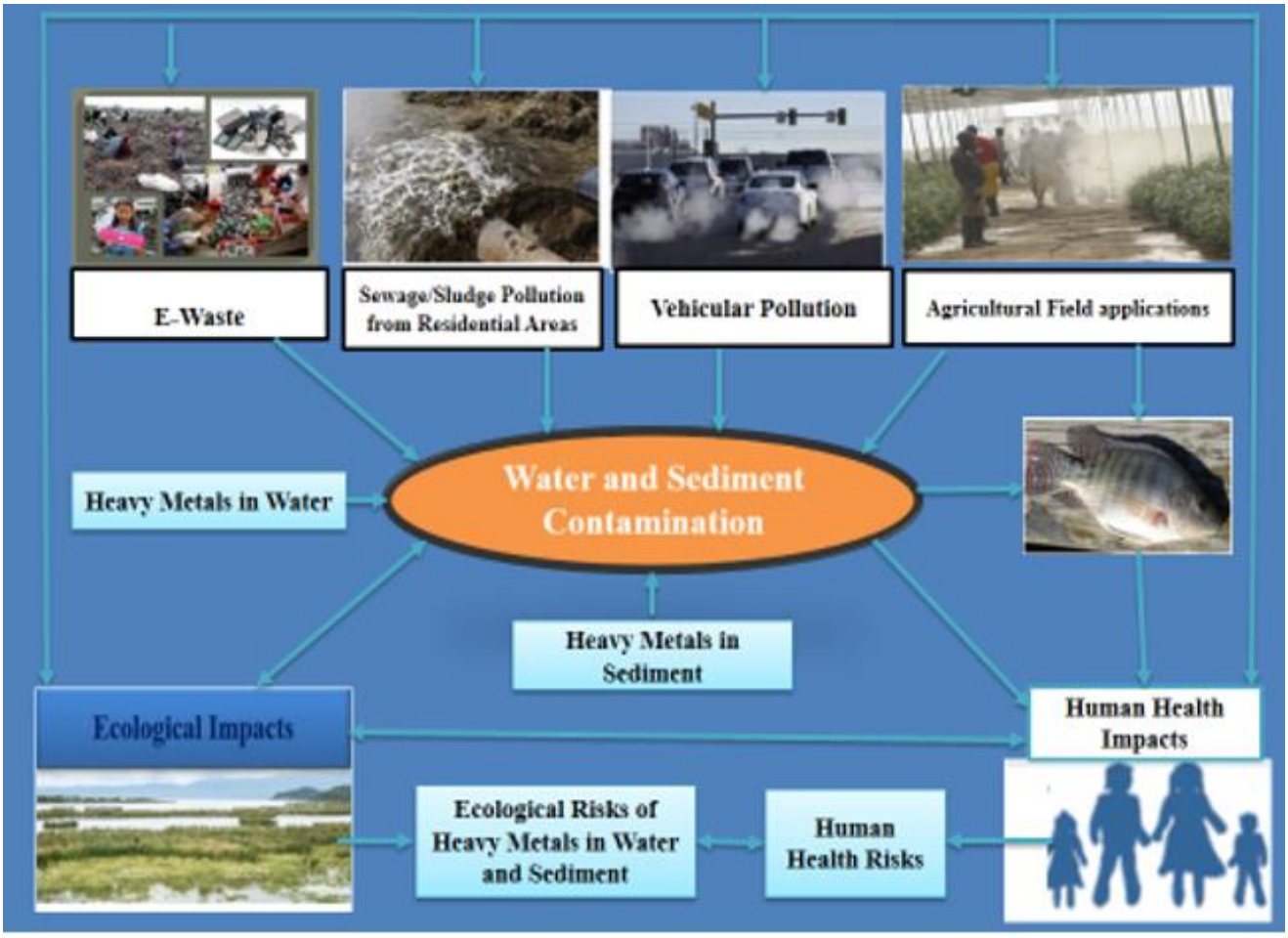
Concentrations of Heavy Metals in water and sediments from streams in the Awetu watershed and fish tissue from the Gilgel Gibe I reservoir, in Jimma zone Southwestern Ethiopia: Ecological and Human Health risks



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Abbreviations and Acronyms

AAS	Atomic Absorption Spectrometry
ANOVA	Analysis of Variance
APHA	American Public Health Association
ATP	Adenosine Triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
BDNF	Brain-Derived Neurotrophic Factor
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CF	Contamination Factor
CR	Cancer Risk
CRIP	Cysteine Rich Intestinal Protein
CSA	Central Statistical Authority of Ethiopia
DNA	Deoxyribonucleic Acid
EU	European Union
EC	European Commission
EDI	Estimated Daily Intake
EDTA	Ethyelene Diamine Tetraacetic Acid
Eir	Ecological Risk Factor and
ERI	Ecological Risk Index
ERL	Environmental Response Liability
FAO	Food and Agricultural Organization
HI	Hazard index

IARC	International Agency for Research on Cancer
ICP-OES	Inductively Coupled Plasma Mass Spectrometry
I_{geo}	Geoaccumulation Index
JECFA	Joint Expert Committee on Food Additives
MAL	Maximum Allowable Limits
mCd	Modified Degree of Contamination
MMT	Methylcyclopentadienyl Manganese Tricarbonyl
MPL	Maximum Permissible Limit
NEMA	National Environment Management Authority
NMDAR	N-Methyl-D-Aspartate Receptor
P_N	Nemerow Pollution Index
PCA	Principal Component Analysis
PLI	Pollution Load Index
SPSS	Statistical Product and Service Solutions package
TCR	Target Cancer Risk
THQ	Target Hazard Quotient
UNEP	United Nations Environmental Program
US EPA	United States Environmental Protection Agency
VGCCs	Voltage-Gated Calcium Channels
WHO	World Health Organization

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Summary

Extended use of substances that contain toxic contaminants like heavy metals, in industries, agriculture, and other human activities leads to the increased release of harmful heavy metals into the environment. Heavy metals are metallic elements that have a relatively high density (> 5 g/cm^3) and can induce toxicity at a low level of exposure. A lot of such substances released into the environment end up in the aquatic systems due to the direct discharges into water bodies and/or through run-off. Consequently, ecological risks due to the accumulation of heavy metals in the ecosystem and human health risks as a result of the consumption of water and foodstuffs contaminated with heavy metals have become serious concerns worldwide. Countries like Ethiopia, with poor pollution regulatory implementation, are among those highly affected. Jimma town, one of the oldest towns in the Southwestern part of Ethiopia, is located within the Awetu watershed that is drained by different streams. These streams and the Gilgel Gibe reservoir receive effluents that contain untreated solid and liquid wastes containing heavy metals that are generated from different industries.

Heavy metals are among the most severe pollutants due to their toxicity, persistence in the environment, bioaccumulation, and bio-magnification in the food chains to higher animals, including humans. The nature, reactivity, and mobility of heavy metals in water, sediments, and biota are crucial for understanding their potential environmental risk. However, the concentrations of heavy metals in the water and sediments of the streams that drain the Awetu watershed and the fish in the Gilgel Gibe reservoir, and their ecological and human health risk is generally unknown.

Therefore, this study was intended to determine the sources and levels of heavy metal contamination and assess the ecological risks in water and sediment from the streams in the Awetu watershed; determine the pollution level and human health risks through fish consumption collected from the Gilgel Gibe I reservoir. The concentrations of heavy metals (arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), and zinc (Zn)) were determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES; ARCOS FHS12) and Atomic Absorption Spectrophotometer (AAS; Perkin Elmer, Waltham, MA).

The concentrations and spatial distributions of heavy metals in water samples were assessed based on Pearson's correlation, hierarchical clustering, and principal component analyses. The contamination levels of heavy metals in sediment were evaluated based on the contamination factor (CF), pollution load index (PLI), and geoaccumulation index (I_{geo}). To evaluate the contamination levels and the potential ecological risks of one or multiple elements to the ecology in water and sediment, modified degree of contamination (mCd), Nemerow pollution index (P_N), ecological risk factor (E_r), and ecological risk index (ERI) were

employed. The human health risks due to ingestion of heavy metals through the consumption of fish were assessed based on the estimated daily intake (EDI), target hazard quotients (THQ), hazard index (HI), and cancer risk (CR).

The concentrations of heavy metals in water samples of the Awetu watershed streams (Awetu, Boye, Dololo, and Kitto) ranged from 18 - 351 µg/L for As, 5 - 19 µg/L for Cd, 232 - 421 µg/L for Cr, 314 - 920 µg/L for Pb and 10 - 16 µg/L for Hg. The highest concentrations of As were detected at Kitto site 3 (K3) (351 ± 177.7 µg/L), Cd at Kitto site 2 (K2) (19 ± 7.3 µg/L), Cr at Dololo site 4 (D4) (421 ± 76.4 µg/L), Pb at Dololo site 4 (D4) (920 ± 327.1 µg/L), and Hg at Dololo site 5 (D5) (16 ± 3.95 µg/L). Analysis of variance results revealed that Cd concentrations were statistically significant to the other metals in Awetu and Dololo streams ($p < 0.05$) and Dololo and Kitto ($p < 0.001$). Dololo stream which receives effluents from Jimma University, maintenance garages, residential and commercial sources, car-wash, and agricultural activities had higher values of heavy metals than the streams located upstream of the watershed.

The concentrations of heavy metals in sediment samples also showed higher concentrations in the center than the upstream of the town, which is predominantly agricultural and grazing catchments. According to the limits determined by the European Community's legislation, the river sediments were contaminated with a contamination factor (CF) ranging from less than unity to six. Particularly, Dololo and Kitto streams showed a much higher degree of contamination ($CF \leq 6$) than Awetu and Boye streams. The geoaccumulation index (I_{geo}) indicated contamination of sites from moderately contaminated to extremely contaminated levels with Cd, Cr, and Pb. Sediment samples also indicated severe contamination levels based on ranges in sediment quality guidelines (SQGs).

Water samples from the center of the town showed higher values of contamination factor (C_f^i) and potential ecological risk factor (E_f^i) by heavy metals than aquatic water permissible limits. Dololo stream (D1, D2, D3, and D4) and Kitto (K1, K2, and K3) were found at a 'toxic' contamination level with a 'severe' ecological risk. Cd, Mn, Ni, Pb, and Sn contributed to the highest ecological risk. Toxic metal contamination in the Dololo stream is attributed to institutions carrying out various anthropogenic activities along the stream bank, such as traditional metal plating, garages, laboratory effluents, carwash, and waste dumping.

The concentrations of As, Cd, Hg, and Pb in the gill, liver, and muscle of *Oreochromis niloticus* and *Labeobarbus intermedius* fish species were studied. Concentrations of the metals varied significantly by tissue type and species. The mean concentrations of As and Cd showed higher values in the liver while Hg and Pb in muscle tissues of *Oreochromis niloticus*. The mean concentrations of As, Cd, Hg, and Pb showed

higher values (0.33 ± 0.22, 0.44 ± 0.12, 0.14 ± 0.13, and 0.04 ± 0.04 mg/kg dw respectively) in the liver than the gill and muscle tissues of *Labeobarbus intermedius* species. The concentration of As, Cd, and Hg exceed the permissible limits set by the EC and FAO/WHO (0.002, 0.05, and 0.5 mg/kg dw respectively). The estimated daily intake (EDI) of heavy metals calculated based on mean fish consumption by Africans indicated human health risks.

In conclusion, a higher concentration of heavy metals was detected in the water and sediments of streams in the Awetu watershed, which confirms that toxic metals are one group of contaminants in the watershed ecosystem. These values were predominantly associated with the type of waste entering the streams. Metal concentrations in the watershed were to the level that can pose a risk to downstream users. The results also showed a strong correlation between heavy metal concentrations and the anthropogenically influenced areas in the present analytical work. The extent of pollution by heavy metals in the stream showed the situation is alarming to the public, environmental authorities, and professionals. Therefore, future pollution control and management plans should be accentuated for the wastes generated from household, commercial and industrial sources and strict regulation of the discharges should be implemented. Moreover, the establishment of waste management systems and stream quality monitoring strategies should be implemented to minimize the deterioration of the aquatic ecosystem. The study shows As and Cd accumulation in the fish species may pose a possible health risk to consumers over time due to the bioaccumulation nature of heavy metals.

Keywords: Awetu watershed, ecological risks, fish, human health risk, heavy metals, sediment, water

Chapter One: General Introduction

1.1 Background

Globally the life of people is intimately intertwined with surface water such as streams or rivers which supply a wide range of ecosystem services (Srebotnjak et al., 2011). Water is vital for all life and human activity and access to fresh water in sufficient amounts and of suitable quality is a precondition to achieve sustainable development. It is therefore at the heart of many international policy objectives of halving poverty to ensure environmental sustainability and water quality management which contributes directly or indirectly to achieving these development goals. Hence, the goods and services that aquatic resources provide to people are fundamental to peace, security, and prosperity (UNEP GEMS/Water, 2006; Chung, 2019).

Surface water pollution has become a key focus of concern all over the world and has many forms. Among all the pollution problems, freshwater resources pollution especially flowing in highly industrialized and urbanized areas, are of great concern. The rapid growth of industrialization and human population and the associated increase in domestic sewage and agricultural development are the main reasons for water quality deterioration in all measures (Hu et al., 2013; Gergen et al., 2015).

Streams/rivers are sources of various biodiversity and support species from all of the major groups of organisms ranging from microbes to higher forms (Pimentel et al., 2004; US EPA, 2009). Despite humanity's reliance on flowing water, human activities have severely degraded the quantity and quality of these water sources worldwide, diminishing their ability to provide valuable ecosystem services and driving species to extinction (Kole et al., 2013; Saha and Paul, 2016). Freshwater sources are changing systems in nature: over the years, decades, centuries, they change in position, bank, bed, water, and community (Huser et al., 2011). However, the way they change, particularly under human impact is to be scrutinized with care. Many human activities interfere with the natural dynamics of river ecosystems in ways that can be examined from human impacts that must be considered against these backgrounds of spatial linkages and temporal variability (Moore et al., 2011).

Various industrial activities have put an increasing burden on the environment by releasing large quantities of heavy metals like arsenic (As), cadmium (Cd), chromium(Cr), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn) and zinc (Zn) that imposed serious damage on the ecosystem (Tak et al., 2013b; Gaur et al., 2014; Dixit et al., 2015). In developing countries, up to 95 % of industrial wastewater is discharged into surface water bodies without any treatment (Pimentel et al., 2014). Industries such as metal plating facilities, mining operations, fertilizer industries, tanneries, battery factories, electronics manufacturers, coal burning, paper, and pulp industries, garages, car washes, pesticides, etc., release heavy metal-containing wastes directly or indirectly into the environment, especially in developing countries (Malm, 2000; Yuan et al., 2013; Ayangbenro and Babalola, 2017).

In the recent 30 years, an increase in global concern over the public health impacts attributed to environmental pollution, in particular, the global burden of disease (Bruggen et al., 2009). Residents, commercials, institutions, industries, hotels, construction and demolition areas, municipal services, treatment plants and sites, agriculture, and street sweepings are the major sources of solid waste generation in the urban areas. These wastes attract birds, rats, flies, and other animals to the dump. Animals feeding at the dump may transmit diseases to a human living in the vicinity (Saha and Hossain, 2011; Brandi and Wilson-Wilde, 2012; Gaidajis et al., 2010; Gergen et al., 2015).

In Africa, rapid and unorganized urban expansion, and agricultural practices coupled with inadequate waste management practice causes significant alterations in the physical environment and increase the accumulation of municipal waste (De Troyer et al., 2016). Most developing towns lack proper liquid and solid waste management regulations for the appropriate disposal of harmful wastes such as toxic or radioactive wastes (Murray et al., 2004; Alamgir et al., 2015; Omwene et al., 2018). Ground, surface, and water from different industrial processing units frequently contain dissolved toxic ions that may arise from operations of dissolution and weathering of minerals, unwise use of agricultural practices, mineral smelting and tannery industry, factories, and other industries (Alemayehu et al., 2017). For instance, natural arsenic pollution of groundwater and, to a much lesser extent, surface water, is known to have exposed more than 140 million people to arsenic (As) concentrations of more than 10 μg/L in at least 70 countries in different parts of the

world, where Ethiopia is among nine countries, with large at-risk populations but no reported cases of pollution (Melak et al., 2017).

Heavy metals; not all are essential to maintain various biochemical and physiological functions in living organisms when in very low concentrations, however, they become noxious when they exceed certain threshold concentrations. Although it is acknowledged that heavy metals have many adverse health effects and persist in the environment, the exposure continues and is increasing in many parts of the world. They are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons (Gegenwart et al., 2008; Nagajyoti et al., 2010; Jaishankar et al., 2014). The most commonly found heavy metals in wastewater include As, Cu, Cd, Cr, Pb, Hg, Ni, and Zn, all of which cause risks for human health and the environment. They may enter the environment by anthropogenic activities like mining, industrial effluents, sewage discharge, pesticides, insecticides, or disease control agents applied to crops (Jaishankar et al., 2014); and from natural pollution sources such as soil erosion, urban runoff, natural weathering of the earth's crust, volcanoes, and many others (Wuana and Okieimen, 2011; Morais et al., 2012).

The maximum permissible concentration of some heavy metals in drinking water, as stated by the Comprehensive Environmental Response Compensation and Liability Act (CERCLA), USA, is 0.01, 0.05, 0.01, 0.015, 0.002, and 0.05 mg/L for As, Cd, Cr, Pb, Hg, and Hg respectively (Chaturvedi et al., 2015). According to Environmental Response Liability (ERL) established by US EPA, the standards for sediments are 46.7, 1.2, 81, 0.15, and 8.2 mg/kg for Pb, Cd, Cr, Hg and As respectively (Hahladakis and Smaragdaki, 2013). The standard for soil, as established by the Indian standards for heavy metals, is 3–6, 135–270, 75–150, 250–500, and 300 – 600 mg/kg for Cd, Cu, Ni, Pb, and Zn respectively (Nagajyoti et al., 2010) that create adverse biological effects beyond the range values. But effluents from various industries (tannery, textile, dyeing, washing, pharmaceuticals, chemicals, paints, steel, ceramic, etc.), and urban sewage discharged in the open water bodies and rivers have already brought the pollution level to an alarming state (Islam et al., 2017). Agricultural sources as a result of long-term application of chemicals contribute a significant amount of heavy metals in the soils and water bodies (Sridhara et al., 2008; Singh et al., 2011; Yadav et al., 2013; Alghobar and Suresha, 2017).

Although there are certain laws and regulations to control industrial pollution, its monitoring system is generally weak and treatment of wastewater from industry is not a priority in low-income countries. Due to this many small to medium-scale industries operating in urban and semi-urban settings dispose of their contaminated effluents directly into urban sewer lines and ultimately enter into open water bodies and streams that bring the heavy metal pollution level at an alarming rate (Yadav et al., 2013; Gebrie et al., 2014; Getaneh et al., 2014; Yang et al., 2016).

Stream waters polluted with different pollution sources used for domestic purposes such as food preparation and drinking purposes is a growing concern for human health. It might also be used for irrigation which derives significant economic activity and supports the livelihood of farmers in the dry season when the scarcity of fresh water is very high. Irrigation by polluted surface water is a common practice in many developing countries (Islam et al., 2017; Woldetsadik et al., 2017). This polluted water can contain municipal, industrial and agricultural runoff (containing heavy metals and agrochemicals) which caused severe ecological and public health problems (Alemayehu et al., 2017; Islam et al., 2017). Several studies have reported a high concentration of heavy metals in soils (Ahmad and Goni, 2010; Alamgir et al., 2015, Chowdhury et al., 2016; Islam et al., 2017) that eventually enters into the nearby water bodies and accumulated in the sediments (Sany et al., 2013) as a result they are regarded as the ultimate sink for metal cations (Islam et al., 2017). This phenomenon compromises the natural inherent capacity of rivers to detoxify the discharged substances and recover to their original quality later overloaded with pollutants potentially hampering their self-purification properties (Mengistie et al., 2016) and this is one of the challenges for developing nations to reach the targets of environmental sustainability set by the Millennium Development Goals (UN, 2009).

Towns in developing countries reveal aspects of the waste-management problem such as piles of uncontrolled garbage, roadsides littered with refuse; streams blocked with rubbish, and inappropriately disposed of toxic waste and disposal sites that constitute a health hazard to residential areas (Khaliq et al., 2014; Yohannes and Elias, 2017). The occurrence of uncontrolled urban sewage farming is a common sight in African cities/towns that expose consumers of such products to poisoning from heavy metals (Assubaie, 2015; He et al., 2018; Yuan et al., 2018). Moreover, abandoned waste dumpsites have been used widely as fertile grounds for cultivating vegetables and studies have indicated these vegetables are capable of accumulating high levels of

heavy metals from contaminated and polluted soils (Arslan et al., 2009) and get poisoned in the food they eat and the water they drink (Tang et al., 2014; Ekhator et al., 2017; Woldetsadik et al., 2017). Heavy metal pollution of the environment, even at low levels, and their resulting long-term cumulative health effects are among the leading health concerns (Khan et al., 2011; Hunt et al., 2012; Yi et al., 2017; He et al., 2018).

Heavy metals from waste dumpsites can accumulate and persist in soils at an environmentally hazardous level (Du Laing et al., 2009; Raulinaitis, 2012; Dixit et al., 2015). This constitutes serious health and environmental concerns because of the phytotoxicity of these metals to the plants and the potential health implications to humans and animals (Hunt et al., 2012; Zeng et al., 2014; Baran and Tarnawski, 2015). Studies have shown municipal refuse and uncontrolled discharge from different sources may increase heavy metal concentration in sediment and surface and underground water (Giouri et al., 2010; Sekabira et al., 2010; Edokpayi et al., 2016). This may have effects on the surface water, sediment, and human health (Wang et al., 2010; Dinis and Fiúza, 2011; Yi et al., 2011).

Ethiopia, one of the developing countries in Africa undeniably is one of the most pressing concerns of urbanization and industrialization as a result of improper solid and liquid waste management (Zinabu and Pearce, 2003). The country with its different geological formations and climatic conditions, has 30 lakes distributed throughout the country, many swamps, marshes, floodplains, and man-made reservoirs. Most of the lakes in the country are found in the rift valley and a large number of rivers flowing on either side of the valley form a drainage network that covers most of the country (Ethiopian Wildlife and Natural History and Wetlands Imternational, 2018). The inland waters constitute a valuable natural resource storing freshwater for domestic consumption, irrigation, some industrial purposes, generating electricity, recreation, domestic animals, and supporting many species of harvestable fish, birds of great tourist attraction as well as several other species (Yewhalaw et al., 2009).

New residential settlements, business complexes, educational institutions including Jimma University, health care facilities, hotels, recreational centers, and automobile workshops are booming up in the town and generate various wastes discharged with no or little treatment to the nearby water bodies. Reports indicate that in Ethiopia many rivers and streams are heavily polluted as they flow through major towns and streams and the Awetu watershed streams are not exceptions

(Van der Bruggen et al., 2009; Ambelu et al., 2013; Haddis et al., 2014).

Streams in the Awetu watershed have long been used for a variety of purposes including the source of public water supply, small-scale irrigation, bathing, washing, livestock watering, and recreation. Nevertheless, poor farming methods, overgrazing, and deforestation which are the most common causes of catchment degradation in the watershed remain serious problems in the stream catchment as well. The streams in the watershed touch Jimma town in different directions and finally enter into Boye wetland and to Gilgel Gibe river.

Recently, Waste Stabilization Ponds (WSPs) system comprising seven ponds have also been designed and constructed at JU different sites to handle the wastes generated by the university having different units with a standard of effluents to be released to the environment. Currently, the campus supports a large number of students in different programs and comprises many academic units‟ laboratories, student services facilities, staff services, conference hall, livestock husbandry, clinic, garage, parking and abattoir which generate wastes of various composition and characteristics. These imply that streams in the Awetu watershed are likely getting contaminants from non-point (surface runoff from the surrounding agricultural lands, municipality as well as soil leaching) and point sources.

Jimma town faces solid and liquid waste management challenges like other developing nations. Many of the town estates are littered with garbage which when eventually collected finds its way into open dumps. The use of open dumps for municipal solid waste in Ethiopia makes environmental pollution highly probable (Ambelu et al., 2013; Haddis et al., 2014). Both surface and ground water remain vulnerable to solid and liquid waste pollution because indiscriminate dumping was chosen for convenience rather than based on environmental safety considerations.

Although there are some studies on the concentration of heavy metals in environmental matrices such as water, sediment, and fish (Melaku et al., 2004; Dsikowitzky et al., 2013; Deribe et al.,2014; Asefa and Beranu, 2016; Bekele et al., 2015; Gerenfes et al., 2019; Kindie et al., 2020) in Ethiopia, there are limited studies in Awetu watershed streams Jimma, southwestern Ethiopia. Moreover, most of the studies cited above had determined only the levels of heavy metals and, did not report associated potential ecological and human health risks. The aim of the present study was, therefore,

to determine the contamination levels of heavy metals in environmental matrices (water, sediment, and fish) and assess possible ecological and human health risks.

1.2 Statement of the Problem

In Ethiopia, despite protection against particular hazardous waste management and disposal control have been publicized in proclamation No.1090/2018 (FDRE Negarit Gazeta, 2018), evidence on the proper liquid and solid waste management is limited (Yazie et al., 2019). Effluents and solid wastes released from urban areas and industrial and agrochemical sources containing heavy metals discharged into the nearby water bodies, thereby contaminating aquatic organisms like fish (Nagajyoti et al., 2010; Dixit et al., 2015; Khan et al., 2017).

Jimma is a town with rapid urbanization in the midst of deteriorating economic, environmental, and health conditions (Asefa et al., 2018). In 2012 approximately 21.2 % of Ethiopia's population live in urban areas (UNEP, 2006; CSA, 2012). The influx of population into the cities/towns in developing countries (like Ethiopia) without matched urban planning has continued and resulted in the variability of environmental problems (Mohiuddin et al., 2011; Haddis et al., 2014). Among others, one of the problems includes the current inability of urban authorities to adequately manage large quantities of the generated solid and liquid waste (UNEP, 2006). The uncontrolled and unmonitored wastes resulted in environmental contamination by heavy metals, a global concern due to their widespread distribution and multiple effects on the ecosystem (Rajeswari and Sailaja, 2014; Azeh Engwa et al., 2019; Bhat et al., 2019). These large amounts of wastes generated comprise an organic material, plastic, paper, metal rubbish, and batteries which are known to be real sources of heavy metal (Sörme and Lagerkvist, 2002; Zhang et al., 2016; Yuan et al., 2018). Furthermore, heavy metals are non-biodegradable and can accumulate in waters and sediments to toxic concentrations that affect plant and animal life (Jan et al., 2015; Azeh Engwa et al., 2019).

Heavy metals found in the aquatic environment may enter the human body via drinking water, may bioaccumulate in fish tissues, or ingest crops grown in the contaminated area through the food chain (Hunt et al., 2012; Jaishankar et al., 2014). Heavy metals such as As, Cd, Cr, Pb, and Hg are cumulative poisons, cause environmental hazards, and are reported to be exceptionally toxic (Athar et al., 2018; Bhat et al., 2019; Janaydeh et al., 2019). These metals might enter into humans and threaten public health through inhalation, ingestion, and dermal penetration particularly by consumption of fish which is an issue of global concern (Gerenfes et al., 2019). These metals are

a major source of oxidative stress in the cell and play an important role in the etiology of diverse human pathologies such as carcinogenesis (Valko et al., 2005; Flora et al., 2008; Sharma et al., 2015). Exposure to heavy metal toxicity leads to brain damage, mental retardation, cerebral palsy, lung cancer, gastrointestinal abnormalities, dermatitis, and death of the unborn fetus (UNEP, 2006; US EPA, 2010). Evaluation of health risks due to ingestion of heavy metals through fish consumption is extremely important in Ethiopia. Because most industries and urban centers discharge untreated wastes directly or indirectly into the nearby water bodies (Asefa and Beranu, 2016; Samuel et al., 2020; Astatkie et al., 2021).

Awetu watershed consists of four streams of which Awetu and Dololo streams are found at the center of the town, Kitto at the margin of the town, and Boye at the downstream and heading to Gilgel Gibe. These streams receive untreated effluents directly or indirectly from the respective nearby small-scale industries like garages, wood processing, Jimma University specialized hospital, and laboratories. The streams which receive these untreated effluents are used by the local communities downstream for domestic and irrigation purposes (Van der Bruggen et al., 2009; Astatkie et al., 2021).

The unrestricted discharge of solid and liquid waste to the nearby water body daily causes a problem to the surroundings and hence causes diseases to both animals and human beings (Van der Bruggen et al., 2009). Studies on the level, contamination status, and ecological risk assessment of heavy metals in surface water and sediment have not been conducted mainly in the Jimma zone, southwestern Ethiopia (Van der Bruggen et al., 2009; Adela et al., 2012; Getaneh et al., 2014; Asefa and Beranu, 2016; Astatkie et al., 2021). On top of that, quantitative data on heavy metals concentrations levels, pollution sources, and ecological and human health risk assessment in the area have hardly been systematically studied so far. Therefore, an assessment of metal pollution in water and sediments in streams in the Awetu watershed is urgently needed. Fish consumption is common in Ethiopia around reservoirs and rivers and increasing rapidly in recent decades is predominantly found in the Jimma zone. Most of the studies determined only the levels of heavy metals but did not report the spatial distribution, pollution sources, and potential ecological and human health risks. Therefore, the present study aimed to determine the concentrations of heavy metals in surface water and sediment to determine the contamination levels of heavy metals in environmental matrices (water, sediment, and fish) and assess possible ecological and human

health risks.

1.3 The Rationale of the Study

WHO estimates that about a quarter of the diseases facing mankind today occurs due to prolonged exposure to environmental pollution (Du Laing et al., 2009; Islam et al., 2017). The general belief that wastes generated from natural and anthropogenic sources are occasionally hazardous to health cannot be overemphasized. Liquid and solid waste indiscriminate dump sites are common in towns in developing countries like Ethiopia. The sites are not only unpleasant aesthetically but also cause environmental pollution, ecological and human health risks as a result of toxic heavy metals pollution (Samuel et al., 2020). These polluted water bodies with toxic heavy metals are used by the residents living around for domestic purposes causing serious health problems and led to a series of human health hazards, disorders, and even death (US EPA, 2004; UNEP, 2006; Morais et al., 2012; Gergen et al., 2015; Järup, 2017).

Within the current trends, impacts on toxic heavy metals pollution are projected to be worsening in the future. However, the effect is across the world, where the developing countries are disproportionately impacted with toxic heavy metal-related problems, mainly due to their limited and neglected treatment facilities. In this aspect, the pollution level of water bodies by heavy metals found near the towns in Ethiopia is worsened by the combined effects of anthropogenic factors and lack of treatment facilities (Islam et al., 2017).

Hence there is an urgent need to quantify the level of heavy metals in the water and sediment compartments and evaluate the ecological and human health risks in the study area. Moreover, the watershed analysis considering stream conditions, level of heavy metals in the stream water and sediment, ecological and health risks posed by heavy metals is minimal in the country and lacking in the southwestern part of Ethiopia.

Therefore, a watershed-based quantification of selected heavy metals in the water and sediments, exploring and comparing with the world standards, assessing the ecological risks as a result of heavy metals in the environmental compartments, and evaluating the human health risks as a result of consuming contaminated fishes with heavy metals. These provide an essential and comprehensive piece of evidence for residents, professionals, and decision-makers to enable interventions for the long-term effects of wastes generated from different anthropogenic sources.

1.4 Significance of the Study

A study concerning heavy metals pollution in environmental matrices is essential because human life is highly dependent on water. Water and sediment are likely sources of heavy metal exposure to humans and hence studying the burden and the exposure sites have great importance for the community. Thus, it provides an insight into the pollution status of the water bodies in the nearby or crossing urban areas and device proper remediation to reduce the toxicity of the particular metals so that people will be safe. This would act as a springboard for other researchers and the researcher himself. The study is expected to address the level of heavy metals from the surface water arising from the different anthropogenic sources, contamination levels of streambed sediment with heavy metals. The values of heavy metals in the water and sediment in the Awetu watershed streams were evaluated for the potential ecological risks. Moreover, fish samples were collected from Gilgel Gibe I reservoir, and the heavy metal contents were evaluated to estimate the human health risks posed by the consumption of fish in the study area. In addition, the findings in this study will be addressed to the respective stakeholders through a consultative meeting, which could contribute to strengthening awareness for solid and liquid waste management actions and the health effects of heavy metals generated from these wastes. Moreover, articles published in reputable journals contribute a piece of information for education and research. As a result, the beneficiaries are the local community in particular and the scientific community around the world in general. On top of that, the current study serves as a benchmark for future research in the field area.

1.5 Research Questions

- What are the concentrations of heavy metals in the water and sediments of Awetu watershed streams?
- What are the possible sources of heavy metals in the surface water and sediments of Awetu watershed streams?
- Do the concentrations of the heavy metals in the water and sediment of streams in the Awetu watershed have an ecological risk?
- Could the consumption of fish harvested from Gilgel Gibe I reservoir cause health risks to humans?

1.6 Objectives

1.6.1 General Objective

To evaluate the contamination level of heavy metals in the water and sediment of streams in the Awetu watershed and determine possible ecological and human health risks from commonly consumed fish in southwestern Ethiopia.

1.6.2 Specific Objectives

- To determine the sources and level of heavy metal contamination in the water of streams in the Awetu watershed, southwestern Ethiopia.
- To determine the contamination of stream sediment with heavy metals in the Awetu watershed of Southwestern Ethiopia.
- To assess the potential ecological risks of heavy metals in water and sediments of streams in the Awetu watershed of Southwestern Ethiopia.
- To assess the human health risk posed by heavy metals in edible fish species obtained from Gilgel Gibe I reservoir of Southwestern Ethiopia.

1.7 Research Hypothesis

The concentrations of heavy metals in the water and sediment in the Awetu watershed streams are not significantly different and edible fishes are not threats to human health.

1.8 Conceptual Framework

The conceptual framework was developed based on the specific objectives of the study (Figure 1.1.) that show the connections of independent and dependent factors. Furthermore, different published articles and reports in the area, and other contributing sources linked to the statement of the problem, study variables, research questions, and specific objectives of the study were assessed to develop a conceptual framework. Different anthropogenic pollution sources, ecological and human health risks of heavy metals were considered for the study. The contaminations of water, sediment, and fish were explored to identify the specific sources of heavy metals pollution. Similarly, the exposure of the environment and humans from different anthropogenic and occupational exposures were assessed to explore the risks of heavy metals. As a whole, the framework provides information on major components included in each research question, sources

of heavy metals pollution, and their ecological and human health risks to provide a consolidated output on the heavy metal pollution of the streams in the Awetu watershed and the downstream.

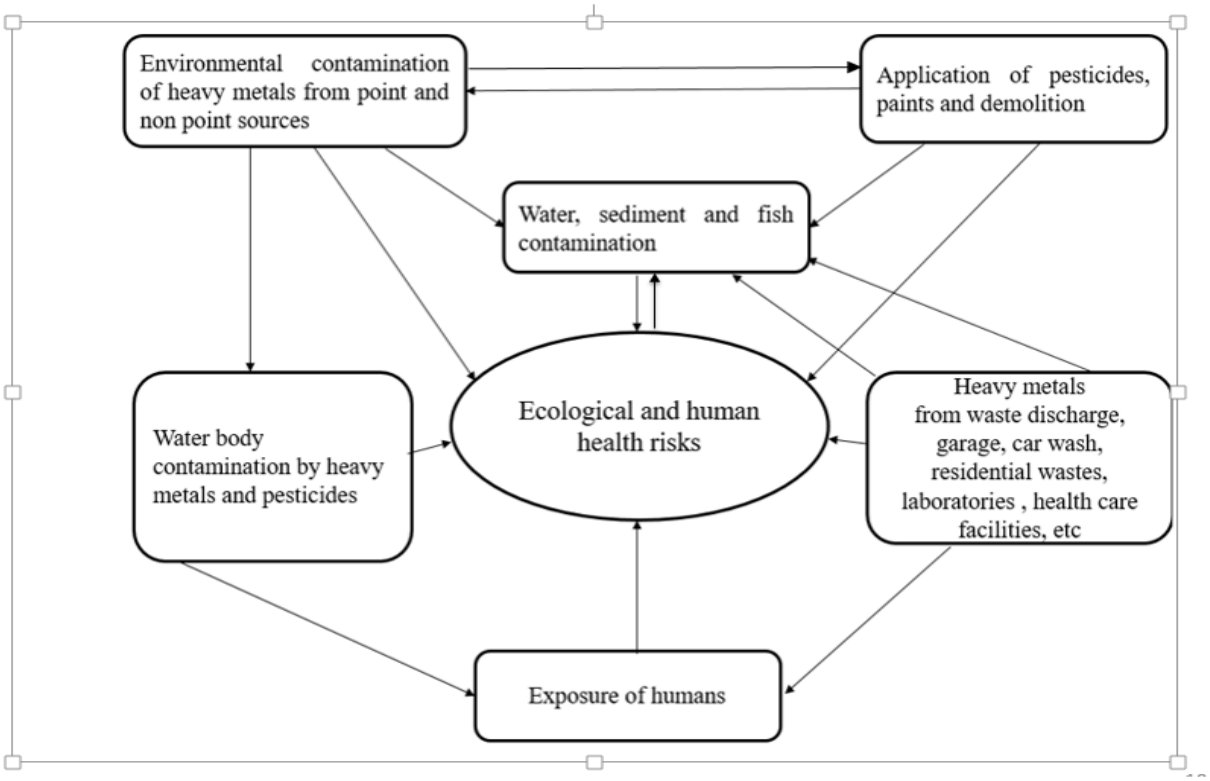

Figure 1. 1. Conceptual framework of the study

1.9 Outline of the PhD Thesis

The dissertation was organized into eight chapters. The background information, the concept of heavy metals, pollution sources of heavy metals, levels of heavy metals in the water, sediment, and fishes, and ecological and human health risks were presented in:

Chapter One: This chapter highlights the background information of heavy metals, pollution sources, and their effect on the environment. Furthermore, the statement of the problem, the rationale of the study, research questions pertinent to the research objectives, the significance of the study, conceptual framework, and outline of the PhD dissertation were described in this chapter.

Chapter Two: Describes the reviewed literature about nature, sources in the environment, toxicity in the environment, effects to the plants and humans, route of exposure, bio-uptake, and bioaccumulation in humans, and mechanisms of toxicity of heavy metals.

Chapter Three: Describes the materials and methods section. The description of the study area, sampling techniques and period, methods of data collection and analysis, including the employed software were presented in this chapter.

Chapter Four: Focuses on the sources and level of heavy metal contamination in the water of streams in the Awetu watershed, southwestern Ethiopia. The pollution sources of heavy metals in the watershed streams, the main anthropogenic activities responsible for heavy metal pollution, and the level of the contaminations in streams in the Awetu watershed are presented in this section (**Article one**).

Chapter Five: Focuses on heavy metal contamination of sediments from Awetu watershed of southwestern Ethiopia: a way of identifying pollution sources. The pollution sources of heavy metals in the watershed streams, the main anthropogenic activities responsible for heavy metal pollution, and the level of the contaminations of sediments in streams in the Awetu watershed are discussed in this chapter (**Article two**).

Chapter Six: Deals with ecological risks posed by heavy metals in the water and sediments in the Awetu watershed. The ecological risks of heavy metals in water and sediments of streams in the Awetu watershed were assessed using the modified degree of contamination, Nemerow pollution index, and ecological risk assessment indices. Moreover, the concentrations of heavy metals in the sediment were compared with sediment quality guidelines **(Article three).**

Chapter Seven: Deals with the concentrations of heavy metals in the two edible fishes harvested from the Gilgel Gibe I reservoir. The human health risks posed by heavy metals were also evaluated from the estimated daily intake of fishes, target hazard quotient, hazard index, and carcinogenic risk (**Article four**).

