INSTITUTE OF HEALTH

FACULTY OF PUBLIC HEALTH

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY

HEAVY METALS IN WASTEWATER AND FISH TISSUES FROM WASTE STABILIZATION POND AND HUMAN HEALTH RISK ASSEEESSMENT, JIMMA, ETHIOPIA

BY: GIRMA BERHANU (BSc, MSc. CANDIDATE)

RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY, FACULTY OF PUBLIC HEALTH, INSTITUTE OF HEALTH, JIMMA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL HEALTH

AUGUST, 2022

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This is to certify that the research paper prepared by Girma Berhanu, entitled: Heavy Metals in Wastewater and Fish Tissues From Waste Stabilization Pond and Human Health Risk Assessment, Jimma, Ethiopia: submitted in partial fulfillment of the requirements for the degree of Master of Science in Environmental Health complies with the regulations of the Jimma University and meets the accepted standards with respect to originality and quality.

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Graduate Program Coordinator

DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been fully acknowledged.

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ABSTRACT

Introduction: Heavy metals are individual metals and metal compounds that can affect human health. These impurities are one of predominant type of water pollutants.

Objective: The present study was conducted to determine concentration of heavy metals in wastewater and fish tissues from Kito Furdisa waste stabilization pond and to assess human health risk in Jimma, Ethiopia.

Methods: Laboratory based cross-sectional study design was employed to determine the concentration of heavy metals in wastewater and fish tissues (gill, liver and muscle). A triplicate wastewater samples and a total of 32 fish samples (*O. Niloticus*) were collected from facultative and maturation ponds. A standard method of procedure was used to collect, digest and analyze samples. Micro Plasma Atomic Emission Spectrometry (Agilent 4210 MP-AES) and Hydride Generated Atomic Absorption Spectrometry (HGAAS, novAA 400P, Germany) were used to detect Cadmium, Lead, Arsenic and Mercury. SPSS version-26 was used for statistical analysis and a paired sample t-test at ($p < 0.01$) was used to test for statistically significant variation of heavy metals concentration between sampling points.

Results: From our findings, the mean concentration of heavy metals in wastewater were in the decreasing order of Cd>Pb >As>Hg, which ranged from (26.53µg/L to 27.66µg/L), (16.13µg/L to 20.67µg/L), (0.375µg/L to 0.387µg/L), (0.097µg/L to 0.346µg/L) in maturation and facultative pond, respectively. Among heavy metals, Hg in wastewater showed a statistically significant difference between sampling points ($p = 0.023$). The concentration of Arsenic (As) recorded in the muscles of fish under study was above the maximum permissible limits (MPL) recommended for human consumption by FAO/WHO which is 0.01 mg/kg. According to the non-carcinogenic and carcinogenic risk assessment, children were more susceptible to heavy metal exposure than adults.

Conclusion: Due to the presence of high levels of these toxic heavy metals, the wastewater is not suitable for fishing purpose in order to avoid bioaccumulation. Generally, it was found that heavy metals showed tissues specific accumulation in this study. The target carcinogenic risk (TR) and THQ estimated in this study revealed that all metals were less than the safe limit. Our results are an indicative of heavy metal contaminations and regular biomonitoring studies in fish are essential in order to prevent excessive buildup of toxic heavy metals in the food chain and human body.

Keywords: Fish Tissues, Human Health Risk, Heavy Metals, Waste Stabilization Pond

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LIST OF ACRONYMS

AAS	Atomic Absorption Spectroscopy
APHA	American Public Health Association
BAF	Bioaccumulation Factor
BOD	Biochemical Oxygen Demand
BW	Body Weights
CSF	Cancer Slope Factor
DO	Dissolved Oxygen
ECDSWA	Ethiopian Construction Design and Supervision Work Authority
EDI	Estimated daily Intake
EFDA	Ethiopian Food and Drug Control Authority
EPHI	Ethiopian Public Health Institute
FAO	Food and Agriculture Organization
FIR	Fish Ingesting Rate
EPA	Environmental Protection Agency
HM	Heavy Metals
WHO	World Health Organization
HHRA	Human Health Risk Assessment
PPM	Parts Per Million
PTDI	Permissible Total Daily Intake
WSP	Waste Stabilization Pond
WWTP	Wastewater Treatment Plants
MDL	Method Detection Limit
MP-AES	Micro-Plasma Atomic Emission Spectroscopy
MPL	Maximum Permissible Limits
SD	Standardized Deviation
US EPA	United States Environmental Protection Agency
RFD	Reference Dose
SPSS	Statistical Package for Social Science
THQ	Target Hazard Quotient

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CHAPTER ONE

1. INTRODUCTION

1.1. Background of the Study

Heavy metals are individual metals and metal compounds that can affect human health. These impurities are one of predominant type of water pollutant. Essential elements such as iron (Fe), copper (Cu) and zinc (Zn) are necessary in biological systems for basic functions as growth (Jennings, 2019). Likewise, the non-essential metals such as nickel (Ni), cadmium (Cd), chromium (Cr) and lead (Pb), are highly toxic at trace amounts in any biological systems ranging from aquatic biota to human along the food chain. Most of the metal ions are carcinogens and lead to serious health issues by forming free radicals. Among several heavy metals arsenic, cadmium, lead, mercury and chromium are considered to be highly toxic. Quantifying levels of any metal of public health concern is important to ensure consumer safety and properly warned of potential health risks associated with fish consumption (Baharom & Ishak, 2015).

Fish is a vital food source for the human body. It is a central component of well-balanced diet, offering low cholesterol level, high-quality proteins, omega-3 fatty acids, healthy source of energy, vitamins and other essential nutrients (Parida et al., 2017). The demand for fish is higher than supply especially, in Ethiopian fasting season. During lent, Christians who abstain from eating meat, milk, and eggs eat fish, since fish is the substitute of meat (Janko, 2013). Fish consumption also serves as one of the main entries through which aquatic pollutants such as heavy metals are bioaccumulated and biomagnified along the food chain.

Health risk due to consumption of food from aquatic ecosystems contaminated with hazardous chemicals including metals has increased globally particularly in developing countries like Ethiopia. The trophic transfer of potentially toxic heavy metals in the human food chains, especially in fish has significant consequences for human health (Ali et al., 2019). The exposure of heavy metals such as Cd, Hg, As and Pb to humans in higher amount or the bio-accumulation of these elements in the human organ systems has become a public concern on human health (Mirzabeygi et al., 2017; Nthunya et al., 2017; Rezaei et al., 2019; Ricolfi et al., 2020). Consumption of unsafe concentrations of heavy metals in food may lead to the disturbance of biological and biochemical processes in the human body (Prabu, 2009). These disorders are characterized by gastrointestinal disorders, stomatitis, tremors, diarrhea, hemoglobinuria, paralysis, vomiting, convulsions, and depression (Jaishankar et al., 2014). Similarly, heavy metals have the ability to disrupt metabolic activity and genetic makeup, or to affect embryonic or fetal development.

Currently, waste water is used for agricultural purposes in many countries of the world. At least 20 million hectares of land are irrigated with untreated or partially treated wastewater that poses the highest risk to the environment and human health (Ruma & Sheikh, 2010; Keraita et al., 2012). Waste Stabilization Pond provides a remarkable method of sustainable wastewater treatment. The effluent of treated wastewater can be reused for irrigation, aquaculture purposes, water conservation, environment, and public health protection. However, the effluent cannot always be reused. Reuse is only being possible if the effluent meets the recommended standards (Desye et al., 2022). In many urban areas of Ethiopia, a large volume of untreated wastewater is released into water bodies that are used for irrigation or agricultural purposes and has significant negative impacts on human health and the environment (Weldesilassie et al., 2011). At present, waste stabilization pond in Jimma provides a treatment process for wastewater generated from student's hostile buildings also used as fish production ponds. To make use of this water for fishing purpose, it is necessary to assess the extent of toxic heavy metal (Cd, Pb, As and Hg) contamination in wastewater and fish inhabiting waste stabilization pond and human health risk to consumer. For this purpose, this research was conducted in wastewater and fish tissues from WSP in Kito Furdisa campus, Jimma University, Ethiopia.

1.2. Statement of the Problem

Food safety is a major public health issues worldwide. During the last decades, the increasing demand of food safety has motivated research regarding the risk related with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins (Mello, 2003).

Currently, the effect of heavy metal pollution on human beings is becoming critical. Heavy metals can cause lethal health effects with various diseases, depending on the nature and quantity of metal, through ingestion, dermal contact, and inhalation pathways. Because of their toxicity, bio-accumulative nature, and persistence in the environment heavy metals contamination in aquatic ecosystems poses a serious threat to human life (Rasool et al., 2016). Thus, study on concentrations and health risk estimation of heavy metals exposure in fish consumption is crucial.

One of the common global scenarios for water reuse comprises beneficial use of treated municipal wastewater and its associated nutrients for aquaculture (Alderson et al., 2015; Kumar & Asolekar, 2016). Such considerations are important because in 2014 more fish for human consumptions came from artificial aquaculture than global fisheries (FAO, 2016), and this trend must continue to meet future global food production demands. In some parts of the world, including Ethiopia, it is common for WSPs to support aquaculture operations. Because relationships between such water reuse practices for fishing purpose and bioaccumulation of emerging contaminants like heavy metals are poorly understood (Brooks et al., 2005; Koba et al., 2018), particularly for WSPs as treatment systems.

Mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) are metals or metalloids of high concern because of their effects on the environment and, particularly, their potential toxicity on the faunas inhabiting there (Vizuete et al., 2018). In the present study, these heavy metals were chosen to be analyzed in fish due to their extremely toxic effects on the aquatic organisms and human health.

Several studies were performed previously in different countries assessing the heavy metals contamination in different environmental compartments such as water, sediment, soil, and foodstuff (Asgedom et al., 2012; Alidadi et al., 2019; Barbieri et al., 2019; Ahmad et al., 2020; Astatkie et al., 2021). However, there is scarcity of data on the heavy metal contamination assessment in fish tissues collected from waste stabilization pond in Jimma, one of the towns in Ethiopia and related safety to consumers. In addition, Ethiopia has no set guideline values on the levels of heavy metals in fish resources and regulation on treated wastewater reuse for fishing.

Therefore, the information available on the heavy metals in wastewater, and fish tissues and the risk they pose to human health in the study area was studied.

1.3. Research Questions

The study intended to answer a couple of research questions: Do wastewater from waste stabilization pond in Jimma suitable for fishing purpose? What is the concentration of Pb, Cd, As, and Hg in wastewater collected from waste stabilization pond in Jimma? What is the levels of accumulation of heavy metals in fish tissues? Do heavy metals causes the non- carcinogenic and non- carcinogenic health effect to consumers?

1.4. Significance of the Study

Heavy metals can pose a serious health implication to all living things in general and humans' beings in particular if accumulated in elevated concentration above maximum acceptable limits. Consumption of unsafe concentrations of food contaminated with heavy metals may lead to the disruption of numerous biological and biochemical processes in the human body (Mengistu, 2021). There is also a concern over bioaccumulation of heavy metal pollutants in the aquatic organisms especially in fish as they are consumed by humans and thus people are at risk of getting exposed to these toxic pollutants (Asgedom et al., 2012). It is already known that the bioaccumulation of heavy metals in fish tends to occur when the water is polluted. The increasing demand of food safety has accelerated researching regarding the risk associated with consumption of foods contaminated with heavy metals (Mansour et al., 2009).

Assessment of heavy metals in fish from contaminated areas can be extremely important in two major aspects: a) from the public health point of view; to evaluate the potential health risks to human associated with consumption of fish from this contaminated ponds to safeguard of human health, and b) from the aquatic environment view point, to improve our knowledge on the biological status of the aquatic ecosystems (Al-Mahaqeri, 2015). The major focus of the present study was about bioaccumulation of heavy metals in fish from wastewater and health risk assessment due to consumption of fishes from waste stabilization ponds. Hence, it forwarded information on bioaccumulation of heavy metals in fishes inhabited in artificial aquatic ecosystem. This study is helpful to researchers as this manmade aquatic ecosystem was not explored as well as data on these edible fish tissues will provide a health initiative for the risk assessment and food safety regulation. It also provided a base line data for the policy makers to develop food safety regulation concerning the consumption of fish collected from artificial aquatic ecosystem like WSP. The findings of the study provided comprehensive information as a baseline reference for future researchers focusing on heavy metals contaminations in aquatic fish and some regulatory actions for Ethiopia. Therefore, this study is paramount important as there is no published information concerning the contamination of fish from the waste stabilization pond in Jimma.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Heavy Metals Concentration in Wastewater

The most common chemical impurities in municipal wastewater include heavy metal cations, hydrocarbons, pesticides, nitrogenous compounds, pharmaceutical residues, detergents and phosphorus. Microbiological contamination sources could be from either animal or human faecal wastes containing different kinds of protozoa, viruses and bacteria, capable of causing diseases in humans (Ohoro et al., 2019). Heavy metals like Chromium (Cr), Cadmium (Cd), Mercury (Hg), Lead (Pb), Nickel (Ni), and Tallium (Tl) are potentially hazardous in combined or elemental forms. Heavy metals are highly soluble in the aquatic environments and consequently they can be absorbed simply by living organisms (Sobhanardakani et al., 2011).

Wastewater is a valuable source of nutrients and organic matter. Meanwhile, it may comprise undesirable chemical metals and pathogens that pose negative environmental and health impacts. The characteristics of treated wastewater and sludge that affect its suitability for land irrigation and beneficial use include the presence of heavy metals, toxic organics compounds, pathogens nutrient and organic matter. Urban effluents of wastewater always comprise trace metals, whereas the concentration in the water is related to the source of the water and activities in the urban environment (Al-khashman et al., 2013). Wastewater generated from factories may comprise heavy metals which through time accumulate in the soil deposits along wastewater stations as well as in aquatic organisms that inhabit such channels. Exposure of humans to contaminated wastewater is often possible particularly in urban highly populated areas or where the wastewater is reused for agricultural activities. However, previous studies have shown that effective reuse of wastewater is a major challenge in many countries of the world (Kalavrouziotis & Koukoulakis, 2016).

According to study conducted by Adeniji, (2020), the concentration of heavy metals in the wastewater sample collected from the three wastewater treatment facilities in Eastern Cape Province, South Africa for Zn, Cu, Cd, Fe, Pb (mg/ L) were; Zn (<DL) at all points, Cu (0.04 ± 0.003, 0.05 ± 0.012 and 0.04 ± 0.002), Cd (0.11 ± 0.001 , 0.11 ± 0.002 and 0.12 ± 0.002), Fe (2.077 ± 0.89, 0.959 ± 0.134 and 0.887 ± 0.159), Pb (<DL at all points) and Zn (<DL) at all points, Cu (0.04 ± 0.002, 0.04 ± 0.003 and 0.04 ± 0.002), Cd (0.11 ± 0.001, 0.11 ± 0.002 and 0.12 ± 0.002), Fe (0.28 ± 0.027, 0.463 ± 0.084 and 0.382 ± 0.028), Pb (<DL at all points) in influent and

effluents of WWTP-A, WWTP-B, WWTP-C respectively. It has been observed that Wastewater Treatment Plant (WWTP)-exhibited better removal efficiency for Fe (86.6%), compared to WWTP-B (34.7%) and WWTP-C (56.9%).

