The BCF of the parasites with respect to the tissues and organs in *C. gariepinus* and *O. niloticus* were found in the decreasing order of Cu>Mn>Fe>Cr>Zn and Zn>Fe>Mn>Cr>Cu, respectively (Table 7). All organs exhibited significant differences ($p<0.05$) in metal accumulation levels in both *C. gariepinus* and *O. niloticus,* except for Cu in *C. gariepinus* and Mn in *O. niloticus,* which showed no significant differences in concentration between the different organs. Furthermore, comparing the bioconcentration capacities of specific organs between the two fish species showed that the parasites of *C. gariepinus* significantly ($p<0.05$) concentrated more Cr and Fe in their gills compared to the gills of *O. niloticus*. However, despite the significant differences, both values were less than 1, indicating poor accumulation. On the other hand, the parasites in *O. niloticus* exhibited significantly higher (t-test, $p<0.05$) concentrations of Mn in their gills and higher concentrations of Zn in their muscle and liver compared to similar organs of *C. gariepinus*. As they all hold values above 1 (Arnot and Gobas, 2006), they exhibit good bioconcentration capacities as well. Both fish species showed similar bioconcentration capacities mainly for Fe except for the gills in *C. gariepinus*. The BCF of the parasites in *C. gariepinus* and *O. niloticus* with respect to the sediments were found in the decreasing order Cu>Zn>Fe>Mn>Cr and Zn>Mn>Fe>Cr>Cu, respectively (Table 8). Comparison between the concentration capacities of the parasites and sediments revealed that Cu and Zn showed significantly better ($p<0.05$) concentration capacities in the parasites found in *C. gariepinus* and *O. niloticus*, respectively than sediments. Since the values obtained are greater than 1, parasites are better bioconcentrators than sediments. Hence, our results confirmed the potential of the parasite, *Contracaecum* sp. to indicate the contamination of Cu in *C. gariepinus* and Zn in *O. niloticus* better than sediments. The variation in the bioconcentration capacities of *Contracaecum* sp. in accumulating metals could be attributed to the low level of the metals in the environment. Furthermore, as they are all essential metals, they could have been metabolized and excreted by the fish leaving the parasite with low levels of concentration. Additionally, the variations and similarities in the BCFs reported can be dependent on the species of the fish, its age and sex (Geyer et al., 2000).

The results of this study found that the muscles of *C. gariepinus*, *O. niloticus*, and *C. carpio* contain Cr levels that exceeded the permissible limit set by WHO/FAO (Table 4). Therefore, to further understand the risk that might take place, other calculations were carried out. All results show that the risk of a non-cancer disease is unlikely as they all are found within the accepted limit (Table 9).

5.2. Growth parameters and parasite infestation in different fish species

The condition of all fish species in this study were calculated by dividing them into different group according to their length and weight. The results reveal that *C. gariepinus* is in poor condition while *O. niloticus* was found in good condition (Table 11). While the presence of parasites was found to reduce the heavy metal accumulation in the host, it was reported to reduce the energy of the host as it keeps on sharing its nutrient (Pienaar, 2020). Additionally, the multiple parasites infection in *C. gariepinus* (*Contracaecum*, *Porrocaecum*, and *Camallanus* sp.) (Figure 2) might result in synergistic effects further building up the effect that would have been encountered if only it was infected by one parasite (Cox, 2001).

A study on the relationship between the mean prevalence of helminth parasites with other variables in *C. gariepinus* and *O. niloticus* revealed that females are more infected than male fish (Abadi Amare et al., 2014; Mgbemena et al., 2020). This is in agreement with the findings of this study for the parasites *Contracaecum* and *Porocaecum* sp. in *C. gariepinus* and *Contracaecum* sp. (Table 12 and Table 13) in *O. niloticus* (Table 15). This was attributed to the difference in the physiological condition between male and female fish especially the low immunity in gravid fish makes them susceptible to infection (Abadi Amare et al., 2014). Furthermore, different studies showed that larger fish are more prone to parasites infection than smaller fish (Steinauer and Font, 2003; Simon et al., 2016; Mgbemena et al., 2020). The change in diet, feeding habit, and even the extent of exposure were some of the reasons reported (Steinauer and Font, 2003; Olofintoye, 2006; Abadi Amare et al., 2014). This is in agreement with the results of this study for all parasites in *C. gariepinus* (Table 12, Table 13, and Table 14). However, the largest mean prevalence was found in the intermediate weight group (80-160g) and small length group (<17cm) for *O. niloticus* (Table 15). Variations where the highest mean prevalence in terms of weight, length and even sex (Hailekiros Gebreegziabher et al., 2020) can occur if the fish has a strong immune system or selected randomly (Akinsanya et al., 2008).