Chapter Eight: Based on the results and discussions of the previous chapters; a general discussion, strengths and limitations, conclusions, and recommendations have been synthesized. In the general discussion section, the overall heavy metals concentrations, major findings in each chapter were highlighted. The strengths and limitations section principally focuses on methodological aspects where possible strong sides and challenges were described. Conclusions consistent with the formulated objectives and major findings were presented. Finally, recommendations in terms of practical and future perspectives were presented. The practical recommendations included the scientific and policy implications, whereas the issues requiring further research were included in the future research perspective section.

Chapter Two: Literature Review

1.1 Heavy Metals

The term "heavy metal" refers to a chemical element having atomic weights between 63.5 and 200.6, and a specific gravity greater than 5.0 g/cm^3 relatively higher density than water (Tak et al. 2013; Ayangbenro and Babalola, 2017; Athar et al., 2018). Cadmium (Cd), chromium (Cr), Lead (Pb), and mercury (Hg) are categorized as toxic heavy metals. Heavy metals also include metalloids like arsenic, with the assumption that heaviness and toxicity are interrelated, which can induce toxicity even at the low level of exposure (Zeng et al., 2014; Baran and Tarnawski, 2015). Others including iron (Fe), copper (Cu), zinc (Zn), aluminum (Al), beryllium (Be), cobalt (Co), and manganese (Mn) are less commonly considered as heavy metals (Jaishankar et al., 2014).

According to Duffus (2002), the term "heavy metals" was reviewed as the user has developed and concluded the classification of metals and their compounds based on their chemical properties. Such a classification would permit interpretation of the biochemical basis for toxicity. It would also provide a rational basis for determining which metal ionic species or compounds are likely to be most toxic. In general, he reported that even if the term "heavy metal" should become obsolete because it has no coherent scientific basis, there will still be a problem with the common use of the term "metal" to refer to metal and all its compounds. This usage implies that the pure metal and all its compounds have the same physicochemical, biological, and toxicological properties.

Heavy metals are very reactive, may chemically or physically interact with the natural compounds, which changes their forms of existence in the environment (Dube et al., 2001) and bioaccumulate in the food web (Channa et al., 2013; Askari et al., 2017; Ayangbenro and Babalola, 2017). Cd, Pb, Hg, and As are widely dispersed in the environment but they have no beneficial effects in humans, and no known homeostasis mechanism to regulate them (Morais et al., 2012; Bhat et al., 2019) and are very toxic to humans and other animals even at low concentrations (Jaishankar et al., 2014). The adverse health effects to humans associated with heavy metals exposure at very low concentrations cause neurotoxic and carcinogenic actions (Muhammad et al., 2011; Jaishankar et al., 2014; Jan et al., 2015; Azeh Engwa et al., 2019).

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by heavy metals. Also, human exposure has risen dramatically

as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications (Nagajyoti et al., 2010; Morais et al., 2012; Al-Jaboobi et al., 2014).

1.2 Sources of Heavy Metals in the Environment

Heavy metals can be generated from natural and anthropogenic sources as shown in Figure. 2.1. Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources (Yuan et al., 2018; Zhang et al., 2018). Environmental pollution is very prominent in point source areas such as mining, foundries and smelters, and other metal-based industrial operations (Manzala et al., 2013; Liu and Ma, 2015; Pan et al., 2018). Heavy metals are naturally present in soil due to weathering process and mineral processing (Sörme and Lagerkvist, 2002; Wu et al., 2014) hence are present in rocks in different chemical forms which are recovered as minerals (Saha and Hossain, 2011; Zeng et al., 2014).

The main anthropogenic sources of heavy metals are industrial activities like mining, refining, smelting effluents, use of chemicals in agriculture, small scale industries, disposal of municipal waste in soil, excess and unsafe application of pesticides (Chopra et al., 2009; Athar et al., 2018) coal burning, petroleum combustion, textiles, plastics, wood preservation, micro-electronics and plants for paper processing and high tension lines (Arruti et al., 2010; Khan et al., 2013), industrial discharge, emissions, transportation, landfills, incineration refuse, traffic and open dumps (Aryal et al., 2017).

Effluents from different sources are the largest contributors to a higher concentration of metals in rivers, ponds, and lakes which consists of untreated or mechanical treated wastewater and materials from the filters of biological treatment plants and waste substances from sewage outfalls that are being discharged into water bodies (Zahra et al., 2014). Moreover, irrigating the crops with contaminated water by industrial effluents and sewage also contaminate soil and vegetables grown in the area (Bridge, 2004). These metals can exert negative effects on human health entering into the body by breathing in polluted air and by ingestion (drinking and eating) or by contact with skin (Kucharzewski et al., 2003).

The distribution of heavy metals in the atmosphere is monitored by the properties of these metals and by the environmental factors (Khlifi and Hamza-Chaffai, 2010). These heavy metals are bioaccumulative and they slowly enter into plants, animals, and human bodies through the air,

water, and by the progression of the food chain. The bioavailability of heavy metals is greatly influenced by sequestration, adsorption, temperature, and phase association. Chemical factors are also important regarding the bioavailability of metals which influence lipid solubility, partition coefficients of water, and complexation kinetics. Biological factors such as physiological and biochemical adaptation, interactions at the trophic level, and species characteristics are also significant factors for bioavailability and uptake of metals (Pilon-Smits, 2005; Kassaye et al., 2017).

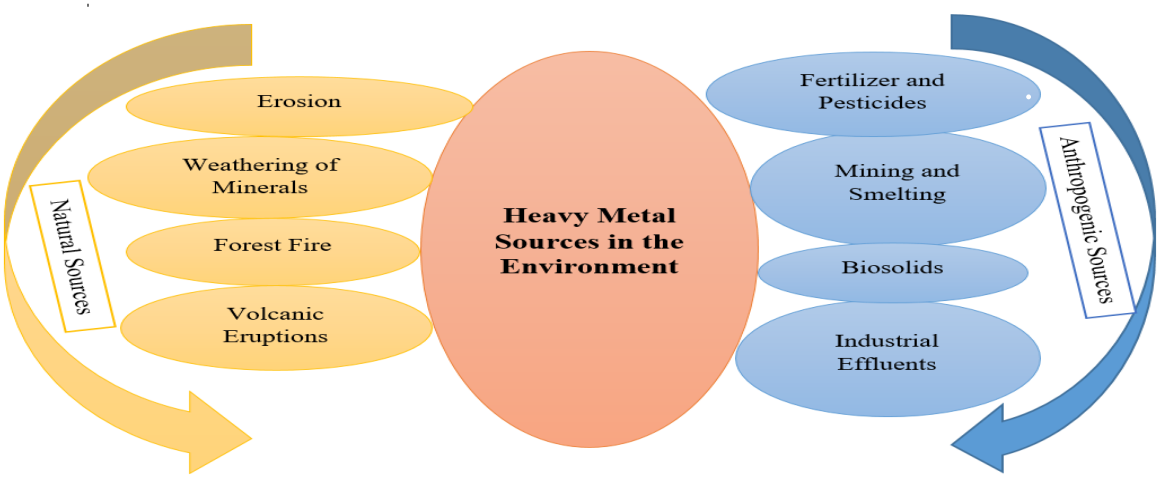

Figure 2. 1. Heavy metal sources in the environment

1.3 Toxicity Potential of Heavy Metals in the Environment

Heavy metals are significant environmental pollutants and their toxicity nature is regarded as the major threat and cause several health risks associated with it. This problem is increasing its significance for ecological, evolutionary, nutritional, and environmental reasons (Nagajyoti et al., 2010). Due to global development in industrial, economic, and agricultural sectors, new challenges are raised especially regarding environmental conservation and protection (He et al., 2005; Ikhuoria and Okieimen, 2000). Due to the industrial revolution, the mixing of heavy metals in soil has been dramatically increased and 90 % of emissions are due to anthropogenic activities that occurred since 1900 AD and now the level of these toxic compounds has increased significantly (Nriagu, 1992).

Heavy metals have vital biological functions with their minimum concentration in addition to their chemical coordination and oxidation-reduction properties. Sometimes they may act as pseudo-

elements of the body but even at certain times they may interfere with metabolic processes and get attached to the protein sites (Jaishankar et al., 2014). The displacement of the specific original metals from their natural binding sites causes the malfunctioning of the cells and ultimately causes toxicity (Bhat et al., 2019).

Heavy metal contamination may occur through atmospheric deposition, leaching, soil erosion, metal corrosion, natural volcanic eruptions, weathering, and metal evaporation from soil and groundwater and sediment re-suspension (Wong et al., 2002; Rule et al., 2006; Cicchella et al., 2016). When released into the environment these substances lead to many toxic effects in the food chain and also on living organisms by bio-magnification and bioaccumulation (Ahmed et al., 2009; Gupta et al., 2009; Deribe et al., 2014). This phenomenon has become a serious problem for all living organisms. Toxicity and heaviness are interrelated (Duffus, 2002), however, toxicity to high levels of heavy metals depends on systemic toxicants which cause multiple organ damage even after exposure to low levels and are classified as carcinogenic (Chung et al., 2016).

1.4 Sources of Heavy Metals Pollution

Heavy metals are naturally present in our environment in the atmosphere, lithosphere, hydrosphere, and biosphere (Sörme and Lagerkvist, 2002; Rule et al., 2006; Astatkie et al., 2021). Although these heavy metals are present in the ecosystem, humans are exposed to various anthropogenic activities (Lucchini et al., 2014; Kayastha, 2015). These heavy metals are present in rocks that are recovered during mining activities as minerals in the earth's crust (Grba et al., 2015). In most ores, heavy metals such as arsenic, iron, lead, zinc, gold, nickel, silver, and cobalt exist as sulfides while others such as manganese, aluminum, selenium, gold, and antimony exist as oxides. Certain heavy metals such as copper, iron, and cobalt can exist both as sulfide and oxide ores. During these mining activities, heavy metals are released from the ore and scattered in an open environment; left in the soil, transported by air and water to other areas. Furthermore, when these heavy metals are used in industries for various industrial purposes, some of these elements are released into the air during combustion or into the soil or water bodies as effluents. Industrial products such as paints, cosmetics, pesticides, and herbicides also serve as sources of heavy metals (Sörme and Lagerkvist, 2002; Wu et al., 2014). Aerial deposition of particles emitted by different human activities, including mining is also the known source of heavy metals in the environment

(Santos-Francés et al., 2017). Afterward, these heavy metals sourced from different environmental compartments may be transported through erosion, run-off, or acid rain to different locations on soils and water bodies

Arsenic (As) is a naturally occurring element that can exist in various allotropes. It is the 20th most abundant element in nature, the 14th most common in seawater, and the 12th most abundant in the human body (Madhukar et al., 2016). Arsenic can be found in over 200 different minerals, the most prevalent of which is arsenopyrite (AsFeS). For its mutagenic, carcinogenic, and teratogenic properties, the name "arsenic" generates a horrifying reaction, and it is dubbed "the king of poisons." Arsenic can enter the human body in a variety of ways, and just a little amount of persistent exposure is required to cause obvious hazardous symptoms. According to the WHO, the acceptable concentration of arsenic in drinking water is 0.01mg/L and the maximum limit of untreated water before being treated for consumption is 0.1mg/L (Li et al., 2013). For an average middle-aged person, the maximum safe limit of arsenic ingestion is 0.22 mg/day (Chatterjee et al., 2017). Humans and other species in the environment are affected by inorganic forms such as arsenite and arsenate compounds. Arsenic enters the body via industrial applications such as smelting and microelectronics. Arsenic from wood preservatives, herbicides, insecticides, fungicides, and paints may contaminate drinking water (Bhattacharya et al., 2007; Smith and Steinmaus, 2009; Sauvé, 2014; Gergen et al., 2015; Bencko and Foong, 2017).

Cadmium (Cd) is a soft, silver-white metal that is found in the earth's crust in the form of zinc, leads, and copper ores. Cadmium chloride and cadmium sulfate are two other water-soluble cadmium compounds (Radulescu et al., 2014). Cadmium is primarily used in the manufacture of paints, pigments, alloys, coatings, batteries, and plastics in the industry (Ismail et al., 2013; Sharma et al., 2015; Yuan et al., 2018). Cadmium makes up three-quarters of the electrode component in alkaline batteries. Cadmium is discharged into the soil, water, and air through industrial operations and from cadmium smelters into sewage sludge, nonferrous metal mining and refining, phosphate fertilizer manufacturing and application, fossil fuel combustion, and waste incineration and disposal (Dixit et al., 2015; Sharma et al., 2015; Astatkie et al., 2021). Cadmium can persist in soils and sediments for decades, accumulating in groundwater and being absorbed by aquatic organisms and plants. As a result, significant human exposure to cadmium can happen through ingestion of contaminated food and beverages, specifically cereals, grains, fruits, and leafy vegetables (Godwill et al., 2015; Unaegbu et al., 2016). Inhalation of cadmium-contaminated

municipal waste, dust, and fumes, as well as inadvertent ingestion of contaminated hands, foods, or cigarettes, are the possible sources of cadmium exposure for humans (Bernard, 2008; Ismail et al., 2013; Basim and Khoshnood, 2016).

Chromium (Cr) is a metal found in petroleum and coal, as well as chromium steel, pigment oxidants, fertilizers, catalysts, oil well drilling, and tanneries for metal plating. Chromium is widely utilized in a variety of industries, including wood preservation, electroplating, metallurgy, paint and pigment manufacturing, chemical manufacturing, tanning, and pulp and paper manufacturing (Zayed and Terry, 2003; Vengosh et al., 2016; Alemu and Gabbiye, 2017). These industries contribute significantly to chromium pollution, which has a detrimental effect on biological and ecological species (Gautam et al., 2014; Mengistie et al., 2016). As a result of human anthropogenic activities, like sewage disposal and fertilizer use, chromium may be released into the environment (Gautam et al., 2014). As a result, these industrial and agricultural practices contribute to chromium contamination in the environment. In recent years, chromium pollution has primarily been caused by hexavalent chromium (Zayed and Terry, 2003; Elangovan et al., 2008; Vengosh et al., 2016).

Copper (Cu) is a heavy metal used to produce copper pipes, cables, wires, copper cookware, copper intrauterine devices, and birth control pills. (Mohiuddin et al., 2011; He et al., 2018). Copper, in the form of copper sulfate, is added to drinking water and swimming pools to avoid algae growth, but an overdose may cause brain and kidney damage (He et al., 2018). It can build in the soil and be taken up by plants as a result of man's anthropogenic and industrial activities. Copper may be found in a variety of foods, including almonds, avocados, wheat germ, and bran. Copper was mostly coupled to organic matter and sulfides, especially in sediments, where the oxidizable component had the highest percentage. Because organic Cu compounds have high stability constant, they form stable complexes with organic materials (Cuong and Obbard, 2006). Cu is a chalcophile element that is mostly associated with sulfides in nature, and it has been demonstrated in other research that its solubility increases under oxidizing circumstances (Hamdaoui, 2009), while the organic fraction accounts for a considerable amount of copper in sediments (Singh et al., 2011).

Lead (Pb), in a dry atmosphere, is a slightly bluish, bright silvery metal. Drinking water, food, cigarette, industrial processes, and domestic sources are the most common sources of lead

exposure (Patrick, 2006; Abarikwu, 2013). Gasoline, house paint, plumbing pipes, lead bullets, storage batteries, pewter pitchers, toys, and faucets all are industrial sources of lead (Thürmer et al., 2002). Industrial operations, as well as automobile emissions, discharge lead into the atmosphere. As a result, it may enter the soil and pass into water bodies, where it can be absorbed by plants, exposing humans to lead through food or drinking water (Wani et al., 2015). Workers in the lead smelting and refining industries, battery manufacturing plants, steel welding or cutting operations, construction, rubber products, and plastics industries, printing industries, firing ranges, radiator repair shops, and other industries that require flame soldering of lead solder are exposed on the job (Patrick, 2006; Adela et al., 2012; Getaneh et al., 2014). Inhalation and ingestion of lead-bearing dust and fumes are the main pathways of lead exposure in these activities (Abarikwu, 2013; Wani et al., 2015). Mean lead concentrations in the air can reach 4,470 g/m^3 during lead smelting and refining; mean airborne lead concentrations of 50 to 5,400 g/m^3 have been recorded during storage battery manufacturing; and an average lead concentration of 1, 200 g/m^3 has been found in the breathing zone of structural steel welders (Patrick, 2006).

Manganese (Mn) is a naturally occurring element that can be found in the air, soil, and water in plenty. Manganese is a critical component of human and animal bodies that are obtained primarily from food and water (US EPA, 2004; Li and Yang, 2018). Inadequate intake or overexposure may result in adverse health effects. Manganese is mostly ingested by humans as a result of the consumption of food. Manganese deficiency in humans appears to be unusual, as manganese is found in a variety of everyday diets (Cima, 2019a). Manganese is required by numerous cellular enzymes and can serve to activate many other enzymes, making it necessary for the proper functioning of humans and other animals (Li and Yang, 2018). Manganese is a necessary nutrient in small levels, but high doses can be hazardous over time. Although there is substantial evidence for the neurological consequences of inhaled manganese in both people and animals, there is limited evidence for the link between oral manganese intake and harmful effects (US EPA, 2012). As Methylcyclopentadienyl manganese Tricarbonyl, this metal is added to gasoline, and so gasoline fumes contain a highly toxic form of manganese (US EPA, 2004; Length, 2007).

Metallic mercury (Hg) is a shining silver-white, odorless liquid metal that, upon heated, turns into a colorless and odorless gas (Huo et al., 2012). Dental amalgams, thermometers, and some batteries contain mercury (Crane et al., 2000; Azeh Engwa et al., 2019). It can be found in the chemical, electrical equipment, automotive, metal-processing, and construction industries, and it

can be inhaled as a gas (Wang et al., 2004). Other anthropogenic activities which cause mercury pollution in humans are municipal wastewater discharges, agriculture, incineration, mining, and industrial wastewater discharges (Guzzi and La, 2008; Syversen and Kaur, 2012; Rahimzadeh et al., 2017).

Nickel is a lustrous white, hard, ferromagnetic metal and occurs naturally in five isotopic forms: 58 (67.8 %), 60 (26.2 %), 61 (1.2 %), 62 (3.7 %), and 64 (1.2 %) (WHO, 2007). Pure nickel is a hard, silvery-white metal with qualities that make it ideal for alloying with other metals (ASTDR, 2005). It's used to make batteries, nickel-plated jewelry, machine components, nickel plating on metallic objects, steel, cigarette smoking, wire, and electrical parts, among other things. Imitation whip cream, unprocessed grains and cereals, commercial peanut butter, hydrogenated vegetable oils, and tainted alcoholic beverages are all examples of foods that include it (Jaishankar et al., 2014). It is released into the air by power plants and trash incinerators (Genchi et al., 2020) will then settle to the ground or fall after reactions with raindrops (Cempel and Nikel, 2006). Nickel is normally eliminated from the air over a long time. When nickel is present in the water streams, it might end up in surface water. The majority of nickel compounds released into the environment will adsorb to sediment or soil particles, rendering them immobile. In the acidic ground, however, nickel is bound to become more mobile and it will often rinse out to the groundwater (Cempel and Nikel, 2006; Genchi et al., 2020).

Tin is a soft, flexible, silvery-white metal that does not quickly rust and resists corrosion thanks to an oxide covering that protects it. It occurs naturally in the Earth's crust at a quantity of about 2-3 mg/kg on average (Boehncke et al., 1999; Ashraf et al., 2012). Tin compounds, both inorganic and organic, can be found in a variety of environmental media. Tin can be found in the environment from both natural and man-made sources. Many soils contain tin, and inorganic tin compounds can be found in dust from windstorms, roadways, and agricultural activities, as well as forest fires and volcanic emissions (Ashraf et al., 2012). Tin releases to the environment can occur as a result of the production, use, disposal, and recovery of tin and tin compounds (Boehncke et al., 1999). The tinfoil was originally a popular food and drug wrapping material, but it has since been superseded by aluminum foil. Tin is mostly used in diverse organic chemicals, with organic tin bonds being the most hazardous to humans (Edokpayi et al., 2016). Despite the risks, they are used in a variety of industries, including the paint and plastic industries, as well as agriculture through pesticides. Even though we are aware of the dangers of tin poisoning, the number of applications

for organic tin substances continues to grow. Organic tin substances have a wide range of effects that are dependent on the type of material present and the organism that is exposed to it. For humans, triethyl tin is the most hazardous organic tin compound (Embiale et al., 2020). It possesses short hydrogen bonds, and as these connections lengthen, a tin compound becomes less hazardous to human health (Lehmler et al., 2018). Tin connections can be absorbed by humans through food, respiration, and skin. The uptake of tin bonds might have both immediate and long-term consequences (Cima, 2019b).

Zinc is a bluish-white, shiny metal that is found in abundance in the environment, with an average concentration of 70 mg/kg in the earth's crust (US EPA, 2010). Around room temperature, it is brittle, but at 100 to 150 °C, it becomes flexible. It is a good conductor of electricity and burns in the air at high red heat, resulting in the formation of oxides in the form of white clouds. Zinc metal is not found naturally; rather, it is found in the +2 oxidation state as sphalerite, smithsonite, and zincite, among other minerals (Dopp and Rettenmeier, 2013). To quantify environmental exposure levels for other metals including cadmium and lead, physiologically based toxicokinetic models have been created. However, no toxicokinetic models for zinc have been created in humans or animals. In the human body, zinc is one of the most prevalent nutritionally necessary minerals (US EPA, 2010). Zinc is found in all organs, tissues, fluids, and body secretions, with skeletal muscle and bone accounting for 86 % of its mass, the skin for 6 %, the liver for 5 %, the brain for 1.5 percent, and the rest dispersed among the other tissues (US EPA, 2010; Levy et al., 2017).

1.5 Route of Exposure, Bio-uptake, and Bioaccumulation of Heavy Metals to Humans

Humans can come into direct contact with heavy metals via eating contaminated foods, drinking contaminated water, inhaling polluted air as dust fumes, or being exposed to them at work (Sörme and Lagerkvist, 2002; Shankar et al., 2014; Yuan et al., 2018). Heavy metal contamination frequently follows this cyclic pattern: from industry to the atmosphere, soil, water, and foods, and finally to humans (Vukovic et al., 2011; Fan et al., 2013; Hameed et al., 2014). These heavy metals can be acquired in a variety of ways. Heavy metals including lead, cadmium, manganese, and arsenic can enter the body through the gastrointestinal tract, or through the mouth, while eating meals, fruits, or vegetables, or when drinking water or other liquids (EC, 2002; Zhao and Babatunde, 2011; Kim, 2012; Chatterjee et al., 2017). Others can be inhaled into the body, while others, like lead, can be absorbed via the skin (US EPA, 2007; Liu and Shi, 2001; Al-Jaboobi et

al., 2014). The majority of heavy metals are transported throughout the body via the bloodstream to the tissues (Florea and Büsselberg, 2006). Lead is taken to the liver and kidney by red blood cells and then redistributed as a phosphate salt to the teeth, bone, and hair (Yuan et al., 2018). Cadmium attaches to blood cells and albumin first, and then to metallothionein in kidney and liver tissue (Bernhoft, 2013; Sharma et al., 2015). Manganese vapor diffuses across the lung membrane to the central nervous system after being transported from the blood to the lungs. Manganese salts that are lipid-soluble are dispersed in the colon for fecal elimination, while water-soluble inorganic manganese salts are distributed in the plasma and kidneys for renal elimination (US EPA, 2004; Li and Yang, 2018). Arsenic is transported through the bloodstream and accumulates in the heart, lungs, liver, kidney, muscle, and neural tissues, as well as the skin, nails, and hair (Navratilova et al., 2013; Askari et al., 2017). Table 2.1 below shows the regulatory limit for selected heavy metals.

Table 2. 1. Regulatory limits of heavy metals in different organizations

Heavy metals	EPA limits in drinking water (mg/L)	OSHA limits in workplace air averaged over an 8-hour (mg/m^3)	FDA limits in bottled water/food (mg/L)
Arsenic	0.01	0.01	0.01
Cadmium	0.005	0.005	0.005
Chromium	0.1	0.005	0.1
Copper	1.3	0.1	1.0
Lead	0.015	0.05	0.005
Manganese	0.05	5	0.1
Mercury	0.002	0.1	0.002
Nickel	0.1	0.5	0.1
Tin	0.002	2	-
Zinc	5	5	5

mg/L- milligram per liter; mg/m^3-milligram per meter cube; EPA-Environmental Protection Agency; OSHA-Occupational Safety and Health Administration; FDA-Food and Drug Administration

1.6 Mechanism and Health Effects of Heavy Metals on Humans

Heavy metals are naturally occurring elements in the ecosystem. They are high-conductivity substances that give up their electrons to create cations on their own. Metals can accumulate in living organisms such as plants and animals and can be found in the atmosphere, the earth's crust, and water bodies. Among the 35 natural existing metals, 23 possess a high specific density above

5 g/cm^3 with atomic weight greater than 40.04 and are generally termed heavy metals (Duffus, 2002; Li et al., 2017). These metals generally termed heavy metals include: antimony (Sb), tellurium (Te), bismuth (Bi), tin (Sn), thallium (Tl), gold (Au), arsenic (As), cerium (Ce), gallium (Ga), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), mercury (Hg), manganese (Mn), nickel (Ni), platinum (Pt), silver (Ag), uranium (U), vanadium (V), and zinc (Zn) (Li et al., 2017; Azeh Engwa et al., 2019). Heavy metals are a class of metals that are renowned not only for their high density but also for their adverse effect on the ecosystem and living organisms (Li et al., 2017). Some of these heavy metals such as Co, Cr, Cu, Mg, Fe, Mo, Mn, Se, Ni, and Zn are essential nutrients that are required for various physiological and biochemical functions in the body and may result in deficiency diseases or syndromes if not in adequate amounts (WHO, 1996) but in large doses, they may cause acute or chronic toxicities. Several natural processes, such as volcanic eruptions, spring waters, erosion, and bacterial activity, as well as manmade activities, such as fossil fuel burning, industrial operations, agricultural activities, and feeding, spread heavy metals throughout the environment (Bradl et al., 2005). Through various processes, heavy metals bioaccumulate in living organisms and the human body, causing adverse health effects. Heavy metals are carried and compartmentalized into body cells and tissues, when they bind to proteins and nucleic acids, damaging them and altering their cellular functions. Heavy metal toxicity has a series of adverse health effects on the human body. It can harm blood constituents, lungs, liver, kidneys, and other critical organs, resulting in mental disorders, as well as cause damage to blood constituents, lungs, liver, kidneys, and other vital organs, resulting in a variety of disease conditions (Jaishankar et al., 2014). In addition, long-term heavy metal accumulation in the body may impede the advancement of physical, muscular, and neurological degenerative processes that mirror diseases like Parkinson's and Alzheimer's (Bradl et al., 2005; Jaishankar et al., 2014). Furthermore, long-term exposure to some heavy metals or their compounds can damage nucleic acids, induce abnormalities, mimic hormones, disturb the endocrine and reproductive systems, and ultimately lead to cancer (Järup, 2017). The various mechanisms that lead to heavy metal toxicity will be given more attention, with a focus on macromolecule and cellular damages, carcinogenesis, neurotoxicity, and the molecular foundation for their noxious effects. Free radicals are known to be formed by several heavy metals, which can cause oxidative stress and other cellular damage. The mechanism of free radical formation in heavy metal is peculiar to its kind (Valko et al., 2005; Gergen et al., 2015; Tchounwou et al., 2012). The

various toxic effects of heavy metals in the human body will be discussed, as well as their signs and symptoms.

Acute or chronic toxicity can result from arsenic exposure. Acute arsenic poisoning can cause damage to blood vessels, gastrointestinal tissue, and the heart and brain, but chronic toxicity, also known as arsenicosis, affects the skin and causes pigmentation and keratosis (US EPA, 2001; Muhammad et al., 2010). Arsenic poisoning can produce nausea and vomiting, as well as a decrease in the production of erythrocytes and leukocytes, damage to blood vessels, an irregular heartbeat, and a prickling feeling in the hands and legs. Skin lesions, lung disease, neurological issues, peripheral vascular disease, diabetes mellitus, hypertension, and cardiovascular disease can also result from long-term contact (Hong et al., 2014). Chronic arsenicosis can cause irreversible alterations in vital organs and, in extreme situations, death. Chronic arsenic exposure has also been linked to the development of cancers such as skin cancer, bladder cancer, lung cancer, liver cancer, and possibly colon and kidney cancers (Zhao and Babatunde, 2011; Hong et al., 2014).

Epigenetic changes and damage to the dynamic DNA maintenance system are among the carcinogenic processes generated by arsenic. Arsenic binds to DNA-binding proteins, disrupting DNA repair pathways and raising the risk of cancer. When arsenic is linked to methyl-transferase, the tumor suppressor gene-coded DNA was suppressed (Bhattacharya et al., 2007; Brandi and Wilson-Wilde, 2012; Rango et al., 2013).

Cadmium has been implicated in promoting apoptosis, oxidative stress, DNA methylation, and DNA damage (Sarkar et al., 2013; Azeh Engwa et al., 2019). Cadmium and its compounds have a variety of health effects in humans, and the inability of the human body to eliminate cadmium exacerbates the health effects of cadmium exposure. Cadmium is re-absorbed by the kidney thereby limiting its excretion. Short-term inhalation of cadmium can result in severe lung damage and respiratory irritation, while ingestion of a greater quantity can result in stomach discomfort, vomiting, and diarrhea. Long-term cadmium exposure causes cadmium deposition in the bones and lungs, which can lead to bone and lung disease (Pedersen et al., 2003; Zhuang et al., 2008; US DHHS, 2012). Cadmium has been linked to bone mineralization in both animals and humans, resulting in osteoporosis (skeletal damage). The “Itai-itai” disease, an epidemic of bone fractures in Japan, has been linked to cadmium exposure (Muhammad et al., 2011; Gaur et al., 2014; Rahman and Singh, 2019). Cadmium is highly toxic to the kidneys, and it accumulates in larger

concentrations in the proximal tubular cells. As a result, cadmium exposure can result in renal dysfunction and illness. Cadmium poisoning can also lead to calcium metabolism problems, the production of kidney stones, and hypercalciuria. Cadmium is also designated by the International Agency for Research on Cancer as a group 1 carcinogen for humans. Smokers are more prone to cadmium intoxication than non-smokers because tobacco is the main source of cadmium uptake in smokers (Bernard, 2008; Tchounwou et al., 2012; Bernhoft, 2013; Sharma et al., 2015).

Chromium is the most toxic species of chromium in its hexavalent form, however other species, such as Chromium (III) compounds, are far less toxic and produce little or no health effects. Chromium (VI) is toxic to the body and can also induce allergic reactions. As a result, inhaling excessive levels of chromium (VI) can irritate the nasal lining and cause nose ulcers. It can also harm sperm and the male reproductive system, causing anemia, irritations, and ulcers in the small intestine and stomach. Chromium-induced allergic responses include significant skin redness and edema. Exposure of extremely high doses of chromium (VI) compounds to humans can result in severe cardiovascular, respiratory, hematological, gastrointestinal, renal, hepatic, and neurological effects and possibly death (Amir et al., 2011; Ali et al., 2013; Gajere, 2015). Cr particularly Cr^{4+} has been shown in in vitro studies to generate free radicals from H_2O_2 (Liu and Shi, 2001). In vivo studies also revealed the presence of chromium-induced free radicals in the liver and blood of animals. The one-electron reduction resulted in the formation of Cr^{5+} intermediates, which were observed. Exposure to chromium compounds can cause ulcers, including nasal septum ulcers, which are frequent among chromate workers. In vivo and in vitro experiments have shown chromate compounds to induce DNA damage in many different ways and can lead to the formation of DNA adducts, chromosomal aberrations, alterations in replication sister chromatid exchanges, and transcription of DNA. In vivo and in vitro studies have revealed that chromate chemicals can cause DNA damage in a variety of ways, including the creation of DNA adducts, chromosomal abnormalities, replication sister chromatid exchange modifications, and DNA transcription (Tchounwou et al., 2012; Bernhoft, 2013). As a result, there is strong evidence that chromium promotes human carcinogenicity, as animals and humans exposed to chromium (VI) in drinking water have developed more stomach tumors.

Copper ions are involved in the creation of reactive oxygen species such as cupric (Cu^{2+}) and cuprous (Cu^{1+}), which can be used in oxidation and reduction reactions. Cu^{2+} can be reduced to Cu^{+} in the presence of biological reductants such as glutathione (GSH) or ascorbic acid, which is

capable of initiating the Fenton reaction, which catalyzes the breakdown of H_2O_2 to generate $OH^{\bullet}$ (Azeh Engwa et al., 2019).

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\bullet} + OH^{-}$$

The $OH^{\bullet}$ radical form is capable of reacting with several biomolecules. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases via oxygen free radicals (Azeh Engwa et al., 2019). Though in vivo studies have not revealed copper-induced oxidation of low-density lipoprotein (LDL), in vitro studies demonstrated LDL oxidation induced by copper (Burkitt, 2001).

Lead poisoning is toxicity caused by lead exposure that affects primarily the gastrointestinal tract and central nervous system in both children and adults (Reda and Ayu, 2016). Lead poisoning can be either acute or chronic. Acute exposure to lead can cause headache, loss of appetite, abdominal pain, fatigue, sleeplessness, hallucinations, vertigo, renal dysfunction, hypertension, and arthritis while chronic exposure can result in birth defects, mental retardation, autism, psychosis, allergies, paralysis, weight loss, dyslexia, hyperactivity, muscular weakness, kidney damage, brain damage, coma and may even cause death (Patrick, 2006; Flora et al., 2012; Wani et al., 2015). Although lead poisoning is preventable, it is nevertheless a severe disease that can harm almost all of the body's organs. Lead toxicity affects the brain's memory and learning processes and is mediated by three different mechanisms (Patrick, 2006; Wani et al., 2015; US DHHS, 2020). Lead can impair learning and memory in the brain by inhibiting the N-methyl-d-aspartate receptor (NMDAR) and can block neurotransmission by inhibiting neurotransmitter release, block the neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs), and reduce the expression of brain-derived neurotrophic factor (BDNF) (Asere et al., 2013; Azeh Engwa et al., 2019). The generation of reactive oxygen species, that changes chromosomal structure and sequence, is believed to cause DNA damage, disrupt the DNA repair system, and disrupt cellular tumor regulatory genes in the lead-induced carcinogenic process (Flora et al., 2012; Abarikwu, 2013; Wani et al., 2015). Lead can disrupt transcription processes by replacing zinc in certain regulatory proteins (Jaishankar et al., 2014; Authman, 2015; Singh et al., 2017). Exposure to elevated levels of lead can cause the plasma membrane of the blood-brain barrier to move into the interstitial spaces leading to edema (Brochin et al., 2008; Abarikwu, 2013). Also, lead exposure can disrupt the intracellular second messenger systems and alter the functioning of the central nervous system. Developing fetuses and children

are most vulnerable to neurotoxic effects due to lead exposure. Many prospective epidemiologic studies in children less than 5 years of age have shown that low-level of lead exposure (5 - 25 μg/dL in the blood) resulted in the impairment of intellectual development which was manifested by the loss of intelligence quotient points (Chowdhury et al., 2016; Azeh Engwa et al., 2019). As such, the Centers for Disease Control (CDC) in the United States has reduced the tolerable amount of lead in children's blood from 25 to 10 μg/dL and recommended universal screening of blood lead for all children (Azeh Engwa et al., 2019).

Manganese is a vital element for the human body, but it has recently become a source of concern due to the poisonous methylcyclopentadienyl manganese Tricarbonyl (MMT) that was added as a gasoline additive. MMT has been associated with the development of a Parkinson's disease-like syndrome of tremor, gait disturbance, postural instability, and cognitive problem and has been reported to be an occupational manganese hazard (US EPA, 2004; Jaishankar et al., 2014; Anyanwu et al., 2018). It is also known to accumulate in the mitochondria of neurons, astrocytes, and oligodendrocytes cells, disrupting ATP synthesis by blocking the mitochondrial respiration chain's F1/F0 ATP synthase or complex 1 (NADH dehydrogenase) (Nwaichi and Dhankher, 2016; Azeh Engwa et al., 2019). Manganese disrupts ATP synthesis, resulting in lower intracellular ATP levels and the production of free radicals, thus increasing oxidative stress and contributing to manganese cellular toxicity (Li and Yang, 2018).

Mercury may easily mix with other elements to generate inorganic and organic mercury. Metal, inorganic, and organic mercury exposure can harm the kidneys, brain, and growing fetus, while methyl mercury is highly carcinogenic (Matta and Gjyli, 2016; Sharma and Singh, 2016; Kobal et al., 2017). Mercury is an element that quickly reacts with other elements to produce inorganic and organic mercury. Metal, inorganic, and organic mercury at high amounts can harm the kidneys, brain, and growing fetus, while methyl mercury is highly carcinogenic (US EPA, 2010; Johnson, 2018). Short-term exposure to metallic mercury vapors at higher levels can lead to vomiting, nausea, skin rashes, diarrhea, lung damage, high blood pressure, etc. while short-term exposure to organic mercury poisoning can lead to depression, tremors, headache, fatigue, memory problems, hair loss, etc. Since these symptoms are also common in other illness or disease conditions, diagnosis of mercury poisoning may be difficult in such cases (US EPA, 2010; Johnson, 2018; Azeh Engwa et al. 2019). Excessive mercury exposure over time can cause erethism, a disorder marked by excitability, hand tremors, memory loss, shyness, and insomnia. Researchers have

linked occupational mercury exposure to measurable decreases in performance on neurobehavioral measures of motor speed, visual scanning, visuomotor coordination, and verbal and visual memory (Johnson, 2018). Dimethyl mercury is a highly poisonous chemical that can permeate the skin through latex gloves, causing central nervous system degeneration and death at very low doses. Mercury exposure during pregnancy can harm the baby, resulting in mental retardation, cerebellar symptoms, retention of primitive reflexes, deformity, and other abnormalities in the progeny (Azeh Engwa et al., 2019).

Human exposure to highly nickel-polluted environments may cause a variety of pathological effects (ASTDR, 2005; Cempel and Nikel, 2006). Chronic bronchitis, impaired lung function, and lung and nasal sinus cancer have all been linked to nickel exposure while working in nickel refineries or processing plants (Cempel and Nikel, 2006; Genchi et al., 2020). Chronic exposure to nickel and its constituents in the human body has been linked to lung fibrosis, kidney and cardiovascular illness, and cancer of the respiratory tract (Tariq et al., 2016). Nickel in soluble and insoluble compounds is classified as Group 1 (carcinogen to humans) by the International Agency for Research on Cancer (IARC), while nickel and alloys are classified as Group 2B by the IARC (possibly carcinogenic to humans) (ATSDR, 2005; Das et al., 2009). Nickel and nickel compounds' potential toxicity is determined by their physicochemical properties, as well as the amount, duration, and route of exposure. Nickel and its compounds can enter the human body through inhalation, ingestion with food, and dermal absorption; however, the riskiest route of exposure to nickel is by inhalation (Beyersmann and Hartwig, 2008).

Nickel has an extensive range of carcinogenic mechanisms which include regulation of transcription factors, controlled expression of certain genes, and generation of free radicals. It was also shown to be implicated in regulating the expression of specific long non-coding RNAs, certain mRNAs, and microRNAs. Nickel can enhance promoter methylation and trigger the downregulation of maternally expressed gene 3 (MEG3), resulting in the upregulation of hypoxia-inducible factor-1, two proteins linked to cancer. Nickel has also been shown to produce free radicals, which contribute to carcinogenic processes (Beyersmann and Hartwig, 2008; Shukla, 2011; Genchi et al., 2020).