The concentration of heavy metals were determined from wastewater samples collected from the Gusii wastewater treatment plant, Kenya by Douglas et al., (2022). The results disclosed that the concentrations of Zinc and Cadmium were below the detection limit for all the sampling sites. The Pb mean concentration of the sampling points ranged from 0.34 ± 0.06 mg/L to 0.86 ± 0.08 mg/L. The facultative pond had the highest Pb mean concentration with 0.86 ± 0.08 mg/L. One-way ANOVA test showed that mean Pb concentration was not significantly different among the sampling stations (F (7, 24) = 1.827; p = 0.128). The mean levels of Cu of the sampling stations also ranged from 0.25 ± 0.05 mg/L to 0.34 ± 0.01 mg/L. One-way ANOVA test indicated that the average Cu concentration was not significant between the sampling stations (F (8, 27) = 0.354; p = 0.935).

The Levels of Mercury (Hg), Lead (Pb), Cadmium (Cd), Chromium (Cr), Nickel (Ni) & Thallium (Tl) were studied in wastewater samples collected from eight sites in open drainage channels at Nairobi industrial area, Kenya. The mean concentrations of heavy metals in wastewater ranged from 0.0001 to 0.015 ppm in an ascending order of Tl <Cd <Hg <Ni <Cr <Pb. The finding obtained the highest Pb levels followed by Cr and the lowest was Tl. The mean concentration of Hg in the wastewater samples was <0.0001 ppm. Pb levels ranged from 0.011 to 0.032 ppm, and this was above the recommended limits of Pb in wastewater set by WHO, Kenya, and US-EPA (Kinuthia et al., 2020).

The distribution of heavy metals (Cr, Cd, Zn, Pb, Fe and Cu) in wastewater samples from the EIZ has been determined by Dagne, (2020). The results recorded from this study revealed overall concentrations of heavy metals (Cr, Cd, Zn, Fe, Pb, and Cu) in the range of (0.20-1.04), (0.04-0.08), (0.07-0.21), (2.89- 5.15), (3.11-45) and (0.30-0.99) in mg/L, respectively. The study also concluded that concentrations of heavy metals (Cr, Cd, Zn, Fe, Pb and Cu) in the wastewater were above the recommended limit of both WHO and FAO. According to (Al-khashman et al., 2013), the mean concentrations for B, Fe, Pb, Cd, Zn, Cu, Mn and Cr in wastewater samples were 320, 170, 78, 23, 109,146, 30 and 3.2 ppb, respectively.

A Study performed in Pakistan observed the level of Heavy Metals in Textile Effluent. Accordingly, the mean concentrations of heavy metals in textile effluents samples for (Mill-1), (Mill-2), (Mill-3), (Mill-4) and (Mill-5) were Cd; 0.175, 0.927, 0.873, 0.165 and 0.123 mg/L; 0.137, 0.131, 0.175, 0.147 and 0.124 mg/L for Pb respectively (Imtiazuddin et al., 2014).

A study conducted in Nigeria, assessed the heavy metals levels in water, sediment and fish collected from UKE stream, documented the mean concentration of metals in the water samples ranged from 0.023 – 7.51 mg/L and for sediments the range was 0.095 – 8.78 mg/g. The heavy metals studied were Pb, Zn, Fe, Cd, Cu, Mn and Hg with mean concentrations of 0.040, 3.19, 7.51, 0.023, 0.95, 0.51 (mg/l) in water and 0.095, 4.79, 8.78, 0.035, 1.34, 0.24, and (mg/ g) in sediment respectively with Hg not detected in both water and sediment samples (Opaluwa et al., 2012).

The concentrations of heavy metals in fresh from the Hashenge Lake in Ethiopia were found to be in the following increasing order of concentrations (µg L^{-1}): Zn (937.5) > Mn (20) > Cd (8.7) > Fe (3.6) > Co (3.5) > Cr (3.4) > Pb (3.3) > Ni (2.3) > Cu (2.1). These comparative analysis of heavy metal concentration in water and fish revealed that Pollution of water bodies is a matter of utmost concern especially if it is surrounded with areas of a wide range of human anthropogenic activities, such as agriculture, that have led to the degradation of the water bodies used for fishing (Asgedom et al., 2012). Another study performed on the assessment of Mn, Pb, Cr and Cd concentrations in Kulufo River water, Arba Minch, Gamo Gofa was observed that, the level of Mn ranges (0.420-520 mg L-1), the level of Pb ranges (0.012-0.023 mg L-1, the level of Cr ranges (0.106-0.201 mg L-1), the level of Cd ranges (0.050-0.108 mg L-1) (Hizkeal, 2019).

2.2. Heavy Metals Concentration in Fish Tissues

Heavy metals may accumulate in the body of an organism directly from the abiotic environment, i.e., water, sediments, and soil or may enter the organism body from its food/prey (Ali et al., 2019). Concentrations of the heavy metals As, Cd, Cu, Hg, Pb, and Zn in fish tissues from the middle coast of Zhejiang Province of China were investigated by Han et al., (2021). It indicated considerable variation in heavy metal concentrations in different tissues. Elevated concentrations of most heavy metals were documented in fish gills. Of all fish samples the study recorded, the highest concentrations of As, Cd, Cr, Hg and Pb were found to be 17, 0.76, 0.836, 0.289 and 1.48 mg/kg wet weight, respectively. Another study in china by Elnabris et al., (2018) has also estimated the mean concentrations of Zn, Ni, Cu, Mn, Pb, and Cd in the muscles of the six fish species provided the following results: Zn 9.05, Ni 0.696, Cu 0.481, Mn 0.480, Pb 0.135, and Cd 0.0139. It indicated to the following ranking: Zn > Ni > Cu > Mn > Pb > Cd. The study done in Bangladesh has reported the mean concentration of heavy metals which were found in fish samples in the range as Cr 3.2039–16.3495 mg/Kg dry wt., Cu 1.5589–4.5848 mg/Kg dry wt., Ni 0.1101–1.9029 mg/Kg dry wt., Pb 0.4373–2.7638 mg/Kg dry wt. and Fe 30.9599–108.780 mg/Kg dry weight (Jothi et al., 2018). The finding obtained from the Atomic Absorption Spectroscopy (AAS) analysis, showed that Pb and Cd were detected in the two fish samples in Palestine (Ejike, 2017). The study recorded Pb (21.40 mg/kg), Cd (1.50 mg/kg) in Clarias gariepinus and Pb (164 mg/kg) and Cd (1.30 mg/kg) in Oreochromis niloticus which were higher than the permissible limit specified by WHO 2003. Bioaccumulation of heavy metals in some tissues of fish from lake in Nigeria was studied, revealed marked variations in the concentrations of heavy metals (Cu, Zn, Pb, and Cd) in muscle, liver, and gills of the fish. Findings showed that the concentrations of the metals in the organs were in the order of liver > gills > muscle. Nonessential metal Pb showed higher bioaccumulation in the liver and Cd recorded higher concentration in gills. Some species of the fish indicated bioaccumulation of Cd in gills (Bawuro et al., 2018). The study conducted on the concentrations of Hg, Pb, and Cd in the muscles of the different fish species collected from Mechraa-Hammadi Dam in Morocco observed the low Cd concentrations. The mean concentrations varied from 0.001mg/kg of wet weight founded in L. macrochirus to 0.005mg/kg of wet weight in E. Lucius. The concentrations of Pb recorded in the muscle of the studied fish, were 0.017mg/kg of wet weight in M. salmoides and 0.115mg/kg of the wet weight obtained in L. macrochirus. The mean concentrations of Hg detected in the muscle of fish, were varied from 0.056mg/kg of wet weight in M. salmoides to 0.287mg/kg of wet weight in E. Lucius (Mahjoub et al., 2021). Heavy metal concentrations were observed as the following increasing order: Cd <Pb <Hg<As, which recorded 0.3 mg/kg for Cd, 0.16 ± 0.05 for Pb, 0.58 ± 0.69 for Hg and 1.52 ± 0.70 for As in the muscles of *O. niloticus* from River Tano in Ghana (Nyantakyi et al., 2021).

The Concentrations of nine metals in the bone and flesh of two fish species (Nile Tilapia and Common Carp) from the Hashenge Lake, Ethiopia have determined by Asgedom et al., (2012). According to the study, accumulation of heavy metals in the bone and flesh samples of Nile Tilapia were: Cu 2.33, 0.85; Pb 0.88, 1.24; Cr 3.02, 0.37; Cd 1.62, 0.58; Mn 6.27, 1.01; Co 6.09, 1.61; Fe 49.59, 64.87; Ni 0.47, 0.41 and Zn 105.57, 24.95 respectively. In most of the fish samples, lead, chromium, cadmium, cobalt, and zinc concentration were found to be above the maximum tolerable values provided by FAO/WHO (Kebede & Wondimu, 2004) have determined distribution of trace elements in muscle and organs of Tilapia, Oreochromis niloticus, from lakes Awassa and Ziway, Ethiopia. The levels of 8 elements in muscle, bone, gill and liver detected by flame atomic absorption spectrophotometer varied, respectively, (mg /kg dry weight): Cd 0.44-1.43, 4.58-4.93, 2.20-2.85, and 1.08-1.90; Pb 1.65-2.69, 39.5-42.3, 17.1-23.1. Results also showed organ specific distribution of trace metals in Tilapia, which has been argued in terms of physiological role in fish and/or the likely influence of anthropogenic origin on lakes.

2.3. Health Risk Assessment from Fish Consumption

A number of heavy metals are identified as being toxic with adverse health effects on human being. These comprise specifically, arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc, as indicated in the United States Environmental Protection Agency (USEPA) Part 503 rule **(**USEPA, 1995). The main fears to human health from heavy metals are linked with exposure to lead, cadmium, mercury and arsenic.

Heavy metal pollution is increasingly recognized as a severe, environmental issue by ecologists; high levels of toxicity, persistence, and potential for accumulation inside human body pose a serious health threat to the inhabitants of urban areas (Karim et al., 2015).

Fish is one of the most important food sources and thus, consumption of trace elements from capture fish, especially toxic elements is one of great concern for human health. The study conducted in Southwestern Nigeria were determined the mean concentration of heavy metals and the health risks resulting from the consumption *C. nigrodigitatus* from Ologe and Badagry lagoons have been estimated based on daily intake (EDI) and target hazard quotient (THQ) (Bassey & Chukwu, 2019). The finding of this study, showed that the target hazard quotient (THQ) of the contaminants decreased in the following order; Pb > Zn > Cd > Fe > Cu > Cr > Ni and the hazard index (HI) risk values were 0.204 and 0.202 during wet and dry season at Ologe lagoon; 0.113 and 0.167 during wet and dry season at Badagry lagoon respectively.

The daily consumption of Cd, Cu, Mn, Ni, Pb and Zn in all fish species in study conducted on Palestinian people of Gaza Strip, Palestine were ranged from Nd-1.0, 2.9–10.6, 4.4–9.7, 5.3–11.40, Nd-6.4 and 43.2– 239.4 lg/day/person, respectively. The average daily intake of metals through fish consumption can be ordered as follows: Zn > Ni > Mn = Cu > Pb > Cd (Elnabris et al., 2018). The estimated weekly intake (EWI) were calculated for Cd, Pb, Cu, Zn consumption from Peninsular Malaysia by Azmi et al., (2019). And obtained the results ranged between 0.01 to 1.42 µg/kg b.wt/week for Cd. The estimated EWI of Pb from eating of various fish types was between 0.03 and 1.02 µg/kg b.wt/week. The evaluated EWI Cu was found between 0.69 and 27.65 µg/kg b.wt/week, Zn ranging from 41.55 to 288.3 µg/kg b.wt/week and Sn were between 5.12 and 103.27

µg/kg b.wt/week. Health risk assessment was calculated on the concentrations of metal in fish muscle and daily fish consumption by people in Machilipatnam Coast, India the (Krishna et al., 2014).

The estimation of potential health risks (both carcinogenic and non-carcinogenic effects) associated with the consumption of fish contaminated with (Pb, As, Ni and Cd) for an individual adult were observed by (Al-Mahaqeri, 2015). In the non-carcinogenic health risk, the As concentrations in the fish muscles have the highest potential as a health risk for fish ingestion followed by the Cd and Ni, with Pb the lowest. The As THQ from the intake of L. poecilopterus, C, striata and P. grootii (seven days and one day/a week) were >1.00. In addition THQ for As from M. frenatus, T. trichopterus and P. schwanenfeldii muscle were higher than 1 for people who eat fish seven days per a week.

2.3.1. Effects of Lead on Human Health

In fact, Pb is considered a non-essential element; it is similar to calcium in metabolism processes and in its mobilization from bone as well as its deposition in bone. Due to the large affinity of Pb for thiol and phosphate-containing ligands, it inhibits the biosynthesis of heme, thus affecting the membrane, permeability of kidney, liver and also brain cells (Al-Mahaqeri, 2015). EPA has identified that lead is a probable human carcinogen. Lead can affect every organ and system in the body. Long-term exposure of adults can cause decreased performance in some tests that measure functions of the nervous system; weakness in fingers, wrists, or ankles; small increases in blood pressure; and anemia. Exposure to high lead concentration can strictly damage the brain and kidneys and eventually cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. The toxicity and carcinogenicity of heavy metals are dose dependent. High-dose exposure leads to sever responses in animal and human which causes more DNA damage and neuropsychiatric disorders (Gorini et al., 2014).

2.3.2. Effects of Mercury on Human Health

The order of increasing toxicity related to different forms of mercury is defined as $Hg^0 < Hg^{2+}$, $Hg^+ < CH_3\text{-}Hg$ (Kungolos et al., 1999). (Yokoyama, 2018) reported that the methylmercury poisoning control measures and the current situation of its effects on fetuses and infants particularly. Mercury toxicity has effects on the rectal system and leads to colorectal cancer. It also has vast effects on the central nervous system leads to brain cancer and lung cancer. High-dose heavy metals exposure, mainly mercury and lead, may induce severe complications such as abdominal colic pain, bloody diarrhea, and kidney failure (Tsai et al., 2017).

2.3.3. Effects of Arsenic on Human Health

Arsenic is a toxic metal for all living organisms including human; its toxicity depends principally on it chemical form (Medeiros et al., 2012). Inorganic arsenic is a known carcinogen and can cause cancer of the skin, lungs, liver and bladder. Lower level exposure can cause nausea and vomiting, decreased production of red and white blood cells, abnormal heart rhythm, damage to blood vessels, and a sensation of "pins and needles" in hands and feet. Consumption of very high levels can maybe result in death. Long-term low level exposure can cause a darkening of the skin and the appearance of small "corns" or "warts" on the palms, soles, and torso (Griswold, 2009). The high concentrations of arsenic in the water and fish species are displaying various clinic pathological conditions including cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neurobehavioral disorders, diabetes, hearing loss, portal fibrosis, hematologic disorders (anemia, leukopenia, and eosinophilia), and carcinoma (Samadzadeh et al., 2013). Abdul et al., (2015) reviewed the health effects of arsenic exposure to human beings. According to this review, the majority of the population expose to this toxic metal through atmospheric air, groundwater, and certain kind of foods.

2.3.4. Effects of Cadmium on Human Health

Chronic toxicity of Cd in children includes damages of respiratory, renal, skeletal and cardiovascular systems as well as development of cancers of the lungs, kidneys, prostate and stomach (WHO, 2011). Exposure of people to Cd includes, eating contaminated food, smoking cigarettes, and working in cadmium-contaminated work places and in primary metal industries (Paschal et al., 2000).