6. CHAPTER SIX: CONCLUSIONS

In this study, the bioconcentration capacity of heavy metals in fish species (*C. gariepinus*, *O. niloticus*, and *C. carpio*) and their associated parasite (*Contracaecum* sp.) was explored. Our findings shed light on their potential as environmental sentinels for heavy metal contamination.

Organ Gill and Sediment Concentration: The organ gill consistently exhibited the highest heavy metal concentrations in all fish species, except *C. carpio* (liver). This was further supported by the MPI values. However, significant difference was found only in *O. niloticus*. Based on MPI values, sediments showed elevated concentrations of heavy metals in sites MM1, MM2, AO, and MR.

Parasite Bioconcentration: *Contracaecum* sp., the most prevalent parasite demonstrated bioconcentration capacity in *C. gariepinus* by accumulating all metals. In *O. niloticus,* the parasite accumulated all metals except for Cu. Significantly different ($p<0.05$) concentration capacity between the two fish species was found in Cr and Fe for the gills and the parasites of *C. gariepinus* and Mn and Zn for gill and (muscle and liver) and the parasites of *O. niloticus*. In sediments, good bioconcentration capacity was recorded by the element Cu and Zn in the parasites of *C. gariepinus* and *O. niloticus,* respectively. Similarly, significantly different ($p<0.05$) concentration capacity between the parasites and the sediments was found in Cu and Zn, by the parasites of *C. gariepinus* and *O. niloticus*.

Health Risk: Although chromium (Cr) levels exceeded permissible limits in fish muscles, the calculated THQ and HI values indicated safe consumption.

Fish Health and Prevalence: *C. gariepinus* showed poor condition while *O. niloticus* and *C. carpio* are in good condition. The poor condition of *C. gariepinus* was attributed to the multiple parasite infection of the fish. Highest mean prevalence of *Contracaecum* sp. was recorded in all variable groups of *C. gariepinus*. In *O. niloticus*, highest mean prevalence was found in the condition factor variable.

Association and Prevalence: The association between mean prevalence and exposed variables revealed that there was a significant relationship between the mean prevalence of the parasite *Contracaecum* sp. and weight of *C. gariepinus*. No association was found between the mean prevalence of *Porrocaecum* sp. and any of the exposed variables. Furthermore, it showed that there was a significant relationship between mean prevalence of *Camallanus* sp., length, and condition factor of *C. gariepinus*. In *O. niloticus*, significant relationship was found between mean prevalence of *Contracaecum* sp. and condition factor.

This study highlighted the prospect of *C. gariepinus*, *O. niloticus* and *Contracaecum* sp. as potential indicators of heavy metal pollution. By taking advantage of their accumulation abilities, we can enhance environmental monitoring and suggest intervention measures to reduce heavy metal pollution in water bodies. These findings pave the way for further investigations and informed decision-making.

7. RECOMMENDATIONS

- Conduct multiple sampling to assess seasonal variations in parasite accumulation patterns.
- If possible, explore the potential of different helminth parasites in accumulating different heavy metals (both essential and non-essential).
- Consider taking *in-situ* parameters like temperature, pH, conductivity, and TDS to understand their impact on metal bioavailability.
- Even though the results indicate safe consumption of the three fish species in this study, the situation might change if *per capita consumption* or the level of heavy metal in the fish increases therefore continuous monitoring is important to ensure the level of heavy metal is well-managed.
- Closely monitor the effluents waste of the Tannery factory into Modjo River and Koka Reservoir.

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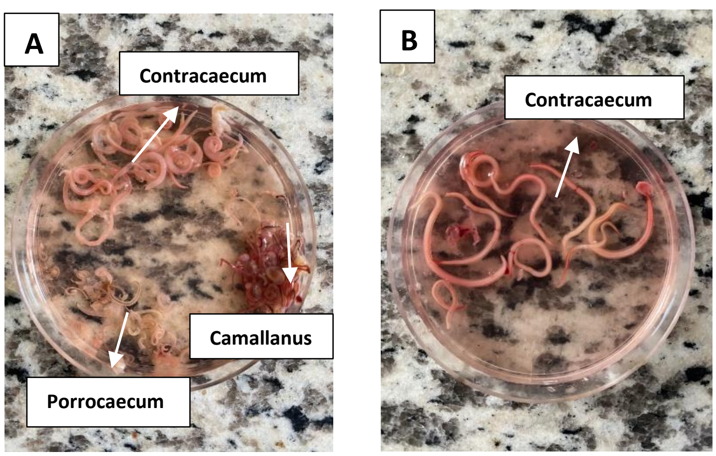
9. APPENDIX

Appendix 1. Laboratory activities carried out at NFALRC, Sebeta

Length and weight measurement of (A) *Clarias gariepinus*, (B) *Oreochromis niloticus*, and (C) *Cyprinus carpio*

Parasite(s) collected from (A) *Clarias gariepinus* and (B) *Oreochromis niloticus*



Appendix 2. Instruments used