Tin compounds, both inorganic and organic, can be absorbed to a degree through inhalation, ingestion, or skin penetration. By either of these pathways, organotin compounds are more readily

absorbed than inorganic compounds, with absorption rising with increasing degrees of alkylation. Tin that has been absorbed is dispersed throughout the body. Tin was identified in kidney, liver, lung, brain, and bone tissue in postmortem samples, with the highest concentration in the latter. There hasn't been any research into the metabolism of inorganic tin compounds. According to animal and in vitro investigations, organic tin compounds are dealkylated/deacylated in sequence. Dealkylation products can be hydroxylated and/or glutathione conjugated, and then metabolized to mercapturic acid derivatives. Tin is excreted mostly through feces and, to a lesser amount, urine. The majority of tin compounds, especially inorganic species, are quickly excreted, but small amounts can be retained in bones for up to a few months. While both inorganic and organic tin compounds can pass the placenta, there is no evidence that these compounds can be absorbed by nursing (ATSDR, 2005; Dopp and Rettenmeier, 2013; Khaliq et al., 2014).

The interactions of the alkyl and aryl moieties with cell membranes, as well as the intracellular reactivity of the alkyl tin cation, cause organotin compounds to be hazardous. Ca^{2+}-dependent and Ca^{2+}-independent reactions are the two types of reactions. Organotin chemicals disrupt Ca^{2+} homeostasis by increasing free intracellular Ca^{2+} concentration, which alters signaling pathways, triggers apoptosis, and promotes cytoskeletal and nuclear protein depolymerization and disintegration, among other things (Butler et al., 2014).

The acute toxicity of inorganic tin compounds is rather low. Following ingestion of canned food contaminated by tin, nausea, vomiting, and diarrhea have been reported. Stomachaches, anemia, and liver and kidney disorders may occur if larger amounts of inorganic tin are ingested. Neurological symptoms (headache, photophobia, altered consciousness, and convulsions) occurred about four days after the intoxication and partially continued in the surviving patients for several years. There are only a few reports on chronic toxic effects of inorganic tin compounds. Chronic inhalation exposure to stannic oxide dust or fumes may cause stannosis, a benign form of pneumoconiosis. Gastrointestinal symptoms may occur after repeated ingestion of inorganic tin compounds. Signs of anemia and gastrointestinal distension are effects observed after chronic exposure of animals to inorganic tin compounds (Nagano et al., 2011).

Zinc appears to be absorbed by both passive diffusion and a saturable carrier-mediated process (US EPA, 2010). The carrier-mediated mechanism appears to be most important at low zinc levels and involves a saturable cysteine-rich intestinal protein (CRIP) (Harmaza and Slobozhanina,

2014). CRIP binds zinc and may act as an intracellular zinc carrier during transmucosal transport. There's additional evidence that CRIP competes with metallothionein for zinc binding. CRIP's zinc-binding ability is limited, and at high zinc concentrations in the intestine, CRIP becomes saturated (US EPA, 2010; Harmaza and Slobozhanina, 2014). At increasing zinc concentrations, metallothionein may play a role in zinc homeostasis. Increased zinc levels, as well as other heavy metals, cause an increase in metallothionein production. Although the precise role of metallothionein in zinc absorption is unknown, it is considered to control zinc availability by sequestering it in intestinal mucosal cells, inhibiting absorption and providing an escape route for excess zinc as these cells are shed and expelled in the feces. As zinc enters the cells of the intestinal mucosa, it is initially associated with CRIP, with only a small fraction binding to metallothionein; however, as zinc concentrations rise, the binding to CRIP becomes saturated, the proportion of zinc bound to CRIP decreases, and more zinc is bound to metallothionein.

Metal fume fever has unknown causes, however, it is assumed to be caused by a combination of variables. Zinc oxide particles have been demonstrated to cause the production of many proinflammatory cytokines, resulting in a chronic pulmonary inflammation that may cause some of the symptoms of metal fume fever, such as impaired lung function and bronchoconstriction. A hypothesized cause is an allergic reaction to zinc particles, which results in an asthma-like response. However, further mechanistic evidence will be needed to fully understand the mechanisms underlying inhaled zinc toxicity (Harmaza and Slobozhanina, 2014).

1.7 Heavy Metal-Induced Carcinogenesis

Heavy metal is known to cause cancer in some people. Heavy metals target some signaling proteins and cellular regulatory proteins involved in apoptosis, cell cycle regulation, DNA repair, DNA methylation, cell proliferation, and differentiation (Jaishankar et al., 2014). As a result, heavy metals may cause cancer by targeting a variety of these proteins. Certain heavy metals' carcinogenic effects have been linked to the activation of redox-sensitive transcription factors such as AP-1, NF-B, and p53 via the antioxidant network's electron recycling. These transcription factors regulate the production of protective genes that cause apoptosis, stop injured cells from proliferating, repair damaged DNA, and provide energy to the immune system (Valko et al., 2005; Morcillo et al., 2016; Azeh Engwa et al., 2019). In the mitogen-activated protein (MAP) kinase pathways, metal signaling of transcription factors AP-1 and NF-B has been reported. The nuclear

transcription factor NF-B is important in modulating inflammatory responses, while AP-1 is involved in cell growth and differentiation (Valko et al., 2005; Patra et al., 2011). The p53 protein is important in cell division as it guards a cell-cycle checkpoint and control cell division (Valko et al., 2005; Azeh Engwa et al., 2019). Inactivation of p53 allows uncontrolled cell division and thus p53 gene disruption has been associated with most human cancers. The transcription factors AP-1 and NF-B are also important in cell proliferation and death, as well as regulating p53. Heavy metal-generated free radicals inside the cell selectively activate these transcription factors, suggesting that exposure to carcinogenic metals may be linked to cell proliferation or cell death. There exist various mechanisms of heavy metal-induced carcinogenesis (Tchounwou et al., 2012).

1.8 Heavy Metal-Induced Neurotoxicity

Some heavy metals such as lead and manganese may affect the brain and cause neurological toxicity as reviewed by (Neal, 2015). The inhibition of N-Methyl-D-Aspartate Receptor (NMDAR) is known to enhance learning and memory mediated by the hippocampus (Florea and Büsselberg, 2006; Neal, 2015; Anyanwu et al., 2018) as this has been confirmed in animal studies in which animals exposed to lead during its developmental process exhibit similar learning deficits comparable to those with the absence or impaired NMDARs (Azeh Engwa et al., 2019). In the hippocampus, NMDAR is a neural receptor that consists of two or more subunits; an obligatory NR1 subunit and one or more subunits from the NR2 particularly NR2A, NR2B, and NR3 families. Lead is a potent, non-competitive antagonist of the NMDAR (Neal, 2015; Azeh Engwa et al., 2019), preferentially with high affinity at a regulatory site on the NR_2A subunit (Asere et al., 2013). This has been further supported in electrophysiological studies in which recombinant receptors for the subunits have shown NR2A-NMDARs to be more potently inhibited by lead than NR_2B-NMDARs (Azeh Engwa et al., 2019). More so, lead has been shown to decrease the content of NR_2A in the hippocampus and also alter the expression of NR1 spliced variants (Neal, 2015; Azeh Engwa et al., 2019) suggesting lead exposure disrupts the normal ontogeny of NMDAR (Neal, 2015).

The biochemical mechanism of heavy metal toxicity has resulted via ingested food or drinking water into the body as they are acidified by the acid medium of the stomach. In this acidic medium, they are oxidized to their various oxidative states (Zn^{2+}, Cd^{2+}, Pb^{2+}, As^{2+}, As^{3+}, Ag^{+}, Hg^{2+}, etc.) which can readily bind to biological molecules such as proteins and enzymes to form stable and

strong bonds. The most common functional group that heavy metals bind is the thio-groups (SH group of cysteine and SCH_3 group of methionine). Cadmium has been shown to inhibit human thiol transferases such as thioredoxin reductase, glutathione reductase, thioredoxin in vitro by binding to cysteine residues in their active sites (Azeh Engwa et al., 2019). The oxidized heavy metal replaces the hydrogen of the SH group and the methyl of the SCH_3 group thereby inhibiting the function of the protein or activity of the enzyme. For example, methylmercury (MeHg) strongly inhibits the activity of l-glutamine d-fructose-6-phosphate amidotransferase in yeast (Naganuma et al., 2000). Heavy metal-bound proteins may be a substrate for certain enzymes. In such situations, the heavy metal-bound protein fits into an enzyme in a highly specific pattern to form an enzyme-substrate complex and thus cannot accommodate any other substrate until it is freed. As such, the product of the substrate is not formed as the enzyme is blocked and therefore, the heavy metal remains embedded in the tissue leading to dysfunctions, abnormalities, and damages in the body. Inhibition of thiol transferases leads to increased oxidative stress and cell damage. For example, toxic arsenic present in fungicides, herbicides, and insecticides can attack–SH groups in enzymes to inhibit their catalytic activities (Azeh Engwa et al., 2019). Also, heavy metal toxicity may be induced by the replacement of a metalloenzyme by another metal ion of similar size. Cadmium displaces zinc and calcium ions from zinc finger proteins and metalloproteins (Azeh Engwa et al., 2019). For instance, cadmium can replace zinc in certain dehydrogenating enzymes, leading to cadmium toxicity. Such replacement can convert the enzyme structurally to an inactive form and completely alter its activity. These heavy metals in their ionic species such as Pb^{2+}, Cd^{2+}, Ag^{+} $Hg^{2+,}$ and As^{3+} form very stable biotoxic compounds with proteins and enzymes and are difficult to dissociate. Heavy metals may also inhibit protein folding. This was first observed when heavy metals such as cadmium, lead, mercury, and arsenite were shown to effectively interfere with the refolding of chemically denatured proteins (Sharma et al., 2008). It was also observed that when protein misfolded in the presence of heavy metals, the misfolded protein could not be rescued in the presence of reduced glutathione or ethylene diamine tetraacetic acid (EDTA) chelator. The order of heavy metals in terms of their efficacy in folding inhibition is mercury > cadmium > lead and correlates with the relative stability of their monodentate complexes with imidazole, thiol, and carboxylate groups in proteins (Tamás et al., 2014). Heavy metal may cause proteins to aggregate as arsenite-induced protein aggregation was observed and shown to be concentration-dependent. Also, the aggregates contained a wide variety of proteins enriched in functions related to

metabolism, protein folding, protein synthesis, and stabilization (Tamás et al., 2014). Saccharomyces cerevisiae (budding yeast) cells were shown to accumulate aggregated proteins after it was exposed to equi-toxic concentrations of cadmium, arsenite, and chromium (Cr (VI)), and the effect of protein aggregation was influenced by heavy metals in this order: arsenic > cadmium > chromium (Azeh Engwa et al., 2019). The in vivo potency of these agents to trigger protein aggregation probably depends on the efficiency of their cellular uptake/export and their distinct modes of biological action (Azeh Engwa et al., 2019).

2.9 Health Effects of Heavy Metal Toxicity on Humans

Heavy metal toxicity can result in a range of health problems. Heavy metals can harm and damage organs such as the brain, kidney, lungs, liver, and blood. Heavy metal poisoning can have immediate or long-term consequences. Long-term heavy metal exposure can lead to degenerative muscular, physical, and neurological diseases such as Parkinson's disease, multiple sclerosis, muscular dystrophy, and Alzheimer's disease. Long-term exposure to some heavy metals has also been linked to the development of cancer (Cai et al., 2015; Doyi et al., 2018). In table 2.2 below, the EPA regulation limits, numerous adverse health effects, and prevalent sources of several heavy metals are summarized.

Table 2. 2. Toxic effects of some heavy metals on human health

Heavy metal	EPA Regulatory Limit (mg/L)	Toxic Effects	Common sources of contaminants	References
As	0.01	Affects essential cellular processes such as oxidative phosphorylation and ATP synthesis, skin damage, or problems with circulatory systems, and may have increased risk of getting cancer	Erosion of natural deposits; runoff from orchards; runoff from glass & electronics production wastes	(US EPA, 2009; Sauvé, 2014)
Cd	5.0	Carcinogenic, mutagenic, endocrine disruptor, lung damage, and fragile bones affect calcium regulations in biological systems, kidney damage	Corrosion of galvanized pipes; erosion of natural deposits; discharge from metal refineries; runoff from waste batteries and paints	(US EPA, 2009; Sharma, 2015)
Cr	0.1	Hair loss, allergic dermatitis	Discharge from steel and pulp mills; erosion of natural deposits	(Gautam et al., 2014)
Cu	1.3	Brain and kidney damage, elevated levels results in liver cirrhosis and chronic anemia, stomach and intestine irritation	Corrosion of household plumbing systems; erosion of natural deposits	(Jaishankar et al., 2014)
Hg (inorganic)	2.0	Autoimmune diseases, depression, drowsiness, fatigue, hair loss, insomnia, loss of memory, restlessness, disturbance of vision, tremors, temper outbursts, brain damage, lung and kidney failure	Erosion of natural deposits; discharge from refineries and factories; runoff from landfills and croplands	(Matta and Gjyli, 2016)
Mn	0.05	Causes adverse metabolic and neuropsychiatric effects	Industrial emissions, combustion of fossil fuels, and re-entrainment of manganese-containing soils	(Perchlorates et al., 1996)
Ni	0.2 (WHO Permissible limit)	Allergic skin diseases such as itching, cancer of the lungs, nose, sinuses, throat through continuous inhalation, immunotoxic, neurotoxic, genotoxic, affects fertility, hair loss	Dust from volcanic emissions, the weathering of rocks and soils, drinking water and food	(Shukla, 2011)
Pb	1.5	Excess exposure in children delays in physical or mental development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, risk of cardiovascular disease, kidney problems, and high blood pressure	Corrosion of household plumbing systems; erosion of natural deposits	(Abarikwu, 2013)
Sn	-	Vomiting, diarrhea, fatigue, and headache	Dust from wind storms, roads, agricultural activities, smelting and refining processes, industries, waste incineration, and burning of fossil fuels	(ATSDR 2005; WHO, 2005)
Zn	0.5	Dizziness, fatigue, etc.	Metal smelters and mining activities, production and use of zinc in brass, bronze, die castings metal, alloys, rubbers, and paints	(US EPA, 2010)

Chapter Three: Methods and Materials

3.1. Description of the Study Area

The study area, Awetu watershed is found in Jimma town, Southwestern part of Ethiopia. It is the largest town in the region located 352 km from Addis Ababa, the capital city of Ethiopia. The town is found with abundant mean annual rainfall between 1800 and 2300 mm which makes this region one of the best watered Ethiopian highland areas. The town has a population density of about 3521 people per km^2 in 2012 and an average population growth rate of 4.9 % per year (CSA, 2012). Awetu watershed is located at 7°40′ N 36°50′ E with an altitude of 1,780 m above sea level, and an average temperature of 18.9 °C (Getahun et al., 2012; Yasin et al., 2015). Awetu watershed mainly contains four streams, of which Awetu is the largest, which divides Jimma town into two while Dololo, Kitto, and Boye streams are tributaries of Awetu stream. Major pollution sources at each sampling site along the course of the Awetu watershed are agricultural activities, small-scale industries, public institutions, chemical and biological laboratories, high vehicle traffic, gas/petrol station, seepages, and residential and commercial areas.

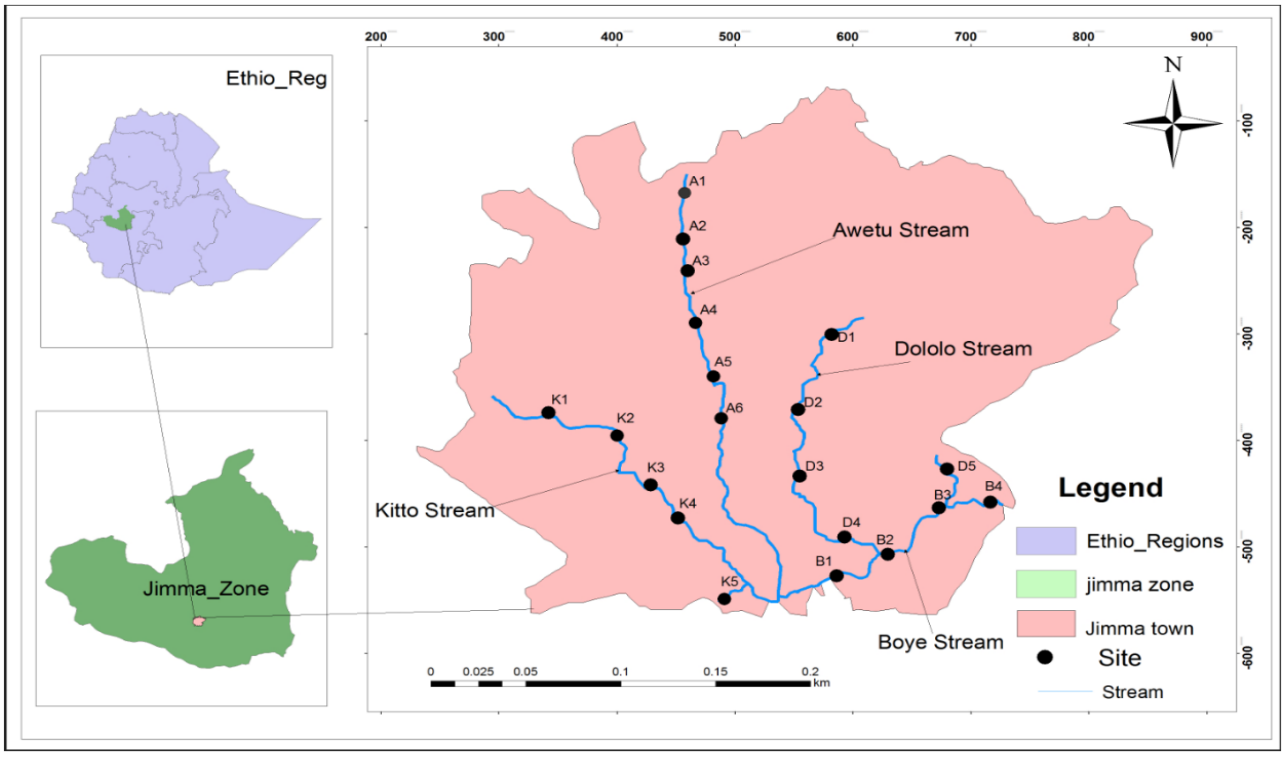

Figure 3. 1. Sampling points in the Awetu watershed streams (Generated from ArcGIS 10.3, ESRI, California, USA)

Gilgel Gibe I reservoir is located in Jimma Zone, Southwest Ethiopia, 260 km from Addis Ababa, the capital of Ethiopia, and 54 km east away from Jimma Town. It was constructed for hydroelectric power generation with World Bank funding at an estimated cost of 2.2 billion Birr on Gilgel Gibe river and has been operating since 2004 to generate 184 MW hydroelectric power. The area has a sub-humid, warm to hot climate, receives between 1,300 and 1,800 mm of annual rainfall, and has a mean annual temperature of 19°C (Yewhalaw et al., 2009). The area is a semi-surrounded basin that receives several rivers in the region and the reservoir has an enormous diversity of fauna-like fishes. The sampling locations were selected based on the discharge of wastes from urban and agricultural sewage into the Gilgel Gibe tributaries. The reservoir formed by the dam receives the incoming water from four major tributaries including Gilgel Gibe, Nada Guda, Nada Qalla, and Nadhi rivers. The reservoir formed by the dam receives the incoming water from four major tributaries including Gilgel Gibe, Nada Guda, Nada Qalla, and Nadhi rivers. Nowadays, the reservoir is also used for fishing and recreation for the surrounding communities (Gure et al., 2019). The map of the study area is shown below in Figure 3. 2.

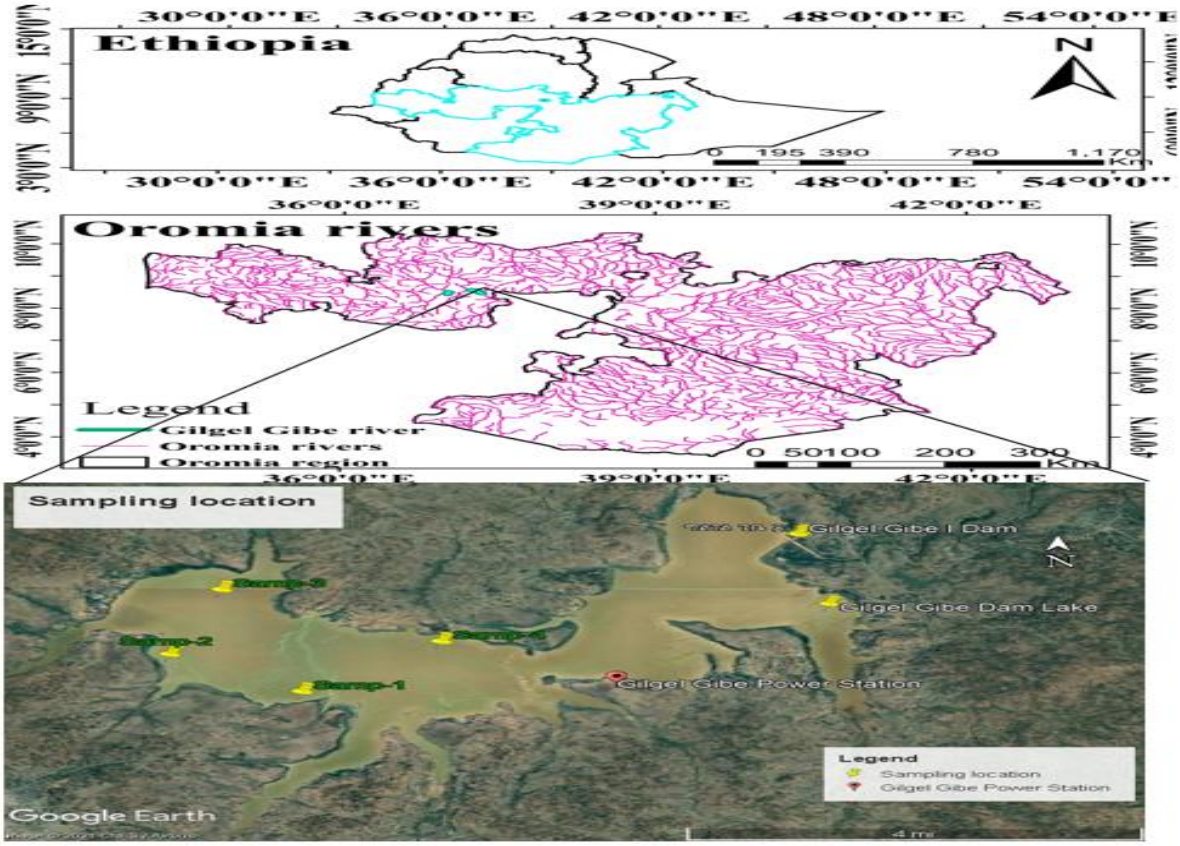

Figure 3.2. Fish sampling points in Gilgel Gibe I reservoir

3.2. Water Quality Parameters

Physico-chemical parameters (temperature, pH, dissolved oxygen, and turbidity) of water samples were measured on-site after collection with a bucket on the spot using a multiparameter probe (Model No. HI 98139, HANNA Instruments Ltd., Germany).

3.3. Reagents and Standards

HNO_3 was obtained from Sigma Aldrich (St. Louis, MO, USA), HCl from Sigma Aldrich (St. Louis, MO, USA), H_2O_2 from Sigma Aldrich (St. Louis, MO, USA). All other chemicals used in this study were ACS reagent grade (Sigma Chemical Co., St. Louis, MO, USA). Double distilled water was used for the preparation of solutions throughout the analysis. Standard solutions; 1000 mg/L of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, and Sn were purchased from Sigma Aldrich and working solutions were made by appropriate dilution as required, immediately before following standard methods (APHA, 1999; Hseu et al., 2002).

3.4. Study Design

The research was carried out in two phases; the first phase was the analytical phase involving the environmental media, sample collection, preparation, and laboratory work. The second phase was the evaluation of the values by comparing with different world standards, guidelines, and computing with multiple indices to evaluate the potential ecological and human health risks.

3.4.1. Water Sample Collection, Preparation, and Determination

Water samples were collected from purposively selected sites along the course of the stream in the Awetu watershed streams where effluents and solid wastes are discharged from different anthropogenic sources. The sampling time was a one-time grab sample in a dry season where the volume of water in the streams was very low. This is purposely chosen because the metal concentrations in the dry season are higher than in the rainy season representing the pollution levels of the streams (Raji et al., 2017). A 1000 mL representative sample was collected from 300 mm depth in white polyethylene plastic bottles. All polyethylene plastic and glassware were cleaned with distilled water, soaked in 10 % m/m nitric acid for 24 hrs, and were rinsed several times with deionized water immediately before use (Taylor and Governor, 2015) and rinsed with the sample water from the stream during sampling. Collected samples were then filtered through

a 0.45 µm membrane filter. The filtrate was then acidified with concentrated HNO_3 to make a pH of < 2. Measured volume (50 mL) of well mixed, acidified samples were taken in a beaker. About 5 mL of concentrated HNO_3 were added and boiled at 130 ^{0}C on a hot plate till the volume came to about 25–30 mL and light color. The addition of HNO_3 and boiling were repeated till the solution becomes light-colored or clear. After cooling, volumes were made to the desired level

Finally, a portion of the digested filtrate samples was sent for metal analyses to the School of Environmental Engineering, Bahir Dar University, and Horticoop Ethiopia Research Laboratory using inductively coupled plasma optical emission spectrometry (ICP-OES) (ARCOS FHS12, Germany) (APHA/WEF/AWWA, 1989).

3.4.2. Sediment Sample Collection, Preparation, and Determination

Submerged surface integrated sediment samples were collected from 20 purposively selected sampling stations in the urban and semi-urban streams of the Awetu watershed. During sample collection, a hand-held GPS was used to identify the locations of the sites (Garmin eTrex®). As described by Decena et al., 2018, each sample constituted a 0-15 cm depth of sediment collected using a stainless steel bottom sampling dredge (grab sampler). The grab sampler was washed with non-ionic detergent and rinsed with distilled water before each use to reduce possible contamination. Sediment samples were placed in a new polyethylene zip-lock bag and placed in a cooler with ice, transported to the laboratory, and kept at 4ºC until analysis. Then, the sediment samples were air-dried and ground using a pestle and mortar after homogenization. Cobles, pebbles, and other coarse debris were manually removed. The grounded samples were sieved in 63 µm (250 mesh size) to obtain fine-powdered particles and placed in a clean polyethylene zip-lock bag. The processed samples were refrigerated at -20 ◦C until metal analysis. Finally, 0.25 g sample was accurately weighed and placed in a dry and clean vessel and digested with 20 mL aqua regia (HNO_3:HCl = 1:3) until the solution turned colorless in an open thermostatically controlled hot plate. The digest samples were heated near to dryness and cooled to ambient temperature. The beaker walls were rinsed with 10 mL deionized water, and 5 mL HCl were added, mixed, and heated. After that, filtration of the sample into a 50 mL volumetric flask using Whatman No. 42 filter paper was made. Then the digest was allowed to cool and transferred into a 100 mL standard flask and filled to the mark with deionized water.

Finally, a portion of the digested filtrate samples was sent for metal analyses to the School of Environmental Engineering, Bahir Dar University, and Horticoop Ethiopia Research Laboratory using ICP-OES (SPECTRO ARCOS Model: ARCOS FHS12, Germany). The calibration curves were obtained from the concentration and absorbance plot and were used to determine the level of heavy metals of each sample. The concentration ranges of standards for each element was: 0 - 20 mg/L for As, Cd, Cr, Hg, and Pb. Data were statistically analyzed using the fitting of a straight line. A reagent blank reading was used to make a necessary correction during the calculation of concentrations.

3.4.3. Fish Sample Collection, Preparation, and Determination

Two commonly consumed fish species namely Nile tilapia (*O. niloticus*) and Labeobarbus (*L. intermedius*) samples were collected from four sampling sites by local fishermen from Gilgel Gibe I reservoir as described in section 3.1. The samples were placed in an insulated cold box immediately transported to the laboratory and stored at -20 ºC. Gills, livers, and muscle tissues of fishes were separately taken using a plastic knife and dried at 60 °C in an oven. Subsequently, the samples were ground in mortar and pestle, sieved in a 2 mm sieve, and ready for the subsequent digestion in a hot plate in a fume hood (Uysal et al., 2009). 1 g of dried sample from each was weighed and transferred into a flask. 10 mL of 65 % nitric acid (HNO_3) and 5 mL of 30 % hydrogen peroxide (H_2O_2) were added to each flask and kept overnight. The flasks were heated to 130 °C until the volume reached 2 to 3 mL and turned clear. The solutions were cooled at room temperature, filtered in Whatman No. 42 filter paper, and diluted to 25 mL with double distilled water (AOAC, 2005; Nigussie et al., 2010).

Analysis of metal concentrations was performed using Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer, Waltham, MA) in Bless AgriFood Laboratory Services, Addis Ababa, calibrated with C-5524 Sigma Standards. The instrument detection limits were 0.001, 0.0015, 0.0005, and 0.01 mg/L for As, Cd, Hg, and Pb respectively. Series of standard solutions were prepared and the calibration concentration ranges were 0 -15 mg/L for As, 0 -15 mg/L for Cd, 0 - 10 mg/L for Hg, and 0 - 20 mg/L for Pb. Absorbance versus concentration was used to obtain a calibration curve for each metal and the concentrations were expressed in milligram per kilogram dry weight.

3.5. Data Management

The heavy metal data were analyzed using Microsoft excel to get the mean and standard error of the mean which was then subjected to statistical tests of significance using ANOVA ($p < 0.05$). The results that show statistical difference were subjected to Tukey's post hoc analysis test (XL Daniel's toolbox version 4.01 for post-ANOVA). This was to determine whether there was any statistical difference between the mean concentrations of the heavy metals in the sites for water, sediment, and fish samples. Tables and graphs were used to present the results.

3.6. Analytical Technique and Accuracy

All the matrixes were analyzed for heavy metals by ICP-OES (SPECTRO ARCOS Model: ARCOS FHS12, Germany) calibrated with C-5524 Sigma Standards). ICP-OES (Spectro Arcos) is an optical emission spectrometer (OES) with a radial observation of inductively coupled plasma (ICP). The features a Paschen-Runge spectrometer mount, employing the Optimized Rowland Circle Alignment (ORCA) technique with 32 linear charge-coupled-devices (CCDs) detectors. The optics are hermetically sealed and filled with argon. Thus, high optical transmission in the vacuum ultraviolet region (VUV) is achieved, allowing the determination of non-metals as well as the use of prominent and interference-free lines in this region. The wavelength range between 130 and 770 nm can be simultaneously analyzed, allowing complete spectrum capture within 2s. Hence, a new measurement is not required even if additional elements or lines are to be determined at a later date. All relevant ICP-OES operating parameters are software controlled, allowing easy selection of the optimum operating conditions (Sarojam, 2010). Analytical conditions for the measurement of the heavy metals in the sample using ICP-OES and AAS were tabulated. The instrument calibration standards were made by diluting standard (1000 ppm) supplied by Sigma-Aldrich. The results were expressed as µg/L for water, mg/kg for sediment, and mg/kg dw for fish samples.

3.7. Quality Assurance and Quality Control

All the standards and reagents used throughout the analysis were analytical grades obtained from Sigma Aldrich. Triplicate samples and reagent blanks were used to ensure the accuracy of the instrument and were calibrated before the samples were run. Deionized ultrapure water was used for the experimental procedure. All glassware and containers were cleaned with 20 % nitric acid, finally rinsed with de-ionized ultrapure water several times, and oven-dried before use.

3.8. Ethical Consideration

Ethical clearance was obtained from the Institutional Review Board (IRB), Jimma University before the actual data collection.

3.9. Dissemination of the Study Results

The final result of this study will be presented to the Jimma University and its copies could be submitted to the Jimma town administration office. Two articles have been published in reputable journals, contributing to sharing a piece of information for the scientific community. The third and the fourth papers are under review in international journals. Besides, the research will be presented at seminars and conferences at both national and internal levels.

Chapter Four:

Sources and Levels of Heavy Metal Contamination in Water from Streams in the Awetu watershed, Jimma, southwestern Ethiopia

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Abstract

Heavy metals contamination of streams remains a threat to humans and biodiversity around the globe. The present study aimed to investigate the contamination source, level, and spatial distribution of heavy metals from streams in the Awetu watershed, southwestern Ethiopia. Surface water samples were collected from 20 sampling sites in December 2019. Water samples were collected in 1000 ml polyethylene bottles previously washed with distilled water, socked in 10 % m/m nitric acid overnight, and were rinsed several times with deionized water immediately before use and rinsed with the sample water from the stream during sampling. Samples were stored in a cooler box at 4 ℃ for transportation to the laboratory. The samples were labeled and acidified with 5 ml nitric acid in the laboratory and were kept at -20 ℃ until the time of digestion for metal analysis. The samples were digested with open acid digestion and the contents of the metal were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES). The concentrations of heavy metals ranging from 314 - 920 µg/L for Pb, 232 - 421 µg/L for Cr, 18 - 351 µg/L for As, 5 - 19 µg/L for Cd, and 10 - 16 µg/L for Hg. The highest concentrations of As were detected at K3, Cd at K2, Pb and Cr at D4, and Hg at D5. Analysis of variance results revealed that the Cd concentrations were statistically significant among all the streams except for Boye. Dololo and Awetu streams found at the center of Jimma town with effluents emanated from Jimma University, garage maintenances, car-wash and agricultural areas had higher values than the streams in the periphery. This study concluded that a higher concentration of heavy metals is associated with the type of waste entering the streams. Heavy metal concentrations in the watershed are to the level that can pose a risk to downstream users. Awareness creation to establish waste management systems and river quality monitoring should be given to the policymakers to implement and minimize the public health risk and deterioration of the aquatic ecosystem.

Keywords: Heavy metals, pollution distribution, surface water pollution, watershed streams

4.1 Introduction

The natural flow of water bodies collected contaminants into one-point collection sites, dams, and reservoirs that can serve as a sink (Ahmed et al., 2009; Gómez-Hortigüela et al., 2013). The unplanned urbanization and industrialization of Ethiopia have adverse effects on the water and sediment quality, as well as the diversity of aquatic fauna. The dumping of municipal waste, untreated waste from various factories, and agrochemicals have reached an alarming situation in open water bodies and streams that continually increases the concentrations of metals and deteriorates the quality of water (Abegaz, 2007; Ali et al., 2016). Due to their non-biodegradability and persistent nature, heavy metals accumulate in aquatic ecosystems, leading to pollution and accumulation to the top consumers through the food chain (Zeng et al., 2014; Akele et al., 2016).

Pollution of aquatic ecosystems with heavy metals is a global problem (Kumar et al., 2019). Least developed countries lack both the equipment and technical capabilities to detect and monitor water quality and are therefore exposed to heavy metal pollution (Nagajyoti et al., 2010; Woldetsadik et al., 2017). Trace amounts of heavy metals are always present in surface waters from terrigenous sources such as weathering of rocks resulting from geochemical recycling in the ecosystems (Mohod and Dhote, 2013; Ji and Jn, 2016). However, excess heavy metals in the water environment might occur through various processes and pathways by anthropogenic activities besides the natural processes (Pekey, 2006; Yuan et al., 2013; Kumar et al., 2020).

Due to poor management and the absence of solid and liquid waste treatment technologies in developing countries, the waste generated from anthropogenic activities could be dumped into the nearby water bodies and rivers crossing towns and their boundaries (Giridharan et al., 2008; Kaufman et al., 2012). Water bodies receive and absorb heavy metals mainly caused by rapid urbanization and industrialization (Du Laing et al., 2009; Rango et al., 2013; Saha and Paul, 2016). A study conducted in Ethiopia showed that stream water pollution with heavy metals sourced from industrial, residential, and agricultural wastes gets higher downstream when it enters Addis Ababa city (Abegaz, 2007; Woldetsadik et al., 2017; Yohannes and Elias, 2017). A similar study conducted in Slovenia, Kazakhstan, and Nile Delta Lagoons shows the quantity of river water can reach alarming levels due to rapid urbanization, insufficient adherence to municipal and industrial wastewater generation, and pollutants from agricultural fields or industries (Vukovic et al., 2011).

The increment of heavy metals in the water bodies due to uncoordinated rapid urbanization with a lack of awareness in the urban rivers also has a serious concern for the sustainable development of the town. The concentration of heavy metals in surface waters becomes relatively high due to significant anthropogenic metal loadings carried by tributary rivers (Li et al., 2011; Gergen and Harmanescu, 2012). Thus, metal pollution in the aquatic environment has owed to its environmental traces, abundance, and persistence (Rango et al., 2013; Ali et al., 2016; Zhang et al., 2016; Zhang et al., 2018). Moreover, water quality characteristics, such as dissolved oxygen, pH, and organic matter content also affect the mobility and availability of these heavy metals in the aquatic environment (Sim et al., 2016).

Heavy metal concentrations in stream water compartments can reveal local and regional pollution (Sekabira et al., 2010). Bottom stream sediments are also sensitive indicators for monitoring contaminants as they act as a sink and a carrier for pollutants in the aquatic environment (Benson and Etesin, 2008). Thus, water analyses play an essential role in evaluating the aquatic environment's pollution status (Moore et al., 2011; Gergen and Harmanescu, 2012). The behavior of metals in natural water is also a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry (Suresh et al., 2012; Islam et al., 2015).

Most urban and semi-urban settings in developing countries have no proper waste management system (Khan et al., 2005). For instance, Jimma town, which has a more than 300,000 population, has no waste management system, where open dumping of solid waste and discharge of untreated wastewater is a daily practice. Previous studies (Ambelu et al., 2013; Alemneh et al., 2017) indicated that the solid and liquid waste dumping had affected the Kitto and Boye water bodies in the Awetu watershed.

Awetu watershed is a considerably large drainage catchment area of land on the southwestern part of Ethiopia, covering numerous streams used as domestic water sources. However, the watershed has experienced extensive agriculture and rapid urbanization in recent years, potential sources of heavy metals pollution. The studied streams receive untreated effluents from agrochemicals, pesticides, car repair garages, car-wash, metal plating, laboratories, waste discharge from Jimma University, and other sources. The high concentration of heavy metals such as arsenic (As),

cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) are discharged into the streams in the Awetu watershed.

Surface water pollution with heavy metals is a significant concern that requires immediate attention through source identification. However, most of the studies conducted on stream pollution focus on the assessment of mean values of heavy metals, thereby lacking information about the level, sources, and spatial distribution of heavy metals in the urban and semi-urban environment. This is highly relevant to devise a pollution control strategy. Despite the widespread environmental deterioration of the urban and semi-urban environment in most low-income countries such as Ethiopia, the identification of pollution sources of heavy metals and their level is hardly available. Hence, this study investigates the pollution sources, levels of contamination, and the sources of heavy metals in the Awetu watershed surface water in southwestern Ethiopia.

4. 2 Methods and Materials

4.2.1 Study Area and Sampling Sites

The study area is located in the urban and semi-urban parts of Jimma town, Southwestern Ethiopia. Jimma town is the largest town in the region, located at 7°40′ N 36°50′ E. It has an altitude of 1,780 m above sea level, with an average temperature of 18.9 °C, and a mean annual precipitation of 1624 mm (Getahun et al., 2012; Yasin et al., 2015). Awetu watershed mainly contains four streams, of which Awetu is the largest one, which divides Jimma town into two while Dololo, Kitto, and Boye streams are tributaries of Awetu stream. Major anthropogenic activities at each sampling site along the course of the Awetu watershed are described in Table 4.1. Samples of surface water were collected from Awetu (A1, A2, A3, A4, A5, and A6), Dololo (D1, D2, D3, D4, and D5), Kitto (K1, K2, K3, K4, and K5), and Boye (B1, B2, B3, and B4) streams. Sample collected from A1 (upstream) was considered as background concentrations of the study area since it is assumed to be free from known anthropogenic heavy metal sources (Figure.3.1).