2.4. Factors Determining the Survival of Fish in Aquatic Ecosystem

The survival and growth of different fish species is also subjective to different range of factors, among them water quality parameters such as Do, PH, T^{O}, EC, turbidity, temperature are a few. Studies demonstrate that a special set of water parameters requirements and ultimate water quality is essential to a healthy, balanced, and functioning aquaculture system (Rakocy, 1990).

According to Gopolang and Letshwenyo, (2018), average pH in the influent and effluent of waste stabilization ponds were 7.03 ± 0.5 and 6.87 ± 0.4 respectively. The effluent pH is within the permissible limit for discharge into other environments whose range is 6 to 9. Extreme pH of wastewater is generally not acceptable because it affects survival of aquatic life including fish. At acidic conditions, heavy metals tend to exist as free metal ions while around neutral conditions, some precipitate as hydroxides or other insoluble species if the appropriate co-ion is available. Another study conducted on Physico-Chemical Analysis and Heavy Metals Concentration in Textile Effluent in Karachi Region in Pakistan reported that pH of different effluent samples appeared to lie between 9 to 11.8 (Imtiazuddin et al., 2014). The study aimed to determine the Physicochemical Parameters of food wastewater recorded the mean pH value ranged from 7.80 to 10.20 (Jingxi et al., 2020). The pH values of the samples ranged from 8.6 to 9.6 with a mean value of 8.96 according to Kumar et al., (2019). At high pH most of the metals become insoluble and accumulate in the sludge and sediments. At low pH most of the metals become soluble and available, therefore could be hazardous to the environment. A Study conducted on Hawasa waste stabilization pond documented that the Mean value of pH was 7.8, which indicated that the treated sewage water is slightly alkaline in nature (Narayanan & Getachew, 2020). The most preferred temperature range for optimal growth of tilapia fish species is 25 to 27 °C, while the ideal pH ranges between 6 and 9 (Dewalle et al., 2017).

According to Mireles et al., (2004) the highest EC was recorded at KN, and the recorded mean value was 3,347.5 dS/m. The lowest was recorded at the site of NL with a measured value of 1,005.6 dS/m. The Electrical Conductivity which measures the salinity hazard ranged between 120.4 mS/s to 151.2 mS/s for the three sampling positions with an averages of 127.9, 123.6 and 123.6 mS/m for position 1, 2 and 3 respectively as observed by Moyo et al., (2015). EC is an indicator of the total dissolved solids in the water bodies. Electrical conductivity (EC) is the most important characteristic in determining the suitability of water for irrigation use and it is a good measurement of salinity hazard to crop as it reflects the TDS in wastewater. EC values of treated wastewater varied from 1100 to 1300 μScm^{-1} (mean value = 1200 μScm^{-1}) while TDS values varied from 545 to 675 mgL^{-1} (mean value = 610 mgL^{-1}) as recorded by (Narayanan & Getachew, 2020). The turbidity fluctuated from 6 to 15 NTU with averages 10.7, 11 and 12.7 NTU at the positions 1, 2 and 3 respectively according to study by Moyo et al., (2015). The turbidity was higher than the FAO recommended turbidity of 5 NTU.

Biological Oxygen Demand (BOD_5) is a measure of the amount of biodegradable organic content of water and used to assess the efficiency of wastewater treatment system, industrial waste and any type of pollution in the water. Al-khashman et al., (2013) recorded the BOD_5 concentration ranged from 19 to 98 mg/L, with an average value of 63 mg/L. BOD $_5$ was higher in summer season than in winter season due to the dilution of rainfall during the rainy season. Dissolved Oxygen (DO) also varied in the range from 3.65 to 4.62 mg/L, with a median value of 4.0 mg/L. The BOD of the effluent had averages of 9.3, 8.8 and 8.3 mg/L for positions 1, 2 and 3 respectively were recorded by (Al-khashman et al., 2013). The FAO guideline recommends a maximum level of 10 mg/L for the BOD.

Evaluation of Treated Municipal Wastewater Quality for Irrigation Purposes was conducted and value was ranged from 62 to 120 mg/L with a median value of 99 mg/L. The chloride value was higher in the summer season than in winter season (Al-khashman et al., 2013). The most common toxicity is from chloride (Cl^-) in the reuse of wastewater. (Cl^-) is not adsorbed or held back by soils, therefore it moves readily with the soil water, is taken up by the crop, moves in the transpiration stream, and accumulates in the leaves. The study by Narayanan & Getachew, (2020) was obtained (Cl^-) ion concentration of the samples varied from 130 to 224 mg/L^{-1} representing slight to moderate degree of restriction on the use of this wastewater in irrigation. The mean values of chloride (Cl^-) ion in textile effluents were 1460, 1818, 1169, 1485 and 1359 mg/L for ($Mill_{-1}$), (Mill-2), (Mill-3), (Mill-4) and (Mill-5), respectively as stated by (Imtiazuddin et al., 2014)**.** It was observed that all the textile Mills effluents had alarmingly high values of chloride contents. High chloride contents are harmful for metallic pipes as well as for agricultural crop if such wastes containing high chlorides are used for irrigation purposes. According to study on Determination of Physicochemical Parameters and Levels of Heavy Metals in Food Waste Water with Environmental Effects, the mean concentrations of nitrate, and phosphate in all the sampling points ranged between 20.15 mg/L and 30.35 mg/L for nitrate, and 10.33 mg/L and 12.65 mg/L for phosphate, respectively (Jingxi et al., 2020).

2.5. Conceptual Framework

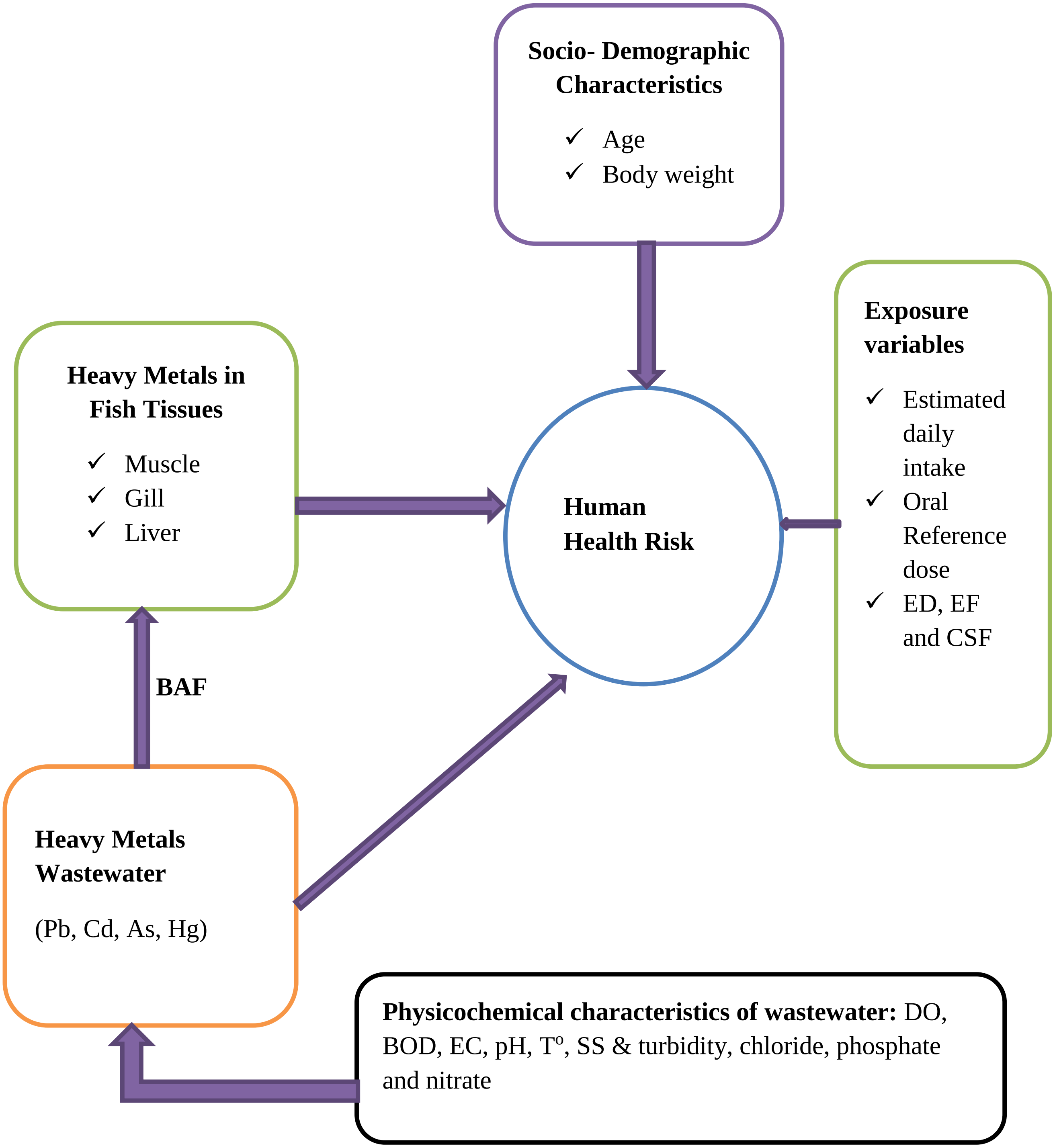

Figure2.1. Conceptual Framework to Determine the Heavy Metals in Wastewater and Fish from Kito Furdisa WSP and Human Health Risk Assessment in Jimma, south west Ethiopia

CHAPTER THREE

3. OBJECTIVE

3.1. General objective

The main objective of this study is the determination of the concentration of heavy metals in wastewater and fish tissues collected from waste stabilization pond and assess human health risk in Jimma, Southwest Ethiopia, 2022.

3.2. Specific objectives

The specific objectives of this study are to:

- Assess physicochemical characteristics of wastewater from WSP
- Determine the level of heavy metals in wastewater collected from WSP
- Investigate the heavy metals accumulation in different fish tissues (muscle, gill and liver)
- Assess human health risk from consumption of fish collected from WSP

CHAPTER FOUR

4. MATERIALS AND METHODS

4.1. Description of the Study Area

The waste stabilization pond system is located in Kito Furdisa Campus of Jimma University, 352 km from capital city Addis Ababa in the Southwest of Ethiopia as shown in Figure4.1.The geographic coordinates are approximately 7°40′ latitude North and 36°50′ longitudes east. The annual mean temperature of the area is 19.3°C (11.5°C–27.1°C) and the annual rainfall is about 1749.1 mm. The waste stabilization pond was laid on 69,236.70 square meters (6.9 hectares). It was designed to serve a population of 40,000 and it contains seven ponds as described in Figure4.1. & Figure4.2. and has the capacity to receive more than 2250 m^3/d raw wastewater (Desye et al., 2022). Wastewater from the student's hostel buildings is first diverted to septic tanks to remove the solids and then diverted to stabilization pond and a series of oxidation and polishing ponds. The domestic sewage/waste as well as runoffs is disposed of in the waste stabilization pond with no pre-treatment, hence increasing concentration of different kinds of pollutants including heavy metals in influent wastewater. The wastewater is treated both by physical and biological treatment to reduce the suspended solids and biochemical oxygen demand to the acceptable levels.

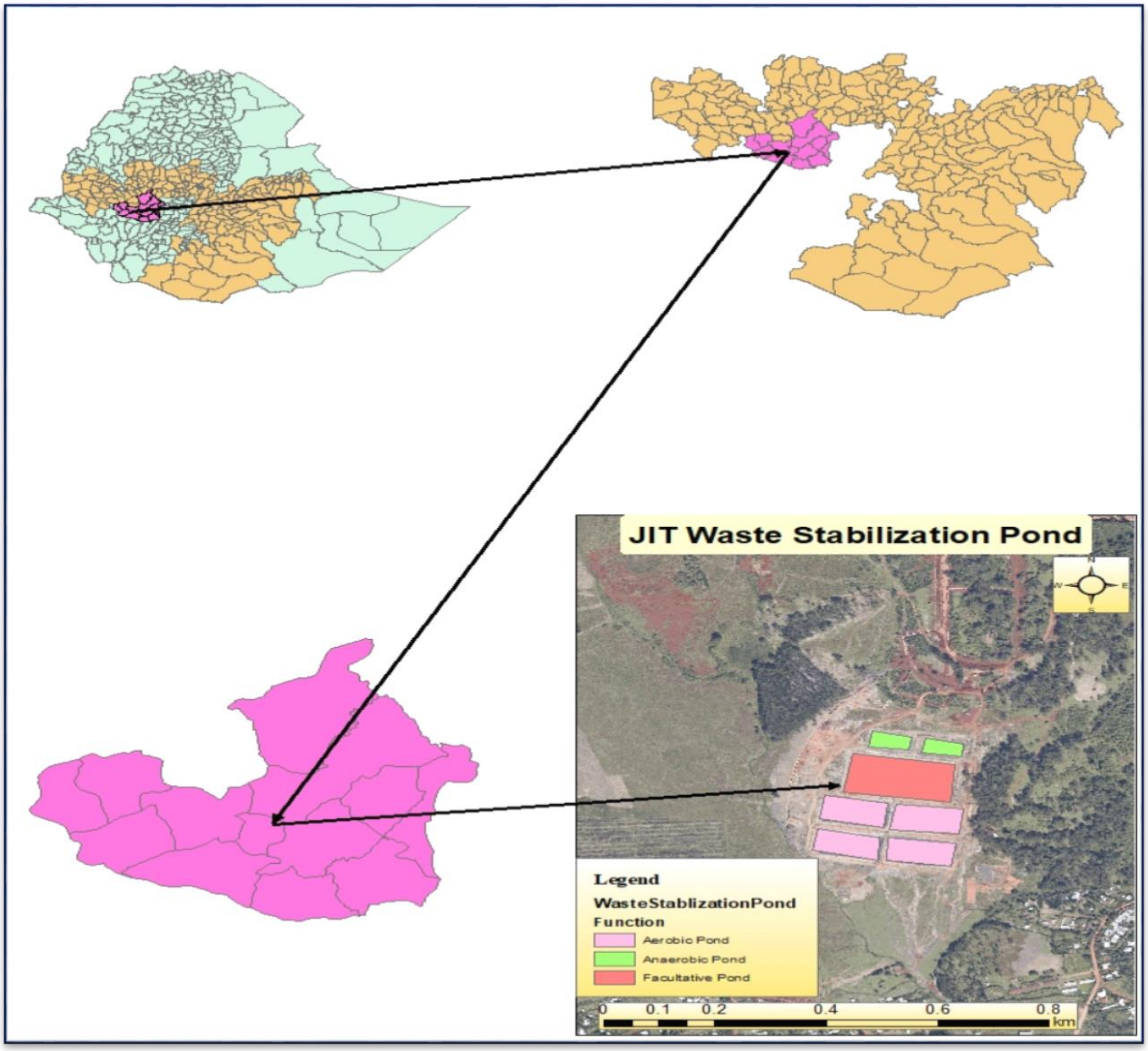

Figure4.1.Location Map of Kito Furdisa Waste Stabilization Pond system in Jimma, Southwest Ethiopia. Source: Google Earth, 2022

Dimension of the Ponds

The waste stabilization pond has three components as indicated in Figure4.2. Each of them has different size and depth, but the facilitative and maturation ponds have the same depth. There are two dimensions of the ponds anaerobic ponds having relatively deeper and smaller in size than the other ponds. The size and the depth are indicated as follows

A= Anaerobic pond (2 in number) = 77.94 (L) x 46.49 (W) x 4.75 (D) meter

F = Facultative pond (1 in number) = 193.83 (L) x101.53 (W) x2.10 (D) meter

M = Maturation pond (4 in number) = 122.83 (L) x65.61 (W) x2.10 (D) meter

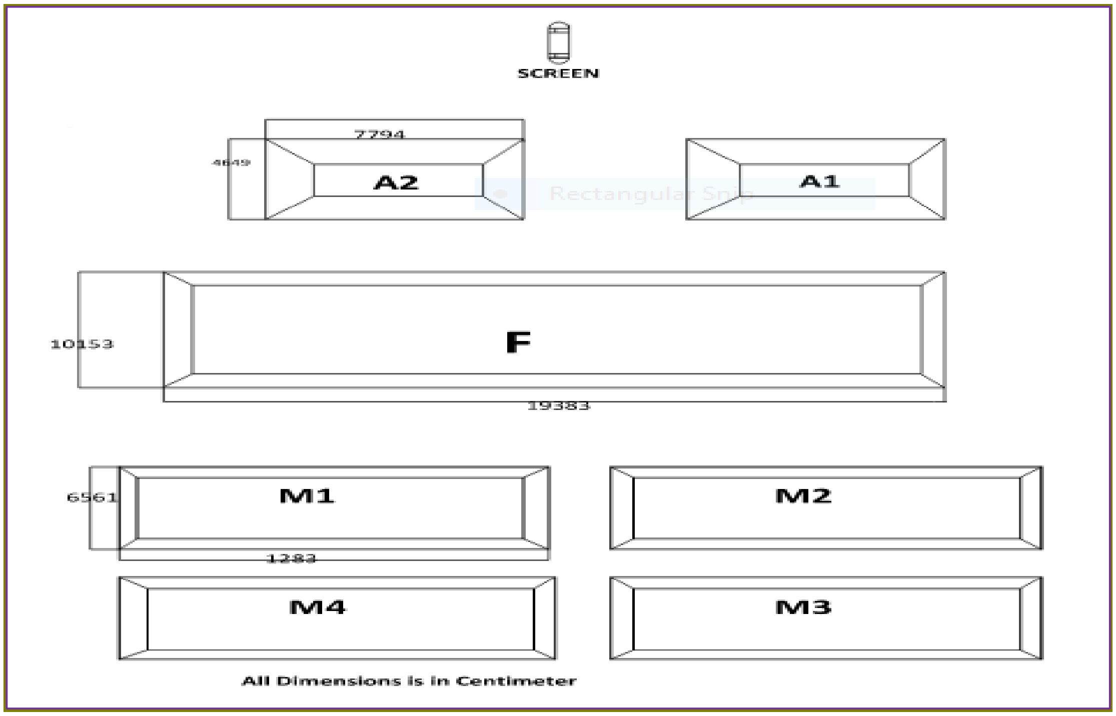

Figure4.2. Dimension of Kito Furdisa waste stabilization Pond system. Source: YOTEK, 2014

4.2. Study Design

Laboratory based cross-sectional study design was employed to assess concentration of heavy metals in wastewater and fish tissues (Muscle, gill and liver) collected from waste stabilization pond. Human health risk assessment models were also used to estimate the human health risk from fish consumption.

4.3. Study Period

The study was conducted from May 05 to 16, 2022.

4.4. Samples Collection

The sampling stations for fish and wastewater samples were purposively selected based on the availability of fish population in WSP. The *Oreochromis Niloticus* fish species has been grown in facultative and maturation ponds of the waste stabilization ponds. The local fisher’s men regularly collect their fish from the facultative and maturation ponds of this wastewater treatment system.

4.4.1. Wastewater Sample Collection

Homogenized wastewater samples were taken from facultative and maturation ponds at surface, middle and bottom of the ponds using Heart Valve water sampler as a sampling procedure followed by Asgedom et al., (2012). Then, the samples were mixed into a single sample to ensure accuracy of representation (Duan et al., 2017). The wastewater samples were collected in triplicates from each point using 500 mL polyethylene bottles (twice rinsed with deionized water and then with samples wastewater). Then, preserved in 2.5 ml concentrated nitric acid to prevent precipitation of metals and growth of algae and stored in an ice box and transported to the laboratory for further analysis.

4.4.2. Fish Sample Collection

A total of 32 *Oreochromis Niloticus* (Tilapia Fish) species were collected from facultative and maturation ponds of waste stabilization pond**.** It was collected from local fishermen by using gill net and wrapped in polyethylene bags, and then ice stored transportation was made to the laboratory for the biometrics, dissection, and collection of fish muscle, gill and liver for Cd, Pb, As and Hg analysis.

4.5. Sample Preparation and Digestion

Wastewater: 100 ml of filtered wastewater samples were digested on a hot plate using 10 ml of Aqua regia (a mixture of HNO_3 and HCl) in the ratio 3:1 inside a fume hood. This was cooled to room temperature, after which another 5 mL concentrated HNO_3 was added. Then 5 ml of H_2O_2 was added. The digested wastewater was cooled down at room temperature and filtered using Whatmann-42 filter paper into a separate 50 ml volumetric flask. The filtrate was made-up to 50 ml with 0.01N nitric acid according to Douglas et al., (2022).

Fish**:** In the laboratory, washing of the samples was performed with tap water for surface cleaning. After cleaning, muscle, gill and liver were isolated and chopped into small pieces using a stainless steel knife. Each tissue was again cleaned with distilled water and dried in an oven at 103°C for 24 hrs. A sample of 2.5 g was placed into Teflon beaker with 5 ml of HNO_3 (70%, Spectrosol), the beaker was then placed on a hot plate and heated at 60°C for 30 min. After allowing the beaker to cool, 10 ml of HNO_3 was added and returned to the hot plate to be heated slowly to 120°C. The temperature was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding 5ml of H_2O_2 (A.C.S.

reagent, Aldrich, UK). The content of the beaker was filtered into a 50 ml volumetric flask. The sample blank was prepared as same as the sample preparation. Next, the digested samples were cooled and filtered through the Whatman No. 42 filter paper. The samples were diluted up to 50 ml with 0.01N nitric acid for analysis.

4.6. Experimental Analysis

4.6.1. Physicochemical Parameters Analysis of Wastewater

Onsite measurement of the pH and temperature, dissolved oxygen (DO), electrical conductivity was performed using a multiparameter probe (HQ40d). Turbidity was measured using a turbidity meter (EUTECH TN-100, Singapore). Chloride, TSS, BOD, nitrate and phosphate were analyzed in Environmental Health Science and Technology Laboratory, Jimma University. The concentrations of nitrate and phosphate were measured by using spectrophotometer (DR 5000) at wave length of 410nm and 690nm respectively. All physicochemical parameters were measured twice a day (Oliveira et al., 2008).

4.6.2. Heavy Metal Analysis

A total of four heavy metals including Pb, Cd, As and Hg were detected in the pre–treated samples of wastewater and fish tissues using Micro Plasma Atomic Emission Spectrometry (Agilent 4210 MP-AES) for Pb and Cd and Hydride Generated Atomic Absorption Spectrometry (HGAAS, novAA 400P, Germany) for As and Hg. The prepared sample was sent to Addis Ababa for laboratory analysis. The absorption wavelength and detection limits of each heavy metal were as follows: 405.781 nm and 0.005 mg/L for Pb, 193.7 nm, 228.80nm and 0.005mg/L for Cd; 253.7nm and 253.65nm and 0.0001 mg/L for Hg; 193.7nm and 0.0001 mg/L for As.

4.7. Bioaccumulation Factors

The bioaccumulation of heavy metals (HM) in the samples was quantified with a bio-accumulation factor (BAF), defined as the ratio of the concentration of a specific heavy metal in the fish tissues to the concentration of the metals in the water (WHO, 1993). The BAF was calculated as mentioned by Kumar et al., (2019).

$$BAF = \frac{\textbf{Concentration of HM in dry tissues of fish } (\mu\text{g /L})}{\textbf{Concentration of HM in pond water } (\mu\text{g/ L})} \qquad 4.\ 1$$

4.8. Human Health Risk Assessment

The potential human health risk due to ingestion of heavy metals through consumption of fish from study area was assessed as non-carcinogenic and carcinogenic health risks. The data and reference values for input parameters and heavy metals contents detected in fish muscle used in human health risk assessment are summarized in Table4.1.

Table4.1. Summary Statistics of Input Parameters in the Human Health Risk Assessment

Parameter	Description(unit)	Children	Adult	Reference
FIR	Fish Ingestion Rate (g/day)	16	30	(Yohannes et al., 2014)
EF	Exposure Frequency (days/year)	365	365	
ED	Exposure Duration (year)	6	65	(WHO, 2015)
BW	Body Weigh (Kg)	15	60	(Walpole et al., 2012), (EPHI, 2016)
AT	Average Time (day/year)	6 × 365	65× 365	(USEPA, 2015)
RfD	Oral Reference Dose (mg/kg/day)	Pb	0.004	(USEPA, 2011)
		Cd	0.001	
		As	0.0003	
		Hg	0.0001	
CSF	Cancer Slope Factor (mg /kg/day)	Pb	0.0085	(OEHHA, 2015)
		Cd	0.38	
		As	1.5	
MC	Heavy Metals contents in fish Muscle (mg/kg)	Pb	0.346 &0.306	Present study
		Cd	0.234 &0.199	
		As	0.018 &0.017	
		Hg	0.033 &0.024	
ARL	Acceptable Risk Level	1×10^{-5}		(Yu et al., 2014)
CF	Conversion Factor	0.208		(Miri et al., 2017)

4.8.1. Estimated Daily Intake Assessment

The Estimated daily intake (EDI) was applied to estimate exposure to potentially toxic trace elements in children and adult via direct ingestion exposure pathway. Estimated daily intake (EDI) of heavy metals from fish consumption was assessed by using metal concentrations in fish samples, daily consumption of fish by the community and body weight by using the formula followed by Yu et al., (2014); Shakeri et al., (2015) and Kumar et al., (2019).

$$EDI = \frac{FIR \times MC \times CF}{WAB} \qquad 4.2$$

Whereas, FIR is the daily fish consumption rate. Although, Ethiopians are traditionally meat eaters, eating practices have lately been shifting in favor of fish in areas and communities where there is regular and sufficient fish supply. In such communities, annual fish consumption can exceed 10kg/person (FAO, 2014). Thus, the daily average fish consumption rate (CR) estimated for adults and children were considered to be 0.03 and 0.016 kg/person/day respectively (Yohannes et al., 2014). This data was obtained from previous study because of absence of national per capita fish consumption data bases in the country.

- MC is the heavy metal concentration in food stuffs (mg/kg dry weight) detected in fish muscle
- BAW – The average body weight which was set by WHO were 60 and 15 kg for Ethiopians adults and children respectively (WHO, 2015).

4.8.2. Daily consumption limit

Based on the carcinogenic effect, maximum allowable daily consumption rate/limit (CR lim) of fish (kg day^{-1}), of the contaminants, was calculated by the following equation (Miri et al., 2017).

$$CRlim = \frac{ARL * BW}{CSF * MC} \qquad 4.3$$

Where ARL and CSF are the maximum acceptable lifetime risk level (in the present study 10^{-5} was used) and cancer slope factor, respectively (Yu et al., 2014).

In case of non-carcinogenic effects of the contaminants, the maximum allowable daily consumption of fish was determined using the following equation (Miri et al., 2017).

$$CRlim = \frac{RfD * BW}{MC} \qquad 4.4$$

Where,

- ✓ CR lim is the maximum allowable daily consumption rate/limit of contaminated fish (kg day^{-1})
- ✓ BW is the mean body weight of consumer population (kg)
- ✓ RfD stands for the oral reference dose (mg/ kg/day) and MC is the metal concentration in the edible part of fish (mg kg^{-1})

4.9. Non carcinogenic risk Assessment

The non-carcinogenic risk was investigated with the target hazard quotient (THQ) which was an estimate of the risk level (non-carcinogenic) due to pollutant exposure. The hazard quotient was determined according to the following equation (Miri et al., 2017).

$$THQ = \frac{EF\times ED\ \times FIR \times CF\times MC}{RfD\times WAB\times ATn} \qquad 4.5$$

The oral reference dose (RfD) for Cd = 0.001, Pb = 0.004, As = 0.0003, Hg = 0.0001 (USEPA, 2011).

Whereas, Oral RfD = Oral Reference dose of chemical (mg/kg/day) based on the upper level of intake for each metal for an adult human with average body weight of 60 kg. HI<1.0 indicates that adverse health effects are not likely to occur. Meanwhile, if the HI is greater than or equal to 1.0, it is probably that adverse health effects will be observed (Abdel-Khalek, 2016).

Exposure to two or more metal pollutants may result in additive and/or collaborating effects. So, the cumulative health risk is evaluated by summing THQ that is known also as the Hazard Index (HI) as follows (Giri & Singh, 2015).

$$HI = (\mathrm{THQ\ (Pb)}\ + \mathrm{THQ\ (Cd)}\ + \mathrm{THQ\ (As)}\ +\ \mathrm{THQ\ (Hg)}) \qquad 4.6$$

The greater value of HI possesses a greater concern. HI above 1 indicates an unfavorable human health effect and suggests the need for possible remedial action.

4.10. Carcinogenic risk Assessment

Target cancer risk (TR) was calculated to indicate carcinogenic risks. The method to estimate TR is also provided in USEPA Region III Risk-Based Concentration Table (USEPA, 2011). The model for estimating TR is shown as follows (Samuel et al., 2020):

$$TR = \frac{MC*FIR*\ CSF*EF*ED}{BW*AT} \qquad 4.7$$

Where, CSF is the cancer slope factor (mg/kg/day) which was 1.5 mg /kg/day for As, 0.0085 mg kg^{-1}/day for Pb, 0.38 for Cd, while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10^{-5} (USEPA, 2010). The TCR was estimated for As, Cd and Pb since these elements may promote both non carcinogenic and carcinogenic effects depending on the exposure dose. As and Cd are known as Group A and Pb is known as Group B carcinogens.

4.11. Variables

4.11.1. Dependent Variables

- ➢ Human Health Risk
- ➢ Concentration of Heavy Metals in Wastewater and Fish tissues

4.11.2. Independent Variables

- ✓ Fish consumption rate
- ✓ Estimated daily intake
- ✓ Socio-demographic characteristics (age, BW)
- ✓ Reference dose
- ✓ ED, EF, R*f*D and CSF
- ✓ Physicochemical characteristics of water (pH, T^o, BOD, DO, EC, SS, turbidity, chloride, phosphate and nitrate)

4.11. Data Management and Analysis

The raw data were coded and entered into a Microsoft Excel spreadsheet. After that, the data were exported to SPSS (version-26) for statistical analysis. Mean values, standard deviations were calculated. Correlation analysis was performed for inter-metallic association and with physicochemical parameter to understand the significance level. Data were analyzed using a paired sample t-test to declare a statistical significant difference between two sampling points. One way ANOVA was used to test whether the concentrations of heavy metals in fish tissues vary depending on the site at $\alpha=0.05$ level of significance. All metal concentrations were reported as micrograms per liter for wastewater and as milligrams per kilogram for fish tissues on dry weight basis.