4.2.2 Sample Collection and Processing

Triplicate water samples were collected from 20 sampling locations from December 1-10, 2019 across the streams in the Awetu watershed (Figure.3.1).). A1000 mL polyethylene (PET) bottles pre-cleaned with nitric acid (10 %) overnight and rinsed three times with deionized water were used to collect surface water samples. Each water sample was collected by submerging the sample

bottles (PET) into the stream at about 300 mm below the surface and 1m away from the edge after rinsing with an open end facing against the current flow direction with the stream water at the time of sampling (Taylor and Governor, 2015). Samples were labeled, immediately transported to the laboratory using a cold box maintained at 4 $^{\circ}$C, filtered through a 0.45 μm Millipore membrane filter, and kept acidified with nitric acid (pH < 2) for 10 days until metal concentrations were determined. The physicochemical properties of the water samples, such as pH, electric conductivity (EC), dissolved oxygen (DO), and turbidity were recorded in-situ, using a calibrated portable multiparameter probe (Hanna LP.2000). The geographic coordinates of each sampling site were recorded using a GPS device (Garmin GPS60).

4.2.3 Sample Extraction and Determination of Heavy Metals

100 mL aliquot of well-mixed water samples were transferred to a beaker. 2 mL of concentrated HNO_3 and 5 mL of concentrated HCl were added. The beakers were covered with a ribbed watch glass and heated in a hot plate until the volume was reduced to 15-20 mL. The beakers were then cooled and the final volumes were adjusted. A portion of the digested filtrate samples was sent to Bahirdar University, Environmental Engineering Laboratory for As, Cd, Cr, Pb, and Hg analyses using inductively coupled plasma optical emission spectrometry (ICP-OES) (ARCOS FHS12, Germany) (APHA/WEF/AWWA, 1989). The absorption wavelength and detection limits of each heavy metal were as follows: 193.7 nm and 0.05 mg/L for As; 226.5 nm and 0.005 mg/L for Cd; 205.6 nm and 0.005 mg/L for Cr; 253.6 nm and 0.05 mg/L for Hg; 220.3 nm and 0.05 mg/L for Pb, respectively. The metal content analyzed is referred to as the acid extractable metals constituting dissolved and weakly sorbed metals on particulates.

4.3 Quality Control and Quality Assurance

Quality control (QC) and quality assurance (QA) were operated using procedural blanks and running twice in each batch of ten samples. A 1000 mg/L standard solution of each metal was purchased from Sigma Aldrich, supplied from BDH (Poole, England), and used for calibration purposes. Mixed working standard solutions containing all metals were prepared. Measurement for each sample concentration was done in triplicate, and the average was taken. The analytical method accuracy was evaluated by measuring samples with known concentrations and comparing the measured values.

4.4 Statistical Analyses

The means and standard deviations of the metal concentrations in water were calculated. The differences of heavy metal concentrations and physicochemical properties among the streams in the watershed were analyzed with a one-way ANOVA, followed by post hoc Tukey tests. The statistical analysis was performed using SPSS version 20 statistical software and a significance level of 0.05 ($p < 0.05$) was considered statistically significant. The relationship between the variables was evaluated based on Pearson's correlation test. The study area and spatial distribution of the sampling sites were mapped using ArcGIS 10.3 (ESRI, Redlands, California, USA). Principal components analysis (PCA) was used as a multivariate statistical method that summarizes the variation of a data set between samples to a set of uncorrelated components and assesses the sources of the heavy metals. This method was performed to determine the differentiation of heavy metals with physicochemical properties based on the elemental level. Since PCA results are sensitive to measurement scales, the original metal contents in water samples were transformed by log (x+1). Cluster analysis (CA) (ward's method) was performed by PAleontological STatistics software (PAST Version 3.25, 1999 – 2019) to determine the relationships between heavy metals in water and environmental variables.

Table 4. 1. Description of streams in the Awetu watershed with their major anthropogenic activities in the sampling sites.

Stream Name	Sampling Code	Major anthropogenic activities of the site
Awetu	A1	Agricultural activities and grazing
	A2	Agricultural activities, grazing, washing clothes, and bathing
	A3	Horticulture, recreational, residential and commercial, vehicle traffic, and agricultural runoff
	A4	Washing, swimming, and fetching water for household consumption
	A5	Vehicle traffic, washing, car washing, and seedling plantation
	A6	Crossing asphalt road, high vehicle traffic, residential area and small scale industries of the town enters into this stream before it.
Dololo	D1	Public institutions, domestic activities, vehicle traffic, hospital, chemical and biological laboratories, and construction sites.
	D2	Car washing, small-scale enterprises like garages, woodwork, and vehicle traffic.
	D3	Commercial area, high vehicle traffic, garages, gas/petrol station, and seepage
	D4	Car washing, gas/fuel station, garages, residential and commercial, and seepage
	D5	Commercial, recreational, vehicle traffic, bus park, gas/petrol station, cement stores, metal works and fabrications, and seepage
Kitto	K1	Institutional wastes, waste stabilization pond, wood and metalwork enterprise, garage, car washing, agricultural activities, and bridge.
	K2	Residential, commercial, garage, seepage, and agricultural activities
	K3	Residential, commercial, seepage, agricultural activities, and airport
	K4	Grazing, agricultural activities, and small scale enterprises like garages
	K5	Solid waste dump sites, horticulture, residential and vehicle traffic
Boye	B1	Car washing, vehicle traffic, residential and commercial area
	B2	Agriculture runoff, irrigation, and residential area
	B3	Irrigation, agricultural runoff, slaughterhouse, and residential area
	B4	Wetland, grazing, agricultural activities, fishing, and recreational

4.5 Results and Discussion

4.5.1 Physico-Chemical Properties of Surface Water

The physicochemical properties of the water samples from streams are presented in Table 4.2. EC ranged from 50.10 – 407.00 µS/cm where the maximum was registered at D1, while the DO ranged

from 3.24 to 7.28 mg/L. The measured DO values at the Boye stream (B1, B2, B3, and B4), Awetu stream (A6), and Dololo stream (D1, D4, and D5) were below 4 mg/L. These show stress to most aquatic animals with severe scarcity as compared to the other sampling points. Turbidity ranges from 8.02 - 302.00 NTU with the highest values at D3 and K5. Among the studied physicochemical properties, pH showed from slightly acidic to slightly alkaline (6.78 – 7.60) in all sampling sites except at K1 which was acidic (pH = 5.82), reflecting the availability of carbonate host in the area (Moore et al., 2011; Radulescu et al., 2014). Comparatively, the pH values from Boye stream sites were lower which might be due to the decrease in dissolved oxygen in the downstream side of the watershed. The water pH predominantly controlled the solubility of heavy metals. A higher pH value can reduce the solubility while a low level enhances the dissolution processes and releases free metal ions into the water column (Singh and Kumar, 2017). The mobility and bioavailability of most heavy metals like As, Cd, Cr, and Pb are principally enhanced within an acidic environment (Caruso et al., 2008). EC and turbidity are the other physicochemical properties that strongly affect surface water quality (De Troyer et al., 2016). The ANOVA performed for EC showed statistically significant differences between Awetu and Dololo, Dololo and Kitto, and Dololo and Boye streams ($p < 0.001$).

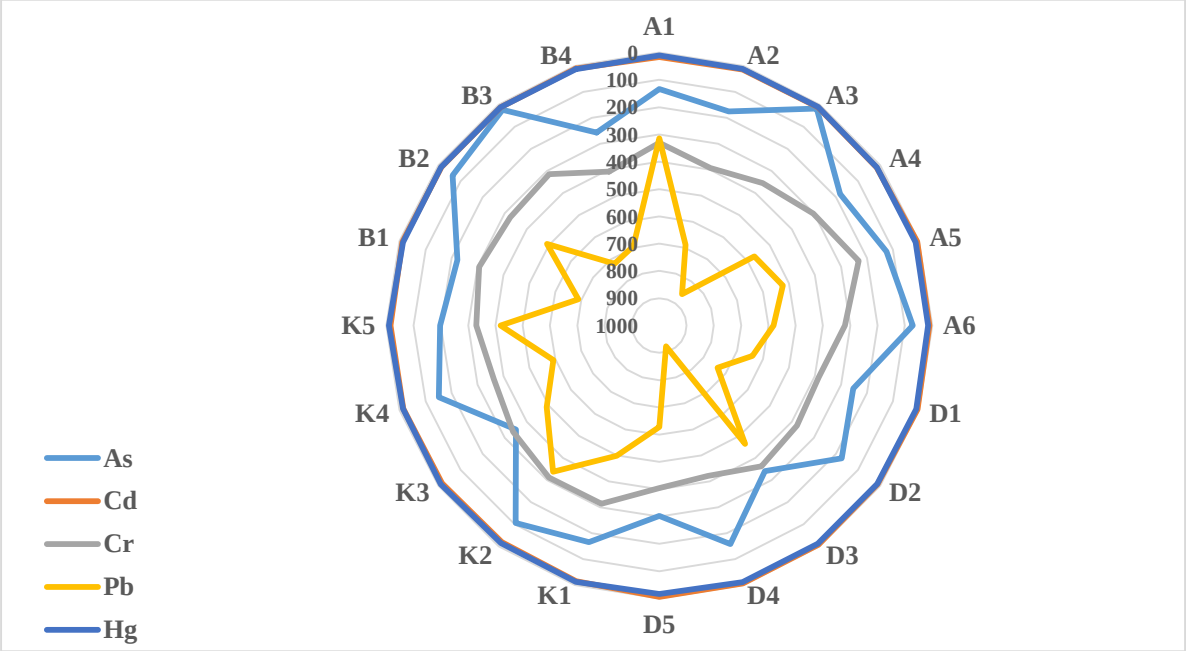

Figure 4. 1. Spatial distribution of heavy metal concentrations (µg/L) in surface water samples in the Awetu watershed streams.

Table 4. 2. Physico-chemical properties of water samples in streams in the Awetu watershed.

Site	Dissolved Oxygen (mg/L) (Average ± SD)	Electric Conductivity (µS/cm) (Average ± SD)	pH (Average ± SD)	Turbidity (NTU) (Average ± SD)
A1	7.09 ± 0.03	62.30 ± 0.46	7.59 ± 0.14	50.50 ± 1.76
A2	6.71 ± 0.17	62.30 ± 0.59	7.60 ± 0.20	52.10 ± 1.50
A3	6.70 ± 0.04	66.30 ± 3.31	7.58 ± 0.02	60.70 ± 0.10
A4	7.28 ± 0.01	69.30 ± 0.83	7.42 ± 0.09	106.00 ± 0.87
A5	7.01 ± 0.04	85.60 ± 1.85	7.05 ± 0.01	71.20 ± 1.06
A6	3.33 ± 0.03	131.40 ± 2.47	7.03 ± 0.02	51.90 ± 1.54
D1	3.59 ± 0.38	407.00 ± 3.21	7.01 ± 0.005	11.97 ± 0.77
D2	4.18 ± 0.32	299.00 ± 1.84	7.43 ± 0.19	26.60 ± 1.01
D3	4.96 ± 0.52	250.00 ± 3.00	7.49 ± 0.09	302.00 ± 1.00
D4	3.80 ± 0.46	313.00 ± 1.62	7.43 ± 0.19	65.30 ± 0.56
D5	3.78 ± 0.54	343.00 ± 2.35	7.40 ± 0.15	14.73 ± 0.95
K1	6.27 ± 0.78	84.40 ± 1.34	5.82 ± 0.26	276.00 ± 1.26
K2	5.99 ± 0.69	82.10 ± 1.56	7.27 ± 0.13	189.00 ± 1.49
K3	5.25 ± 0.23	90.10 ± 2.94	7.03 ± 0.09	128.00 ± 0.87
K4	5.20 ± 0.16	201.00 ± 2.20	7.23 ± 0.09	8.02 ± 0,90
K5	4.96 ± 0.65	250.00 ± 1.80	7.49 ± 0.06	302.00 ± 0.96
B1	3.49 ± 0.51	93.40 ± 2.12	6.79 ± 0.08	54.90 ± 0.47
B2	3.24 ± 0.10	87.30 ± 1.41	6.85 ± 0.04	19.40 ± 0.60
B3	3.79 ± 0.15	87.80 ± 1.68	6.88 ± 0.08	31.80 ± 0.26
B4	3.68 ± 0.11	105.30 ± 1.77	6.78 ± 0.09	26.40 ± 0.44

SD = Standard Deviation, mg/L = milligram per liter, µS/cm = micro siemens per centimeter and NTU = nephelometric turbidity unit

4.5.2 Heavy Metal Concentrations in Surface Water Samples

The mean concentrations of heavy metals in stream waters followed a decreasing Pb > Cr > As > Cd > Hg. The concentrations of heavy metals were compared with world river water (WRW) (Khan et al., 2005), US EPA (US EPA, 2004), irrigation water guideline values (FAO/WHO, 2001), and the background concentration of the study (Table 4. 3). The mean values of heavy metals were higher than WRW (Khan et al., 2005), US EPA, and TRV (US EPA, 2004), indicating severe contamination of the streams. The highest mean concentrations of As (246 ± 80 µg/L), Cr (390 ± 23 µg/L), and Pb (678 ± 167 µg/L) were detected at Dololo stream, Cd at Kitto (16 ± 2 µg/L), and Hg with very low values in all the streams. The highest concentration of Pb was observed at D4 (920 µg/L), which is much higher than the legal limits set by the US EPA in WRW (3 µg/L).

In developing countries, leaded gasoline is still commonly used, which significantly increases the amount of Pb in urban soils due to its non-degradability nature (Naveedullah and Hashmi et al., 2013) which is eventually disposed of to the nearby water bodies. Pesticides, car washing at the side of the streams, and lead pipe from the city's old and corroded water distribution line were the other causes of the elevated concentration of Pb (Sörme and Lagerkvist, 2002; Haiyan and Stuanes, 2003; Getaneh et al., 2014). Therefore, the most likely sources of Pb pollution are industrial processes, smelting, fumes from high traffic loads, and atmospheric deposition (Flora et al., 2012; Zeng et al., 2014). Because of its intrinsic chemical characteristics, Pb can also be found in association with other elemental pairs inherently linked with each other (Patrick, 2006).

The concentration of Cr ranged from 232 to 421 µg/L, with an average of 344.6 ± 45.6 µg/L. At D4, the confluence point of the Dololo and Kochi streams showed the highest concentrations of Cr (Figure. 4.1). The main reasons for higher Cr are the discharge of untreated waste from chemical laboratories, construction remnants, deposition of household and municipal wastes, infrastructural encroachment, construction and demolition activities, and dust emissions from automobile exhaust fumes (Rule et al., 2006; Gergen and Harmanescu, 2012; Khan et al., 2017; Umaya, 2017). Moreover, these wastes are directly released to the water bodies without preliminary treatment, hence the value was by far greater than the world river water standards (US EPA, 2004; Rajiv et al., 2010) as shown in Table 4. 3. The persistent nature of Cr (VI) accumulates in the food chain, which reaches harmful levels in living things over time, resulting in severe health hazards (Jaishankar et al., 2014). The higher concentrations of Cr significantly inhibit the activity of microorganisms and pose a serious threat to the health of the environment, humans, and animals (Mengistie et al., 2016; Ayangbenro and Babalola, 2017).

Cd concentrations range from 5 to 19 µg/L, with an average value of 12 ± 4 µg/L which surpass the permissible limit set by the US EPA criteria for water quality, World Rivers, and FAO guideline values for irrigation water (FAO/WHO, 2001; US EPA, 2004; Khan et al., 2005). The highest concentration of Cd was detected at K2, where the area has been associated for several decades with intensive cropping with high inputs of agrochemicals such as phosphate fertilizer. Welding, fertilizer, surface runoff and deposition, and solid waste disposal are the other sources of Cd, contributing to the leaching of the nickel-cadmium battery to the nearby water bodies (Sharma et al., 2015; Ayangbenro and Babalola, 2017). The Cd released from these sources reaches

aquatic ecosystems that later easily affect humans through the food chain, drinking water, and breathing. Under the US EPA (2004) cancer guidelines, Cd was identified as a potential human carcinogen; acute and chronic exposure leads to adverse health effects both for humans and animals (Dokmeci et al., 2009). The ANOVA performed for heavy metals concentrations showed statistically significant differences for Cd between Awetu and Dololo ($p < 0.05$), Dololo and Kitto ($p < 0.001$). The ANOVA test revealed that DO showed a statistically significant difference between Awetu and Dololo, and Awetu and Boye stream ($p < 0.05$) (Table 4. 4).

Table 4. 3. Mean values of heavy metal concentrations in water (mean ± SD) in (µg/L) in the Awetu watershed streams.

Sites	As	Cd	Cr	Pb	Hg
A1	133 ± 40.3	16 ± 4.3	331 ±13.6	314 ± 278.9	10 ± 2.05
A2	175 ± 1.7	14 ± 2.3	393 ± 48.4	689 ± 96.1	11 ± 1.05
A3	18 ± 155.3	11 ± 0.7	355 ± 10.4	858 ± 265.1	10 ± 2.05
A4	181 ± 7.7	15 ± 3.3	302 ± 42.6	569 ± 23.9	14 ± 1.95
A5	124 ± 49.3	6 ± 5.7	232 ± 112.6	524 ± 68.9	12 ± 0.05
A6	71 ± 102.3	10 ± 1.7	320 ± 24.6	581 ± 11.9	15 ± 2.95
D1	253 ± 79.7	5 ± 6.7	386 ± 41.4	641 ± 48.1	10 ± 2.05
D2	173 ± 0.3	11 ± 0.7	376 ± 31.4	736 ± 143.1	12 ± 0.05
D3	341 ± 167.7	8 ± 3.7	363 ± 18.4	464 ± 128.9	12 ± 0.05
D4	158 ± 15.3	7 ± 4.7	421 ± 76.4	920 ± 327.1	11 ± 1.05
D5	303 ± 129.7	6 ± 5.7	404 ± 59.4	628 ± 35.1	16 ± 3.95
K1	165 ± 8.3	15 ± 3.3	313 ± 31.6	498 ± 94.9	12 ± 0.05
K2	106 ± 67.3	19 ± 7.3	312 ± 32.6	338 ± 254.9	14 ± 1.95
K3	351 ± 177.7	16 ± 4.3	338 ± 6.6	491 ± 101.9	11 ± 1.05
K4	151 ± 22.3	14 ± 2.3	363 ± 18.4	592 ± 0.9	13 ± 0.95
K5	197 ± 23.7	16 ± 4.3	330 ± 14.6	419 ± 173.9	10 ± 2.05
B1	222 ± 48.7	11 ± 0.7	306 ± 38.6	689 ± 96.1	13 ± 0.95
B2	64 ± 109.3	13 ± 1.3	325 ± 19.6	492 ± 100.9	12 ± 0.05
B3	23 ± 150.3	11± 0.7	315 ± 29.6	721 ± 128.1	11 ± 1.05
B4	257± 83.7	10 ± 1.7	407 ± 62.4	694 ± 101.1	12 ± 0.05
BC	133	16	331	314	10
WRW	0.02	0.01	1	1	0.07
US EPA	1	2	11	3	1.8
TRV	150	5	11	3	2

SD is the standard dev, BC - Background Concentration (this study), TRV - Toxicity Reference Value for freshwater proposed by US EPA (US EPA, 2004), United States Environmental Protection Authority (US EPA, 2004), WRW - World River Water (Khan et al., 2005).

The highest concentration of As is detected at K3 (351 ± 177.7 µg/L), D3 (341 ± 167.7 µg/L), and D5 (303 ± 129.7 µg/L). This might be K3, which is the place where the agricultural plantation was found and maximum agricultural activities and extensive use of arsenic trioxide pesticides are predominantly applied. Sites D3 and D5 are located in the center of the town, where pesticides, insecticides, herbicides, pigments, and the use of wood preservatives containing which are possible

sources of arsenic contamination. At the pH value of 7.49, the highest concentration of As (341 µg/L) suggests that higher pH is more conducive to the mobilization of As (Bencko and Foong, 2017). More significantly, it persists in pollution due to the persistent presence of As in water bodies, which imposes detrimental effects on different aquatic and terrestrial species and eventually affects human health (Chatterjee et al., 2017).

The mean Hg concentration was 12.1 ± 1.7 µg/L ranging from 10 to 16 µg/L, where the highest concentration was detected at D5. This might be due to the elemental mercury found in dental amalgam, the emission of fossil fuels, batteries, and the incineration of medical waste generated from laboratories, dental clinics, and inorganic mercury from the aquatic environment (Paraquetti et al., 2004). Through plants and livestock, soil polluted by mercury or the redistribution of contaminated water may reach the food chain (Rice et al., 2014). It can bioaccumulate once Hg has entered the food chain and cause adverse effects on human health (Nagajyoti et al., 2010).

The concentration of heavy metals in Awetu water streams showed trends correlated with source contribution and anthropogenic activities around the streams and their tributaries. This might be due to the effluents discharged from Jimma University, car repair garages, and car-wash. Because of the regular use of household items, such as cleaning materials, toothpaste, and cosmetics, the discharge of domestic wastewater also might increase Pb concentrations (Nagajyoti et al., 2010; Rak, 2015). The broad inter-and intra-site variations are due in part to real changes in the environment. The direct solid and liquid waste discharged at various locations from different industrial, municipal and domestic activities significantly affects the heavy metal condition of the watershed streams. The numerous tributaries also contribute to heavy metals' elemental concentration. It could be also hypothesized the downstream locations of the streams in the Awetu watershed would be more marked by contamination of the streams in the Awetu watershed with heavy metals. Minimum concentrations due to percolation and dilution factors are also found in downstream streams in the Awetu watershed. The mobility and possible trace effects of heavy metals in a specific environment are typically governed by their existing chemical forms (Baran and Tarnawski, 2015).

Heavy metal concentrations found in stream water samples were spatially varied with anthropogenic activities. The concentrations and distributions of heavy metals in the water samples were potentially influenced by the physicochemical properties, such as DO, EC, pH, and turbidity.

High levels of Pb and Cd were observed in some specific areas, very close to garages, smelting, motor-vehicle exhaust fumes, and from corrosion of lead pipework which indicates the source might be mainly from these sources (Patrick, 2006; Gowd and Govil, 2008; Getaneh et al., 2014).

Table 4. 4. ANOVA analysis for heavy metal concentrations and physicochemical properties between streams in the Awetu watershed.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
As	Between Groups	51342.000	3	17114.000	2.281	.118
	Within Groups	120038.200	16	7502.388		
	Total	171380.200	19			
Cd	Between Groups	186.250	3	62.083	9.034	**.001**
	Within Groups	109.950	16	6.872		
	Total	296.200	19			
Cr	Between Groups	14384.417	3	4794.806	3.062	.058
	Within Groups	25054.383	16	1565.899		
	Total	39438.800	19			
Pb	Between Groups	127212.967	3	42404.322	1.973	.159
	Within Groups	343830.833	16	21489.427		
	Total	471043.800	19			
Hg	Between Groups	.150	3	.050	.015	.997
	Within Groups	54.800	16	3.425		
	Total	54.950	19			
DO	Between Groups	29.584	3	9.861	5.209	.011
	Within Groups	30.288	16	1.893		
	Total	59.873	19			
EC	Between Groups	199461.875	3	66487.292	32.755	**.000**
	Within Groups	30447.483	15	2029.832		
	Total	229909.358	18			
pH	Between Groups	1.498	3	.499	4.094	.025
	Within Groups	1.951	16	.122		
	Total	3.450	19			
Turbidity	Between Groups	21713.097	3	7237.699	1.034	.404
	Within Groups	112002.176	16	7000.136		
	Total	133715.272	19			

Tukey HSD test revealed that the concentration of Cd was significantly (p-value = 0.001) higher at Kitto while the minimum was registered at the Dololo stream. A higher concentration of DO was recorded at the Awetu stream which might be due to flow turbulence as oxygen will get a

chance to diffuse into the water. On the contrary, the lower oxygen levels are registered at Dololo, an immediate outlet of Jimma University and Boye, where the stream passes through the wetland. The level of EC in the Dololo stream is significantly higher than the other streams, which might be attributed to the waste discharge from Jimma University, car maintenance garages, and car-wash which contains different ions. Figure. 4.2 demonstrates the difference between streams at a 95 % confidence level.

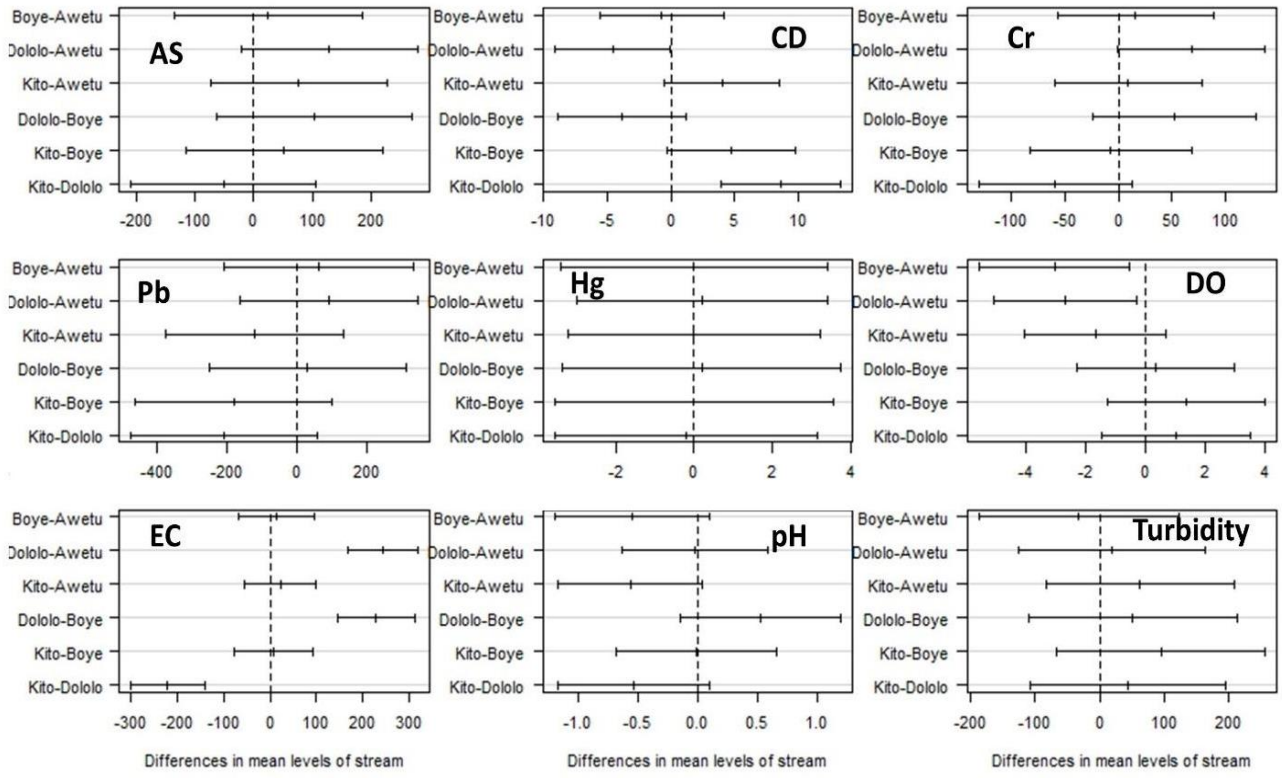

Figure 4. 2. Tukey HSD test output showing the 95% stream-wise confidence level of heavy metals

The elemental association has also been evaluated by Pearson correlation coefficient (r) and the results are presented in Table 4.5 which shows that elemental pairs Pb/Cr, ($r = 0.490$, $P < 0.01$) and Pb/Cd, ($r = 0.536$, $P < 0.01$) were significantly correlated with each other, whereas the rest of elemental pairs showed no significant correlation with each other. The elemental association may signify that each paired element have an identical source or common sink in the streams (Sekabira et al., 2010). Metal and physicochemical associations showed pairs As/EC are correlated with each other, whereas the rest were not significantly correlated.

The correlation analyses performed showed possible common characteristics of heavy metals in surface water. Pb & Cr and Pb & Cd were correlated with each other, indicating that primarily anthropogenic sources such as traffic and industrial activities contribute to contamination (Ji and Jn, 2016; Yuan et al., 2018). The significant positive correlations between As and EC confirm the considerable share of EC with the binding of heavy metals and might be attributed to anthropogenic impacts (Alghobar and Suresha, 2017).

Table 4. 5. Pearson correlation coefficient matrix for heavy metals and physicochemical characteristics in Awetu watershed stream waters (n = 20).

	As	Cr	Pb	Cd	Hg	DO	EC	pH	Turbidity
As	1								
Cr	0.37	1							
Pb	-0.15	**0.49***	1						
Cd	-0.19	-0.28	**-0.54***	1					
Hg	0.10	-0.10	-0.10	-0.07	1				
DO	-0.09	-0.36	-0.25	0.37	-0.07	1			
EC	**0.62****	0.30	0.06	-0.14	-0.06	-0.23	1		
pH	0.01	0.32	0.18	-0.13	0.02	0.22	0.09	1	
Turbidity	0.26	-0.23	-0.39	0.24	0.07	**0.44***	0.05	-0.23	1

**. Correlation is significant at the 0.01 level (2-tailed).*. Correlation is significant at the 0.05 level (2-tailed).

Hierarchical multivariate CA was performed to find out the relationships between heavy metal source distributions in the stream water of the Awetu watershed. The specific anthropogenic activities for each sampling site are described in Table 4.1 above. From the dendrograms, two cluster groups were identified based on the various sources of heavy metals as shown in Figure. 4. 3. All the twenty sampling stations were grouped into two statistically meaningful clusters at Euclidean distance < 0.5. Distance metrics are based on the Euclidean distance single linkage method (nearest neighbor). This dendrogram indicates sites (B2, D3, A1, A4, A5, A6, K1, K2, K3, K4, and K5) as cluster 1 and sites (A2, A3, B1, B3, B4, D1, D2, D4, and D5) as cluster 2 which have significant similarity of the concentrations of heavy metals with each other. Their close association with each other controls the concentrations of heavy metals in the clusters. This relation

is due to the topography, the possible pollution source, and the dilution factor of heavy metals in the stream water.

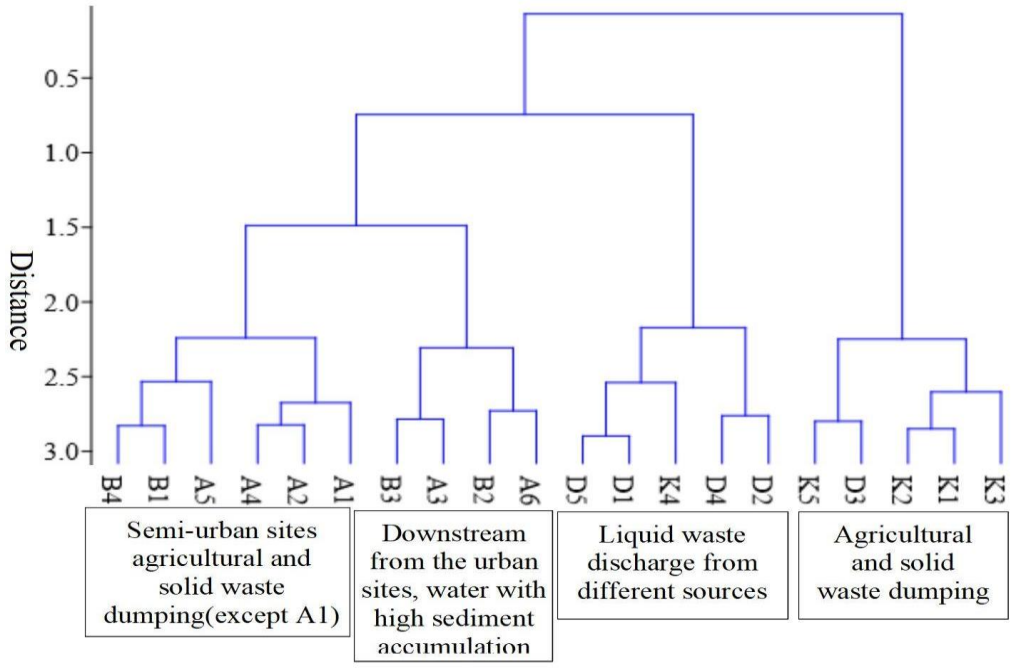

Figure 4. 3. Dendrogram of hierarchical clustering analyses showing the relevant association among the parameters in waters of Awetu watershed streams.

Results from the principal component analysis (Figure. 4.4) indicate that sites with high turbidity nearly have a higher concentration of cadmium which is located in the semi-urban section of Jimma town. In comparison, those sites located in the middle of the town with high electrical conductivity had a higher concentration of chromium and lead. High arsenic concentration was found at a confluence point of the two streams, Awetu stream after crossing the town and Kitto stream from a semi-urban environment. Perhaps, arsenic is released from a waste dumping site located adjacent to the Awetu stream before the confluence point of the two streams. The loadings of the variables and correlation between variables and the PC scores are indicated in Table 4. 6.

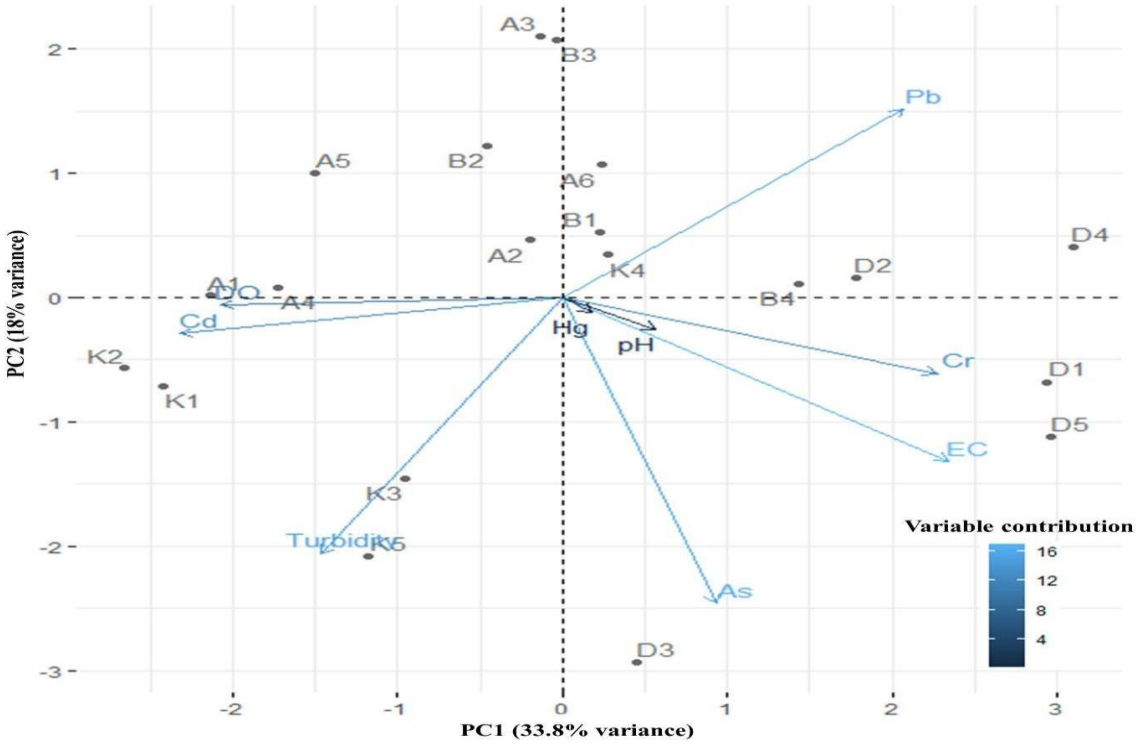

Figure 4. 4. PCA biplot of heavy metal and physicochemical characteristics of water samples from Awetu watershed, Southwestern Ethiopia.

Table 4. 6. The loadings of the variables and correlation between variables and the PC scores.

	Component		
	1	2	3
DO	-0.685	0.019	0.488
EC	0.774	0.434	0.033
pH	0.187	0.084	0.760
Turbidity	-0.481	0.679	-0.004
As	0.307	0.811	-0.077
Cd	-0.766	0.096	0.165
Cr	0.751	0.201	0.325
Pb	0.682	-0.500	0.158
Hg	0.060	0.039	-0.625
Extraction Method: Principal Component Analysis			
a. 3 components extracted.			

4.6 Conclusion

This study provides new information on concentrations of As, Cd, Cr, Pb, and Hg in the surface water in the Awetu watershed streams. Heavy metal concentrations were relatively high beyond surface water quality standards. From all the measured heavy metals, Pb shows the highest, whereas Hg concentration remained the lowest. The highest concentrations of heavy metals were found at the center of the town where the maximum anthropogenic activities are practiced. The main sources of heavy metals were assumed from the waste discharge from Jimma University laboratories and dental clinic, car maintenance garages, car-wash, agrochemicals (phosphate fertilizers), pesticides, the emission of fossil fuels, batteries, and the incineration of medical wastes. Accordingly, lower concentrations were detected downstream of the watershed due to slower water flow and sedimentation. Main sources of As and Pb were assumed to be from laboratories, smelting and carwash activities and Cd from agricultural activities as the uncontrolled effluents are disposed of to the nearby water bodies even without preliminary treatment. The water in the area requires remediation as per environmental quality criteria and regular monitoring of heavy metals. Due to surface water being laid above the sediments in the aquatic systems, studying the contamination level of heavy metals in the sediments of the streams in the Awetu watershed are very important

Chapter Five:

Heavy Metal Contamination in Sediments of Streams in Awetu Watershed, Jimma, Southwestern Ethiopia.

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Abstract

Surface sediment samples were collected from different streams of the Awetu watershed in southwestern Ethiopia. Sediment samples were analyzed for As, Cd, Cr, Pb, and Hg levels using inductively coupled plasma optical emission spectrometry. The mean values of heavy metals contamination showed 623.32 ± 291.65 mg/kg for As, 151.09 ± 111.5 mg/kg for Cd, 375 ± 212.03 mg/kg for Cr, 2005.94 ± 954.99 mg/kg for Pb and 4.64 ± 0.59 mg/kg for Hg. The mean heavy metal concentration in the streams followed the decreasing order of Pb > As > Cr > Cd > Hg. As, Cr and Pb are detected at high concentrations with mean values of 623.32, 375.00, and 2,005.94 mg/kg respectively. The lowest level of heavy concentration (3.6 mg/kg) was recorded for Hg. The contamination factor (CF) of all the studied heavy metals ranged from a low degree (CF < 1) to a very high degree (CF ≥ 6) of contamination. Mainly, Dololo and Kitto's streams show a very high degree of contamination (CF ≥ 6) than Awetu and Boye streams. Specifically, As, Cd and Cr in the Dololo and Kitto streams have significantly elevated concentrations than others. Geo-accumulation index (I_{geo}) shows low to moderate contamination level with As, Pb, and Hg; uncontaminated to heavily contaminated by Cr; and moderate to extreme contamination by Cd. Dololo and Kitto streams receive wastewater effluents from teaching institutions, garages, car washing discharges, and wood and metal enterprises which might be the possible sources of heavy metal contamination.

Keywords: Awetu Watershed, Contamination, Ethiopia, Heavy Metals, Sediment

5.1 Introduction

The quality of water is affected by human-induced or natural activities in the upstream watershed (Sany et al., 2013). As a result of the natural flow of the water, most pollutants are drained into a one-point collection site, such as reservoirs that can serve as a sink for different pollutants (Nowrouzi and Pourkhabbaz, 2014). Due to its potential and toxic environmental and public health effects and the ability to accumulate, heavy metal contamination of the aquatic ecosystems is becoming a potential global problem (Sharma et al., 2015).

Sediment contamination with heavy metals could be either from natural geogenic sources or sourced from anthropogenic activities (Giouri et al., 2010). The benthic environment of aquatic ecosystems receives and absorbs heavy metals from natural weathering, erosion, industrial wastes, and atmospheric deposition (Jaishankar et al., 2014). Anthropogenic activities, such as industrial and agricultural discharges, inappropriate disposal of industrial wastes, dumping of domestic and municipal wastes, faulty drainage systems are some of the causes for heavy metal contamination of aquatic ecosystems (Hahladakis and Smaragdaki, 2013; Islam et al., 2015a).