4.12. Data Quality Assurance

To maintain the quality of the data instruments were calibrated, blank measurements and triplicate analysis were used. Stock standard solutions 1000 mgL^{-1}, containing 2% HNO_3 of the metals Pb, Cd, Hg and As (Buck Scientific Puro-Graphic) were used. The calibration curve was determined using serial dilutions. All reagents used in the experiments were analytically pure. Samples were then digested without delay and analyzed by HGAAS and MP-AES following documented procedures. Each determination was based on the average values of triplicate samples.

4.13. Ethical Consideration

Ethical permission to undertake the study was obtained from research review board (RRB) office, Jimma University. Official letter of cooperation obtained from Environmental health Science and Technology Department was given to Kito Furdisa campus, Jimma University, and EFDA, and ECDSWA, Addis Ababa for laboratory assistance.

4.14. Dissemination Plan

Data were analyzed and based on the results; conclusion and recommendation was made. Finally, written documents was submitted to Jimma University, the Department of Environmental Health Sciences and Technology, and Jimma Zone health office for further action and the benefits of the community to alleviate the identified gaps. It will be deposited at the Jimma University Library to be made available to borrow under rules of library. The manuscript of this research work will be published on reputable journal.

CHAPTER FIVE

5. RESULTS

5.1. Physicochemical Characteristics of the Wastewater

The assessment of various physicochemical parameters, namely pH, temperature, turbidity, BOD, DO, conductivity, TSS, chloride, nitrate, and phosphate were carried out by using standard methods as described in APHA (APHA, 1926). The results of physicochemical parameters of the wastewater samples investigated in the present study are depicted in Table5.1. The average values of pH and DO were increasing from influent to effluent of the pond, where as another parameters showed decreasing order along wastewater treatment process.

Table5.1.The Level of Physicochemical Characteristics in Wastewater samples from Kito Furdisa WSP in Jimma, Southwest Ethiopia, 2022

	Sampling Site					
	Anaerobic Pond		Facultative Pond		Maturation Pond	
Parameters	Average	St. D	Average	St. D	Average	St. D
PH	7.44	0.18	10.51	0.05	8.84	0.07
Temperature (℃)	24.5	0.87	25.06	0.115	24.76	0.057
DO (mg/L)	1.11	0.01	2.65	0.01	5.2	0.01
EC(µS/cm)	1199.67	27.43	421.33	0.577	250.33	0.577
Turbidity (NTU)	238.67	23.03	68.3	0.53	16.85	0.708
BOD (mg/L)	416.93	4.58	223.23	4.13	101.57	3.78
TSS (mg/L)	160	30	40	10	16.67	5.77
Chloride (mg/L)	124	2	54.67	1.16	28	2
Phosphate (mg/L)	122.92	4.8	37.03	0.88	9.15	0.39
Nitrate (mg/L)	0.55	0.001	0.37	0.001	0.195	0.002

*Std. D = Standard Deviation, mg/L = milligram per liter, µS/cm = micro Siemens per centimeter and NTU = Nephelometeric Turbidity Unit, ℃= Degree Celsius

5.2. Linearity Assessment for the Studied Heavy Metals

Calibration curves for Cd, Pb, As and Hg were obtained by using standard solutions prepared from their respective stock solutions. The correlation coefficients of metals were found to be from 0.996 - 0.999, which indicate strong relationship. The correlation coefficients of the elements were determined using prepared standards versus their corresponding absorbance. Finally, a quality results were obtained from samples analysis for each heavy metals using HAAS and MP-AES. The prepared standard concentrations and the corresponding correlation coefficients of the calibration curve for each metal in wastewater and fish tissues are illustrated in the following figures.

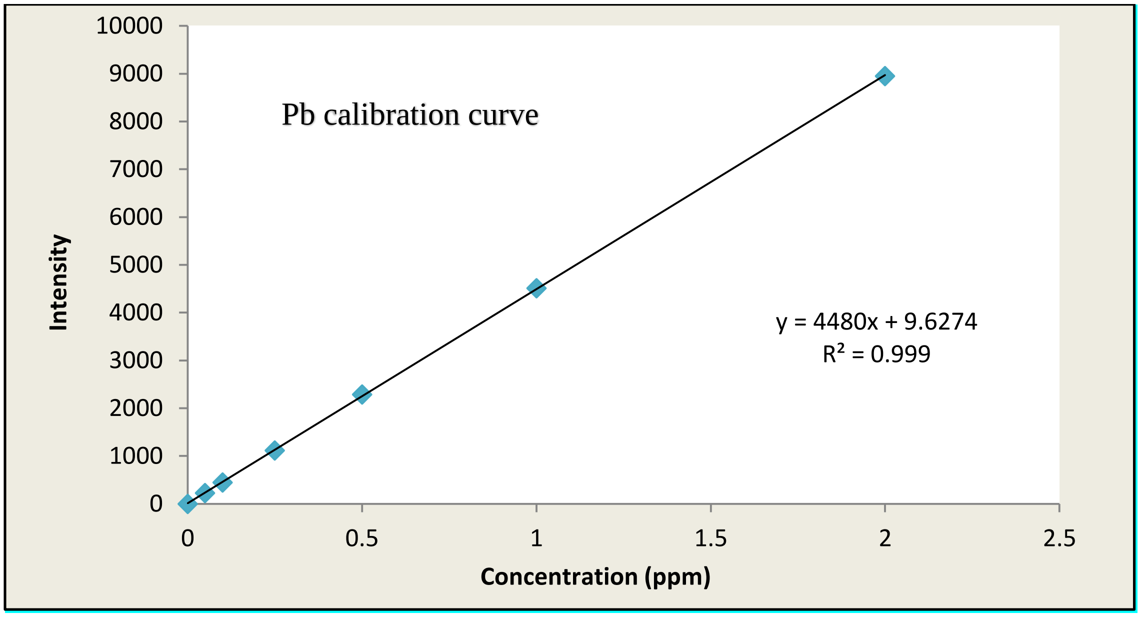

Figure5.1.Lead Standard Concentration versus Intensity at (405.781nm)

The result of the linearity test showed that it was quite linear as it produced a regression equation of $y = 4480x + 9.6274$ with a coefficient correlation (r^2) of 0.999 as depicted in Figure5.1.The test was performed using standard solution contained lead.

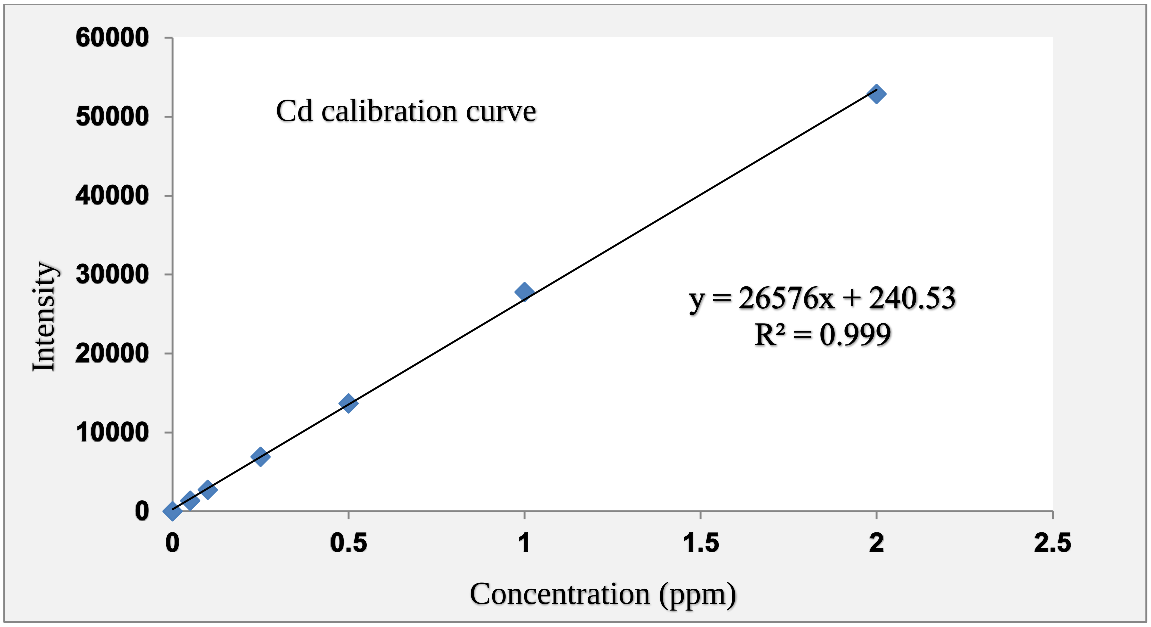

Figure5.2. Cadmium Standard Concentration versus Intensity at (228.802nm)

The result of the linearity test showed that it was quite linear as it produced a regression equation of y = 2657x + 240.53 with a coefficient correlation (r^2) of 0.999, as shown in Figure5.2. The test was performed on a standard solution contained Cadmium.

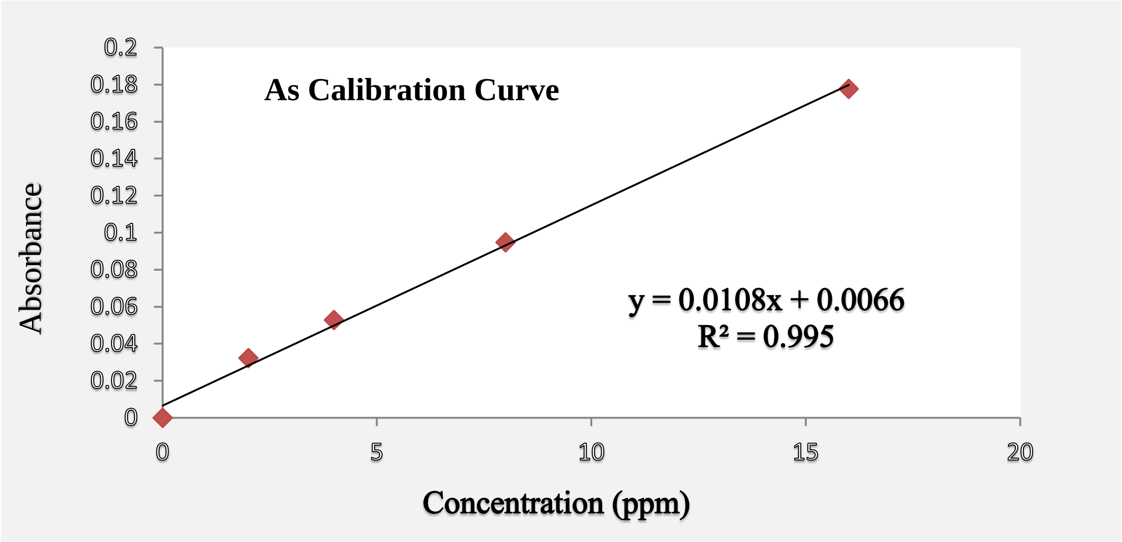

Figure5. 3. Arsenic Standard Concentration versus Absorbance at (193.7nm)

The result of the linearity test showed that it was quite linear as it produced a regression equation of y = 0.0108x + 0.0066 with a coefficient correlation (r^2) of 0.996, as shown in Figure5. 3. The test was performed on a standard solution contained Arsenic.

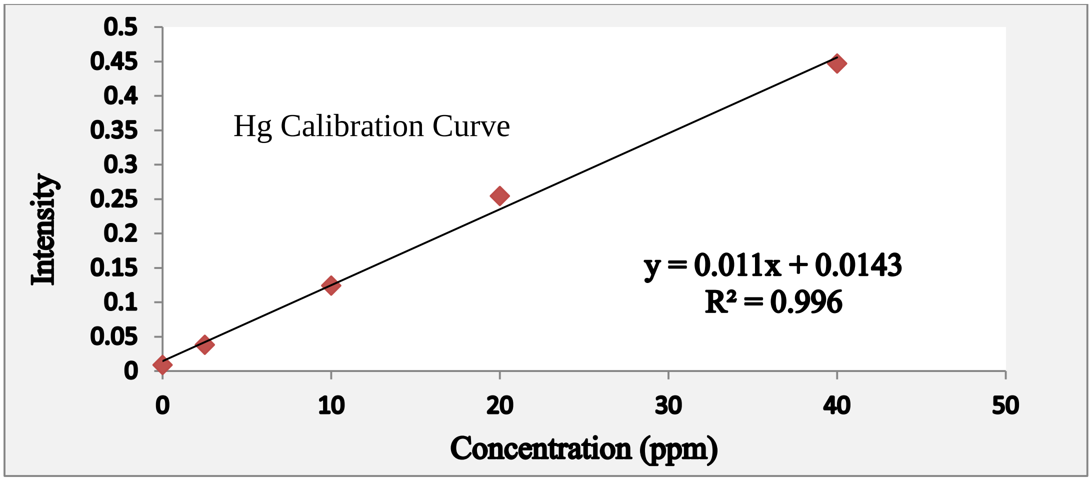

Figure5.4. Mercury Standard Concentration versus Absorbance at (253.652nm)

The result of the linearity test indicated that it was quite linear as it produced a regression equation of y = 0.011x + 0.0143 with a coefficient correlation (r^2) of 0.996, as showed in Figure5.4. The test was done on standard solution contained mercury.

5.3. Heavy Metals Concentration in the Wastewater Samples

The results for heavy metals concentration in wastewater samples from Kito Furdisa Waste stabilization pond are presented in Table5.2. Heavy metals were found to be in the following decreasing order of concentrations (µg/ L): Cd> Pb > As > Hg in both facultative and maturation ponds, showing Cd as the metal with highest concentration throughout and Hg as the least. Maximum level of all heavy metals was obtained from facultative pond. Moreover, the concentrations of Cd and Pb were above the permissible limit set by (WHO, 2004) and (USEPA, 2015), which could pose a huge threat to human health and the natural environment.

Table5.2.Mean and Standard Deviation of Heavy Metals Concentration (µg/L) in Wastewater from Kito Furdisa WSP in Jimma Compared to the International Standard, 2022

Sampling Points	Parameter	Pb	Cd	As	Hg
Facultative Pond	Mean	20.67	27.66	0.387	0.349
	St. Dev.	2.081	1.527	0.124	0.013
Maturation Pond	Mean	16.13	26.53	0.375	0.197
	St. Dev.	0.321	0.568	0.103	0.042
International Standards	(WHO, 2004)	10	3	10	1
	(USEPA, 2015)	15	5	-	-

5.4. Statistical Analysis of Heavy Metals and physicochemical parameters in the Wastewater

In the statistical correlation among the heavy metals in the wastewater between the two ponds, Cd exhibited a negative correlation with As ($r^2 = -0.314$, $p < 0.05$) and a positive correlation with Hg ($r = 0.239$ at $p < 0.05$), as presented in Table5.3. Pb showed a strong positive correlation with Hg ($r^2 = 0.779$) and a moderately positive correlation with As and cadmium ($r^2 = 0.229, 0.415$). The positive correlations obtained between some metallic elements prove that they may have similar accumulation behaviors or originate from the same environmental sources of pollution (Tytła, 2019).