Several studies indicated that heavy metal concentration in stream sediments is relatively high due to significant anthropogenic metal loadings carried by tributary rivers (Li et al., 2011). As a result, surficial sediments may serve as a metal puddle that can release metals to the overlying water that could potentially adversely affect the riverine ecosystems (Evans et al., 2003). It is well-known that the mobility and availability of heavy metals in aquatic environments are primarily affected by physicochemical parameters of water, such as pH, dissolved oxygen, and organic matter content (Sim et al., 2016).

Due to the absence of waste treatment facilities in towns and the discharge of wastes into the nearby water bodies, rivers located near towns are often suffering from heavy metal contamination (Giridharan et al., 2008). A study conducted by Mekonnen and his colleagues (2014) on the Akaki river that crosses Addis Ababa city showed severe contamination of the stream water with heavy metals sourced from industrial, residential, and agricultural wastes. The quality of the water gets worsened as heavy metals bioaccumulate in algal blooms in the downstream section of the water bodies where wastes are discharged from the upstream (Melaku et al., 2007).

Other studies of sediment and water pollution assessment in Ethiopia and the region showed that

the concentration of heavy metals rapidly increases when it entered Addis Ababa city (Woldetsadik et al., 2017), Kombolcha city (north-central part of Ethiopia) (Zinabu et al., 2019), but significantly decreased downstream of the towns. This decrement of heavy metal downstream of the town might be due to heavy metals adsorbed into the sediment portion of the river (Khan et al., 2017). Bottom stream sediments are sensitive indicators for monitoring contaminants as they can act as a sink and a carrier for pollutants in the aquatic environment (Benson and Etesin, 2008). The presence and behavior of metals in the natural water is a function of the substrate sediment composition, the suspended sediment composition, and the water chemistry (Suresh et al., 2012). Heavy metals are potentially accumulated in sediments and aquatic organisms in the freshwater environment and subsequently transferred to man through the food chain. As a result, the concentration of heavy metals in aquatic ecosystems is usually scrutinized by measuring their concentrations in sediments (Ekeanyanwu et al., 2010; El Bouraie et al., 2010).

Nowadays, various indices are developed to assess the level of sediment contamination and ecological risk. Geo-accumulation index (I_{geo}), enrichment factor (EF), contamination factor (CF), and pollution load index (PLI) methods have been commonly used for geochemical standardization approaches (Müller, 1979). The presence of heavy metals in stream sediments has a vital role in evaluating the aquatic environment requires immediate attention (Li and Zhang, 2010). However, information on metal concentrations in stream sediments at a spatial scale along a course of streams in the urban and semi-urban environment is very limited. Hence, this study addresses existing research gaps and provides valuable information regarding the spatial distributions of selected heavy metals in the urban rivers around the Awetu watershed catchment. This paper discusses the concentrations of heavy metals (As, Cd, Cr, Pb, and Hg) in the surface sediments of four streams around the Awetu watershed catchment, comparing with the SQGs, and assesses the heavy metal contamination using the I_{geo}, CF, and PLI methods.

Table 5. 1. Sampling stations, code, GPS coordinates, and major anthropogenic activities in the Awetu watershed streams.

Stream Name	Site Code	GPS Coordinate	Major anthropogenic activities of the site
Awetu	A1	36º 49' 51.15" E 7º 42' 28.14"N	Agricultural activities and grazing
	A2	36º 49' 52.39"E 7 º42' 4.03"N	Agricultural activities, grazing, washing clothes, and bathing
	A3	36º 49' 52.89"E 7º 41' 43.38"N	Horticulture, recreational, residential and commercial, vehicle traffic, and agricultural runoff
	A4	36º 49' 57.27"E 7 º41' 13.20"N	Washing, swimming, and fetching water for household consumption
	A5	36º 50' 5.95"E 7 º40' 46.11"N	Vehicle traffic, washing, car washing, and seedling plantation
	A6	36º 50' 9.79"E 7 º40' 15.47"N	High vehicle traffic, residential area, and small scale industries
Dololo	D1	36º 50' 50.67"E 7º 40' 45.52"N	Public institutions, vehicle traffic, hospital, chemical and biological laboratories, and construction sites.
	D2	36º 50' 41.99"E 7º 40' 0.28"N	Car washing, small-scale enterprises like garages, woodwork, and vehicle traffic.
	D3	36º 50' 41.65"E 7º 39' 44.82"N	Commercial area, high vehicle traffic, garages, gas/petro station, and seepage
	D4	36º 51' 4.81"E 7º 39' 22.63"N	Car washing, gas/fuel station, garages, residential and commercial, and seepage
	D5	36º 51' 43.74"E 7º 40' 2.46"N	Commercial, vehicle traffic, bus park, gas/petrol station, cement stores, metal works and fabrications, and seepage
Kitto	K1	36º 48' 34.68"E 7º 40' 23.97"N	Grazing, institutional wastes, waste stabilization pond, wood and metalwork enterprise, garage, car washing, agricultural activities, and bridge.
	K2	36º 49' 15.04"E 7º 40' 19.80"N	Residential, commercial, garage, seepage, and agricultural activities
	K3	36º 49' 32.86"E 7º 39' 54.87"N	Residential, commercial, seepage, agricultural activities, and airport
	K4	36º 49' 53.70"E 7º 39' 31.91"N	Grazing, agricultural activities, and small scale enterprises like garages
	K5	36º 50' 18.86"E 7º 39' 1.56"N	Solid waste dump sites, horticulture, residential and vehicle traffic
Boye	B1	36º 50' 46.97"E 7 º38' 57.16"N	Car washing, vehicle traffic, residential and commercial area
	B2	36º 51' 23.16"E 7º 39' 19.66"N	Agriculture runoff, irrigation, and residential area
	B3	36º 51' 44.99"E 7º 39' 38.48"N	Irrigation, agricultural runoff, slaughterhouse, and residential area
	B4	36º 52' 12.42"E 7º 39' 42.30"N	Wetland, grazing, agricultural activities, fishing, and recreational

5.2. Methods and Materials

5.2.1 Study Area and Site Description

Awetu watershed encompasses Jimma town and the surrounding urban and semi-urban regions of southwestern Ethiopia. The streams under the Awetu watershed are receiving pollution from different sources, such as different laboratories of Jimma University, Jimma Medical Center garages and car washing points, untreated Jimma town municipal effluent, agricultural non-point source discharges, and animal feedlots. The watershed mainly contains four different streams, of which Awetu is the largest, which divides Jimma town into two while Dololo, Kitto, and Boye are tributaries of Awetu stream. The major land-use activities in the Awetu watershed along with the site code and the coordinate points are listed in Table 5.1. Sampling sites were mapped using ArcGIS 10.3 for Desktop (ESRI, Redlands, California, USA). Twenty surface integrated sediment samples were collected from each sampling point (Figure.4.1).

5.2.2 Sediment Samples Collection and Preparation

Submerged surface integrated sediment samples collected from 20 sampling stations in the urban and semi-urban streams of the Awetu watershed are used. As described by Decena et al., 2018, each sample constituted a 0-15 cm depth of sediment collected using a stainless steel bottom sampling dredge (grab sampler). The different grain sizes of the sediments were considered and homogenized to keep the uniformity of samples collected from each sampling station (Figure.5. 1). The grab sampler was washed with detergent and rinsed with distilled water before each use to reduce possible contamination. Sediment samples were placed in a new polyethylene zip-lock bag and placed in a cooler with ice, transported to the Environmental Health Laboratory of Jimma University within a few hours of sampling, and kept at 4ºC until analysis. During sample collection, a hand-held GPS was used to identify the sites (Garmin eTrex®). The sediment samples were air-dried and ground using a pestle and mortar after homogenization. Cobles, pebbles, and other coarse debris were manually removed. Finally, the grounded samples were sieved to pass through 63 µm to obtain fine-powdered particles and placed in a clean polyethylene zip-lock bag. The processed samples were refrigerated at -20 ◦C until further analysis.

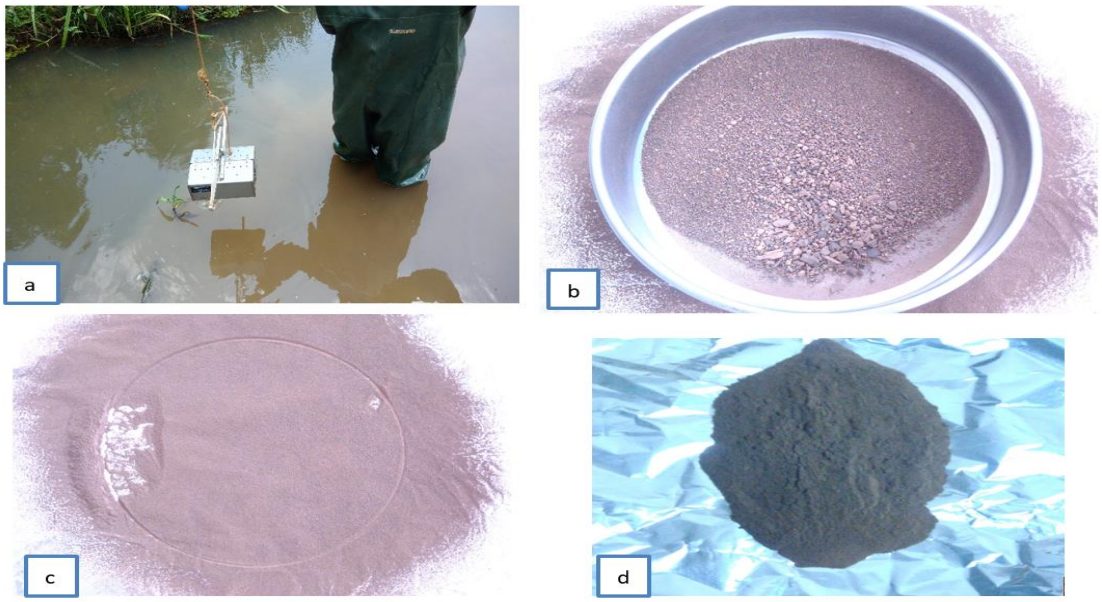

Figure 5. 1. Sediments sample collections and preparations in the Awetu watershed streams.

5.2.3 Analysis of Sediment Samples

A 0.25 g sediment sample was accurately weighed and placed in a dry and clean Teflon microwave digestion vessel and digested with 20 mL aqua regia (3 HNO_3:1 HCl v/v) until the solution turned colorless in an open thermostatically controlled hot plate. The digest samples were heated near to dryness and cooled to ambient temperature. The digestion beaker walls were rinsed with 10 mL deionized water, and 5 mL HCl were added, mixed, and heated. After that, filtration of the sample into a 50 mL volumetric flask using Whatman No. 42 filter paper was made. Then the digest was allowed to cool and transferred into a 100 mL standard flask and filled to the mark with deionized water. The digested samples were subjected to metal analysis using inductively coupled plasma optical emission spectrometry (ICP-OES) (SPECTRO ARCOS Model: ARCOS FHS12, Germany) (UNEP, 2006). The calibration curves obtained from concentration and absorbance were used to determine the level of heavy metals of each sample. Data were statistically analyzed using the fitting of a straight line ($r > 0.999$). A blank reading was used to make a necessary correction during the calculation of concentrations.

5.2.4 Data Analyses

Different contamination indices were used to evaluate the findings with different standards. The contamination status of sediment was assessed based on the geo-accumulation index (I_{geo}), contamination factor (CF), and pollution load index (PLI) (Graça et al., 2002).

5.2.4.1 Contamination Factor (CF)

CF was used to determine the simple and effective tool in monitoring the level of heavy metal contamination at each site using the following formula (Graça et al., 2002).

$$CF = \frac{C\ sample}{C\ background} \quad \text{...} (1)$$

Where C_{sample} is the mean metal content in sample sediment, $C_{\text{background}}$ is the mean natural background value of the metal. The natural background sample was collected from A1, which is about 10 km from the main areas where maximum anthropogenic activities are performed assuming similar geogenic factors. This area is assumed to be free from the known anthropogenic source of selected heavy metals. The ratio of the measured concentration to the natural abundance of a given metal had been proposed as the index CF being classified into four grades for monitoring the pollution of a single metal over some time (Ali et al., 2016): low degree ($CF < 1$), moderate degree ($1 \leq CF < 3$), considerable degree ($3 \leq CF < 6$), and very high degree ($CF \leq 6$). Thus, the CF values can monitor the enrichment of a given metal in sediments over a while.

5.2.4.2 Pollution Load Index (PLI)

PLI is used to evaluate sediment quality. PLI of the combined approaches of the five heavy metals was calculated according to (Islam et al., 2017). The PLI is defined as the n^{th} root of the multiplications of the contamination factor of the target heavy metals (CF).

$$\text{PLI} = \text{n} \sqrt{(\text{CF1} \times \text{CF2} \times \text{CF3} \times \ldots \times \text{CFn})} \quad \text{...} (2)$$

Where CF_1 is the concentration of the first metal, CF_2 is the concentration of the second metal, CF_3 is the concentration of the third metal and CF_n is the concentration of metal n^{th}, and n = the total number of studied heavy metals in the sample. PLI = 0 indicates excellence; PLI =1 suggests the presence of only a baseline level of pollutants and PLI > 1 indicates progressive deterioration of the site and estuarine quality (Tomlinson et al., 1980). The PLI evaluated the overall toxicity status of the sample and its contribution to the contribution of the five metals.

5.2.4.3 Geo-Accumulation Index (I_{geo})

The degree of contamination from the heavy metals could be assessed by measuring the geo-accumulation index (I_{geo}). The index of geo-accumulation has been widely used for the assessment of sediment contamination (Islam et al., 2014). To characterize the level of heavy metal

contamination in sediment samples, geo-accumulation index (I_{geo}) was calculated using the equation:

$$Igeo = Log2[\frac{Cn}{1.5\ Bn}] \quad \text{.....(3)}$$

Where; Cn is the content of measured metal ‘‘n’’ in the samples, Bn is the crustal shale background content of the metal ‘‘n’’, the constant of 1.5 is introduced to minimize the variation of background values due to lithogenic origins, and I_{geo} is a quantitative index of metal enrichment or contamination levels.

Geo-accumulation index (I_{geo}) values were interpreted as $I_{geo} \leq 0$ uncontaminated; $0 \leq I_{geo} \leq 1$ - uncontaminated to moderately contaminated; $1 \leq I_{geo} \leq 2$ - moderately contaminated; $2 < I_{geo} \leq 3$ – moderately to heavily contaminated; $3 \leq I_{geo} \leq 4$ - heavily contaminated; $4 \leq I_{geo} \leq 5$ - heavily to extremely contaminated; and $5 < I_{geo}$ - extremely contaminated (Müller, 1979).

5.2 5 Statistical Analyses

Heavy metal concentration data were log-transformed to reduce the variability and minimize outliers. R statistical package (R Core Team, 2019) was used to undertake a paired permutation test evaluating the presence of a statistically significant difference between the streams. To determine heavy metal concentrations variability among sediment samples between the urban and semi-urban sites, a one-way ANOVA was applied. Pearson’s correlation was made to examine the correlation between heavy metals and selected physicochemical properties. A probability of 0.05 was considered as a level of significance.

5.2.6 Quality Control and Quality Assurance

The quality of the analytical results is assured by laboratory quality assurance and quality control methods. These were implemented by pre-cleaning laboratory materials with 10 % HNO_3, use of standard operating procedures as indicated in section 5.2.3 above. Analysis of blanks was also done to ensure the quality of data by determining the analysis of a sample without the analyte. Calibration with the standard is the technique of plotting the instrument response against a series of samples with known concentrations of the analyte (standards). Each heavy metal was analyzed in three replicates, and the results were presented as mean. In this study, the metal contents were measured according to dry weight to ensure consistency.

5.3 Results and Discussion

5.3.1 Description of Anthropogenic Activities

Heavy metals concentration in stream sediment indicates the magnitude of pollution in water ecosystems and the capacity to accumulate contaminants (Nowrouzi and Pourkhabbaz, 2014). Speciation and bioavailability of heavy metals in aquatic ecosystems are strongly dependent on pH, and values < 4 increase the toxicity of heavy metals (Edokpayi et al., 2016). The average pH values determined varied between 5.82 and 7.60, reflecting the availability of carbonate hosts in the area with pH greater than 7 (Moore et al., 2011). The mobility of metal is also affected by pH, adsorption/desorption processes, salinity, sulfur, and carbonates (Giouri et al., 2010). Bottom sediments with a higher concentration of organic matter also influence the solubility and mobility of heavy metals in aquatic ecosystems (Ullah and Haque, 2010). The formation of potential mobile metal dissolved organic carbon complexes under oxidizing conditions prevents metals from co-precipitation with or adsorbing to oxides of metallic ions (Du Laing et al., 2009).

5.3.2 Heavy Metal Distribution

Heavy metal concentrations in sediments of the Awetu watershed catchment channelized streams with different world standards are summarized in Table 5.2. The present study exposed all the heavy metals in stream sediment that were found above the permissible limit set by WHO and EU (EU, 1993). A significant difference in the concentration of As, Cr, Cd, and Pb between streams was identified ($p < 0.01$).

The mean values of As showed 623.32 ± 291.65 mg/kg (ranged from 183.60 to 1102.80 mg/kg). The highest concentrations of As were observed in D2, D3, K1, and K2 sampling sites where it is the center of the town at which maximum anthropogenic activities are experienced. This might be sourced from the burning of fossil fuels, the use of arsenical fungicides, herbicides and insecticides in agriculture, and wood preservatives in woodwork enterprises The highest value of As is perceived at lower pH (5.82). The study supports this; pH is the most critical factor controlling arsenic speciation under oxidizing conditions and $H_2AsO_4^-$ is dominant at a lower value (pH < 6.9) (Smedley and Kinniburgh, 2001). The interchange in oxidation and solubility of arsenic species affects the environmental behavior and subsequent transportation of arsenic species in the aquatic ecosystems (Shankar et al., 2014; Chatterjee et al., 2017).

The mean values of Cd showed 151.09 ± 111.5 mg/kg (ranged from 4.40 to 303.20 mg/kg). The highest values of Cd are found at K1, D2, and D3 sampling sites. This might be due to the

institutional wastes, garages, car-washing, and agricultural activities where phosphate fertilizer has been in use upstream of K1. The values at D2 and D3 sites could be from the commercial waste, high vehicle traffic, garages, gas/petrol stations, and seepage from chemical laboratories and public institutions. The high level of Cd in the sediment compared to its levels in the water is to be expected for sediments that have been described as a sink or reservoir for pollutants in water (Topi et al., 2012). The concentration of Cd from all sites is significantly different from the background concentrations (23.2 mg/kg). It also significantly differs World River Sediment background concentrations by EC (3 mg/kg), PEL and CFSG (3.53 mg/kg), and WRSB (0.25 mg/kg) (Table 5. 2).

The mean values of Cr showed 375 ± 212.03 mg/kg (ranged from 4149.20 to 807.20 mg/kg). The highest values of Cr are found at the K1 sampling site, which could be attributed to the direct influence of effluents coming from institutional wastes like seepage of laboratory and electroplating (Baig et al., 2013; Song et al., 2000). The high level of Cr (VI) in sediment is expected more than water, mainly in the mobile environment but has low mobility under moderately oxidizing and reducing conditions and nearly neutral pH (Decena et al., 2018) and is discharged into the nearby natural water bodies without treatment.

The mean values of Pb showed 2005.94 ± 954.99 mg/kg (ranged from 485.60 to 3748.80 mg/kg). The highest values of Pb were recorded in Dololo (D1-D5), and Kitto (K1-K3) streams. This might be due to intensive anthropogenic activities like garages, gasoline stations, chemical laboratories, and construction industries. The other factors contributing to higher Pb concentrations might be due to the waste generated from public and private institutions released to the adjacent water bodies without treatment and old water pipelines (Adela et al., 2012). These values exceed the background concentrations of this study (952 mg/kg), EC (300 mg/kg), PEL and CFSG (91.3 mg/kg) and WRSB (48 mg/kg) guideline values (Khan et al., 2005). Exposure to elevated concentrations of Pb through drinking water or food may damage the kidney, increase blood pressure, and anemia (Basim and Khoshnood, 2016). Pb is a threat to public health even at very low concentrations because it is usually bioaccumulated in the body. Specifically, it is essentially harmful to children under six and causes mental and physical retardation (Kathuria, 2017).

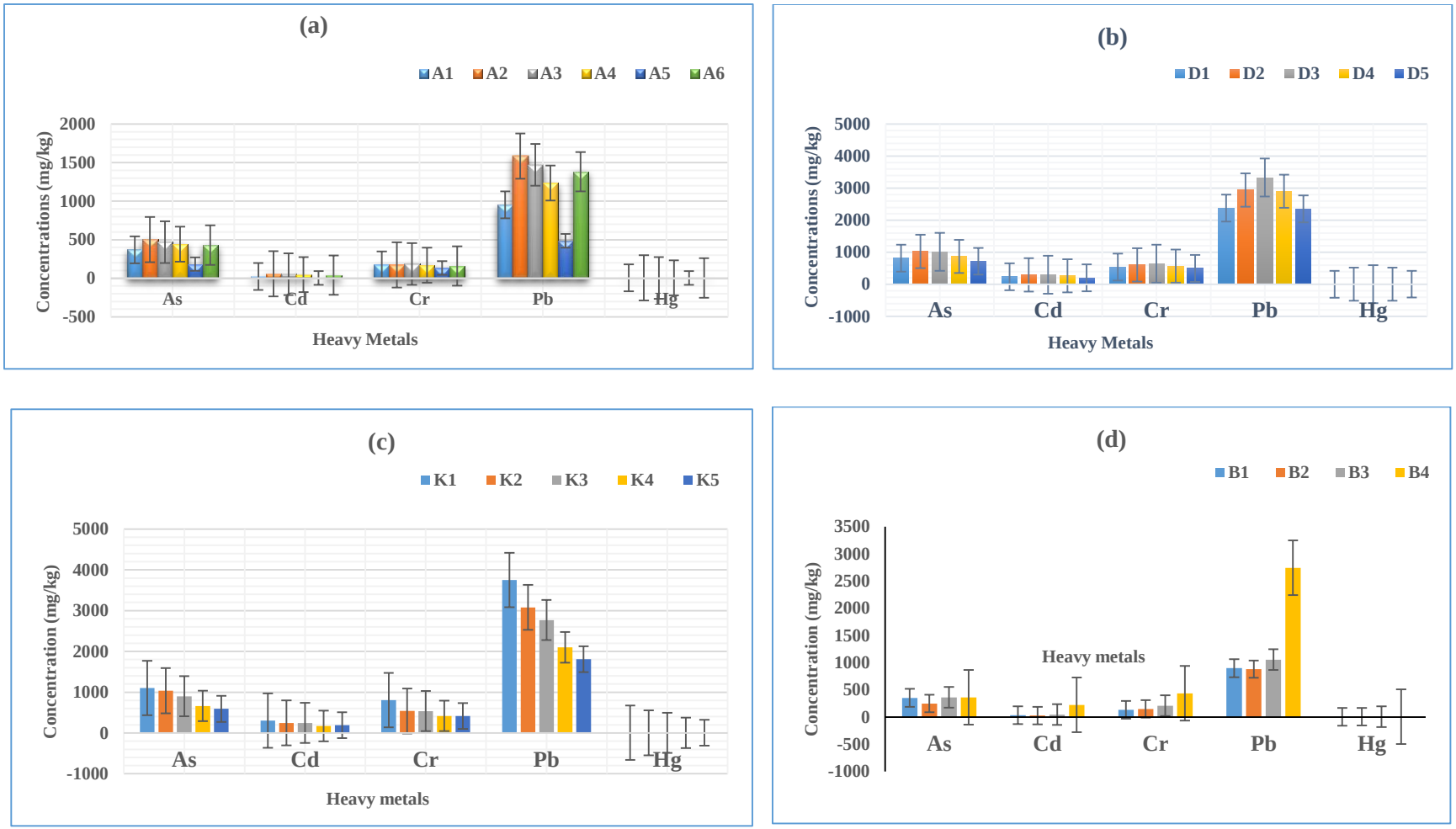

Figure 5. 2. Mean metals contents in sediments samples in mg/kg (mean ± SD) in the Awetu watershed in Awetu (a), Dololo (b), Kitto (c), and Boye (d) streams.

The mean values of Hg showed 4.64 ± 0.59 mg/kg (ranged from 3.6 to 6 mg/kg). The highest value of Hg is recorded D2 (6 mg/kg) followed by B3 (5.6 mg/kg) and D4 (5.2 mg/kg). This might be due to runoff and erosion from the surrounding catchment and solid wastes like municipal and medical incinerations. As shown in Table 5. 2, these values exceeded the background concentrations of this study (4.4 mg/kg), PEL (0.486 mg/kg) and WRSB (0.4 mg/kg).

The mean heavy metal concentration in the streams followed the decreasing order of Pb > As > Cr > Cd > Hg. As, Cr and Pb are detected at high concentrations with values of 623.32, 375.00, and 2,005.94 mg/kg respectively and the minimum concentration found is Hg with a value of 3.6 mg/kg. The heavy metals concentrations of sediment samples from individual streams showed high variability whereas the Dololo stream showed the highest value for all studied heavy metals except Hg. This might be due to the specific pollution sources of heavy metals might be different. The concentration of heavy metals in all stream sediments are by far higher than the Sediment Quality Guidelines (SQG) values set by World Rivers, EC and PEL (Khan et al., 2005), US EPA water quality criteria (Hahladakis and Smaragdaki, 2013), Canadian Freshwater Sediment Guidelines (Gergen et al., 2015), and World River Sediment Background Concentration (WRSB) concentration and background concentration of this study. Such contaminated environmental resources could be hazardous to humans, wildlife, and highly toxic to aquatic life (Olafisoye et al., 2013). The trend lines are in Figure 5.3 show the highest values in the sampling stations were observed in Pb.

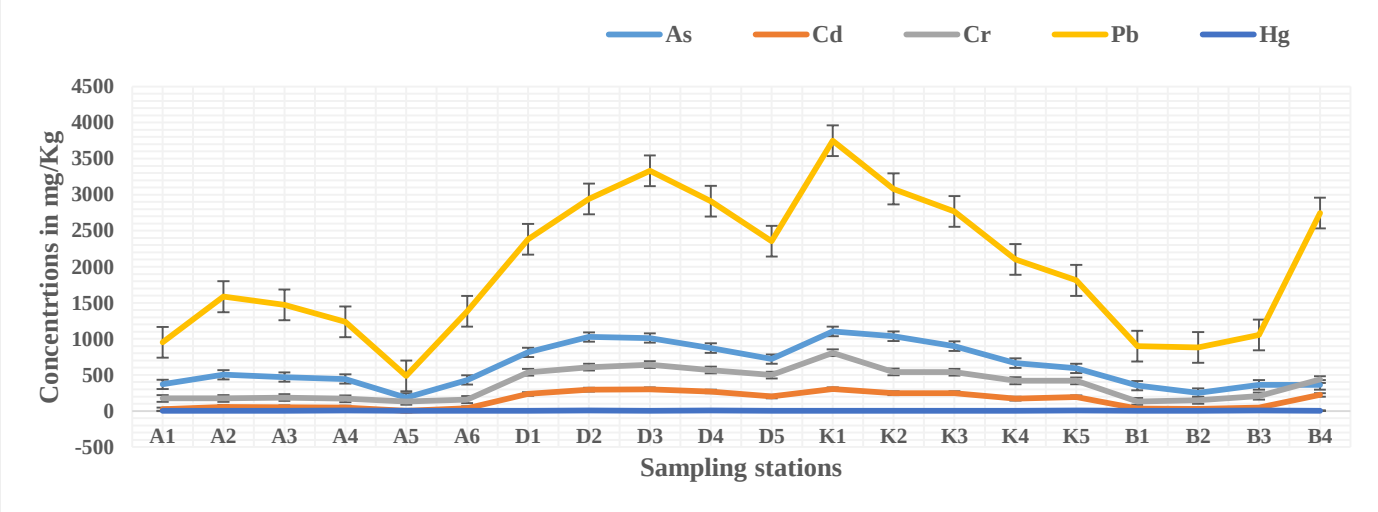

Figure 5. 3. Mean metal contents in mg/kg (mean ± SD) in sediments samples of the Awetu watershed streams.

Table 5. 2. Heavy metal concentrations (mg/kg) of sediment samples in comparison with different world standards.

Heavy metals	Sampling Stations																			[b]EC	[c]PEL	[d]WRSB
	A1	A2	A3	A4	A5	A6	D1	D2	D3	D4	D5	K1	K2	K3	K4	K5	B1	B2	B3	B4			
As	369.2±254.12	503.2±120.12	470 ±153.32	444 ±179.32	183.6 ±439.72	430.4 ±192.92	813.2 ±189.88	1026±402.68	1011.6±388.28	873.2 ±249.88	720.8±97.48	1102.8 ±479.48	1037.2 ±413.88	899.6±276.28	664 ±40.68	591.6 ± 31.72	351.2±272.12	249.2±374.12	362.8±260.52	362.8±260.52	20	17	1
Cd	23.2 ±127.89	57.2 ± 93.89	52.8 ± 98.29	47.2 ± 103.89	4.4 ± 146.69	40.12 ± 110.97	236.4 ± 85.31	295.6 ±144.51	30 2 ±150.91	269.6 ±118.51	201.2 ±50.11	303.2 ±152.11	247.2 ±96.11	247.6 ±96.51	171.2 ±20.11	192 ±40.91	33.6 ±117.49	28 ±123.09	45.2 ±123.09	224 ±105.89	3	3.53	0.25
Cr	174 ± 201.0	173.6 ± 201.4	186.8 ± 188.2	169.6 ± 205.4	133.6 ± 241.4	158.8 ± 216.2	537.2 ± 162.2	607.2 ± 232.2	643.2 ± 268.2	567.6± 192.6	498.8 ± 123.8	807.2 ± 432.2	540.8 ± 165.8	537.6 ± 162.6	419.6 ± 44.6	418.8 ± 43.8	132.8 ± 242.2	149.2 ± 225.8	207.6 ± 167.4	436 ± 61.0	ND	90	55.5
Pb	952 ± 1053.94	1585.2 ± 420.74	1472.4 ± 533.54	1235.6 ± 770.34	485.6 ± 1520.34	1382 ± 623.94	2381.6± -375.66	2940.8 ± -934.86	3330.8 ± -1324.86	2908.4 ± -902.46	2354.8 ± -348.86	3748.8 ± - 742.86	3080.4 ± -1074.46	2767.6 ± -761.66	2100.4 ± -94.46	1811.6 ± 194.34	900 ± 1105.94	880.8 ± 1125.14	1056 ± 1125.14	2744 ± -738.06	300	91.3	48
Hg	4.4 ± 0.24	4.8 ± 0.16	4.4 ± 0.24	5.2 ± 0.56	4.8 ± 0.16	4.4 ± 0.24	4.4 ± 0.24	6 ± 1.36	3.6 ± 1.04	5.2 ± 0.56	4.4 ± 0.24	4 ± 0.64	4.8 ± 0.16	4.4 ± 0.24	4 ± 0.64	5.2 ± 0.56	4 ± 0.64	4.4 ± 0.24	5.6 ± 0.96	4.8 ± 0.16	20	0.486	0.4

Background concentration of this study (A1), [b] *European Commission (EC),* [c] *Probable Effects Level (PEL)* (Khan et al., 2005) *and Canadian Freshwater Sediment Guidelines (CFSG)* (Shafie et al., 2015) *and* [d] *World River Sediment Background Concentration (WRSB)*

Table 5. 3. Contamination categories are based on a geo-accumulation index (I_{geo}), pollution load index (PLI), and contamination factor (CF) (Varol, 2011).

I_{geo}				PLI			CF	
Class	Value	Classification	Level	Value	Contamination	Level	Value	Level
0	< 0	Uncontaminated	1	CF < 1	Low degree	1	PLI = 0	Excellent
1	0 - 1	Uncontaminated to moderately Contaminated	2	CF = 1 - 3	Moderate degree	2	PLI = 1	Baseline
2	1 - 2	Moderately contaminated	3	CF = 3 - 6	Considerable degree	3	PLI > 1	Progressive deterioration
3	2 - 3	Moderately and heavily Contaminated	4	CF > 6	Very high degree			
4	3 - 4	Heavily contaminated						
5	4 - 5	Heavily to extremely contaminated						
6	> 5	Extremely contaminated						

The concentration of heavy metals alone does not provide sufficient information on the mobility and potential toxicity of contaminants or their potentially harmful effects on the environment, because different chemicals can inactivate and promote synergistic effects. I_{geo}, CF, and PLI determine the potentially harmful effect of heavy metals in the environment. These indices provide a basis for assessing the effects of sediment-associated contaminants in sediment compared to the values concerning each index (Moore et al., 2011).

5.3.3 Comparision of Contamination Level of the Streams with Different Pollution Indices

Based on the I_{geo} values, As and Pb made moderate pollution (0), Cr moderately to heavily polluted, Cd extreme pollution, Hg unpolluted to moderately polluted in the Dololo stream (Table 5.4).

Table 5. 4. I_{geo} values for heavy metals in sediments of streams with the class of pollution in brackets in the Awetu watershed.

Streams	As	Cd	Cr	Pb	Hg
Awetu	- 0.47 (0)	0.11 (1)	- 0.64 (0)	- 0.27 (0)	- 0.49 (0)
Dololo	1.61 (1)	7.5 (6)	2.19 (3)	1.95 (2)	0.72 (1)
Kitto	0.63 (1)	2.74 (3)	1.09 (2)	0.92 (1)	- 0.56 (0)
Boye	- 0.74 (0)	1.25 (2)	- 0.17 (0)	- 0.03 (0)	- 0.49 (0)

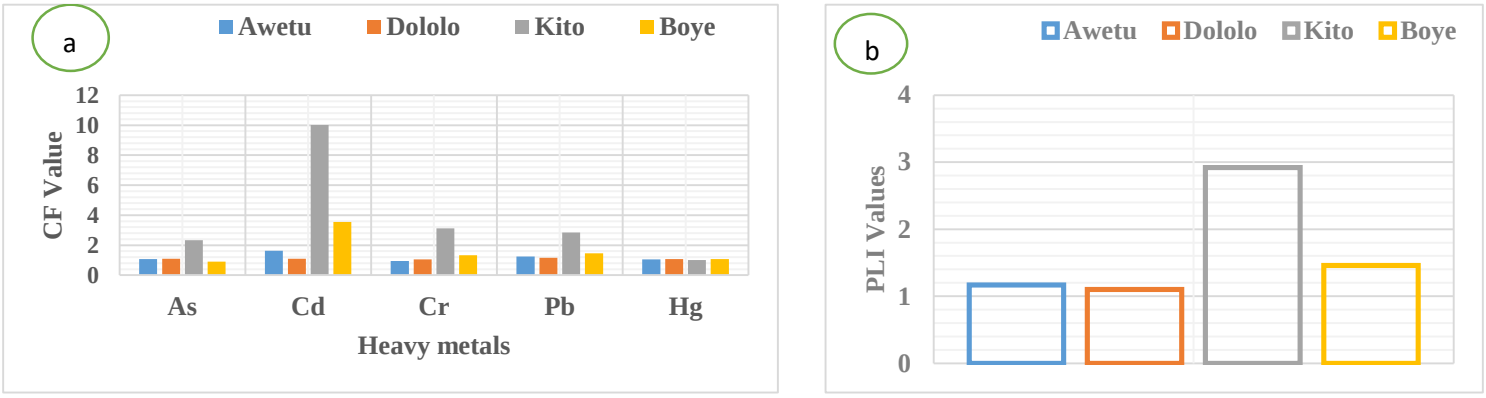

Figure 5. 4. The results of CF (a) and PLI (b) in the Awetu watershed streams

The CF and PLI values are indicated in Figure.5.4. These values for heavy metals in sediments are essential to monitoring the enrichment of a given metal over time (Ali et al., 2016). The CF results of As indicated, in all streams except Boye (0.9) showed a moderate degree of pollution ($1 \leq CF < 3$) with the values of 1.08, 1.09, and 2.33 for Awetu, Dololo, and Kitto streams respectively. This might be due to the concentrations of heavy metals in these streams being higher than the background concentrations. The CF of Cd in Awetu and Dololo streams are 1.62 and 1.1, respectively, which shows the sediment is in the status of a moderate degree of pollution ($1 \leq CF < 3$). The CF of Cd in the Boye riverine wetland is 3.56, which shows a considerable degree ($3 \leq CF < 6$), and the Kitto stream is 10.01, which shows a very high degree of pollution ($CF \geq 6$). This might be due to the Kitto stream being predominantly polluted by Cd metal. The CF of Cr in the Awetu stream is 0.95, which indicates the sediment is in a low degree of pollution ($CF < 1$) while the CF of the Kitto stream is 3.13, which indicates the sediment is in a considerable degree of pollution ($3 \leq CF < 6$). Dololo and Boye streams with the CF values of 1.06 and 1.33 respectively show a moderate degree ($1 \leq CF < 3$). The CF values of Pb in Awetu, Dololo, Kitto, and Boye streams are 1.25, 1.17, 2.84, and 1.47 respectively, which shows these streams are in a moderate degree of pollution ($1 \leq CF < 3$) by Pb. The CF values of Hg in Awetu, Dololo, Kitto, and Boye streams are 1.06, 1.07, 1.02, and 1.07 respectively, indicating streams are in a moderate degree of pollution ($1 \leq CF < 3$). The CF of heavy metals greater than 6 showed the enrichment of a given metal in sediments greater variation between the metal concentrations and the background values.

The PLI values calculated for each stream revealed the decreasing order of contamination: Kitto > Boye > Awetu > Dololo. All the studied streams were found to be polluted (PLI > 1), suggesting the Kitto stream receives a huge amount of metallic discharge inputs from anthropogenic sources.

The I_{geo} of As shows practically unpolluted in Awetu and Boye, which shows a very low value (< 0), unpolluted to moderately polluted in Kitto (0-1), and moderately polluted in Dololo (1-2). This might be due to the difference in pollution dynamics of the studied heavy metals and I_{geo} is a quantitative index of metal enrichment or contamination levels. The I_{geo} values calculated for each stream of the studied metals are also indicated in Table 5. 4. Accordingly, the concentration level of As indicates unpolluted to moderately polluted status in Awetu and Boye (0 -1) and moderately polluted in Dololo and Kitto streams (1-2). Cd indicates unpolluted to moderately polluted status in Awetu (0 -1), moderately polluted in Boye (1-2), polluted moderately to heavily polluted in Kitto (2 - 3), and extremely polluted in Dololo streams (> 5). Cr shows practically unpolluted in Awetu and Boye (< 0), moderately polluted in Kitto (1-2), and moderately to heavily polluted in Dololo streams (3 - 4). Pb shows practically unpolluted in Awetu and Boye (< 0), unpolluted to moderately polluted in Kitto (1-2), and moderately polluted in Dololo (1-2) streams. Hg shows practically unpolluted in Awetu, Kitto, and Boye streams (< 0) and unpolluted to moderately polluted in the Dololo stream (0 -1). The anthropogenic pressures largely contribute to the observed heavy metal concentrations rather than the mineralogical composition of the crust along the watershed. The higher values of I_{geo} were observed in samples from the Dololo stream as compared to other streams, which might be due to the stream being found in the center of the town where the maximum magnitude of anthropogenic pressures is observed. However, both factors are likely to be the most significant cause of the observed differences. When compared with results from other developing nations, the concentrations measured in this study are much higher than in rural and urban streams (Bai et al., 2011).