Metals and physicochemical parameters associations showed, Hg was correlated with all physicochemical parameters and association was statistically significant at 2-tailed ($p<0.01$), whereas the rest are not significantly correlated. Pb also showed a strong positive correlation with pH, EC, turbidity, BOD, Cl^-, PO_4^{-3} and NO_3^-, and the association was statistically significant at 2-tailed ($p<0.05$). Moreover, Hg and Pb showed strong negative correlation with DO. The significant positive correlations of Hg and Pb with another physicochemical properties may confirm that considerable share of wastewater properties with adsorption and oxidation of trace metals. The lack of a significant correlation between trace metals and other wastewater properties might be caused by the compositional variety controlling heavy metals. Paired Sample t-test analysis revealed no significant variation showed for the heavy metals being studied except for Hg, which demonstrated a substantial statistical difference between facultative and maturation ponds ($t= 6.450$, $p=0.023$).

Table5.3. Pearson correlation coefficient matrix for heavy metals and physico-chemical characteristics in WSP in Jimma, 2022

	Pb	Cd	As	Hg	pH	T°	DO	EC	Turb	BOD	TSS	Cl^-	$PO4^{-3}$	NO_3^-
Pb	1													
Cd	0.415	1												
As	0.229	-0.314	1											
Hg	0.776	0.328	0.228	1										
pH	.909*	0.528	0.053	.933**	1									
T°	0.743	0.118	0.209	.933**	.850*	1								
DO	-.879*	-0.518	-0.062	-.948**	-.989**	-.895*	1							
EC	.880*	0.517	0.057	.946**	.989**	.895*	-1.000**	1						
Turb	.885*	0.514	0.076	.950**	.991**	.894*	-1.000**	1.000**	1					
BOD	.874*	0.491	0.079	.961**	.990**	.903*	-.999**	.998**	.999**	1				
TSS	0.632	0.203	-0.026	.936**	.859*	.901*	-.869*	.869*	.868*	.888*	1			
Cl^-	.869*	0.469	0.101	.949**	.972**	.926**	-.995**	.995**	.994**	.993**	.864*	1		
PO_4^{-3}	.875*	0.542	0.028	.939**	.990**	.882*	-.999**	.999**	.999**	.997**	.866*	.992**	1	
NO_3^-	.884*	0.515	0.064	.948**	.991**	.893*	-1.000**	1.000**	1.000**	.999**	.870*	.994**	.999**	1

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

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

5.5. Heavy Metals Concentration of in the Fish Tissues

5.5.1. Biometric data of the fish samples

Biometric data, such as length and weight of *Oreochromis Niloticus* fish collected from Kito Furdisa waste stabilization pond were measured. The average length and weight of *O. Niloticus* (n=32) collected from facultative and maturation pond were 24.76 ± 0.64 cm and 108.33 ±7.89 g, 16.54 ± 1.68cm and 82.33 ± 4.67g respectively. It can be used to provide information concerning their biological health and environment.

5.5.2. Heavy Metals Concentration in the Fish tissues

Heavy metals concentration expressed as (mg/Kg dry weight) were detected in the muscle, liver, and gill of *O. Niloticus* collected from waste stabilization ponds, using HAAS and MP-AES. In the liver, the *O. Niloticus* accumulated the highest concentration of Pb, which was ranged from 0.339mg/Kg to 0.366mg/Kg and gill exhibited the highest value of cadmium which was 0.345 mg/Kg to 0.406 mg/Kg form facultative and maturation pond respectively, while the muscle showed the lowest concentration of all heavy metals. It can be noticed that, different organs exhibited different patterns of accumulation as follows: Gill: Cd > Pb> Hg> As, liver: Pb> Cd > Hg> As, muscle: Pb > Cd> Hg> As from both ponds.

The results of analysis of variance showed significant differences in metal concentrations in the different tissues at ($p<0.05$). Of this mercury was highly selective to be accumulated in all types of fish tissues and showed statistically significant difference between all tissues at ($p<0.05$). The concentration of Cd showed statistically significant differences between liver and muscle at ($p<0.05$), whereas, As and Pb are not showed statistically significant difference between fish tissues. It gives an indication about the accumulation efficiency for any particular metal in fish tissues.

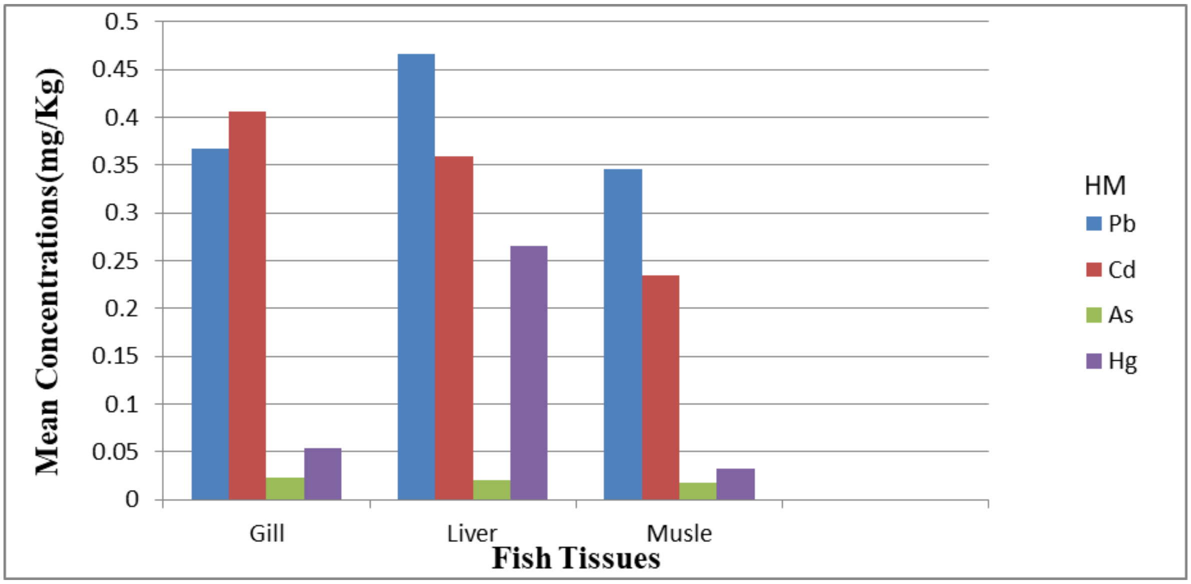

Figure5.5. Mean Concentration of Heavy Metals in the Tissues of *O. Niloticus* collected from Facultative Pond of Kito Furdisa WSP Jimma, Ethiopia, 2022

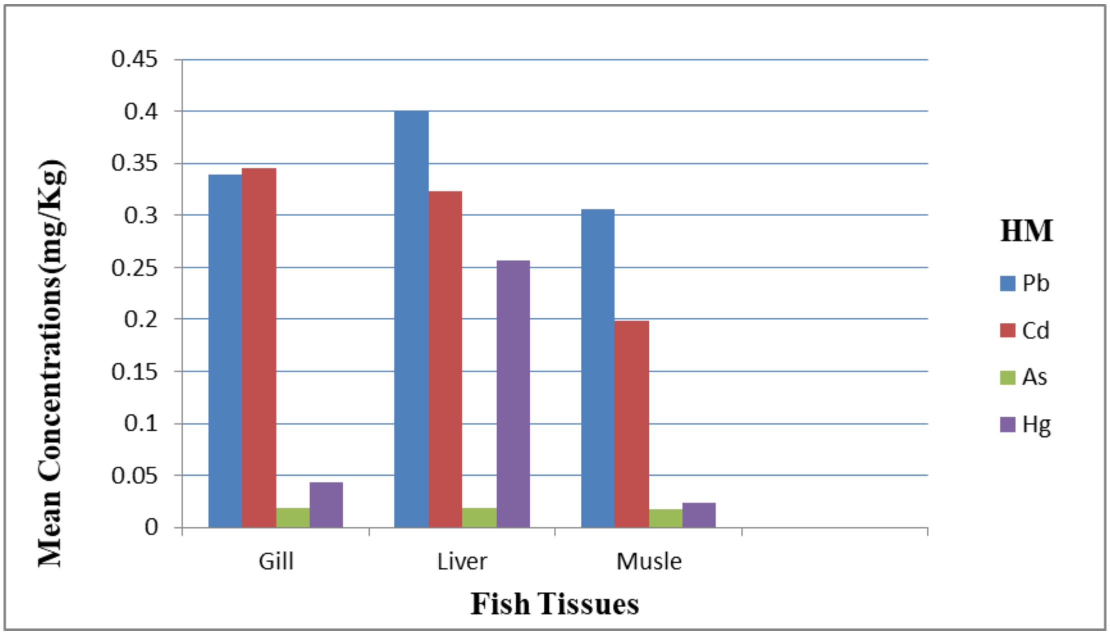

Figure5.6. Mean Concentrations of Metals in Tissues of *O.Niloticus* from Maturation Pond of Kito Furdisa WSP in Jimma, Ethiopia, 2022

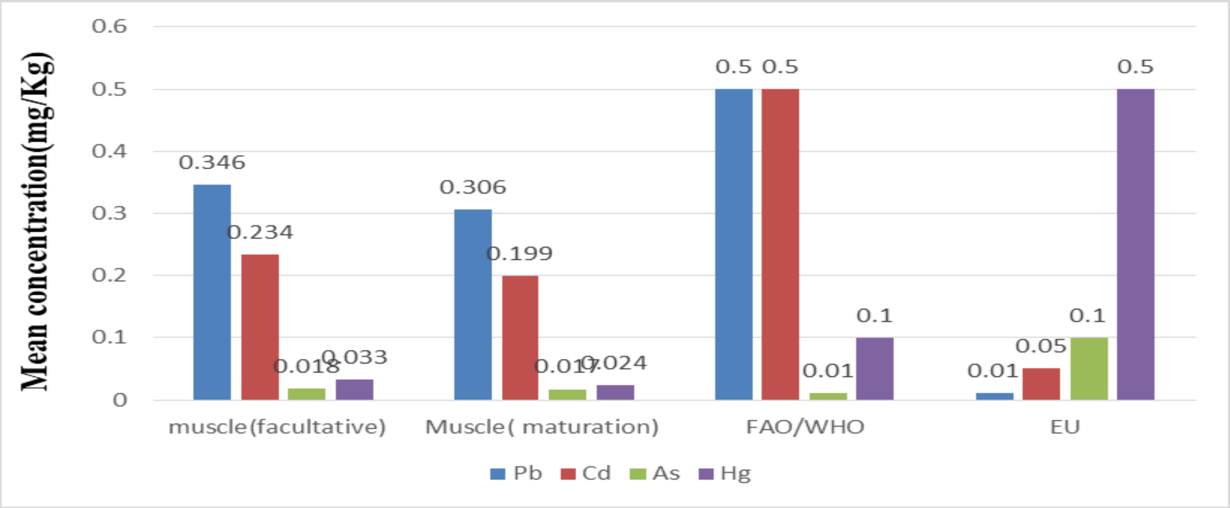

Figure5.7. Comparison of Heavy Metal Levels in Fish Muscle with Standard Guideline Values

In the present study, Pb and Cd were recorded higher concentration in *O. niloticus* muscle. However, it is below FAO/WHO guideline permissible limits. The concentration of As recorded in the muscles of studied fish was above the maximum permissible limits (MPL) recommended for human consumption by FAO/WHO , which is 0.01 mg/kg weight (FAO/WHO, 2013).

5.6. Bioaccumulation Factors of Heavy Metals in Fish Tissues

Bioaccumulation of heavy metals in fish tissues collected from Kito Furdisa Waste stabilization pond were calculated by equation 4.1 and presented in Table5.4. The maximum BAF of Hg in the liver of *O. niloticus* were calculated, which was 1302.5 and 764.73 at maturation and facultative ponds respectively. It can be seen that the bioaccumulation factors were increased in the order of Cd< Pb <As <Hg for all tissues. This is a clear indication that the bioaccumulation factors of Hg in all *O.Niloticus* tissues were higher compared with other metals.

Table5.4.Bioaccumulation Factor (BAF) of Heavy Metals in Tissues of *O. Niloticus* from Kito Furdisa waste stabilization in Jimma, Southwest Ethiopia, 2022

Site	Parameter	Pb	Cd	As	Hg
Facultative Pond	Water/Gill	17.75	14.67	59.17	155.2
	Water/Liver	22.54	12.97	51.94	764.73
	Water/Muscle	16.73	8.46	46.25	94.5
Maturation Pond	Water/Gill	21.02	12.77	46.13	221.83
	Water/Liver	21.08	14.2	49.87	1302.5
	Water/Muscle	18.97	7.51	45.41	120.3

5.7. Human Health Risk Assessment

5.7.1. Estimated Daily Intake

The EDI of Heavy metals was evaluated according to the average concentrations of each metal in fish muscle and the respective daily consumption rate. The daily intake of arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) through consumption of fish muscle from waste stabilization pond by adults and children consumers was estimated by equation 4.2. The estimated daily intake (EDI) and daily consumption limits values are presented in Table5.5.The results are expressed as per unit body weight per day (mg/Kg/day). The highest metal intake through fish consumption corresponded to Pb which was estimated from 3.18×10^{-5} mg/Kg/day to 3.5×10^{-5} mg/Kg/day for adult consumers and 6.78×10^{-5} mg/Kg/day to 7.19×10^{-5} mg/Kg/day for children.

Table5.5.The Estimated Daily Intake (EDI) of Heavy Metals in Muscle of *O. Niloticus* from Kito Furdisa Waste Stabilization Pond in Jimma, Southwest Ethiopia, 2022

Heavy Metals		EDI(mg/Kg/Day)		CRlim NonCarcinogenic		CRlim Carcinogenic	
		Children	Adults	Children	Adults	Children	Adults
Facultative pond	Lead	7.19×10^{-5}	3.59×10^{-5}	0.17	0.69	0.05	0.2
	Cadmium	5.19×10^{-5}	2.4×10^{-5}	0.06	0.26	0.002	0.007
	Arsenic	3.97×10^{-6}	1.86×10^{-6}	0.25	1	0.006	0.024
	Mercury	7.25×10^{-6}	3.4×10^{-6}	0.05	0.18	NA	NA
Maturation pond	Lead	6.78×10^{-5}	3.18×10^{-5}	0.2	0.78	0.06	0.23
	Cadmium	4.42×10^{-5}	2.08×10^{-5}	0.07	0.3	0.002	0.03
	Arsenic	3.7×10^{-6}	1.77×10^{-6}	0.26	1.05	0.05	0.02
	Mercury	5.25×10^{-6}	2.39×10^{-6}	0.06	0.2	NA	NA

5.7.2. Non Carcinogenic Risk Assessment

Non-carcinogenic THQs and hazard index of the 4 trace metals through consumption of the fish muscle from WSP in Jimma, were estimated by equation 4.5. The target hazard quotient (THQ) of the pollutants decreased in the following order: Hg > Cd > Pb > As. Target hazard quotient is a ratio between potential exposure to a given trace metal and its oral reference dose. It is used to assess potential health risk associated with long-term exposure to dietary trace metals. The hazard index (HI) risk values were 0.069 and 0.156 at facultative pond and 0.059 and 0.126 at maturation pond for adults and children respectively during study period as shown in Table5.6. Hazard index is the numerical sum of the computed THQ values. The contribution of individual THQ values to the HI was evaluated and the results showed that Hg contributed more than 49.27% to the combined THQ through this ingestion exposure pathway of edible muscle. Therefore, for the non-carcinogenic risks, more attention should be paid to Hg, pollution in the study area. When analyzed, the risk quotient for each metal in each exposure pathways was less than one, which is an indication that the heavy metals do not impart non carcinogenic effect independently.

Table5.6.Target Hazard Quotient (THQ) and Hazard Index (HI) of Heavy Metals from consumption of *O. niloticus* from Kito Furdisa waste stabilization pond, 2022

Site	Category	THQ (Pb)	THQ (Cd)	THQ (As)	THQ (Hg)	HI
Facultative Pond	Adult	0.009	0.024	0.0062	0.034	0.069
	Child	0.019	0.052	0.0132	0.073	0.156
Maturation Pond	Adult	0.00795	0.0207	0.0059	0.0239	0.059
	Child	0.0169	0.044	0.0123	0.0525	0.126

5.7.3. Carcinogenic Risk Assessment

Risk was estimated by calculating the incremental probability of an individual developing cancer over a life time as a result of exposure to a potential carcinogen metals using 4. 7 and results are shown in Table5.7. According to the values for Pb, As and Cd were less than the safe limit of 1×10^{-4}, revealing that there was no carcinogenic risk when both adults and children were exposed to only Pb, As or Cd. Comparing the risk levels, the value for Cd was larger than for As and Pb, implying that Cd was the main pollutant for carcinogenic risk. Comparing the results of risk for adults and children, children have showed more carcinogenic risk than adults, implying that children were more sensitive and vulnerable to heavy metals in fish muscle.

Table5.7.Target Cancer Risk (TCR) of Heavy Metals Due to Consumption of *O. Niloticus* from Kito Furdisa Waste Stabilization Pond in Jimma, Southwest Ethiopia, 2022

Site	Category	Target cancer risk (TCR)		
		Pb	Cd	As
Facultative Pond	Adult	3×10^{-7}	9.2×10^{-6}	2.79×10^{-6}
	Child	6×10^{-7}	1.97×10^{-5}	5.95×10^{-6}
Maturation Pond	Adult	2.7×10^{-7}	7.9×10^{-6}	2.65×10^{-6}
	Child	5.7×10^{-7}	1.68×10^{-5}	5.5×10^{-6}

CHAPTER SIX

6. DISCUSSION

The pH values in study area ranged from 7.44 ± 0.18 to 10.51± 0.046. This might be due to increased algal activity in facultative and maturation ponds as CO_2 is consumed during photosynthesis by algae. This finding was supported by study of Jingxi et al., (2020), which observed the pH value of the samples ranged from 7.80 to 10.20 in Food Waste Water. Another study also observed that the pH of solution exerts strong influence as regards to the concentration of heavy metals in solutions. Heavy metals are soluble in acidic solutions and can be precipitated in basic media (Pavlović et al., 2007). Among the heavy metals, only Hg and Pb were statistically significantly associated with pH; at 2- tailed ($p < 0.01$) level. The chemistry of water systems, specifically heavy metals, is much affected by pH and vice versa. Solubility of metals depends on pH (Lei et al., 2010). Temperature is basically important for its effect on other properties of wastewater including heavy metals. The average temperature of wastewater under study was ranged from 24.50 ± 0.87 to 25.06 ± 0.115 across wastewater treatment system. The most preferred temperature range for optimal growth of tilapia fish species is 25 to 27 °C, while the ideal pH ranges between 6 and 9 (Dewalle et al., 2017).

The results obtained for dissolved oxygen was increased from 1.11 ± 0.01 to 5.20 ± 0.01 from anaerobic to maturation ponds of the treatment plants. This indicated that the waste stabilization pond system showed removal efficiency on organic matter. This finding is relatively higher than (0.675±0.01mg/L), the study conducted in Hawassa, Ethiopia (Beyene & Redaie, 2011). The possible reason for this discrepancy might be due to the nature of the raw wastewater, the type of oxidation pond, and environmental factors (Butler et al., 2017). The standard for sustaining aquatic life is stipulated at 5mg/l and concentration below this value adversely affects aquatic biological life, while concentration below 2mg/l may lead to death for most fishes (Chapman, 2021). In this regards, the pond under study has created a favorable conditions for some fish species to be grown in waste stabilization ponds.

The Electrical conductivity values of wastewater samples were 1199.67 ± 27.43 μScm^{-3} for Anaerobic,421.33 ± 0.577 μScm^{-3} for facultative pond, 250.33 ± 0.577 μScm^{-3} for maturation pond. The conductivity of water is a useful indicator of its salinity or total salt content and the current study have recorded that it was much higher in the influent wastewater. This result is not surprising, since wastewater from industrial sewage and domestic often contain high level of dissolved salts. The mean Electrical conductivity values for anaerobic ponds of the treatment plant were higher than the WHO guideline values of 1000 µScm^{-3} for the discharge of wastewater through channel into stream (Simpi et al., 2011).

Nitrate levels were recorded as 0.55±0.001mg/L to 0 .195±.002 mg/L in influent and effluent of wastewater treatment system respectively. It is an essential nutrient for many photosynthetic autotrophs and in some instances, functions as a growth-limiting nutrient. It is used by algae and other aquatic plants to form plant protein which, in turn, can be used by animals to form animal protein. Nitrate is a major ingredient of farm fertilizers and is necessary for plant uptake and is essential for plant growth. Nitrates are the indirect source of food for fish, is may increase the fish population. This study observed the nitrate levels in wastewater, which is below water quality guideline established by EFD for the protection of aquatic life is 50 mg/l (EEPA, 2003). However, if algae grow too widely, oxygen levels will be reduced and fish will die. The levels of phosphate in all three ponds of waste stabilization system are higher than the WHO limit of 5 mg/L.

The results of the present study showed that Cd concentrations in wastewater were above the permissible limit, which is 3µg/L set by (WHO, 2004). It could be due to anthropogenic activities such as Welding, metal coating and smelting as well as surface runoff and deposition and leachate from solid waste disposal which are significant sources of cadmium within waste stabilization pond. Other sources of Cd includes old galvanized pipes and new plastic (PVC) pipes, solders and other metal fittings, tobacco products, combustion of coal, incineration of sewage sludge, rechargeable batteries, detergents, body care (Ali et al., 2019 and Bekele, 2021). This finding is lower than the value ranged from 110 ± 0.00µg/L to 120 ± 0.002µg/L studied by Adeniji, (2020) in Wastewater from Selected Municipal Treatment Plants in Eastern Cape Province, South Africa and 80 ±0.00 µg/L to 40 ±0.00 µg/L studied Bekele, (2021), in Wastewater around Eastern Industrial Zone, Central Ethiopia. The discrepancy might be due to the nature of wastewater, treatment technologies, anthropogenic activities and environmental factors. Cadmium is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes learning disabilities and hyperactivity in children (Hunt, 2003).

The Pb mean concentration of the wastewater ranged from 16.13±0.321µg/L to 20.67±2.08µg/L in maturation and facultative pond respectively. This values exceeded acceptable limit, which is

10µg/L set by (WHO, 2004). The high levels of Pb in wastewater can be attributed to greater solubility characteristics and effluents from institution (that's higher learning and research institution), student's clinics, mechanical workshops, and laboratory. Some studies have also shown that lead dominates most of the commonly used metal products, cables and pipelines, paints and pesticides and this can be the reason for the higher levels in the wastewater sample (Tyagi, 2014). The finding of present study, is in line with the study recorded 11 to 32 µg/L for Pb in wastewater from open drainage channels in nairobi, Kenya (Kinuthia et al., 2020). However, it is lower than the value recorded by Douglas et al., (2022), which is 340 ± 0.06 µg/L to 860 ± 0.08 µg/L and 124 µg/L to 147 µg/L were observed by Kinuthia et al., (2020) in textile effluent in Karachi Region of Pakistan. The variation might be due to the nature of wastewater and the treatment technologies used to treat influent wastewater.

In this study, As concentration in the wastewater samples was recorded 0.375± 0.124µg/L and 0.387 ±0.103µg/L from maturation and facultative respectively, which is below acceptable limit of 10µg/L set by (WHO, 2004). Arsenic is recognized as one of the most alarming chemical in the environment, exhibiting heavy toxicity even at very low concentrations (Olmedo et al., 2013). The mean Hg concentration in wastewater sample was 0.349 ± 0.013µg/L and 0.197± 0.042µg/L in facultative and maturation pond respectively. High levels of Hg 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. Major sources of mercury contamination include anthropogenic activities such as agriculture, municipal wastewater discharges, mining, incineration, and discharges of industrial wastewater (Chen et al., 2012).

In present study, the concentration of As recorded in fish muscle was above maximum permissible limits (MPL) recommended for human consumption by FAO/WHO, which is 0.01 mg/kg weight (FAO/WHO, 2013). Comparable result of As has been recorded in fish in coastal waters of Ghana (Gbogbo et al., 2018), which was (0 - 0.04 mg/kg). The finding was found lower than reported 1.52 ± 0.70 mg/kg for As in the muscles of *O. niloticus* from River Tano in Ghana (Nyantakyi et al., 2021). Another study also observed As had the highest concentrations and the most prevalent metal in all evaluated organs of all fish species (Al-Mahaqeri, 2015). This might be due to bioavailability of metal and its specific chemical properties. This Arsenic affects the human body, especially the blood which can disturb the bone marrow and change the composition of blood cells, on the liver causing central necrosis and cirrhosis of the liver. The effect of arsenic on the kidneys is vessel damage, tubules and glomerular kidneys. The effect of arsenic on the cell system can cause destruction to cell mitochondria which causes a decrease in cell energy resulting cell death (Medeiros et al., 2012).

Fish of the present study indicated the lowest concentration of heavy metals in muscle. Pb and Cd were accumulated mainly in the liver and gill. Highest concentration of Hg was observed specifically in liver. The bioaccumulation patterns of a certain pollutant in the aquatic biota depend mainly on the uptake and removal rates of this pollutant. The external tissues of gill showed high accumulation pattern in case of all metals and this may be attributed to the anatomical location of these tissues which allow their direct and continuous contact with the external pollutants. Gills are the main route of metal ion exchange from water (Qadir & Malik, 2011) as they have very large surface areas that facilitate rapid diffusion of toxic metals (Dhaneesh et al., 2012). Therefore, it is suggested that heavy metals accumulated in gill are mainly concentrated from water. This is in agreement with the El-moselhy et al., (2019), the studied fish tend to accumulate Pb, Mn and Cd in gills. Whereas, the excessive metal accumulation in liver tissues are associated with the detoxification, transformation and excretion processes that occur in hepatic tissues (Abdel-Khalek et al., 2016).

The bioaccumulation factor was evaluated in relation to the concentration of the aqueous metal at which the studied fish inhabits. The bioaccumulation factor for different heavy metals from wastewater to the fish tissues was highest in the liver. The relatively higher BAF of metals may be due to their role as an activator of numerous enzymes present in fish. Gills, liver tissues were observed to be active bioaccumulators for all metals, since these tissues have a considerable mass in which the accumulated metals may be detoxified, regulated or excreted. This findings were coincided with that obtained by Jayaprakash et al., (2015), they exhibited that the highest BAF values of Pb were observed in the gills and liver tissues. The magnitude of bioaccumulation of heavy metals for gill, liver and muscle of O. Niloticus, were higher for Mercury and lower for cadmium. It is already known that the bioaccumulation of heavy metals in fish tends to occur when the water is polluted. From present study, it can be clearly seen that great variations among concentrations of heavy metal in all tissues and showed different affinity capabilities for accumulation. Moreover, muscle accumulates the lowest levels of heavy metal which indicated muscles are not an active site for metal biotransformation and accumulation. The accumulation of a specific metal depends to a large degree on the presence of the metal ion in the water column, the physiological role of each element, and the preference of an element to bind to or replace some elements in the tissues (Asgedom et al., 2012).

The human health risk assessment for the metals was done based on assumption that the waste stabilization pond system in the present study is the major source of fish to the communities surrounding the WSP. The highest Pb intake was observed in consumers of *O niloticus* from Kito Furdisa WSP. The PTDI of Pb values according FAO/WHO is 0.004mg/kg/day of body weight. All the EDI values of Pb were below this permissible limit. The EDI values obtained in this study were generally lower compared to the findings (Moslen & Miebaka, 2017) recorded EDI values 0.85×10^{-3} for Pb and 0.063×10^{-3} for Cd in *Sarotherodon melanotheron* for the Health Risk Assessment of Consumption of Fish from an Estuarine Creek in the Niger Delta Nigeria. Abubakar et al., (2015) also observed EDI values of 5.83×10^{-3} for Pb, and 1.162×10^{-3} for Cd from imported frozen *Scomber scombrus* species sold in Nigeria.

In this study, the THQ and HI for all metals were less than 1 indicating that all examined fish muscle is safe for consumption, and possible health risk related with non-carcinogenic effect is relatively low. This indicated that adverse health effects are not likely to occur due to ingestion of 30g and 16g of fish per day for adults and children respectively from study area. The estimated THQ and HI are higher in the children than adults, which might be due to the fact that children have higher intake of chemicals per unit of body weight than adults (WHO, 2015). Particularly the THQ of Hg was significantly higher in both groups of people than other metals. Due to the low RfD value of Hg, this metal showed a higher non-carcinogenic risk than other metals. Another study also reported the highest THQ values for Hg which is 0.287- 2.016 from the consumption of fish muscle from Ethiopian Rift-Valley Lake (Hawassa) and a Neighboring Stream (Boicha) (Samuel et al., 2020).

The highest TCR value was obtained due to exposure of Cd in this study. The carcinogenic risk of Cd was $1.97 \text{x } 10^{-5}$ for children and $9.2 \text{ x } 10^{-6}$ for adult, which are within the acceptable range. CR values lower than 10^{-6} denote the metals' negligible exposure, whereas 10^{-6} to 10^{-4} means the acceptable range, and higher than 10^{-4} indicates the terrible exposure (Baki et al., 2018)**.** The results of the present study revealed that the potential carcinogenic risks are still within the acceptable risk levels, which introduced by (USEPA, 2011). Therefore, it can be inferred that there is no potential carcinogenic health risk from ingestion of Cd, Pb and As through the consumption of fish muscles from waste stabilization pond. Besides, Cd is responsible for endocrine malfunctioning, which can cause the failure of the essential organ such as the kidney and brain. Moreover, long term contamination of Cd may cause the dysfunction of the blood circulatory system, bone softening and prostate. Cadmium has been recognized as being carcinogenic to both humans and animals (Buha et al., 2018). They also reported that long-term Cd exposure could promote breast cancer. Early life low levels of Cd exposure are related to lower child IQ in 5-year-old girls and boys and 10-year-old children with Cd exposure are associated with lower ingeniousness, especially in boys (Kippler et al., 2012).

CHAPTER SEVEN

7. CONCLUSION AND RECOMMENDATION

7.1. Conclusion

Heavy metals such as Cd, Pb As and Hg were detected in wastewater and fish tissues collected from kito Furdisa waste stabilization pond in Jimma, southwest Ethiopia. The concentrations of four heavy metals in the wastewater and fish tissues collected from WSP were recorded as in the decreasing order Cd > Pb > As > Hg in wastewater. From the present study, it can be clearly seen that great variations among concentrations of heavy metal in all fish tissues and this may be due to different affinity of heavy metals for accumulation. Generally, heavy metals showed organ specific accumulation in this study. The concentration of Pb and Cd in the wastewater studied were above the set limits by WHO and USEPA. Due to the presence of high levels of toxic heavy metals, this wastewater is not suitable for fishing purpose in order in order to minimize human exposure and health risks. The estimation of non-carcinogenic risk (THQs and HI) conducted in this study indicated no adverse health effects from the consumption of fish. The target cancer risk estimated indicated that Pb, As and Cd are still within the acceptable risk levels, which introduced by (USEPA, 2011). All of the results in risk assessment implied that in the same exposure environment, children are more sensitive and vulnerable (at risk of developing health problems related to heavy metals) than adults; thus, more attention should be given to children to avoid the harmful effects of pollutants. This indication call for great concerns and highlight the requirement for constant monitoring of the waste stabilization pond in order to safeguard the health and lives of people associated with it.