The box and whisker plot (Figure. 5.5) indicates that relatively higher heavy metal concentrations were identified in the Dololo and Kitto streams, except for Hg. Pairwise permutation test using R statistical package (R Core Team, 2019) showed no significant concentration difference between Awetu and Boye, and Dololo and Kitto streams for As, Cd, and Cr concentrations. These streams are known to receive wastewater discharges from Jimma University compasses. For example, the Dololo stream receives untreated wastewater discharge from the main campus and the Jimma

Medical Center. Similarly, the Kitto stream receives the wastewater effluent from the waste stabilization pond of Jimma Institute of Technology. These campuses have also different laboratories which might be the source of these contaminants. Though the further investigation of tracing which exact laboratory source from these campuses is crucial, this might be the possible reason why these two differently located streams showed a similar pattern of heavy metal contamination.

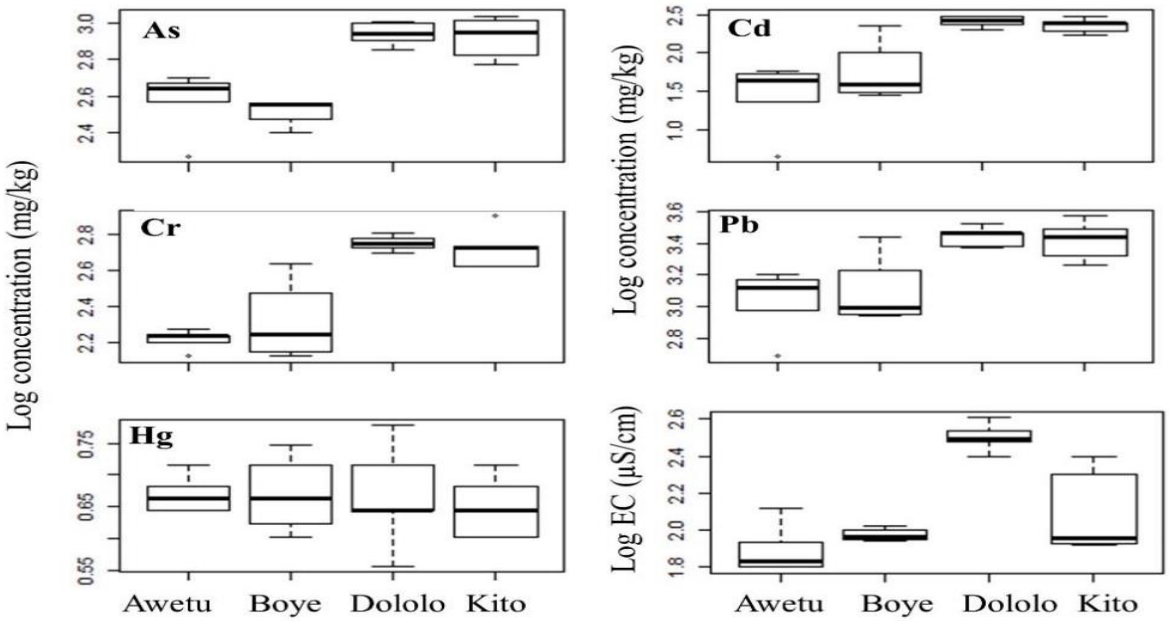

Figure 5. 5. Box and whisker plot of heavy metals concentrations and level of electrical conductivity (EC) along with the streams in the Awetu watershed.

The interrelationship among metals in sediment of the aquatic environment provided important information on sources and pathways of heavy metals. The result of bivariate correlations between parameters is shown in Table 5. 6. The elemental pairs, As and Cd ($r = 0.901$, $P < 0.01$); As and Cr ($r = 0.916$, $P < 0.01$); As and Pb = 0.912, $p < 0.01$); Cd and Cr = 0.978, $p < 0.01$); Cd and Pb = 0.963, $p < 0.01$); Cr and Pb ($r = 0.958$, $P < 0.01$) showed significant positive correlation. This result might indicate similar pollution sources of two or more heavy metals. The elemental association may signify that each paired elements have a common source in the stream sediments discharged from municipal wastes and agricultural inputs (Bhuyan et al., 2017). Metal and physicochemical associations show pairs EC/As ($r = 0.467$, $p < 0.05$), EC/Cd ($r = 0.484$, $p < 0.05$) and EC/Cr (0.448, $p < 0.05$) are correlated with each other, whereas the rest are not significantly correlated. The positive correlations of heavy metals concentration of the sediment with EC might

be attributed to anthropogenic impacts (Alghobar and Suresha, 2017). Turbidity has correlated with As ($r = 0.574$, $p < 0.01$), Cr ($r = 0.513$, $p < 0.05$) and Pb ($r = 555$, $p < 0.05$). DO and pH are negatively correlated with all studied heavy metals in the stream sediment, which significantly affects heavy metals (Sekabira et al., 2010). The concentrations of heavy metals were significantly correlated with pH (negatively), turbidity (positively) except for Hg (negatively).

Table 5. 5. Correlation between heavy metals, DO, EC, pH, and Turbidity in sediment (n = 20).

	DO	EC	pH	Turbidity	As	Cd	Cr	Pb	Hg
DO	1								
EC	- 0.227	1							
pH	0.224	0.087	1						
Turbidity	**0.444***	0.046	- 0.227	1					
As	- 0.040	**0.467***	- 0.054	**0.574****	1				
Cd	- 0.218	**0.484***	- 0.170	0.441	**0.901****	1			
Cr	- 0.166	**0.448***	- 0.268	**0.513***	**0.916****	**0.978****	1		
Pb	- 0.094	0.411	- 0.172	**0.555***	**0.912****	**0.963****	**0.958****	1	
Hg	- 0.241	- 0.121	- 0.121	- 0.151	- 0.209	- 0.220	- 0.186	- 0.235	1

*, ** Correlation is significant at 0.05 and 0.01 level respectively

The absence of a significant correlation between the heavy metals and pH might be due to variation in sediment composition that implies minerals are the only factors controlling the binding of heavy metals (Ali et al., 2016). Other studies also showed that there is a significant variation in the concentrations of heavy metals based on the type of waste discharge at different sites (Nagajyoti et al. 2010). Our findings showed elevated heavy metal concentration than many other published studies (Table 5.7). This indicates how much the aquatic environment and probably the biota is at risk of elevated heavy metal contamination.

Table 5. 6. Correlation between heavy metals in sediment (n = 20).

	As	Cd	Cr	Pb	Hg
As	1				
Cd	**0.901****	1			
Cr	**0.916****	**0.978****	1		
Pb	**0.912****	**0.963****	**0.958****	1	
Hg	-0.065	-0.018	-0.084	-0.103	1

**. Correlation is significant at the 0.01 level.

Besides the teaching institutions' wastewater discharge, run-off and waste discharges from different sources, such as car washing facilities, hotels, and garages are directly entering the stream system. This could be the main reason for the extremely exceeded Pb concentration than the other studies done in China, Turkey, and Bangladesh. Similarly, (Liao et al., 2017) found that the concentrations of heavy metals were up to 120 fold higher than the background concentration, which was mainly sourced from mining activities. However, in our case, multiple sources of aquatic pollution might have contributed to these very high concentrations of heavy metals.

Table 5. 7. Comparison of heavy metal concentrations in sediment samples of this study with the other studies.

Study area	Mean concentrations of heavy metals in the Sediment (mg/Kg dw)					Source of contamination identified	References
	As	Cd	Cr	Pb	Hg		
Awetu watershed stream, Jimma zone, southwestern Ethiopia	623.3	151.1	375.0	2005.9	4.6	Uncontrolled municipal and commercial wastes discharged and solid waste dumping sites	This study
Tisza River and its tributaries, Serbia	*	*	11.37	19.13	*	Industrial activities, municipal sewage discharges, and agricultural inputs	(Manojlovic, 2009)
Yangtze River intertidal zone, China	*	0.261	78.9	27.3	*	Not mentioned	(Zhang et al. 2009)
Yilong Lake Wetland, China	15.46	0.76	86.73	53.19	*	Industrial discharges, coal and lead mining areas, and domestic effluents	(Bai et al., 2011)
The northern part of Lake Babrukas, Lithuania	24.59	31.79	76.97	7.25	10.25	Municipal wastewater discharge	(Raulinaitis, 2012)
Butrinti Lagoon, Albania	*	0.125	56.5	31.2	0.16	Pedo-geological processes and human activity	(Topi et al., 2012)
Upper Tigris River, Turkey	5.90	3.02	135.81	380.4	*	Copper mine plant	(Varol and Şen, 2012)
Jinjiang River, China	9.6	1.6	32.2	95.1	0.08	Residential, industrial, and agricultural pollutant loads	(Chen et al., 2013)
Turag, Buriganga, and Shitalakha rivers, Bangladesh	35	17	695	356	*	From residents and household waste and sewage treatment facility	(Islam et al., 2014)
Korotoa River of Bogra district urban area, Bangladesh	25	1.2	109	58	*	Domestic raw sewage, household waste, and industrial wastes	(Islam et al., 2015)
Karnaphuli River, Bangladesh	81.1	2.01	20.3	43.7	*	Industrial effluent and domestic sewage discharge	(Ali et al., 2016)
Lake Edku, Egypt	*	*	113.1	44.6	*	Run-off from agricultural fields	(Ahmed, 2017)
Jiaozhou Bay rivers, China	7.7	0.159	69.3	20.2	*	Industrial and domestic waste discharge	(Xu et al., 2017)
Zhelin Bay, China	*	0.063	23.07	35.7	*	Not mentioned	(Gu, 2018)
Mustafakemalpas¸ Turkey	154	8.78	516	65.6	*	Mining and industrial activities	(Omwene et al., 2018)
Miyun District, China	15.1	0.18	31.5	33.01	*	Not mentioned	(Pan et al., 2018)

*No data

5.4 Conclusion

Different useful guidelines and indices have been employed for the evaluation of sediment pollution in Awetu watershed streams, Jimma zone, southwestern Ethiopia. The contamination of studied heavy metals (As. Cd, Cr, Pb, and Hg) showed at various degrees. The concentration of the metals in sediments decreased in the order of Pb > As > Cr > Cr > Hg. The concentrations of heavy metals in the streams of Awetu watershed sediments were remarkably high and varied among sampling points. The data analyses by I_{geo}, CF, and PLI values showed contamination of the sediments by all the studied heavy metals which exceeded the limits of average world concentration. A significant interelement association indicates that these metals were derived from similar sources and also moving together. As a result, the higher concentration level of heavy metals in sediment poses a risk of water pollution during sediment disturbance or changes in sediment chemistry, which eventually intermix and transfer easily with the water in the hydraulic movement. The results suggested that special attention must be given to the issue of heavy metal pollution since a considerable portion of elements in sediments is likely to be re-distributed to the water column. Therefore, public awareness creation, ecosystem management, policy interventions should be implemented and forced to have waste management options before releasing to the environment.

Chapter Six:

Ecological Risk Assessment based on Heavy Metals Concentrations in Streams from the Awetu Watershed, Jimma, southwestern Ethiopia.

Abstract

Heavy metals in excess have been and continue to be dumped into Ethiopia's freshwater environments in excess. However, there is a scarcity of data on the ecological risk assessment of heavy metals from water and sediments for the inhabitants in Ethiopia's southwestern region. Water and sediment samples were collected from streams (Awetu, Boye, Dololo, and Kitto) in the Awetu watershed for this study. An Inductively Coupled Plasma Optical Emission Spectrometer was used to analyze the samples for heavy metals (As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn) (ICP OES). Sediments had substantial contamination levels based on ranges in sediment quality guidelines (SQGs). Heavy metals were found at higher levels in water samples, creating a potential ecological risk. The Nemerow pollution index, potential ecological risk index, and modified degree of contamination were used to assess the synergistic impacts of heavy metals in the study area. The Dololo and Kitto streams, located in the heart and perimeter of Jimma town, were judged to have ‘heavy' contamination and ‘severe' ecological risk, respectively. The metals Cd, Mn, Ni, Pb, and Sn were shown to become the most harmful to the environment compared to the other metals. Institutions carrying out a variety of anthropogenic activities along the stream bank, including traditional metal plating, garages, laboratory effluents, extensive agriculture, carwash, uncontrolled waste disposal, and urban population growth, are attributed to heavy metal contamination in the Dololo stream. In conclusion, heavy metals have seriously polluted the streams of the Awetu watershed. As a result, continual pollution control strategies and management plans should be adopted, and rigorous limitations on the discharge of solid and liquid wastes from anthropogenic sources should be enforced.

Keywords: Heavy metals, multi-elemental indices, risk assessment, sediment quality guidelines

6.1 Introduction

The discharge of potentially hazardous heavy metals by rapid industrialization, intense agricultural practices, and significant population increase harms the aquatic environment, particularly in developing countries. Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr)), copper (Cu), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), and zinc (Zn) represent a severe threat to fish, invertebrates, and humans (Rabee et al., 2011; Yi et al., 2011; Nawab et al., 2018).

The widespread use of heavy metals in a range of commercial, agricultural, residential, and technological applications has resulted in a significant increase in human exposure. Heavy metals are dumped in large quantities into rivers, where they can collect in water and sediment, bio-magnify in the aquatic food chain, and cause sub-lethal effects or death in local fish populations (Tchounwou et al., 2012). Heavy metals are rapidly deposited and tightly bound to fine-grain sediments as a result of residual anthropogenic activities, and they last a long time (Simpson and Spadaro, 2016). Heavy metals are receiving a lot of attention because of their persistence and bioaccumulative tendency in the food chain, as well as their negative effects on aquatic ecosystems and humans (Altan et al., 2016; Yuan et al., 2018).

Heavy metals that remain in the sediments of fine gain may be detached and released adversely affecting the quality of water (Gupta et al., 2009; Khan et al., 2017). For sediments, this happens more frequently, because large environmental changes such as pH, temperature, and alteration of sedimentary deposits by living organisms will change the condition quickly (Islam et al., 2013). Heavy metals are highly mobile from anthropogenic than lithogenic sources which affect aquatic species adversely due to its nature of bioavailability (Vu et al., 2017). In the water portion, heavy metals that are adsorbed to suspended sediments reduce the concentration, are inactive in the sediment system, and are often considered conservative contaminants (Giri and Singh, 2014; Lundy et al., 2017; Yi et al., 2017). But they could be released into the water in response to certain disturbances (Shafie et al., 2015) causing potential risk to ecosystems (Moore et al., 2011).

Bottom sediments provide ecosystems and benthic fauna with a food supply, and heavy metals can be harmful to marine lives directly or indirectly. The bioaccumulation and bioconcentration of heavy metals in the food web resulted from their effect outside the water ecosystem (Zhuang et

al., 2008; Deribe et al., 2014; Jiang et al., 2018). The analysis of the distribution of heavy metals in water and sediments adjacent to residential areas can also be used to investigate anthropogenic ecosystem impacts and helps to determine the threats posed by the discharge of human waste (Matta and Gjyli, 2016).

Heavy metals diluted rapidly in the water systems due to low solubility and transported with hydrologic gradients for long distances and settled on the bottom sediments (Dir et al., 2014; Jiang et al., 2018; Zhang et al., 2018). In the meantime, under favorable environments, heavy metals adsorbed in the sediments could be released into overlying waters and may enter the food chain, thereby posing health risks to human consumers (Yi et al., 2017; He et al., 2018). Sediments found at the bottom of the water body might act as an indicator of the degree of contamination and serve as a monitoring tool to detect historical and recent pollution in the surrounding area (Benson et al., 2016).

The pollution status and ecological risk can be assessed from the total metal concentration and several approaches have been developed and optimized (Brady et al., 2015). Mostly, calculating contamination factor (C_f), enrichment factor (E_f), and geo-accumulation factor (I_{geo}) of a single element are the common methods of evaluating metal toxicity and ecological risk in sediments (Adebiyi and Ezeh, 2015; Islam et al., 2015; Liu and Ma, 2015). Heavy metals are most likely to cause synergistic environmental impacts as a single element may not be appropriate in their contamination and risk assessment (Duodu et al., 2016; Vu et al., 2017). Hence, multi-element indices such as modified degree of contamination (mC_d) and Nemerow pollution index (P_N) can be employed to calculate the synergistic effects of heavy metals (Hakanson,1980; Yan et al., 2016), and synergistic ecological risk can also be shown quantitatively by the potential ecological risk index (Nawab et al., 2018).

Jimma, the oldest town in the southwestern part of Ethiopia, is in the rapid expansion of urbanization, industrialization, and intensive agricultural practices. But there are still no waste management facilities where huge quantities of contaminants from different anthropogenic sources are discharged daily inappropriately to the nearby water bodies (Getahun et al., 2012; Ambelu et al., 2013). For a long time, the town has been the center for a variety of different small-scale industries expanded carelessly on the outskirts of the water bodies and this effluent enters even

without preliminary treatment. A huge amount of contaminated liquid and solid waste discharge is received by the streams contained in the watershed from the industrial plants operating along its banks (Haddis et al., 2014). Broad public attention has been drawn to the condition of the streams in the Awetu watershed, contributing to the need to determine the degree of contamination and the ecological risk posed by heavy metals and consistent measures to be implemented to ensure the stream supports the health of residents and the environment. Based on the concentrations of heavy metals in the surface water and sediments, the ecological risks posed by these heavy metals will be assessed and discussed in this chapter.

6.2 Methods and Materials

6.2.1 Description of the Study Area

The present study aimed to investigate the pollution level and ecological risk assessment of heavy metals in the streams in the Awetu watershed that are found at the center and periphery of Jimma town, the largest town in the southwestern part of Ethiopia. The town is found at 7° 40' N 36° 50' E and has an altitude of 1,780 m above sea level, average annual rainfall of 1624 mm, and an average temperature of 18.9 ° C (Getahun et al., 2012; Astatkie et al., 2021). The watershed consists mainly of four streams, the largest of which is Awetu, which divides Jimma town into two, while Dololo, Kitto, and Boye streams are tributaries and found at the periphery of the town. Samples of surface water and sediments were collected from six sites from Awetu, five sites from Dololo, five sites from Kitto, and four sites from Boye streams. The streams directly received liquid wastes discharged from public and private institutions and solid wastes from municipal discharge along the stream banks. But, Awetu and Boye streams are still used as reliable water sources for domestic purposes for Jimma town residents. Industrialization and urbanization have resulted in significant stream contamination and altered the existence and chemistry of the streams (Lin et al., 2016).

6.2.2 Sample Collection and Preparation

A total of 40 (20 water and 20 sediments) samples were collected from 20 different sampling stations in December 2019 across the Awetu watershed channelized streams; Awetu, Dololo, Kitto, and Boye (Figure 4.1). This study used standard methods of water sampling (US EPA, 2013) and sediment sampling (US EPA, 2007). Each water sample was collected by submerging the sample container into the stream at about 100 mm below the surface and the center of the river after rinsing

the bucket with an open end facing against the current flow direction (Astatkie et al., 2021). The samples were then transferred into acid-cleaned 100 mL polypropylene bottles. One mL of ultrapure nitric acid was added to each polypropylene bottle to achieve a pH of ~1(Islam et al., 2015a).

At each sampling point, composite sediment samples were collected from the stream bed at a depth of 0-5 cm using a portable Ekman Dredge grab sampler. Collected sediment samples were collected in bags and finally brought to the laboratory and kept in dark conditions below 4 °C until analysis.

6.2.3 Heavy Metal Analysis

Water samples were filtered through a 0.45 μm millipore membrane filter and kept acidified with nitric acid (pH ~2). 0.25 g sediment sample was precisely weighed and placed in a clean beaker and digested with 20 mL aqua regia (3 HNO_3:1 HCl v/v) until the solution turned clear in a hot plate. The digest was heated near to dryness and cooled to ambient temperature. The walls of the beaker were rinsed with 10 mL of de-ionized water and 5 mL HCl, mixed, heated again, and filtrated into a 50 mL volumetric flask using Whatman No. 42 filter paper. The filtrate was cooled, transferred to a 100 mL flask, and filled with de-ionized water up to the mark. The portion of digested samples was subjected to metal analysis using ICP-OES (SPECTRO ARCOS Model: ARCOS FHS12, Germany) (Sarojam, 2010). The calibration curves were obtained for concentration vs absorbance and confirmed with correlation coefficient (R^2), which ranges from 0.996 - 0.999 (US EPA, 2000). All reagents used were of analytical grade and water used in all analyses was de-ionized. The MDLs for sediment samples (mg/kg dw) were as follows: As (0.1), Cd (0.09), Cr (0.1), Cu (0.1), Hg (0.01), Mn (0.05), Ni (0.09), Pb (0.1), Sn (0.1) and Zn (0.3). The MDLs for water samples (mg/L) were as follows: As (0.003), Cd (0.005), Cr (0.005), Cu (0.0009), Hg (0.0005), Mn (0.001), Ni (0.001), Pb (0.002), Sn (0.007) and Zn (0.003).

6.2.4 Risk Assessment Methods

6.2.4.1 Modified Degree of Contamination (mC_d)

The mC_d of heavy metal is an important indicator used to quickly and efficiently assess the severity of the contamination of a site and calculated as:

$$mCd = \frac{1}{n}\sum_{i=1}^{n} \quad C_f^i, \ \ where \ \ C_f^i = C^i \ / C_{ref}^i \qquad (1)$$

Where C^i is the heavy metal concentration in sediment and water samples; $C^i_{ref\ is}$ the reference value of the element (Turekian and Wedepohl, 1961; Hakanson, 1980) and C_f^i the contamination factor of each element.

6.2.4.2 Nemerow Pollution Index (P_N)

The P_N reflects the comprehensive effects of heavy metals (Vu et al., 2017) and can be used to infer heavy metal pollution at a particular site (Duodu et al., 2016) and can be calculated as:

$$P_N = \frac{\sqrt{\bar{Cf}^2 + C^2 fmax}}{2} \quad \ldots\ldots (2)$$

Where $\bar{Cf}$ the arithmetic means of contamination factor of all heavy metals, and C_{fmax} is the maximum contamination factor among the heavy metals.

6.2.4.3 Ecological Risk Assessment (ERA)

Risk assessment of surface water and sediment consists of three major steps which may be further subdivided in some jurisdictions (US EPA, 2000; Yi et al., 2011): problem formulation; analysis (exposure and effects and communities in the field (Saha and Hossain, 2011). The risk index (RI) evaluates the potential ecological risk of one or several elements (Hakanson, 1980). The RI is calculated as risk characterization. The overall intent of any ERA is to evaluate the risk to populations.

$$RI = \sum_{i=1}^{n} E_f^i, \qquad where\ E_f^i = C_f^i \times T_f^i \quad \ldots\ldots (3)$$

Where C_f^i is the contamination factor, T_f^i the toxicity response coefficient of each element (As = 10, Cd = 30, Cr = 2, Cu = Pb = Ni = 5, Zn = 1 and Hg = 40) (Hakanson, 1980; Islam et al., 2015; Lu et al., 2015) and E_f^i the potential ecological risk factor of each element (Yi et al., 2011; Vu et al., 2017).

Table 6. 1. Modified degree of contamination, Nemerow Pollution Index, and Ecological risk index of heavy metals (Pejman et al., 2015).

Class	mC_d	Contamination degree	P_N	Contamination degree	RI	Ecological risk
0	< 1.5	Unpolluted	< 1	Unpolluted	< 110	Low risk
1	$1.5 \leq mC_d < 2$	Slightly polluted	$1 \leq P_N < 2.5$	Slightly polluted	$110 \leq RI < 200$	Moderate risk
2	$2 \leq mC_d < 4$	Moderately polluted	$2.5 \leq P_N < 7$	Moderately polluted	$200 \leq RI < 400$	Considerable risk
3	$4 \leq mC_d < 8$	Moderately-heavily polluted	≥ 7	Heavily polluted	≥ 400	Severe risk
4	$8 \leq mC_d < 16$	Heavily polluted				
5	$16 \leq mC_d < 32$	Severely polluted				
6	≥ 32	Extremely polluted				

6.2.5 Statistical Analysis

The statistical analyses were performed using SPSS version 20. Data were subjected to ANOVA to analyze the difference in heavy metal content between the sites. Probability less than 0.05 ($p < 0.05$) were considered as statistically significant. The relationship between the variables was evaluated based on Pearson's correlation analysis. The spatial distribution of heavy metals was mapped using ArcGIS 10.3 (ESRI, Redlands, California, USA).

6.3 Results and Discussion

6.3.1 Heavy Metal Contamination in Water and Sediment

The concentration of heavy metal in sediment and water samples in the streams in the Awetu watershed are summarized in Table 6.2 and Table 6.3 respectively. Mean concentrations with the highest values of Cd, As, Cr, Cu, Ni, Sn, Mn, and Pb of sediment samples were observed in SK1, Hg in SD2, and Zn in SD3. Mean metal concentrations were higher in the Dololo and Kitto streams than Boye and Awetu streams. This may be due to Kitto and Dololo streams crossing the center of the town, where maximum wastes are released from various anthropogenic sources. The mean concentrations of heavy metals in the sediments were higher than the water in the streams. This might be heavy metals easily precipitate and bind strongly to sediments, but may detach from sediments and release them back to the column of water, which adversely affects the quality of water (Simpson and Batley, 2007; Ahmed et al., 2009; Yard et al., 2015). The mean concentrations of heavy metals downstream have smaller values than upper reaches. This might be due to the downward hydraulic movement of the stream's water, settlement of suspended sediments comprising heavy metals, and amalgamation of the other pollutants in the lower reaches.

The mean concentrations of heavy metals in water samples were: 173.3 ± 94.97, 11.7 ± 3.95, 344.6 ± 45.56, 49.8 ± 8.96, 12.05 ± 1.70, 361.45 ± 281.82, 177.6 ± 48.77, 592.9 ± 157.45, 393.1 ± 79.41 and Zn 74.7 ± 25.1 µg/L for As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn respectively. The highest mean concentrations of all studied heavy metals except As, Cd, Ni, and Zn are found in the Dololo stream, where maximum anthropogenic activities are compared to the other three streams. Dololo and Kitto were the most severely contaminated sites, significantly higher than the other two streams ($P < 0.05$). Moreover, the Dololo stream shows the highest concentrations of Pb, As, and Cr as compared to Awetu, Kitto, and Boye streams. This might be due to the stream receiving

effluents from Jimma University being directly discharged to this stream. In waters, compared with reports from other studies, the concentration of As, Cd, Cr, Cu, Hg, Pb, and Zn in all the four streams were higher than Lake Caizi, Southeast China (Jiang et al., 2018), Cd, Cr, Pb, Zn, and Ni in Houjing river (Veu et al., 2017), and As, Cd, and Ni in the upper Indus river in Pakistan (Nawab et al., 2018).

The mean concentrations of heavy metals were higher at Dololo and Kitto streams than Awetu and Boye streams, particularly for Pb. This might be due to the discharge of the metal-plating industry, garages, small-scale industries, public and governmental institutions (Adela et al., 2012; Getaneh et al., 2014). Moreover, the concentrations of Pb in the sediment samples were compared with findings of other studies around the world and showed extremely higher values than those reported for the Lake Caizi in Southeast China (Jiang et al., 2018), Korotoa river of Bogra district in Bangladesh (Islam et al., 2015), Shur river in Iran (Karbassi et al., 2008), and Houjing River in Taiwan (Vu et al., 2017) indicating serious pollution.

Awetu stream receives discharges from residential, commercial, garages, and processing centers of metal-surface-coating. Dololo, situated at the exact center of the town, receives wastes from Jimma University specialized hospital, laboratories, workshops, electroplating, garages, student cafeterias, demolition building, and high-traffic runoff areas at Jimma University. Kitto, situated on the outskirts of the town, receives discharges from the campus of the Jimma institute of technology, Jimma university huge enterprise, the open solid waste dumping site of Jimma town, traditional processing centers for metal surface coating, and Jimma AbaJifar airport. Boye, situated at the downstream reach of the watershed of Awetu, receives discharges from residential, several small-scale metal-processing, small-scale, electroplating, and abattoir. All the streams are bordered by a residential area with some marketplaces and houses, which are sources of pollution introduced by leachate and illegal discharge. Waters originating from all these upstream sources were collected and mixed downstream of Awetu and finally to Boye, making this site the most likely cause of heavy metal contamination. The correlation analyses show common characteristics of heavy metals in water and sediment. The interelement association has been evaluated by Pearson correlation coefficient (r) and the results were presented in Table 6.6 for water and Table 6.7 for sediment samples.

Mn concentrations in sediment samples were higher at Dololo and Kitto streams, particularly at SD3, SD4, SK1, and SK3 sampling sites. Mean concentrations of heavy metals in both sediment and water samples in the middle were relatively higher than in the lower and upper. This might be due to the main interim of the liquid and solid waste dumped in the center of the town. But the upstream and downstream have lower concentrations due to lower anthropogenic activities in the upstream and movement and deposition of suspended sediments and combined with local pollution in the lower reaches. Mn concentrations were compared to Conguime river in Ecuadorian Amazon (González-Merizalde et al., 2016), the upper part of the Korbevačka River basin in Serbia (Živković et al., 2019) and Palizada River in Mexico (Montalvo et al., 2014) and were two to thirty-five times higher than this study.

6.3.2 Ecological Risk Assessment of Heavy Metals in Water

The ecological risk assessment of heavy metal has been established for sediments but not for water so far. The most common method used is target hazard quotient and target cancer risk (Hassaan et al., 2016). However, recently Vu et al., (2017) calculated potential ecological risk and the contamination factors for heavy metals in water and Canadian Council of Ministers of the Environment (CCME, 2007; Vu et al., 2017). This study used water quality reference values for aquatic life and irrigation as the streams are involved in agriculture and waste dumping sites for small-scale industries found in the periphery of the water bodies.

The mean values of heavy metals in comparison to the potential ecological risk factors in aquatic life and irrigation in Awetu watershed streams are summarized in Table 6. 4. These findings were one to four orders higher than the low reference values in the guidelines (CCME, 2007). These values suggest that metal contamination and ecological risk might be major distress for future pollution control and management plans to monitor and prevent the risk posed by heavy metals. Fluctuations in hydraulic conditions through time lead to the release of heavy metals from sediments to the water column hence the values in sediments are greater than the water.

Heavy metal accumulation in terrestrial and aquatic environment sediments can be quantified by modified degree of contamination which could widely be applied to evaluate individual pollution and enrichment impact of groups of heavy metals (Cheng et al., 2012; Vu et al., 2017). The P_N index can be used to evaluate the inclusive pollution status of sediments with all the heavy metals

(Yan et al., 2016). Since different heavy metals may have impacts on one site, this method could provide a reasonable interpretation of the heavy metal pollution at each site as a whole. These metals may accumulate to a high concentration level and lead to ecological damage (Brady et al., 2015). The ecological risk posed by heavy metals in sediments can be used to calculate the geo-accumulation index (Yi et al., 2011) and the ecological risk index (Hakanson, 1980).

6.3.3 Ecological Risk Assessment of Heavy Metals in Sediment

This study compared heavy metal concentrations to sediment quality guidelines to reflect the contamination of the streams in the Awetu watershed streams. Among the studied heavy metals Pb showed the worst heavy metal contamination in the streams in the Awetu watershed, as its concentrations in sediments exceeded the referenced different SQGs, which is understandable since its concentrations were severe. In the natural environment, heavy metals may co-exist and their combined effect triggered negative impacts (Brady et al., 2015). Due to single-element indices not adequately reflecting the synergistic effects of heavy metals (Duodu et al., 2016), this study is mainly focused on the combined effects. Hence multi-element indices such as modified degree of contamination (mC_d) and Nemerow pollution index (P_N), indicate the synergistic effect of diverse contaminants at a site (Yan et al., 2016). The Canadian Council of Ministers of the Environment (CCME) and the Australian and New Zealand Environment and Conservation Councils issued guidelines that set lower and upper limits for various heavy metals (CCME, 1995; ANZECC and ARMCANZ, 2000). The ecological effects of individual heavy metals in sediments can be considered to be “rarely observed” if its concentration drops below the lower limit (Lin et al., 2016) and the upper-limit concentrations show ecological effects “apparently observed”. When the concentrations of heavy metals are equal or greater than the lower limit but below the upper limit, their effects will be “rarely observed” (Lin et al., 2016; Vu et al., 2017). The average concentration of heavy metals of this study was found higher than the reference risk values found in Taiwan EPA’s upper limit (Vu et al., 2017) and CCME’s probable effect level (CCME, 1995).

Table 6. 2. Mean values of heavy metal concentrations (mg/kg) of sediment samples from Awetu watershed streams.

	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
Sites	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
SA1	369.2 ± 254.12	23.2 ± 127.89	174 ± 201	37.2 ± 29.56	4.4 ± 0.24	340.4 ± 2245.86	136 ± 171.82	952 ± 1053.94	395.6 ± 771.664	720 ± 104.28
SA2	503.2 ± 120.12	57.2 ± 93.89	173.6 ± 201.4	51.6 ± 15.16	4.8 ± 0.16	588.8 ± 1997.46	148.4 ± 159.42	1585.2 ± 420.74	728 ± 439.264	408.4 ± 415.88
SA3	470 ± 153.32	52.8 ± 98.29	186.8 ± 188.2	53.2 ± 13.56	4.4 ± 0.24	431.2 ± 2155.06	123.6 ± 184.22	1472.4 ± 533.54	714 ± 453.264	303.6 ± 520.68
SA4	444 ± 179.32	47.2 ± 103.89	169.6 ± 205.4	44.4 ± 22.36	5.2 ± 0.56	435.6 ± 2150.66	137.6 ± 170.22	1235.6 ± 770.34	617.6 ± 549.664	1042 ± 217.72
SA5	183.6 ± 439.72	4.4 ± 146.69	133.6 ± 241.4	21.6 ± 45.16	4.8 ± 0.16	280.8 ± 2305.46	105.2 ± 202.62	485.6 ± 1520.34	240.8 ± 926.464	524 ± 300.28
SA6	430.4 ± 192.92	40.12 ± 110.97	158.8 ± 216.2	34 ± 32.76	4.4 ± 0.24	415.6 ± 2170.66	94.4 ± 213.42	1382 ± 623.94	542.4 ± 624.864	1088.4 ± 264.12
SD1	813.2 ± 189.88	236.4 ± 85.31	537.2 ± 162.2	135.2 ± 68.44	4.4 ± 0.24	3418.8 ± 832.54	510.8 ± 203	2381.6 ± 375.66	1668.4 ± 501.136	1406.8 ± 582.52
SD2	1026 ± 402.68	295.6 ± 144.51	607.2 ± 232.2	86 ± 19.24	6 ± 1.36	4426.4 ± 1840.14	533.2 ± 225.4	2940.8 ± 934.86	1726.4 ± 559.136	1103.6 ± 279.32
SD3	1011.6 ± 388.28	302 ± 150.91	643.2 ± 268.2	113.6 ± 46.84	3.6 ± 1.04	5618.4 ± 3032.14	616 ± 308.2	3330.8 ± 324.86	2604.4 ± 437.136	1692.4 ± 868.12
SD4	873.2 ± 249.88	269.6 ± 118.51	567.6 ± 192.6	82.4 ± 15.64	5.2 ± 0.56	5521.2 ± 2934.94	563.6 ± 255.8	2908.4 ± 902.46	1921.2 ± 753.936	838.8 ± 14.52
SD5	720.8 ± 97.48	201.2 ± 50.11	498.8 ± -123.8	73.2 ± 6.44	4.4 ± 0.24	4568.4 ± 1982.14	405.2 ± 97.38	2354.8 ± 348.86	1260 ± 92.736	1039.6 ± 215.32
SK1	1102.8 ± 479.48	303.2 ± 152.11	807.2 ± 432.2	152.8 ± 86.04	4 ± 0.64	6725.2 ± 4138.94	717.6 ± 409.8	3748.8 ± 742.86	2762 ± 1594.736	653.6 ± 170.68
SK2	1037.2 ± 413.88	247.2 ± 96.11	540.8 ± 165.8	116 ± 49.24	4.8 ± 0.16	2406.4 ± 179.86	427.6 ± 119.8	3080.4 ± 1074.46	1866.8 ± 699.536	839.6 ± 15.32
SK3	899.6 ± 276.28	247.6 ± 96.51	537.6 ± 162.6	94.4 ± 27.64	4.4 ± 0.24	6674.8 ± 4088.54	368 ± 60.18	2767.6 ± 761.66	1738.4 ± 571.136	744.8 ± 79.48
SK4	664 ± 40.68	171.2 ± 20.11	419.6 ± 44.6	68.4 ± 1.64	4 ± 0.64	2781.6 ± 195.34	403.2 ± 95.38	2100.4 ± 94.46	1285.2 ± 117.936	1120.8 ± 296.52
SK5	591.6 ± 31.72	192 ± 40.91	418.8 ± 43.8	58.4 ± 8.36	5.2 ± 0.56	5442 ± 2855.74	366 ± 58.18	1811.6 ± 194.34	1022.8 ± 144.464	1118.4 ± 294.12
SB1	351.2 ± 272.12	33.6 ± 117.47	132.8 ± 242.2	29.6 ± 37.16	4 ± 0.64	304.4 ± 2281.86	113.2 ± 194.62	900 ± 1105.94	497.2 ± 670.064	574.4 ± 249.88
SB2	249.2 ± 374.12	28 ± 123.09	149.2 ± 225.8	27.2 ± 39.56	4.4 ± 0.24	336.4 ± 2249.86	76.4 ± 231.42	880.8 ± 1125.14	445.2 ± 722.064	400 ± 424.28
SB3	362.8 ± 260.52	45.2 ± 105.89	207.6 ± 167.4	28 ± 38.76	5.6 ± 0.96	504.4 ± 2081.86	155.2 ± 152.62	1056 ± 949.94	654.44 ± 512.824	433.2 ±391.08
SB4	362.8 ± 260.52	224 ± 72.91	436 ± 61	28 ± 38.76	4.8 ± 0.16	504.4 ± 2081.86	155.2 ± 152.62	2744 ± 738.06	654.44 ± 512.824	433.2 ± 391.08

Table 6. 3. Mean values of heavy metal concentrations (µg/L) of water samples from Awetu watershed channelized streams.