7.2. Recommendation

- It is highly recommended that regular biomonitoring of heavy metal contaminants in fish is essential in order to prevent excessive buildup of toxic heavy metals in the food chain and human body.
- Top management authorities and policymakers should take in consideration the current metal and metalloids status in the artificial aquatic ecosystem to provide a healthful environment.
- Health institutions, public and private organizations must have a continuous communication about risk and benefit of fish consumption in order to control the quality and improve the balance between risk and benefit of the fish consumption towards human health.
- National based policy and regulations should be developed regarding the wastewater reuse for fishing purpose
- Institutions should plan and design integrated wastewater treatment system. A rational design approach views wastewater treatment and aquaculture as a single system to be optimized for maximum fish production and wastewater treatment.
- Further studies are recommended in the study area on the fish tissues, sludge, and wastewater samples for other persistence toxic elements such as Chromium, nickel, Tallium and other environmental contaminants including pharmaceuticals and pathogenic microorganisms that were not addressed due to financial and time constraints.
- Regulatory bodies must carry out routine surveillance of foodstuff, involving taking of samples of potentially contaminated product, followed by laboratory analysis to determine the levels of the metal in question.
- Waste stabilization pond is not efficient to treat selected chemical substances. To adequately treat wastewater and make it suitable for reuse in aquaculture, it requires adequate preliminary treatment like septic tank to reduce the incoming pollutant loading, desludging of the pond, additional treatment, and frequent monitoring and maintenance of the pond.
- Awareness creation should be given to consumers on safe quantities of fish from contaminated area to minimize the public health risk.

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Annex I: Laboratory Analytical Procedure

Procedure for wastewater analysis

1. In the laboratory, 100 ml of each wastewater sample was measured using a measuring cylinder and then transferred into 250 ml separate beakers.
2. A wastewater sample was digested by adding 10 ml of aqua regia to 100 ml of mixed sample in a beaker inside a fume hood.
3. The mixture was slowly boiled on a hotplate until the volume reduced to about 20 mL.
4. This was cooled to room temperature and another 5 mL concentrated HNO_3 was added.
5. Then 5 ml of H_2O_2 was added and beaker walls was rinsed with distilled water.
6. The digested solution of each sample was then filtered using Whatman No. 42 filter paper and transferred into a separate 100 ml volumetric flask.

2. Analytical Procedure for Analysis of fish sample

1. 2.5 g of sample (dry weight) placed into Teflon beaker with 5ml of HNO_3 (70%, Spectrosol)
2. The beaker was then placed on a hot plate and heated at 60°C for 30 min.
3. After allowing the beaker to cool, 10 ml of HNO_3 was added and returned to the hot plate to be heated slowly to 120°C.
4. Increased the temperature to 150°C, and the beaker was removed from the hot plate when the samples turned black.
5. The beaker was removed and allowed to cool, and then added 5 mL of H_2O_2
6. Repeat the H_2O_2 additions until the samples are clear
7. The content of the beaker was filtered into a 50 ml volumetric flask. The sample blank was prepared as same as the sample preparation. Next, the digested samples was cooled and filtered through the Whatman No. 42 filter paper.
8. The samples were diluted up to 50 ml with 0.01N nitric acid for analysis.

Laboratory procedure for physicochemical parameters

❖ Total Suspended Solids: Gravimetric Method

1. 100ml of wastewater was measured
2. Clean filter paper was dried at 103℃ in an oven.
3. The dried filter paper was cooled in desiccator and weighed
4. Sample was filtered using suction filter funnel

5. After filtration the filter paper contained TSS was dried at103℃ for 1hr.
6. Then cooled in desiccator
7. The final weight(filter paper with TSS) and was recorded
8. Calculation

$$\mathrm{mg\ suspended\ solids/L} = \frac{(\mathrm{A}-\mathrm{B})\mathrm{x}1000}{\mathrm{ml\ sample}}$$

Where: A= Weight of filter + dried residue, mg

B= Weight of filter, mg

❖ Chloride: Argentometric Method

1. 25ml of wastewater was measured
2. A color comparison blank was prepared by placing distilled water in a similar flask and the Volume must be equal to that of the sample
3. 1 mL of potassium dichromate indicator solution was added to the blank and the sample; and Mix
4. To the color comparison blank carefully added from a burette drop by drop silver nitrate titrant until the yellow color changes to a brownish tinge.
5. mL silver nitrate titrant consumed were recorded.
6. If the sample turns yellow, gradually add silver nitrate titrate from a burette. Shake the Flask continuously and continue adding the titrant until the sample turns the same
7. mL silver nitrate titrant consumed were recorded
8. Calculation:

$$\mathrm{mg}\frac{\mathrm{Cl}}{\mathrm{L}} = \frac{(\mathrm{A}-\mathrm{B})\ \mathrm{X}\ \mathrm{N}\ \mathrm{X}\ 35{,}450}{\mathrm{mL\ of\ sample}},$$

Where A= mL titration for sample

B= mL titration for blank, and

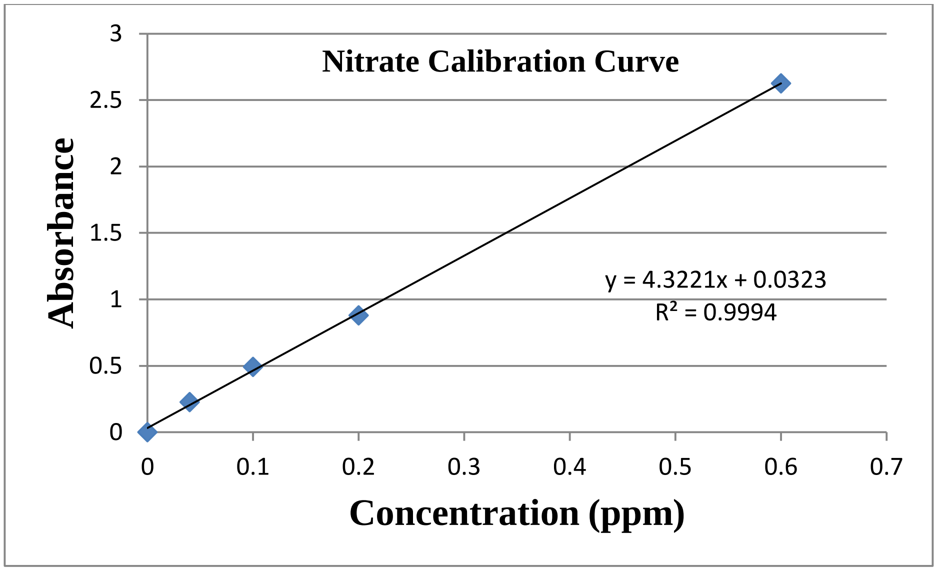
N= normality of silver nitrate

mg NaCl/L = (mg Cl/L) x 1.65

❖ Nitrate: Phenoldisulfonic Acid Method

1. The chloride content of the water sample was determined and Treated with an equivalent amount of silver sulfate solution (1mL for l mg C1) to precipitate the chlorides.
2. The precipitated chloride was removed by filtration
3. 20ml of wastewater sample was taken and evaporated using hot plate Until dryness
4. After cooling 2ml of phenoldisulfonic acid and rub the content using glass rod.
5. After rubbing add 20ml of distilled water for dilution purpose
6. 7ml of NH_4OH was added to the sample in anuntil maximum yellow color is developed.
7. any resulting flocculent hydroxides was removed by filtration
8. The filtrate of clear solution was transferred to a 50-mL volumetric flask. Rinse the dish, glass rod and filter paper with distilled water, adding the rinsing to the flask or cylinder until all the colored solution has been transferred.
9. Dilute to the 50- mL mark with distilled water, and mix thoroughly
10. Measure the absorbance at a wave length of 410 nm against a blank prepared from the same volumes of reagents as used for the samples.
11. Calibration curve was constructed in the range 0-2 mg/L NO_3 – N by adding 0, 0.04, 0.1, 0.2 and 0.6 of standard nitrate solution to separate evaporating dishes and treating them in the same way as the sample.

$$\text{mg/L } NO_3\text{-N} = \frac{\mu g\ NO_3\text{-N}}{\text{mL sample}}$$

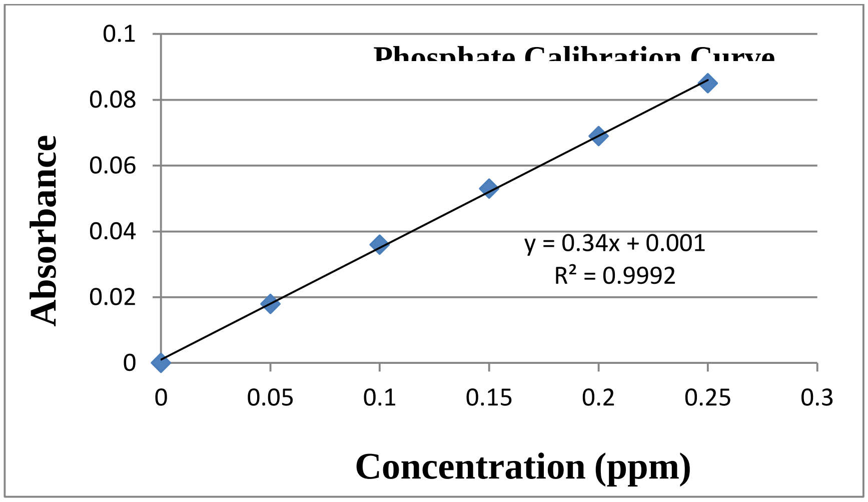


❖ Orthophosphate:

Stannous Chloride Method

1. 10ml of wastewater sample was measured by measuring cylinder
2. Filled measuring cylinder with distilled water to 50ml mark
3. 0.05 ml of phenolphthalein indicator solution was added, then the sample turns pink, added strong acid solution drop wise until the color is discharged
4. 2 mL acid- molybdate solution was added to each of the standards and sample
5. 0.25 mL of stannous chloride solution to each of the standards and sample
6. Blue color observed; after 10 minute the concentration of orthophosphate in mg/L Was measured by using R500 spectrophotometer at wavelength of 6900nm.
7. Calculation: L PO_4^3 = µg phosphate

   mLof sample



Annex II: List of Materials Used During Study

- Distilled water
- Detergent
- Tap water
- Ice-cooled box
- Stainless steel knife
- Polyethylene bag
- Polyethylene bottles
- Pipette
- Spatula
- Mortar and Pestle
- HNO_3 (70% Spectrosol, BDH, England)
- H_2O_2 (30% Riedel- de Haen)
- 70% alcohol
- Different sizes of volumetric flasks
- Beakers, pipette
- Filter funnels
- Graduated cylinder
- Portable multiparameter probe

Annex III: statistical analysis output (SPSS output)

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Conc.Pb.F	20.67	3	2.082	1.202
	Conc.Pb .M	16.133	3	.3215	.1856
Pair 2	Conc.Cd .F	27.67	3	1.528	.882
	Conc.Cd .M	26.533	3	.5686	.3283
Pair 3	Conc.As .F	.38733	3	.124001	.071592
	Conc.As .M	.37567	3	.103055	.059499
Pair 4	Conc.Hg .F	.39967	3	.077887	.044968
	Conc.Hg. M	.13100	3	.032187	.018583
Pair 5	PH.F	10.5167	3	.04619	.02667
	pH.M	8.8433	3	.07024	.04055
Pair 6	Temp.F	25.067	3	.1155	.0667
	Temp.M	24.767	3	.0577	.0333
Pair 7	Do.F	2.6500	3	.01000	.00577
	Do.M	5.2000	3	.01000	.00577
Pair 8	Ec.F	421.33[a]	3	.577	.333
	Ec.M	250.33[a]	3	.577	.333
Pair 9	Turbidity.F	68.300	3	.5292	.3055
	Turbidity.M	16.8500	3	.70873	.40919
Pair 10	BOD.F	223.233	3	4.1308	2.3849
	BOD.M	101.567	3	3.7873	2.1866
Pair 11	TSS.F	40.00	3	10.000	5.774
	TSS.M	16.67	3	5.774	3.333
Pair 12	Chloride.F	54.67	3	1.155	.667
	Chloride.M	28.00	3	2.000	1.155
Pair 13	Phophate.F	37.0333	3	.88008	.50811
	Phophate.M	9.1500	3	.39000	.22517
Pair 14	Nitrate.F	.37500	3	.001000	.000577
	Nitrate. M	.19500	3	.002000	.001155

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Conc.Pb	Between Groups	.006	2	.003	1.003	.464
	Within Groups	.009	3	.003		
	Total	.015	5			
Conc.Cd	Between Groups	.034	2	.017	16.689	.024
	Within Groups	.003	3	.001		
	Total	.037	5			
Conc.As	Between Groups	.000	2	.000	2.532	.227
	Within Groups	.000	3	.000		
	Total	.000	5			
Conc.Hg	Between Groups	.066	2	.033	810.870	.000
	Within Groups	.000	3	.000		
	Total	.066	5			

Paired Samples Test

		Paired Differences					T	Df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	Conc.Pb.F - Conc.Pb .M	4.5333	2.3459	1.3544	-1.2943	10.3609	3.347	2	.079
Pair 2	Conc.Cd .F - Conc.Cd .M	1.1333	2.0551	1.1865	-3.9718	6.2384	.955	2	.440
Pair 3	Conc.As .F - Conc.As .M	.011667	.050521	.029168	-.113834	.137167	.400	2	.728
Pair 4	Conc.Hg .F - Conc.Hg. M	.268667	.072141	.041651	.089458	.447875	6.450	2	.023
Pair 5	PH.F - pH.M	1.68333	.11372	.06566	1.40083	1.96584	25.637	2	.002
Pair 6	Temp.F - Temp.M	.3000	.1732	.1000	-.1303	.7303	3.000	2	.095
Pair 7	Do.F - Do.M	-2.55000	.01732	.01000	-2.59303	-2.50697	-255.000	2	.000
Pair 9	Turbidity.F - Turbidity.M	51.45000	.60025	.34655	49.95890	52.94110	148.462	2	.000
Pair 10	BOD.F - BOD.M	121.6667	.9238	.5333	119.3719	123.9614	228.125	2	.000
Pair 11	TSS.F - TSS.M	23.333	5.774	3.333	8.991	37.676	7.000	2	.020

Pair 12	Chloride.F - Chloride.M	26.667	3.055	1.764	19.078	34.256	15.119	2	.004
Pair 13	Phophate.F - Phophate.M	27.88333	.92154	.53205	25.59410	30.17256	52.407	2	.000
Pair 14	Nitrate.F - Nitrate. M	.180000	.002646	.001528	.173428	.186572	117.838	2	.000

Annex IV: Photo taken during study

Figure1: photo shows Fish samples collected from Kito Furdisa waste stabilization pond, Jimma, 2022

Figure 2. photo during fish samples prepareation in laboratory, 2022

Figure 3. photo during wastewater sample collection and measurement onsite and in laboratory

Figure3. photo taken during sample digestion and analysis, 2022