	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
Sites	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
WA1	133 ± 40.3	16 ± 4.3	331 ± 13.6	22 ± 12.15	10 ± 2.05	43 ± 318.45	122 ± 55.6	314 ± 278.9	337 ± 56.1	123 ± 48.3
WA2	175 ± 1.7	14 ± 2.3	393 ± 48.4	42 ± 7.85	11 ± 1.05	34 ± 327.45	212 ± 34	689 ± 96.1	416 ± 22.9	62 ± 12.7
WA3	18 ± 155.3	11 ± 0.7	355 ± 10.4	45 ± 10.9	10 ± 2.05	37 ± 324.45	263 ± 85	858 ± 265	334 ± 59.1	91 ± 16.3
WA4	181 ± 7.7	15 ± 3.3	302 ± 42.6	36 ± 1.85	14 ± 2	46 ± 315.45	111 ± 66.6	569 ± 23.9	416 ± 22.9	81 ± 6.3
WA5	124 ± 49.3	6 ± 5.7	232 ± 113	55 ± 20.9	12 ± 0.05	456 ± 94.55	121 ± 56.6	524 ± 68.9	234 ± 159.1	71 ± 3.7
WA6	71 ± 102.3	10 ± 1.7	320 ± 24.6	35 ± 0.85	15 ± 3	145 ± 216.45	177 ± 0.6	581 ± 11.9	292 ± 101.1	60 ± 14.7
WD1	253 ± 79.7	5 ± 6.7	386 ± 41.4	35 ± 0.85	10 ± 2.05	2591 ± 2229.6	198 ± 20	641 ± 48.1	385 ± 8.1	42 ± 32.7
WD2	173 ± 0.3	11 ± 0.7	376 ± 31.4	34 ± 0.15	12 ± 0.05	946 ± 584.55	188 ± 10	736 ± 143	436 ± 42.9	59 ± 15.7
WD3	341± 167.7	8 ± 3.7	363 ± 18.4	50 ± 15.9	12 ± 0.05	344 ± 17.45	207 ± 29	464 ± 128.9	539 ± 146	37 ± 37.7
WD4	158 ± 15.3	7 ± 4.7	421 ± 76.4	40 ± 5.85	11 ± 1.05	623 ± 261.55	250 ± 72	920 ± 327	390 ± 3.1	78 ± 3.3
WD5	303 ± 129.7	6 ± 5.7	404 ± 59.4	29 ± 5.15	16 ± 4	515 ± 153.55	257 ± 79	628 ± 35.1	465 ± 71.9	58 ± 16.7
WK1	165 ± 8.3	15 ± 3.3	313 ± 31.6	30 ± 4.15	12 ± 0.05	34 ± 327.45	163 ± 14.6	498 ± 94.9	298 ± 95.1	25 ± 49.7
WK2	106 ± 67.3	19 ± 7.3	312 ± 32.6	29 ± 5.15	14 ± 2	25 ± 336.45	102 ± 75.6	338 ± 254.9	324 ± 69.1	35 ± 39.7
WK3	351 ± 177.7	16 ± 4.3	338 ± 6.6	28 ± 6.15	11 ± 1.05	64 ± 297.45	174 ± 3.6	491 ± 101.9	364 ± 29.1	30 ± 44.7
WK4	151 ± 22.3	14 ± 2.3	363 ± 18.4	29 ± 5.15	13 ± 0.9	1032 ± 670.55	198 ± 20	592 ± 0.9	385 ± 8.1	36 ± 38.7
WK5	197 ± 23.7	16 ± 4.3	330 ± 14.6	27 ± 7.15	10 ± 2.05	65 ± 296.45	117 ± 60.6	419 ± 173.9	471 ± 77.9	45 ± 29.7
WB1	222 ± 48.7	11 ± 0.7	306 ± 38.6	38 ± 3.85	13 ± 0.9	50 ± 311.45	181 ± 3.4	689 ± 96.1	458 ± 64.9	33 ± 41.7
WB2	64 ± 109.3	13 ± 1.3	325 ± 19.6	34 ± 0.15	12 ± 0.05	30 ± 331.45	157 ± 20.6	492 ± 100.9	445 ± 51.9	53 ± 21.7
WB3	23 ± 150.3	11 ± 0.7	315 ± 29.6	23 ± 11.15	11 ± 1.05	50 ± 311.45	213 ± 35	721 ± 128	345 ± 48.1	42 ± 32.7
WB4	257 ± 83.7	10 ± 1.7	407 ± 62.4	22 ± 12.15	12 ± 0.05	99 ± 262.45	141 ± 36.6	694 ± 101	528 ± 135	433 ± 0.3

Table 6. 4. Comparison of mean values of heavy metals in this study with the potential ecological risk factors in aquatic life and irrigation.

Elements	Average water heavy metal content (mg/L)	AWPL[a] (mg/L)	Aquatic Life				Irrigation		
			$C_f^i A$	Grade of contamination	$E_f^i A$	Grade of ecological risk	IWPL[b] (mg/L)	$C_r^i I$	Grade of contamination
As	0.173	50	0.012	Low	0.12	Low	100	0.00173	Low
Cd	0.012	1.8	0.012	Low	0.12	Low	10	0.00012	Low
Cr	0.345	2	0.004	Low	0.04	Low	100	0.00345	Low
Cu	0.050	4	0.001	Low	0.01	Low	200	0.00050	Low
Hg	0.012	0.1	0.048	Low	0.48	Low	2	0.00012	Low
Mn	0.361	-	-	-	-	-	-	-	-
Ni	0.178	150	-	-	-	-	200	0.00178	Low
Pb	0.593	7	0.008	Low	0.08	Low	200	0.00593	Low
Sn	0.393	-	-	-	-	-	-	-	-
Zn	0.075	30	0.0004	Low	0.004	Low	1000	0.000747	Low

[a]Aquatic life water permissible limits and [b]Irrigation life water permissible limits (CCME, 2007), except Ni (NRMMCA, 2011) and Hg (ANZECC and ARMCANZ. 2000)

In the natural environment, heavy metals may co-exist and their combined effect triggered negative impacts (Brady et al., 2015). Due to single-element indices not adequately reflecting the synergistic effects of heavy metals (Duodu et al., 2016), this study is mainly focused on the combined effects. Hence multi-element indices such as modified degree of contamination (mC_d) and Nemerow pollution index (P_N), indicate the synergistic effect of diverse contaminants at a site (Yan et al., 2016).

The results of the mC_d and P_N indices calculations at the sampling sites are shown in Figure 6.1. Although the calculation and classifications of the two indices differ, their results are similar. The sampling sites in Awetu and Boye streams which are similarly impacted by heavy metals were found to be much less severely contaminated than Dololo and Kitto streams, which explains Awetu and Boye streams were classified as 'Class 2' in mC_d and 'Class 3' in P_N calculations. But Dololo and Kitto streams were the most impacted by heavy metals, classified as 'Class 4' in mC_d, and all the four streams are classified as 'Class 3' in P_N calculations. The element contributing most to mC_d at Dololo and Kitto streams was Pb followed by Mn and Sn. However, all four streams were grouped into one class in P_N calculations. This variance was due to the difference in the specificity of the indices' two classification systems. Because mC_d classification is more specific, heavy metal contamination was better categorized. The categorization of heavy metal contamination based on

mC_d and P_N calculations agreed relatively well with the intensity of heavy metal concentrations, where Kitto and Dololo streams were found to have the highest accumulation of heavy metals.

This study also employed the potential ecological risk index (RI) to assess the potential impact of heavy metal contamination on the ecosystem of the streams in the Awetu watershed. Dololo (SD1, SD2, SD3 & SD4 sites) and Kitto (SK1, SK2 & SK3 sites) streams were determined to be likely to face 'severe' ecological risk (Class 2 and Class 3) in both mC_d and P_N values illustrating that degree of contamination do not always represent potential for ecological risk. While Awetu (SA1, SA2, SA3, SA4, SA5 & SA6 sites) and Boye (SB1, SB2, & SB3; except SB4 site) streams were less exposed to the ecological risk of heavy contamination (Class 1). Dololo and Kitto face ecological risks posed by Cd, Mn, Ni, Pb, and Sn. Therefore, it showed Cd. Pb and Mn pose the most significant ecological risk to the Awetu watershed streams.

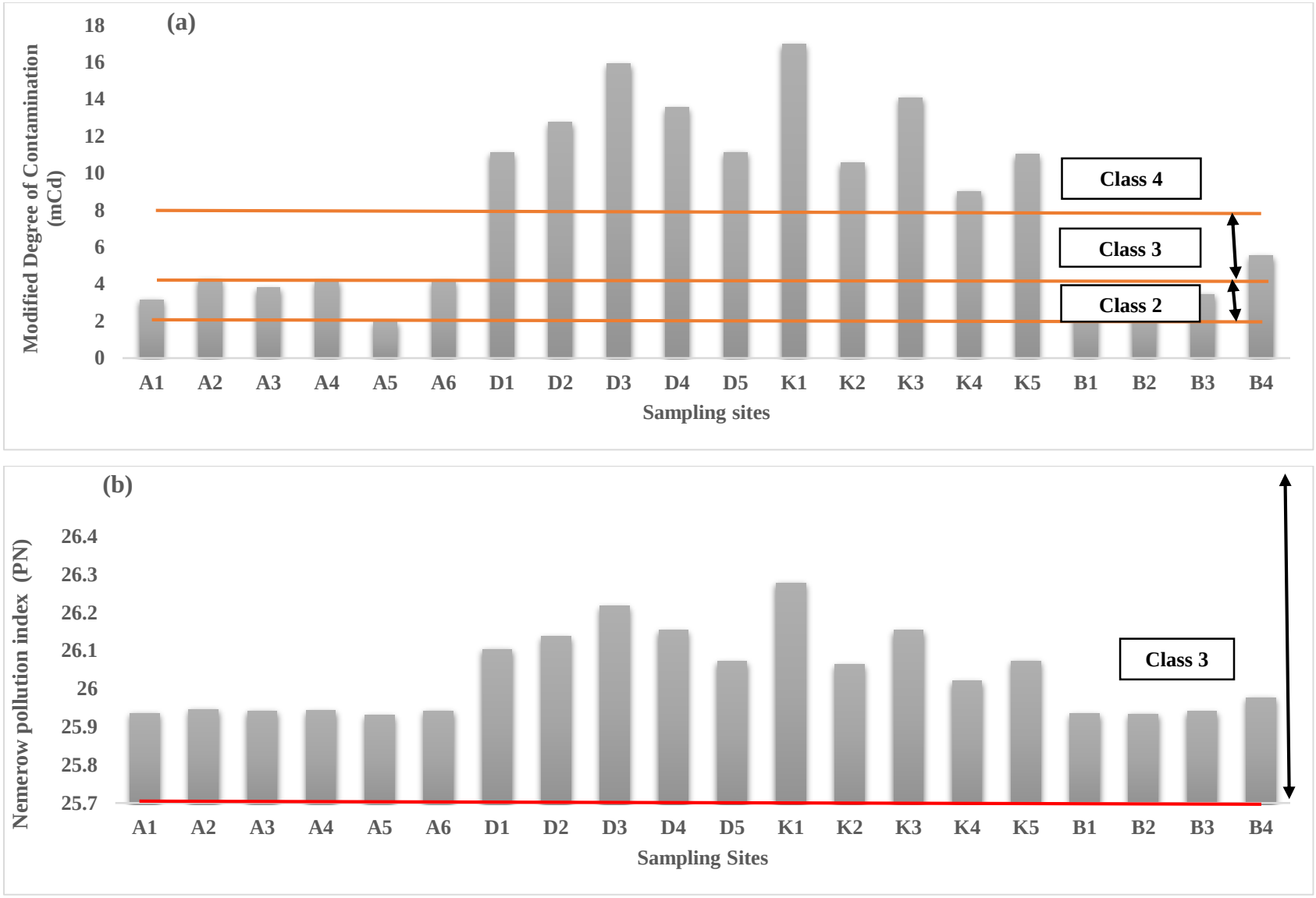

Figure 6. 1. Modified degree of contamination (mC_d) (a) and Nemerow pollution index (P_N) (b) of heavy metals in the streams in the Awetu watershed, Jimma, southwestern Ethiopia.

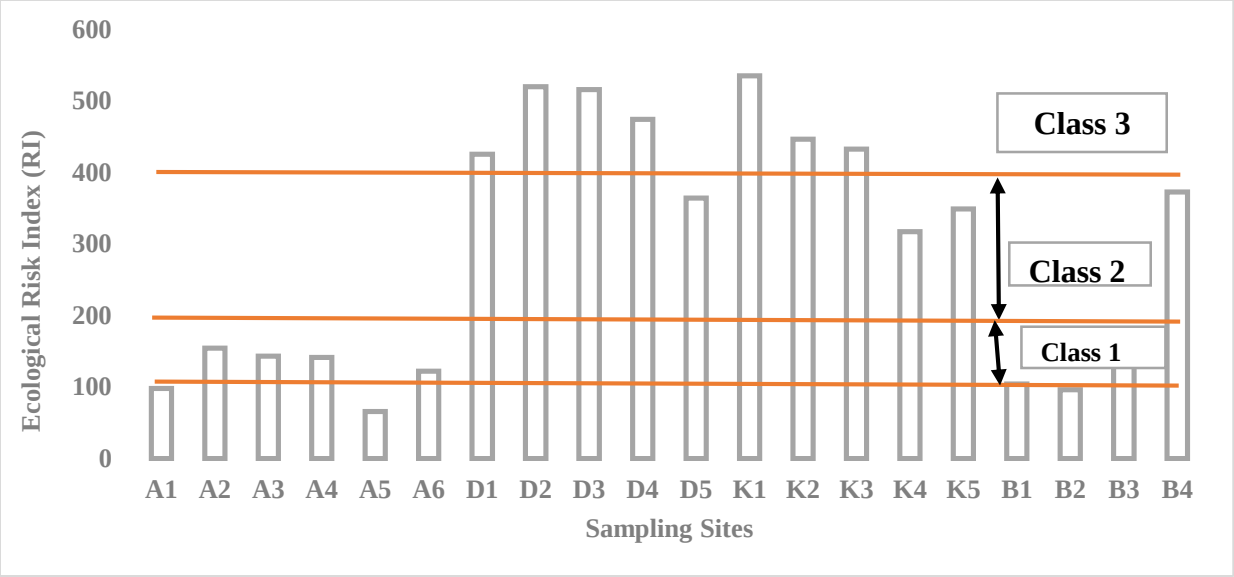

Figure 6. 2. Ecological risk index (RI) of heavy metals in sediments in the streams in the Awetu watershed, Jimma, Ethiopia.

Table 6. 5. Comparison of the mean values of heavy meals of this study with different sediment quality guidelines (mg/kg).

Elements	Mean value of this study	Taiwan EPA[a]	CCME ISQG[b]	CCME PEL[c]	ANZECC & ARMCANZ Low[d]	ANZECC & ARMCANZ High[e]	NOAA ERL[f]	NOAA ERM[g]
As	623.32	33	5.9	17	20	70	8.2	70
Cd	151.09	2.5	0.6	3.5	1.5	10	1.2	9.6
Cr	375	233	37.3	90	80	370	81	370
Cu	66.76	157	35.7	197	65	270	34	270
Hg	4.64	0.87	0.17	0.486	0.15	1	0.15	0.71
Mn	2586.26	-	-	-	-	-	-	-
Ni	307.82	80	-	-	21	52	20.9	51.6
Pb	2005.94	161	35	91.3	50	220	46.7	218
Sn	1167.264	-	-	-	-	-	-	-
Zn	824.28	387	123	315	200	410	150	410

[a]*Taiwan Environmental Protection Agency (Taiwan EPA)'s sediment quality guideline upper and lower limits (Vu et al., 2017)*

[b,c] *Canadian Council of Ministers of the Environment (CCME)'s interim sediment quality guideline (ISQG) and probable effect level (PEL) (CCME, 1995)*

[d,e] *Australian and New Zealand Environment and Conservation Council (ANZECC) & Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ)'s low and high levels (ANZECC and ARMCANZ, 2000)*

[f,g] *National Oceanic and Atmospheric Administration (NOAA)'s effects range-low (ERL) and effects range-median (ERM) (Long et al., 1995)*

Table 6. 6. Correlation matrix for heavy metals in waters of streams in the Awetu watershed.

	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
As	1									
Cd	-0.19	1								
Cr	0.37	-0.28	1							
Cu	0.40	-0.27	0.08	1						
Hg	0.09	-0.07	-0.10	-0.01	1					
Mn	0.24	**-0.54***	0.35	0.01	-0.15	1				
Ni	0.04	**-0.54***	**0.61****	0.16	-0.04	0.25	1			
Pb	-0.15	**-0.54***	**0.50***	-0.15	-0.10	0.22	**0.73****	1		
Sn	**0.57****	-0.12	**0.57****	0.41	0.04	0.02	0.15	0.11	1	
Zn	0.11	-0.12	0.32	-0.10	-0.06	-0.13	-0.17	0.18	0.33	1

**. Correlation is significant at the 0.01 level and *. Correlation is significant at the 0.05 level.

Table 6. 7. Correlation matrix for heavy metals in sediment samples of streams in the Awetu watershed.

	As	Cd	Cr	Cu	Hg	Mn	Ni	Pb	Sn	Zn
As	1									
Cd	0.90**	1								
Cr	0.92**	0.98**	1							
Cu	0.92**	0.82**	0.87**	1						
Hg	-0.07	-0.02	-0.08	-0.24	1					
Mn	0.84**	0.86**	0.88**	0.77**	-0.09	1				
Ni	0.93**	0.91**	0.95**	0.90**	-0.10	0.89**	1			
Pb	0.91**	0.96**	0.96**	0.84**	-0.10	0.79**	0.88**	1		
Sn	0.96**	0.90**	0.94**	0.93**	-0.20	0.86**	0.96**	0.92**	1	
Zn	0.54*	0.52*	0.49*	0.51*	-0.17	0.51*	0.59**	0.43	0.53*	1

**. Correlation is significant at the 0.01 level and *. Correlation is significant at the 0.05 level.

6.4 Conclusion

The study showed the level of heavy metals pollution and their ecological risks for the streams in the Awetu watershed. Based on sediment quality guidelines (SQGs), modified degree of contamination (mC_d), Nemerow pollution index (P_N), and Ecological risk index (RI) findings, the concentrations of heavy metals exceeded standard values, indicating ' moderately ' to ' frequently observed ' threats by heavy metals contamination. The computed results of mC_d, P_N, and RI, multi-element indices used to reflect synergistic effects, agreed with each other in the determination of ‘heavy’ contamination and ‘severe’ ecological risk at Dololo and Kitto streams. Cd, Mn, Ni Pb, and Sn might pose a significant potential ecological risk in the study area. The finding revealed that the primary pollution source for heavy metals in water and sediments in the Dololo stream might be sourced from traditional metal plating, garage works, laboratory effluents, carwash, and municipal discharge from residential and commercial areas. Future pollution control and management plans should focus on discharge from these pollution sources situated alongside the streams.

Chapter Seven:

Human Health Risk Assessment of Toxic Heavy Metals from *Oreochromis Niloticus* and *Labeobarbus Intermedius* Fishes Consumption, Southwestern Ethiopia

Abstract

Toxic metals released from different sources to the nearby water bodies may be accumulated in the aquatic ecosystem. The bio-concentration of toxic metals into living organisms may lead to cumulative effects through the food web once its concentration reaches significantly above the normal. The objective of this study was to determine the concentrations of toxic metals from two abundant fish species; *Oreochromis niloticus* and *Labeobarbus intermedius* in the Gilgel Gibe I reservoir of southwestern Ethiopia and evaluate potential risks to the health of consumers. The concentrations and the human health risks of As, Cd, Hg & Pb were assessed in the gill, liver, and muscle of fish species. The mean concentrations of As (0.25 ± 0.04 mg/kg dw) and Cd (0.54 ± 0.14 mg/kg dw) was higher in the liver organ than those of Hg (0.09 ± 0.1 mg/kg dw) and Pb (0.06 ± 0.04 mg/kg dw) in the muscle tissues of *Oreochromis niloticus*. In *Labeobarbus intermedius* species, mean concentrations of As (0.33 ± 0.22 mg/kg dw), Cd (0.44 ± 0.12 mg/kg dw) in the liver were higher than those of Hg (0.04 ± 0.04 mg/kg dw) in liver and Pb (0.13± 0.15 mg/kg dw) in the gill. Concentrations of toxic metal in fish samples varied significantly ($p < 0.05$) by tissue types and species. The mean concentrations of As (0.18 ± 0.12 mg/kg), Cd (0.44 ± 0.21 mg/kg), Hg (0.13 ± 0.09 mg/kg), and Pb (0.10 ± 0.07 mg/kg). The values of As and Cd exceed the acceptable limits set of As (0.002 mg/kg) by JECFA and Cd (0.05 mg/kg) by WHO/FAO. But the values of Pb and Hg show lower values than the acceptable limits set of Pb (0.30 mg/kg) and Hg (0.05 mg/kg) by WHO/FAO. Concerning estimated daily intake (EDI), all the studied heavy metals showed higher values than the respective PTDI values hence causing a health risk to the public. Hence, frequent monitoring of these toxic metals is important to safeguard consumers from chronic illnesses.

Keywords: Bioaccumulation; Heavy metal; Labeobarbus intermedius; Oreochromis niloticus

7.1 Introduction

The ecological and economic importance of surface waters to society is vital (He et al., 2018). River waters and reservoirs, on the other hand, are particularly susceptible to pollution since they are inherently open, easily accessible, and frequently used in agricultural, industrial, and municipal processes. Toxic metals are normally found in very low amounts in aquatic environment deposits. However, as a result of various human activities, the concentrations have risen dramatically (Khan et al., 2016; He et al., 2018). Because of their persistence and toxic nature to living organisms, pollution of aquatic environments by toxic metals originating from various anthropogenic activities is now a worldwide problem (Aytekin et al., 2019; Shafiuddin et al., 2019). Studies indicated that bio-accumulation of toxic metals is a vital concern of aquatic pollution (Soliman and Nasr, 2015; Rumisha et al., 2017; Doyi et al., 2018). As, Cd, Pb, and Hg are among the most toxic metals that demand more attention due to their toxicity to the ecosystem, particularly to humans (Khan et al., 2016).

Recently, in different parts of the world the interest in consuming aquatic animals, predominantly fish, has increased due to low saturated fats and high protein content (Rumisha et al., 2017; Esilaba et al., 2020). Fishes are considered an immediate medium and higher trophic level organisms eaten by humans due to the bioaccumulative nature of toxic metals from aquatic ecosystems. They're used as a bio-indicator of aquatic environments and toxic metal concentration in water and sediments (Yi et al., 2017). Toxic metals found in the fish body is also a function of pH, temperature, and alkalinity of the water environment (Abubakar et al., 2015; Isa et al., 2015; Soliman and Nasr, 2015). The higher concentration of heavy metals in the water and sediment in Awetu watershed streams end up with the lower reaches (Gilgel Gibe I reservoir) of the watershed and lead to the contamination of fish species.

The most important commercially available fish species for residents in many parts of Ethiopia are *Oreochromis niloticus* and *Labeobarbus intermedius*. These species are commercially significant, accounting for a significant portion of the overall locally cultivated seafood production in Ethiopia's southwestern region. As a result, identifying toxic metal concentrations in fish consumed in the region and comparing them to FAO/WHO and EC guideline values for edible animals is important (EFSA, 2011; FAO/WHO, 2011).

Gilgel Gibe I hydroelectric reservoir where *Oreochromis niloticus and Labeobarbus intermedius* fish species harvested are polluted by extensive agricultural practices and high tanker traffic loads. However, data are scarce regarding the level of toxic metals of these economically and ecologically important fish species and the health risks to consumers. Therefore, this study investigated the level of toxic metals in two important fish species and their potential risk to fish consumers in southwestern Ethiopia.

7.2 Methods and Materials

7.2.1 Description of the Study Area

Gilgel Gibe I reservoir is located in Jimma Zone, Southwest Ethiopia, 260 km from Addis Ababa, the capital of Ethiopia, and 54 km east away from Jimma Town. It was constructed for hydroelectric power generation with World Bank funding at an estimated cost of 2.2 billion Birr on Gilgel Gibe river and has been operating since 2004 to generate 184 MW hydroelectric power. The area has a sub-humid, warm to hot climate, receives between 1,300 and 1,800 mm of annual rainfall, and has a mean annual temperature of 19°C (Yewhalaw et al., 2009). The area is a semi-surrounded basin that receives several rivers in the region and the reservoir has an enormous diversity of fauna-like fishes. The sampling locations were selected based on the discharge of wastes from urban and agricultural sewage into the Gilgel Gibe tributaries. The reservoir formed by the dam receives the incoming water from four major tributaries including Gilgel Gibe, Nada Guda, Nada Qalla, and Nadhi rivers. The reservoir formed by the dam receives the incoming water from four major tributaries including Gilgel Gibe, Nada Guda, Nada Qalla, and Nadhi rivers. Nowadays, the reservoir is also used for fishing and recreation for the surrounding communities (Gure et al., 2019). The map of the study area is shown in Figure 3. 2.

7.2.2 Sample Collection and Preparation

Two commonly consumed fish species namely *Oreochromis niloticus* and *Labeobarbus intermedius* samples (n = 20) were collected from four sampling sites by local fishermen from Gilgel Gibe I reservoir in December 2020. The samples were placed in an insulated cold box and immediately transported to the laboratory and stored at -20 ºC. Gills, livers, and muscle tissues of fishes were separately taken using a plastic knife and dried at 60 °C in an oven. Subsequently, the samples were ground in mortar and pestle, sieved in a 63 µm sieve, and ready for the subsequent digestion in a fume hood (Uysal et al., 2009). 1 g of dried and powdered sample was weighed and

transferred into a flask. 10 mL of 65 % nitric acid (HNO_3) and 5 mL 30 % hydrogen peroxide (H_2O_2) were added to each flask and kept overnight. The flasks were heated to 130 °C until the volume reached 2 to 3 mL and turned clear. The solutions were cooled at room temperature, filtered in Whatman No. 42 filter paper, and diluted to 25 mL with double distilled water (AOAC, 2005).

7.2.3 Heavy Metals Analysis

Analyses of As, Cd, Hg, and Pb were performed using a flame atomic absorption spectrophotometer (Thermo Jarrell Ash). The detection limits As, Cd, Hg, and Pb were 0.001, 0.0015, 0.0005, and 0.01 mg/L, respectively. Glass vessels were precleaned thoroughly using detergent and soaked in 20 % nitric acid (HNO_3) overnight rinsed in double-distilled water. The experimental reagents and standards used during the study were of analytical grade obtained from Sigma Aldrich. The AAS instrument was calibrated with a series of standard solutions prepared in the concentration range of each element. A series of standard solutions of As were prepared from arsenic trioxide (As_2O_3) (0.005, 0.01, 0.02, 0.03 and 0.05 mg/L), Cd from $CdCl_2$. $2.5H_2O$ (0.0005, 0.001, 0.002, 0.003 and 0.005 mg/L), Hg from CH_3HgCl (0.0005, 0.001, 0.002, 0.003 and 0.005 mg/L), and Pb from Pb $(NO_3)_2$ (0.005, 0.01, 0.02, 0.03 and 0.05 mg/L). A calibration curve of absorbance versus concentration was obtained and used to quantify As, Cd, Hg, and Pb content in the commonly consumed fish species. Triplicate samples were used to ensure the precision of the instrument and a reagent blank and the standard were run after every 3 samples. Heavy metal concentration was expressed in milligram per kilogram dry weight to minimize the errors associated with varying moisture compositions of the fish tissues.

7.2.4 Human Health Risk Assessment

Risks posed by toxic metals to humans from fish consumption were estimated from estimated daily intake (EDI) and target hazard quotient (THQ) calculations. Different studies revealed that exposure to different toxic metals from similar sources may cause cumulative effects (Hassaan et al., 2016; Kacholi and Sahu, 2018; Kawser et al., 2016). Thus, THQs values of As, Cd, Hg, and Pb could be added to obtain HI which shows the cumulative health risk of toxic metals in the frequently eaten fishes.

7.2.4.1 Estimated Daily Intake (EDI)

To see the exposure of humans to toxic metals, the EDI value is calculated from the concentration of toxic metals and consumed fish data. The EDI values depend on both the number of toxic metals

in fish and consumed fish amounts (US EPA, 2000). The EDI can be calculated using equation 1 and expressed in mg/kg body weight/day.

$$EDI = \frac{Cm \times DI}{BW} \quad \text{(1)}$$

Where C_m is toxic metal mean concentration in fish muscle tissue (mg/kg); DI is the fish consumed amount (g/day), and BW is the average body weight for an average African adult (60.7 kg) (Esilaba et al., 2020). And the values of EDI are compared with Provisional Tolerable Daily Intake (PTDI) values of As (0.002 mg/kg/day), Cd (0.0607 mg/kg/day), Hg (0.004 mg/kg/day), and Pb (0.2168 mg/kg/day) (FAO/WHO, 2011) to decide whether the results exceeded the daily recommended values resulted in health risks.

Table 7. 1. Tolerable values of heavy metals in fish (mg/kg) (Kortei et al., 2020).

Heavy metals					
As	Cd	Hg	Pb	Organization	References
-	0.05	-	0.2	Directive 2005/78/EC	(EU, 1993)
-	0.5	0.0005	0.5	FAO/WHO	(FAO/WHO, 2011)
-	0.18	-	0.12	IAEA-407	(UNEP, 2004)
0.002	-	0.0016	-	JECFA	(Boiret et al., 2017)
-	0.05	-	0.2	TFC	(Kortei et al., 2020)
-	0.3	-	0.3	UNEP	(UNEP, 2004)

Directive 2005/78/EC – European Commission; FAO/WHO – Food and Agriculture Organization/ World Health Organization; IAEA-407 – International Atomic Energy Agency; JECFA – Joint FAO/WHO Expert Committee on Food Additives; TFC – Turkish Food Codes; UNEP – United Nations Environmental Program.

7.2.4.2 Target Hazard Quotient (THQ)

THQ is employed to express the non-carcinogenic risk potential from fish consumption. THQ is the ratio of an EDI value to oral reference dose (RfD) (mg/kg/day) (Liu et al., 2018; Huang et al., 2019). If THQ >1, the population exposed is likely to develop adverse health effects (Yabanli and Alparslan, 2015; Yap et al., 2015; Peters et al., 2018). According to the New York State Department of Health (NYSDOH), if 5 < THQ > 1, the risk is low; 10 < THQ > 5, the risk is moderate; and if THQ > 10, the risk is high (US DHHS, 2007).

$$THQ = \frac{EDI}{RfDo} \quad \text{(2)}$$

Where RfDo is the oral reference dose (mg/kg/day) which is an acceptable intake of a toxic element for an adult. According to (US EPA, 2000; Yabanli and Alparslan, 2015), the oral RfDo for As, Cd, Hg, and Pb were 0.014, 0.001, 0.0001, and 0.004 mg/kg/day, respectively. THQs of As, Cd, Hg, and Pb were summed to generate a HI using an equation followed by Yap et al., (2015).

7.2.4 3 Hazard Index (HI)

HI is a degree of the probable risk of adverse health effects. When HI > 1, the probability of adverse human health effects and the necessity for further action (Piracha, 2015; Moslen and Miebaka, 2017). HI can be computed from THQ as follows:

$$HI = \sum_{i=1}^{n} THQi \quad \text{..... (3)}$$

7.2.4.4 Carcinogenic Risk (CR)

CR shows the possibility of an individual developing cancer by exposure to potential carcinogen over a lifetime and the equation used for estimation of the cancer risk (US EPA, 2012; Saha et al., 2016) is:

$$CR = CSF \times EDI \quad \text{..... (4)}$$

Cancer slope factor (CSF) is the carcinogenic slope factor (As = 1.5 mg/kg/day and Pb = 0.0085 mg/kg/day (US EPA, 2012). Acceptable carcinogens risk level ranges from 10^{-4} to 10^{-6} showing risk of developing cancer over a human lifetime is 1 in 10,000 and 1 in 1,000,000 respectively (Yuet Ping et al., 2013).

7.2.5 Quality Assurance and Quality Control

All the standards and reagents used throughout the analysis were analytical grades obtained from Sigma Aldrich. Triplicate samples and reagent blanks were used to ensure the accuracy of the instrument and were calibrated before the samples were run. Double distilled water was used in all experimental works throughout the study to ensure the reliability of the results. Glass vessels were pre-cleaned thoroughly using detergent and soaked in 20 % nitric acid (HNO_3) overnight as well as rinsed with double-distilled water.

7.2.6 Data Analysis

Statistical analysis was done using SPSS version 23.0. A one-way ANOVA test was performed to assess the significant differences among tissues of fish samples. Results were presented as mean ± SD for each of the measured concentrations of toxic metals.

7.3 Result and Discussion

7.3.1 Heavy Metal Concentrations in Fish Tissues

Toxic metals can be accumulated in fish tissues and are commonly found in the higher level of the aquatic food chain which causes adverse human health effects (Aytekin et al., 2019; Samuel et al., 2020). Toxic metals are commonly taken by the fish from food and water and stored in target tissues (Bawuro et al., 2018). In this study, the concentrations of selected toxic metals in two fish species (*Oreochromis niloticus and Labeobarbus intermedius*) were investigated to show the burden of contamination sources in the study areas.

Table 7.2 presents a summary of the average concentration of the selected toxic metals in the gill, liver, and muscle of fish species across sampling sites. Toxic metals were detected in both fish species from all the sampling sites in a concentration order of Cd > As > Hg > Pb.

Oreochromis niloticus fish species showed higher mean values of As (0.25 mg/kg dw) and Cd (0.54 mg/kg dw) in liver organs and smaller concentrations of Hg (0.06 mg/kg dw) and Pb (0.09 mg/kg dw) in muscle tissues. In the organs of *Oreochromis niloticus* fish species; As concentration ranged from 0.11 mg/kg dw in muscle to 0.25 mg/kg dw in the liver, Cd ranged from 0.32 mg/kg dw in muscle to 0.54 mg/kg dw in the liver, Pb ranged from 0.09 mg/kg dw in muscle to 0.18 mg/kg dw in the liver, and Hg ranged from 0.06 mg/kg dw in muscle to 0.17 mg/kg dw in the liver.

Labeobarbus intermedius fish species have greater concentrations of toxic metals in liver organs than the muscles and gills. In the liver organ of *Labeobarbus intermedius* species; As concentration ranged from 0.12 mg/kg dw in gill to 0.33 mg/kg dw in the liver, Cd ranged from 0.41 mg/kg dw in muscle and gill to 0.44 mg/kg dw in the liver, Pb ranged from 0.13 mg/kg dw in gill to 0.15 mg/kg dw in muscle, and Hg ranged from 0.04 mg/kg dw in gill and liver to 0.15 mg/kg dw in muscle.

This might be attributed to the nature and type of wastes discharged to the specific sampling sites. The higher concentrations of As and Cd were detected in the liver than the muscle and gill. This might be due to the accumulative nature of the liver which acts as the major site for homeostasis (Reynders et al., 2006) than the muscle (Iwegbue, 2008). It is also observed that the mean values of As, Cd, Hg, and Pb in all organs of the two species surpass their respective permissible limits (FAO/WHO, 2011; Boiret et al., 2017) which confirms the degree of pollution of the bio-system of the reservoir.

The metal concentration differences between the two fish species may be due to different biotopes, metabolic action, and feeding habits which are principally controlled by the habitation, capacity of metal accumulation, and types of organisms (Desta et al., 2008; Aytekin et al., 2019). From the quantitative results of four metals, Cd had the maximum value in fish species followed by As, Hg, and Pb. This might be due to the higher pollution levels of Cd in the upper catchment of the reservoir. The quantity of Cd and Pb in fishes can serve as an indicator of the environmental levels. The concentrations of As and Cd are higher than Hg and Pb, this could be revealed as these toxic metals play a vital role in respiratory and enzymatic actions in fishes. The result also demonstrated that there are small variations in the mean concentrations of As and Cd in both the fish species while the variations in Hg and Pb are higher.

Table 7. 2. Average concentrations of toxic metals (mg/kg dw) in *Oreochromis niloticus* and *Labeobarbus intermedius* fish species collected from the Gilgel Gibe I reservoir.

Fish Species	Organs/ Tissues	Toxic metals			
		As	Cd	Pb	Hg
Labeobarbus intermedius	Gill	0.12 ± 0.09	0.41 ± 0.43	0.13 ± 0.15	0.04 ± 0.04
	Liver	0.33 ± 0.22	0.44 ± 0.12	0.14 ± 0.13	0.04 ± 0.04
	Muscle	0.17 ± 0.04	0.41 ± 0.20	0.15 ± 0.08	0.15 ± 0.06
Oreochromis niloticus	Gill	0.14 ± 0.04	0.53 ± 0.11	0.13 ± 0.05	0.16 ± 0.02
	Liver	0.25 ± 0.04	0.54 ± 0.14	0.18 ± 0.03	0.17 ± 0.04
	Muscle	0.11 ± 0.03	0.32 ± 0.04	0.09 ± 0.1	0.06 ± 0.04
Permissible limits (FAO/WHO, 2011)		-	0.05	0.30	0.5
European Union (EU, 1993)		-	0.05 - 0.1	0.05	0.05
JECFA (JECFA, 2019)		0.002	-	-	0.0016

*NA – Not applicable

The concentration of toxic metals in a different part of the tissues of *Oreochromis niloticus* was in the order of liver > muscle > gills for As while the concentration of Cd was in the order of liver > muscle and equal to gill. For Hg, the values in the muscle > liver > gill while for Pb muscle > liver

is equal to gill. But, the concentrations of As, Cd, Hg, and Pb in *Labeobarbus intermedius* fish species show the same order of; liver > gills > muscle. In *Oreochromis niloticus*, the levels of As and Cd in the liver were significantly greater than those in the gills and muscles ($P < 0.05$). In *Labeobarbus intermedius*, the concentrations of As, Cd, Hg, and Pb in the liver were significantly higher than in the muscle and gills ($p < 0.05$). The results revealed that the orders of the toxic metal concentrations vary between different fish tissues. This might be due to the physiological functions of the body parts and the nature of the fish. Moreover, the concentrations of the toxic metals in *Oreochromis niloticus and Labeobarbus intermedius* tissues differed significantly, and As, Cd, Hg, and Pb values were higher in the livers than in gills and muscle tissues. From the analysis of variance (ANOVA) results, toxic metal concentrations in the internal organs of the two fish species showed significant differences ($p < 0.05$) in the liver, gill, and muscle of the fish species. In *Labeobarbus intermedius* fish species, higher concentrations of toxic metals were detected in the liver and gill organs than in muscle tissues. This result is supported by a study done by (Kebede and Wondimu, 2004), which revealed that the gill and liver are active tissues metabolically and have the tendency to accumulate high levels of toxic metals.

The values of As and Cd are higher in the liver while Cd and Hg were found in the gills of *Oreochromis niloticus* fish species. The higher As and Cd values were found in the liver and muscle while higher values of Cd and Pb were found in the gills of *Labeobarbus intermedius* fish species. In general, substantial differences were observed among the mean values of toxic metals in both fish species and organs (tissues).

The higher values of toxic metals in the liver organ might be attributed to it being the main target for toxic metals and making the liver the most important storage, fish metabolism, and target organ in aquatic organisms and it is an organ that is responsible for detoxification of any foreign substances. The metal bioaccumulation in the liver may be associated with its role in the metabolic processes of chemicals to maintain life in a living organism (Zhao et al., 2012; Deribe et al., 2014; Bawuro et al., 2018). This is supported by several studies showing the liver accumulates higher metal values than the muscle (Küpeli et al., 2014; Aytekin et al., 2019) which acts as a detoxifying organ by storing toxic metals. The toxic metal concentrations in gills of both fish species were considerably higher than muscles hence fish gills are the main organs for excretory and respiratory functions and usually reflect the levels of metal in the environment. Because gill is in direct contact

with water and highly absorbed toxic metals (Aytekin et al., 2019), and is considered as having a higher susceptibility to metals. Because fish gills have a larger surface area with water and absorb the right amount of toxic metals (El-Moselhy et al., 2014), the amount of toxic metals in gills shows the toxic metals in the water (Bawuro et al., 2018).

The fish muscle is the main edible part, but toxic metals are hardly accumulated and are considerably lower than gill and liver in *Labeobarbus intermedius* fish species. This may be due to its lower affinity to metals absorption through body contact as its levels in the liver and gills. This might be good for consumers as the value is lower in the muscles of the fish. The levels Cd and Pb in the tissues of *Oreochromis niloticus and Labeobarbus intermedius* were lower than those obtained in the study done in Lakes Awassa, Ziway, and Hashenge, Ethiopia (Kebede and Wondimu, 2004; Asgedom et al., 2012).

There are significant differences of toxic metals of this study with similar studies conducted in Ethiopia (Asefa and Beranu, 2016; Gerenfes et al., 2019; Kindie et al., 2020), Nigeria (Bawuro et al., 2018), Oman Sea (Sadeghi et al., 2020), Bangladesh (Ahmed et al., 2015), and China (Zhao et al., 2012). In all listed studies the liver shows higher values than the muscle and gill of fish species. These differences might be due to specific activities like crop farming, irrigation activities, and the resulting pollution level of the water. Fish are commonly migratory species and occasionally settle in one location, however toxic metal values in fish organs give evidence of exposure to a polluted aquatic environment (Bawuro et al., 2018) could be used to estimate the health status of the environment from collected fish species.

Table 7. 3. Average heavy metals concentrations in tissues of fish species from Gilgel Gibe I reservoir in comparison with other studies in Ethiopia.

No	Sources	Fish Parts	Concentrations (mg/kg dw)				References
			As	Cd	Pb	Hg	
1.	Gilgel Gibe I Reservoir	Liver	0.25 - 0.33	0.44 - 0.54	0.14 - 0.18	0.04 - 0.17	This study
		Gill	0.12 - 0.14	0.41 - 0.53	0.13	0.04 - 0.16	
		Muscle	0.11 - 0.17	0.32 - 0.41	0.09 - 0.15	0.06 - 0.15	
2.	Tendaho reservoir	Liver	-	0.16 - 0.32	0.41 - 2.45	-	(Asefa and Beranu, 2016)
		Muscle	-	0.18 - 0.23	0.33 - 0.52	-	
3.	Tana lake	Liver	0.98 - 4.94	0.7 – 1.63	2.65 - 6.12	-	(Kindie et al., 2020)
		Muscle	ND - 3.83	BDL	BDL	-	
4.	Abaya lake	Liver	-	0.017 – 0.027	0.13 -1.63	-	(Gerenfes et al., 2019)
	Chamo lake	Liver	-	0.017 - 0.027	0.13 - 1.63	-	
5.	Hawassa and Ziway lakes	Liver	-	1.08	2.70 - 3.37	-	(Kebede and Wondimu, 2004)
		Muscle	-	0.44 - 1.43	1.65 - 2.69	-	
6.	Abaya lake	Muscle	-	0.37	ND	-	(Bekele et al., 2015)
7.	Hashenge lake	Muscle	-	0.58	1.24	-	(Asgedom et al., 2012)
8.	Koka and Ziway lakes	-	-	-	-	0.64	(Deribe et al., 2014)

ND = not detected; - = not measured; BDL = Below Detection Limit

7.3.2 Human Health Risk Assessment

Humans are exposed to contaminants mostly through food and fish, which have a high potential for accumulating a variety of pollutants, including toxic metals. The most important body parts eaten by humans that accumulate harmful metals are fish muscles. The levels of toxic metals identified in all tissues of *Oreochromis niloticus and Labeobarbus intermedius* fish species are detected above the recommended limits by FAO/WHO and EU (FAO/WHO, 2011; Aytekin et al., 2019).

The mean concentrations of Cd (0.18 mg/kg) and Pb (0.10 mg/kg) were below recommended limits (Cd = 0.5, Pb = 2 mg/kg) by FAO/WHO in seafood (Bogdanović et al., 2014; Aytekin et al., 2019), signifying lower accumulation in both *Oreochromis niloticus and Labeobarbus intermedius* fish species. But the mean concentrations of As and Cd detected in muscle tissues of both fish species were not within safe limits for human consumption.

The health risk to humans due to exposure to toxic metals was assessed from the common fish species sold and consumed in southwestern Ethiopia. The EDI, THQ, and HI of As, Cd, Hg, and Pb in commonly consumed fish species, were computed by assuming the average consumption of an African adult which is 27 g/day of *Labeobarbus intermedius* and 23 g/day of *Oreochromis niloticus* fish species (Esilaba et al., 2020). The EDI of As, Cd, Hg, and Pb by the populations

around the study area, is presented in Table 7. 4. The results show that the trends of the calculated EDI reduced in the following order Cd > As > Hg > Pb both for *Oreochromis niloticus* and *Labeobarbus intermedius*

Table 7. 4. EDI of As, Cd, Hg, and Pb of fish species consumed in Jimma, southwestern Ethiopia.

Fish species	Fish intake, g/day	Toxic metals intake (mg/kg/day)			
		As	Cd	Hg	Pb
Oreochromis niloticus	27	0.092	0.17	0.065	0.03
Labeobarbus intermedius	23	0.078	0.19	0.058	0.04
PTDI (For an adult weighing 60.7 kg)		0.00042	0.001	0.00023	0.00357

PTDI - Provisional tolerable daily intake (mg/kg/day) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 60.7 kg is the average body weight of an African adult.

The EDI value of Cd in *Oreochromis niloticus* fish (0.17 mg/kg/day) is much higher than the PTDI value (0.001mg/kg/day) for an adult. But the daily intake Cd via consumption of fish was lower than the daily intake reported in developing countries; Ethiopia (Dsikowitzky et al., 2013), Bangladesh (Ahmed et al., 2015), Colombia (Pinzón-Bedoya et al., 2020), Democratic Republic of Congo (Squadrone et al., 2016), Egypt (El-Sadaawy et al., 2013), Ghana (Kwaansa-Ansah et al., 2019), Iran (Miri et al., 2017; Majlesi et al., 2018), Nigeria (Moslen and Miebaka, 2017), and Saudi Arabia (El-Bahr and Abdelghany, 2015). This might be due to the minimum feeding habit of fish in the Jimma zone.

The EDI value of Pb in *Labeobarbus intermedius* fish species (0.04 mg/kg/day) was considerably greater than the PTDI value (0.00357 mg/kg/day) for an adult. Likewise, daily Pb intake from commonly consumed fish was lower or similar to the daily intake reported for adult consumers in other developing countries; Ethiopia (Dsikowitzky et al., 2013), Colombia (Pinzón-Bedoya et al., 2020), Egypt (El-Sadaawy et al., 2013), Ghana (Kwaansa-Ansah et al., 2019), Iran (Majlesi et al., 2018), Kenya (Esilaba et al., 2020), Nigeria (Moslen and Miebaka, 2017), Palestine (Elnabris, 2013), and Saudi Arabia (El-Bahr and Abdelghany, 2015). Hence Pb may pose a serious health risk to consumers due to the likelihood of bio-accumulation over some time (Esilaba et al., 2020).

The highest EDI value of As in *Oreochromis niloticus* fish species (0.092 mg/kg/day) was much higher than the PTDI value (0.00042 mg/kg/day) for an adult. As daily intake from consumption of fish was lesser or similar to the daily intake reported for adults in Ethiopia (Dsikowitzky et al., 2013) and Colombia (Pinzón-Bedoya et al., 2020).

The daily intake of Hg (0.065 mg/kg/day) in *Oreochromis niloticus* fish was far higher than the PTDI value (0.00023 mg/kg/day) (Esilaba et al., 2020). Daily Hg intake from consumption of fish was lower or similar to the daily intake reported for adult consumers in other countries; Colombia: 0.00008 mg/kg/day (Pinzón-Bedoya et al., 2020), Iran: 0.000057 mg/kg/day (Majlesi et al., 2018), Poland: 0.00062 mg/kg/day (Kuras et al., 2017).

THQs and HI values were determined to estimate the non-carcinogenic effects of toxic metals from commonly consumed fish species harvested from the Gilgel Gibe I reservoir. The trend of THQ values for consumption of fish declined in the order of Hg > Cd > Pb > As for both *Oreochromis niloticus and Labeobarbus intermedius* species. Calculated THQ values of As, Cd, Hg, and Pb were all less than 1, indicating toxic metal's health effects are uncertain to occur. But the bio-accumulative nature of heavy metals might pose health risks in the future.

HI values of As, Cd, Hg, and Pb in this study were 0.834 for *Oreochromis niloticus* and 0.786 for *Labeobarbus intermedius* fish species. Consumers of *Oreochromis niloticus* had higher values than *Labeobarbus intermedius* fish species, showing substantial health risks for consumers posed by the possible additive effects of As, Cd, Hg, and Pb.

The CR values due to the exposure of As and Pb via consumption of selected fish species were 2.55×10^{-4} and 3.4×10^{-7} respectively, which were slightly greater than the studies (Gbogbo et al., 2018; Kortei et al., 2020) indicating fishes were polluted. Generally, the values of CR lower than 10^{-6} are considered insignificant, above 10^{-4} are considered to be detrimental, and lying in between 10^{-6} and 10^{-4} are considered as the tolerable range (US EPA, 2005). In this study, CR for As was greater than the undesirable range indicating the risk of developing cancer as a result of exposure via consumption of fish.

7.4 Conclusion

The concentrations levels of toxic heavy metals from the two commonly consumed fish species found in the Gilgel Gibe I reservoir differ among species and tissues. The concentrations are in the order of liver > muscle > gill for *Oreochromis niloticus* and liver > gill > muscle for *Labeobarbus intermedius* species as the liver is the major metabolic organ in any living organisms including fish and may accumulate a higher amount compared to other tissues. The distribution of toxic metal concentrations in both species follows: Cd > As > Hg > Pb; indicating Cd is the common pollutant. The daily intake of As, Cd, Hg, and Pb through consumption of the studied fish species were within recommended guideline limits set by WHO/FAO. THQ values of As, Cd, Hg, and Pb suggest no health risk to 2 commonly consumed fish species. However, long-term exposure to these metals might pose serious health risks hence needs greater attention. The CR for As was greater than the acceptable range which indicates a likelihood of developing cancer due to exposure to consumers. Therefore, rigorous measures should be implemented to monitor and mitigate traces of toxic metals and the associated human health risks particularly the long-term risk from fish consumption in the Jimma zone, southwestern Ethiopia.

Chapter Eight: General Discussion, Conclusion, and Future Prospects

8.1 General Discussion

In developing countries, cities/towns are exposed to liquid and solid wastes due to uncontrolled discharge from natural and anthropogenic sources. Discharge from residential and commercial refuse, roadsides littered public institutions, streams blocked with rubbish, and inappropriately disposed of toxic waste and disposal sites are the most important anthropogenic sources of heavy metals pollution (Abegaz, 2007; Clement Akan et al., 2012; Islam et al., 2015b). Solid wastes are released to the abandoned waste dumpsites and extensively used as fertile grounds for cultivating vegetables through irrigation but leached to the surrounding water bodies (Akpan-Idiok et al., 2012; Mahmood and Malik, 2014; Gupta et al., 2019) and effluent discharges from these sources cause various environmental and health hazards.

Studies in different parts of the globe have shown municipal and commercial wastes increase the heavy metal concentration in surface water and sediments (Singare et al., 2013; Ji and Jn, 2016) and have effects on crops and human health (Olafisoye et al., 2013; Isa et al., 2015; Soliman and Nasr, 2015). Food items (crops, vegetables, and fish) harvested from these polluted soils and water bodies can accumulate high levels of heavy metals. These heavy metals are sourced from different environmental compartments and can accumulate and persist in water, sediment, and biota at an environmentally hazardous level (Moore et al., 2011; Sim et al., 2016; He et al., 2018). They cause pollution and hazard even at low levels. Their resulting long-term cumulative health effects on plant and animal life are among the leading health concerns all over the world (Abubakar et al., 2015) due to their non-biodegradable and bio-accumulative nature (Vukovic et al., 2011; Reda and Ayu, 2016).

Continuously, heavy metals cause increasingly environmental and human health risks globally due to the discharge from industries, urban centers, and agricultural activities. Developing countries like Ethiopia are victims of such risks due to lack of awareness of waste management practices, absence of preliminary treatment facilities, and poverty. Hence, to solve ecological and human health risk problems, studies on contamination levels of heavy metals in water, sediment, and fishes are very crucial (Samuel et al., 2020).

However, streams found in the Awetu watershed, southwestern part of Ethiopia face challenges from the discharge of wastes from different public and commercial institutions which contain heavy metals. The polluted effluents drain directly into streams and further downstream into Boye and finally to Gilgel Gibe I reservoir. The wastes generated from these sources are dumped into the nearby water bodies arbitrarily making environmental pollution highly probable as they are used for drinking and other domestic purposes.

The concentration of heavy metals in streams in the Awetu watershed streams showed trends correlated with source patterns with anthropogenic activities and their tributaries. This might be due to the waste discharge from Jimma University, car repair garages, and car-wash. Because of the regular use of household items, such as cleaning materials, toothpaste, and cosmetics, the discharge of domestic wastewater also might increase heavy metal concentrations (Nagajyoti et al., 2010; Rak, 2015). This is due to the direct solid and liquid waste discharged at various locations from different industrial, municipal, and domestic activities which significantly affects the heavy metal condition of the watershed streams. Minimum concentrations of heavy metals were also observed in the downstream part of the streams which might be due to the dilution of the metals to the water. Of course, the mobility and possible trace effects of heavy metals in a specific environment are typically governed by their existing chemical forms (Baran and Tarnawski, 2015).

The concentrations of heavy metals found in stream water samples were spatially varied with anthropogenic sources and were potentially influenced by the physicochemical properties, such as DO, EC, pH, and turbidity (Rajeshkumar et al., 2018). High levels were observed in some specific areas, very close to garages, smelting, motor-vehicle exhaust fumes, and from corrosion of lead pipework which indicates the source of these elements could be mainly from a point source pollution (Patrick, 2006; Gowd and Govil, 2008; Getaneh et al., 2014). The inter-element associations of elements have shown that some elemental paired had an identical source or common sink in the streams (Sekabira et al., 2010).

The concentrations of heavy metals in the stream sediment of the streams were also studied hence its concentration is the measure of pollution in water ecosystems and capacity to accumulate contaminants (Giouri et al., 2010; Sekabira et al., 2010; Nowrouzi and Pourkhabbaz, 2014). Bottom sediments with a higher concentration of organic matter also influence the solubility and

mobility of the heavy metals in the aquatic ecosystems (Du Laing et al., 2009). The formation of potential mobile metal dissolved organic carbon complexes under oxidizing conditions prevents metals from co-precipitation with or adsorbing to hydroxides of metallic ions (Giouri et al., 2010; Gao et al., 2013).

The mean concentrations of heavy metals in sediments were compared between streams and different world sediment quality guideline values. The values are by far higher than the Sediment Quality Guidelines (SQG) values set by World Rivers, EC and PEL (Khan et al., 2005), US EPA water quality criteria (Hahladakis and Smaragdaki, 2013), Canadian Freshwater Sediment Guidelines (ANZECC and ARMCANZ, 2000), and World River Sediment Background Concentration (WRSB) and background concentration of this study. The values at the center of the town showed the maximum load where different anthropogenic activities are practiced.

However, the heavy metal concentrations of water and sediments don't provide sufficient information on the mobility and potential toxicity of contaminants or their potentially harmful effects on the environment because different chemicals can inactivate and promote synergistic effects. A potential harmful effect of heavy metals in the environment is determined by I_{geo}, CF, and PLI indices which provides a basis for assessing the effects of sediment-associated contaminants in sediment as compared to the values for each index (Müller 1979; Moore et al., 2011).

The PLI values demonstrate the decreasing order of contamination: Kitto > Boye > Awetu > Dololo. All the studied streams were found to be polluted (PLI > 1), suggesting inputs from anthropogenic sources. The I_{geo} of As shows practically unpolluted in Awetu and Boye, which shows a very low value (< 0), unpolluted to moderately polluted in Kitto (0-1), and moderately polluted in Dololo (1-2). The anthropogenic pressures largely contribute to the observed heavy metal concentrations rather than the mineralogical composition of the crust along the watershed. The higher values of I_{geo} were observed in samples from the Dololo stream as compared to other streams, which might be due to the kind and magnitude of anthropogenic pressures on the areas and differences in industrial and household waste management. However, both factors are likely to be the most significant cause of the observed differences. When compared with results from other developing nations, the concentrations measured in this study are much higher than in rural and urban streams (Giouri et al., 2010; Sekabira et al., 2010).

The interrelationship among metals in sediment of the aquatic environment provided important information on sources and pathways of heavy metal. The result of correlations between heavy metals acquiesced with the results of PCA that confirm some new relations between parameters which shows a significant variation in the concentrations based on the type of waste discharge at different sites (Nagajyoti et al., 2010).

Understanding the distribution, potential ecological risk, and sources of emission are crucial for the management of these heavy metals in the environment. Heavy metals easily precipitate and bind strongly to sediments, but may detach and be released into the water column which adversely affects the quality of water and living organisms (Simpson and Batley, 2007; Ahmed et al., 2009). Therefore, their presence in higher concentrations in water and sediments causes problems in the ecology of the water systems. In this study, Dololo and Kitto streams were the most severely contaminated sites as compared to Awetu and Boye streams.

Based on the values of heavy metals in water and sediment, the ecological risk assessment of toxic metal has been established. Accordingly, toxic metal contamination level and ecological risk calculation findings for water in the streams in the Awetu watershed were one to four orders higher than the low reference values. These values suggest metal contamination and ecological risk might be major distress for future pollution control and management plans to monitor toxic metal to prevent the risk. Fluctuations in hydraulic conditions can at times lead to the release of heavy metals from sediments to the water column hence the values in sediments are greater than the water.

The results of the mC_d and P_N indices indicated that Awetu and Boye streams were similarly impacted less severely and contaminated than Dololo and Kitto streams by toxic heavy metals which explains the reason the two streams were classified as 'Class 2' in mCd and 'Class 3' in P_N calculations. Dololo and Kitto streams were the most impacted by toxic metals, classified as 'Class 4' in mCd and all the four streams are classified as 'Class 3' in P_N calculations.

This study also employed the potential ecological risk index (RI) which is a multi-element index, to assess the potential impact of toxic metal contamination on the ecosystem of the streams in the watershed. Awetu and Boye streams were less exposed to the ecological risk of toxic contamination (Class 1), while Dololo and Kitto were determined to be likely to face 'severe'

ecological risk (Class 2 and Class 3), all per mCd and P_N calculations. Dololo stream (D1, D2, D3, and D4 sites) and Kitto stream (K1, K2, and K3 sites), however, were found most likely to be exposed to ecological risk, illustrating that degree of contamination does not always represent potential for ecological risk.

Heavy metal contamination is also considered one of the most significant sources of human health risks due to the consumption of food from aquatic ecosystems contaminated with hazardous chemicals. The increasing demand for food safety research has accelerated regarding the risk associated with the consumption of food contaminated by toxic heavy metals. The concentrations of heavy metals in the gill, liver, and muscle tissues of two fishes (*Labeobarbus intermedius* and *Oreochromis niloticus*) were studied and the potential human health risks were predicted.

The level of most heavy metals in the liver of the *Oreochromis niloticus* fish species was significantly higher than in muscles and gills tissues in this study which is in line with other studies (Küpeli et al., 2014; Aytekin et al., 2019). This is due to the liver acts as detoxifying by storing heavy metals. High metal accumulation capabilities make the liver the most important storage and target tissue in aquatic organisms (Jezierska and Witeska, 2006). The results show that metals concentrated in the liver of fishes in both *Oreochromis niloticus* and *Labeobarbus intermedius* species accumulate the highest concentration of Cd and As. Heavy metal concentrations in the gill were significantly higher than muscles hence fish gills are critical organs for excretory and respiratory functions. Gills usually reflect the levels of metal in the environment. This organ is in direct contact with water, it may absorb the metals present in the water. One of the reasons for the susceptibility of fish to metals is due to high absorption in the gills. Fish muscles are the primary edible parts, but muscles are not the tissues where heavy metals are accumulated especially in *Labeobarbus intermedius* fish species. In this study, the metal levels in muscle in *Labeobarbus intermedius* species were significantly lower than in liver and gill tissues. The results show that lower metal concentrations are observed in the muscles when compared to the gills, while the liver showed relatively higher values.

The concentrations of heavy metals recorded on the fish compared with other studies to assess the level of metal pollution in the Gilgel Gibe I reservoir. Accordingly, the levels Cd and Pb in the fish species of both *Oreochromis niloticus* and *Labeobarbus intermedius* were lower than the study

in Lakes Awassa and Ziway (Kebede and Wondimu, 2004) and Hashenge Lake (Asgedom et al., 2012). Hence food that humans eat is the most common route of exposure to pollutants, fishes have a strong potential for accumulating many pollutants including metal. The fish muscle is the most important part of the fish to be eaten by a human. Regarding the effect of metals on human health, the muscle tissue of fish is explored more than other organs because it is the part usually consumed by humans.

The EDI values were also compared with PTDI values of heavy metals in the two fish species. The value shows similar values and might not pose a problem to the consumers hence the EDI value should be greater than the PTDI value to cause risk. THQ is used to assess potential health risks associated with long-term exposure to dietary (Javed and Usmani, 2016; Moslen and Miebaka, 2017). Computed THQ values of As, Cd, Hg, and Pb were all below 1, suggesting that health effects associated with these heavy metals are unlikely to occur. However, the trend of THQ values for consumers of fish species decreased in the order of Hg > Cd > Pb > As for both *Oreochromis niloticus* and *Labeobarbus intermedius* fish species.

The HI values were 0.834 for *Oreochromis niloticus* and 0.786 for *Labeobarbus intermedius* fish species. *Oreochromis niloticus* fish consumers had higher HI values than *Labeobarbus intermedius* consumers, suggesting significant health risks for these consumers posed by the possible additive effects. The CR values of As and Pb due to exposure from the consumption of targeted fish species were 2.55×10^{-4} and 3.4×10^{-7} respectively, indicating that the fishes were polluted. Generally, the values of CR lower than 10^{-6} are considered as negligible, above 10^{-4} are considered to be undesirable, and lying in between 10^{-6} and 10^{-4} are considered as an acceptable range (US EPA, 2005). Hence, CR for As was higher than the un-acceptable range (greater than 10^{-4} to 10^{-6}) indicating the risk of cancer and repeated fish consumption was of concern.

8.2 Strengths and Limitations of the Study

Among the strengths, in areas like the Awetu watershed found in the southwestern part of Ethiopia with variable anthropogenic activities, studies are inadequate to address heavy metal pollution problems to make decisions. As a result, the use of an integrated approach of water, sediment, and fish samples to indicate environmental pollution are very significant. Besides, the use of watershed-based analysis contributes to addressing local-scale conditions and is ideal for an integrated and sustainable intervention action to solve the problem. Furthermore, integrating the concept of water, sediment, and fish to address the pollution level of heavy metals is very important to contribute complete information for the community, professionals, and decision-makers to act accordingly. Hence waste management needs the full involvement of all stakeholders to solve the pollution problem as the sources are the residents.

Moreover, the pollution sources of heavy metals are indicated in the framework of this study where the potential sources are pinpointed. Such a framework is essential to understand the details of pollution dynamics within a specified area differentiate system-driven problem-solving strategy to remediate and identify pollution sources and provides combined pieces of evidence to support a decision and policy options. Furthermore, concurrently analyzing the human and ecological components of a watershed ecosystem is plausible for comprehensive decision-making that could support sustainable management interventions of environmental pollution. Additionally, the use of multiple data sources makes the data more powerful.

As a limitation of this study, an effort was made to perform sequential extraction of heavy metal speciation in environmental media in different physicochemical fractions of material using specific solvents. These methods have been used widely in determining specific chemical forms of heavy metals in a range of environmental media which include sediments and waste materials. However, it was not achieved due to the chemical and instrument problem. Moreover, the human health risks assessment would be comprehensive and attractive if other food items consumed by the residents were studied and qualitative data were included to study the actual daily intake of the residents.

Finally, this dissertation is the compilation of a wide range of literature that led to repetitions of background information, methodological, and discussion sections. A lot of effort was made to reduce many of the repetitions. However, to link between chapters and protect the loss of information some repetitions, mainly in the method sections were still unavoidable.

8.3 Conclusions

The discharge of solid and liquid wastes from different sources into the water bodies increased ecological and human health risks globally. These wastes are mainly generated from anthropogenic activities such as small-scale industries, agricultural activities, health care facilities, laboratories, etc. These ecological and human health risks posed as a result of these anthropogenic activities are very common in developing countries like Ethiopia due to lack of awareness and poor waste management practices. Studies on the contamination levels of heavy metals in the water, sediment, and fishes are very limited where they are very important to know the magnitude of environmental pollution problems and prevent ecological and human health problems. Thus the finding provides new information on concentrations of heavy metals in stream water and sediments of the Awetu watershed at various levels.

The finding showed the highest concentrations of heavy metals in both water and sediment were found at the center of the town where maximum anthropogenic activities and uncontrolled effluents are disposed of to the nearby water bodies without preliminary treatment. The main sources of the waste discharge might be from Jimma University laboratories, dental clinics, car maintenance garages, car-wash, agrochemicals (phosphate fertilizers), pesticides, the emission of fossil fuels, batteries, and the incineration of medical wastes. Lower concentrations of heavy metals in water samples were detected downstream due to slower water flow and sedimentation. Common anthropogenic pollution sources resulting from rapid urbanization and inappropriate waste discharge were relatively beyond surface water quality standards, demonstrating a considerable potential environmental risk. It was also observed that the heavy metal concentrations in the streams of Awetu watershed water and sediments were remarkably high and varied among sampling points. As a result, the higher concentration of heavy metals in water and sediment poses a risk of water pollution. Heavy metals in stream beds distribute under sediment disturbance and/or changes in sediment chemistry which eventually intermix and transferred easily with the water in the hydraulic movement.

The contamination level and ecological risks posed as a result of higher concentrations of heavy metals were calculated based on I_{geo}, CF, PLI, mCd, PN, and RI and SQG values. Stream water and sediments show higher levels of heavy metals contamination which exceed the limits of world

average concentration. The finding shows the toxic metal concentrations in the water and sediment of the streams are highly likely to present a high risk to the ecosystem. Hence, regular monitoring of the water quality is needed to identify any change in the quality and mitigate the outbreak of health problems and the detrimental impacts on the aquatic ecosystem.

Human health risks posed by heavy metals were investigated in tissues of two edible fish species of Gilgel Gibe I reservoir. Accordingly, the concentrations of heavy metals varied among species and tissues. The metal concentrations in different tissues differed in the general order of liver > muscle > gill for *Oreochromis niloticus* species and liver > gill > muscle for *Labeobarbus intermedius* species. The result showed that the liver accumulates higher concentrations of heavy metals in comparison to muscle and gills in the studied fish species. EDI, THQ, HI, and CR are used to assess consumers' exposure to heavy metals via the consumption of fish. EDI values were within FAO/WHO recommended limits. Hence, THQ values may not pose a serious health risk to fish consumers. CR for As was higher than the unacceptable range indicating the risk of cancer.

Finally, this study justifies the need for further studies to ascertain the long-term effects of contaminants and waste dumping sites and investigations on water chemistry. The water in the area requires remediation as per environmental quality criteria and regular monitoring of heavy metals. Strengthening integrated waste management systems and river quality monitoring should also be implemented in the watershed streams to minimize the health effects and deterioration of the aquatic ecosystem.

8.4 Recommendations and Future Prospects

8.4.1 Recommendations

To solve the existing pollution problems, ecological and human health risks posed by heavy metals in the Awetu watershed streams and the downstream, the following practical and policy recommendations should be considered based on the research findings:

- Public and private institutions should get awareness on how to manage effluents generated from the garage maintenances, car wash facilities, and health facilities prior releasing to the nearby water bodies and should monitor and control regularly;
- Higher public institutions like universities are using wet laboratories and extensively used highly concentrated standard solutions containing heavy metals should be treated properly;

- Government authorities, policymakers, environmental health professionals, and residents should be aware of the toxicity nature of heavy metals and strict laws and regulations should be enforced at all levels to manage solid and liquid wastes specially released to the nearby water bodies without preliminary treatment;
- Toxic heavy metal accumulation mainly in fish consuming inhabitants should be done to carry out substantial public health interventions;

8.4.2 Future Prospects

Although the Ethiopian government has made considerable efforts to establish waste management strategies, much more effort is needed to identify the potential sources of heavy metals in the proper remediation technologies. As a result, future researchers consider integrated solid and liquid waste management is essential to consolidate national scale pieces of evidence.

- Biological samples of people living in streams in the Awetu watershed should be taken for analysis to determine heavy metal levels.
- Samples of the air and the other water sources not tested in this study should also be analyzed to ascertain the level of heavy metal.
- Sequential extraction of heavy metals from different environmental compartments should be analyzed to give more precise and accurate values.
- Food sources grown in the area from the irrigated areas should be analyzed to determine heavy metal levels.

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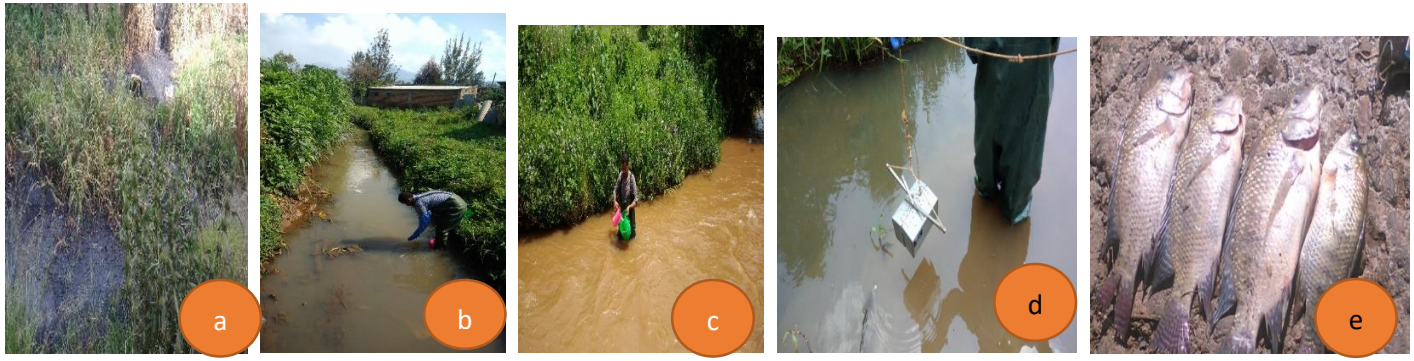
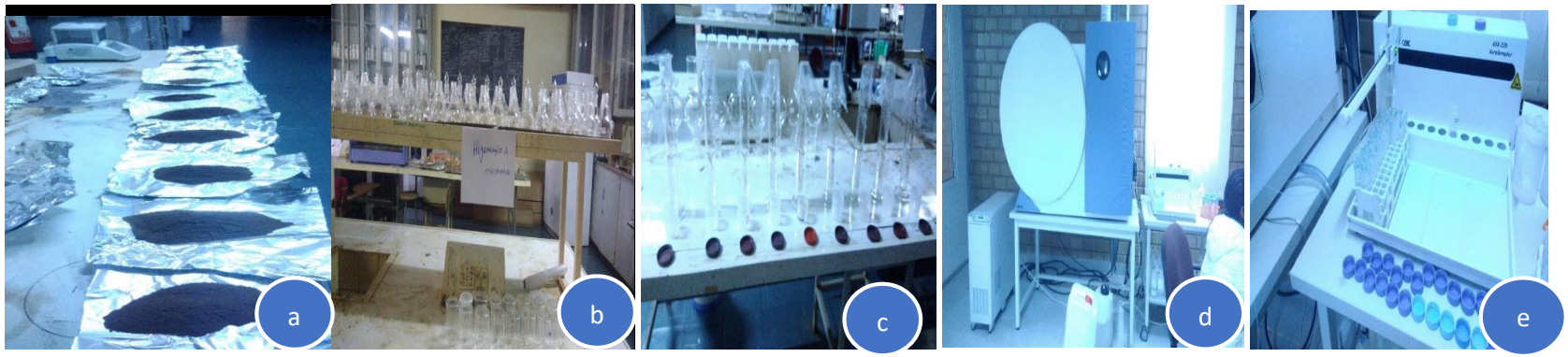
Appendices

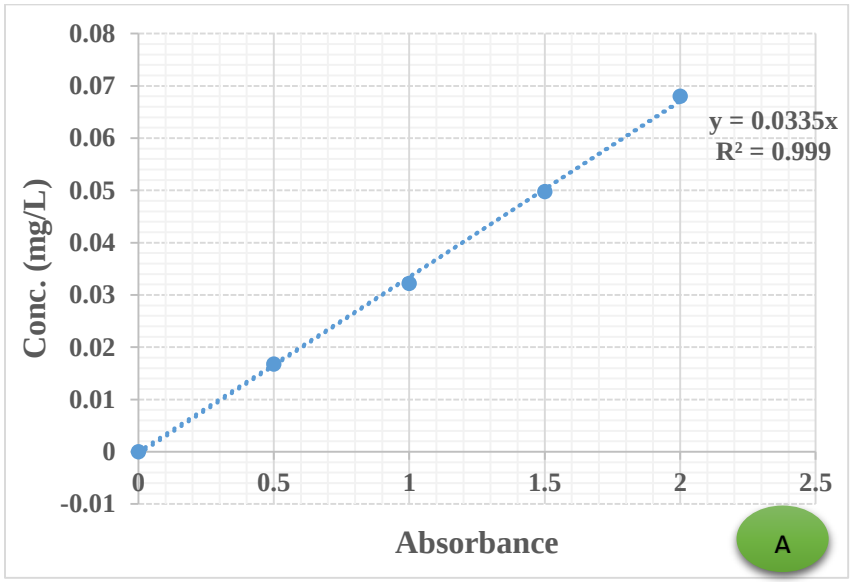
Appendix I. Pictures of field sample collection some sampling points (a, b, c &d), on-site water sample collection (b & c), sediment sample collection, and fish samples (e)

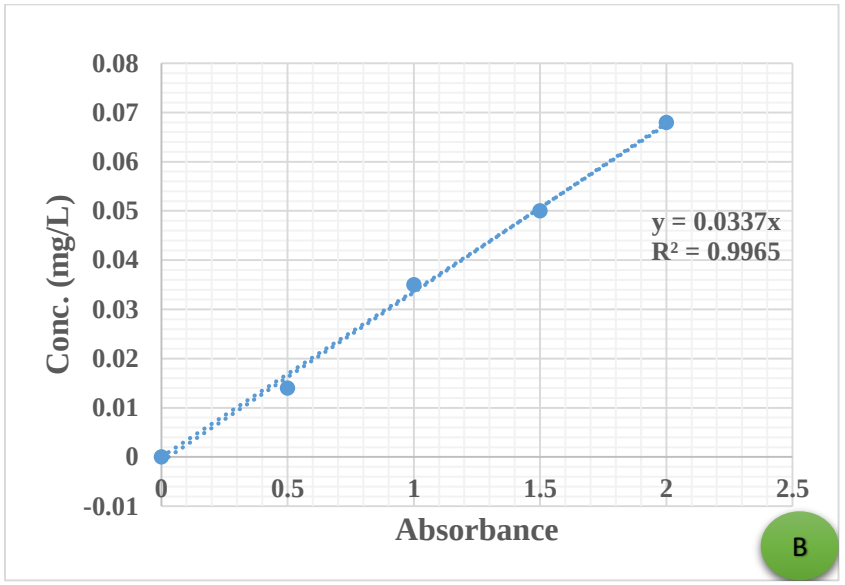


Appendix II. Pictures of samples preparation in the laboratory and sample analysis in the ICP-OES instrument; sample preparation in the laboratory (a, b & c) and sample analysis (d & e).



Appendix III. Calibration curves of heavy metals drawing concentrations (mg/L) versus absorbance (A for As, Hg, Zn, Mn, Ni, and Sn) and (B for Cd, Cr, Pb, and Cu).





Curriculum Vitae

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Education and Training

PhD Candidate in Environmental Health, Jimma University, since 2016.

Sept. 19, 2009 – Oct. 05, 2011	Master of Science in Environmental Technology at Jimma University, Ethiopia.
Sept. 2008 - June 2009	Higher diploma- teaching and action research skills certificate, Jimma University, Ethiopia.
Oct. 02, 2002 – July 20, 2006	Bachelor of Science Degree in Chemistry, Jimma University, Ethiopia.
Jan. 2012-June, 2012	Modelling and qualitative research certificate from Jimma University.
August 29-Sept.01, 2016	Collaborative Innovation Design for Recurrent Effects of Drought (CRID4CRID) Co-creation Workshop Hosted by Horn of Africa Resilient Lab (HoA-RILab)
December 2017	Scientific writing, publication ethics, e-resources, and reference Management Software, Certificate from Jimma University.
January & February 2017	Project coordinator and supervisor in the Medline National Survey Of CBN, UNICEF-WASH in Ethiopia in collaboration with North Carolina, USA.
Sept. 2018 – Dec.2020	Project coordinator in BIDERSE Project funded by EU and GIBF.
Feb. 2014 – Sept.1019	Coordinator of laboratory in the Department of Environmental Health Sciences and Technology, Jimma University.
Mother Tongue	Amharic
Other languages	English: Excellent in listening, reading, writing, and speaking

Publications

1. **Higemengist Astatkie Gebrie**, Dejene A. Tessema and Argaw Ambelu (2014). Elevated blood lead levels among unskilled construction workers in Jimma, Ethiopia. Journal of Occupational Medicine and Toxicology, **9**:**12.** https://doi.org/10.1186/1745-6673-9-12.
2. Fekadu Melak, Argaw Ambelu, **Higemengist Astatkie,** Gijs Du Laing and Esayas Alemayehu (2019). Freeze desalination as point-of-use water defluoridation technique, Applied Water Science. https://doi.org/10.1007/s13201-019-0913-0.
3. Menberu Yitbarek, Kisi Abdeta, Abebe Beyene, **Higemengist Astatkie**, Dessalegn Dadi, Gashaw Desalew, B.Vander Bruggen (2019). Experimental evaluation of sorptive removal of fluoride from drinking water using natural and brewery waste diatomite. Process Safety and Environmental Protection **128, 95–106**. https://doi.org/10.1016/j.psep.2019.05.052.
4. Yohannes Desalegn Wirtu, Fekadu Melak, Menberu Yitbarek, **Higemengist Astatkie** (2020). Aluminum coated natural zeolite from water defluoridation: A mechanistic insight. Groundwater for sustainable development **12 (2021) 100525**. https://doi.org/10.1016/j.gesd.2020.100525.
5. **Higemengist Astatkie**, Argaw Ambelu, Embialle Mengistie Beyene (2021). Sources and level of heavy metal contamination in the water of Awetu watershed streams, southwestern Ethiopia. Heliyon 7(2021) e06385. https://doi.org/10.1016/j.heliyon.2021.e06385.
6. **Higemengist Astatkie**, Argaw Ambelu and Embialle Mengistie (2021). Contamination of Stream Sediment with Heavy Metals in the Awetu Watershed of Southwestern Ethiopia. Front. Earth Sci. **9:658737.** https://doi.org/10.3389/feart.2021.658737
7. Jafer Siraj, Seblework Mekonen, **Higemengist Astatkie** and Abera Gure (2021). Organochlorine pesticide residues in tea and their potential risks to consumers in Ethiopia. Heliyon **7** (2021) **e07667**. https://doi.org/10.1016/j.heliyon.2021.e07667
8. Seblework Mekonen, Mohammedgezali Ibrahim, **Higemengist Astatkie,** Aynalem Abreha (2021). Exposure to organochlorine pesticides as a predictor of breast cancer: A case-control study among Ethiopian women. PLOS ONE **16 (9): e0257704**. https://doi.org/10.1371/journal. pone.0257704

Publications under Review

1. **Higemengist Astatkie**, Embialle Mengistie, Argaw Ambelu (2020). Heavy Metals in Waters and Sediments of Awetu Watershed Streams in Southwestern Ethiopia: An Ecological Risk Assessment
2. **Higemengist Astatkie**, Seblework Mekonnen, Embialle Mengistie, Argaw Ambelu (2021). Human Health Risk Assessment of Toxic Metals in Fishes (*Oreochromis niloticus* and *Labeobarbus intermedius*) from Gilgel Gibe I Reservoir Southwestern, Ethiopia
3. Fekadu Melak and **Higemengist Astatkie** (2021). An approach to the shelf-life study of traditional Ethiopian alcoholic beverage, Tella.

Presentations

- Indigenous point of use wáter treatment technology: Conical Clay Pot (CCP) Innovation competition in TechCon 2016, at Massachusetts Institute of Technology, Boston, USA.

Projects Won and Involved

- A research grant award from Jimma University with project title of Shelf-life study of traditional alcoholic beverage, *Tella* (2011).
- Mega research grant award from Jimma University with project title of Risk Assessment of Coffee Processing Effluent on Human, Cattle, and Aquatic Biota in the Receiving Water Bodies: a One Health approach, Institute of Health, Jimma University (2016).
- Small grant award from International Foundation of Science (IFS), Sweden (2021)

Membership and Committee Participation

- Chemical Society of Ethiopia
- Ethiopian Environmental Health Professionals Association (EHPA)
- Ethiopian Public Health Association (EPHA)
- Life Member of Red Cross Society of Ethiopia
- MSc curriculum developmental committee member in the Department of Environmental Health Science and Technology, Jimma University, Ethiopia
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