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COMPARATIVE EVALUATION OF PATHOLOGICAL LESIONS IN FISH COLLECTED FROM DIFFERENT EFFLUENT SITES IN LAKE BATU AND LAKE HAWASSA, ETHIOPIA

MVSc THESIS

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MVSc THESIS

Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Veterinary Science in Veterinary Pathology

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Entitled**:**

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And recommend that it be accepted as fulfilling the thesis requirement for the degree of:

Masters of Veterinary Science in Veterinary Pathology

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Final approval and acceptance of the thesis dissertation is contingent upon the submission of its final corrected copy to the candidate‟s major department.

STATEMENT OF THE AUTHOR

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LIST OF ABBREVIATIONS

APHA	American Public Health Association
H&E	Hematoxylin and Eosin
Mg/L	Milligram/Liter
MMC	Melanomacrophages Center
O. niloticus	*Oreochromis niloticus*
OBBN	Ontario Benthos Biomontoring Network
OR	Odds Ratio
PM	Post Mortem
SM	Smooth Muscle
TDS	Total Dissolve Solid
UG/L	Microgram/Liter),

ABSTRACT

A cross sectional study was conducted from November 2022 to May 2023 with the objective of comparatively evaluating gross and microscopicl lesions in fish collected from different effluent sites that were identified based on water chemical analysis in lake batu and lake hawassa. Water and fish samples were collected from effluent and reference sites. Mean (±SD) of water quality parameters were statistical different for a mmonia, nitrate, nitrite, silica, total dissolved solute (TDS) and alkalinity by sampling sites ($P < 0.05$). Mean for ammonia, nitrite, silica and alkalinity were found significantly higher ($P<0.05$) in Lake Batu water samples compared to Lake Hawassa. Means of all analysed water quality parameters were found significantly higher ($P < 0.05$) in effluent site of Lake Batu compared to reference sites. Similarly, except nitrite the mean water quality parameters were found significantly higher ($P < 0.05$) in effulent site compared to reference site for Lake Hawassa. Based on logistic regression analysis (OR) fish collected from lake Batu were 2.46 times more likely to develop gross gill lesions ($P<0.05$) ; and 1.8 times likely to develop gross skin lesions ($P<0.05$) than fish collected from lake Hawassa. Regarding sites of fish collection, fishes collected from effulent sites were 2.95 times mor likely to develop gross gill lesions ($P<0.05$); 4.67 times more likely to develop gross liver lesions ($P<0.05$) and 2.98 times more likely to develop gross skin lesions ($P<0.05$) compared to reference site in lakee Batu. For Lake Hawassa; fish collected from effulent site were 2.9 times more likely to develop gross gill lesions and 2.98 times more likely to develop gross skin lesions ($P<0.05$) in effulent sites compared to reference site. Based on microscopic lesions grading; la mellar necrosis for gill, multifocal hepatocelular necrosis and biliary epithelium necro sis for liver, and epidermal cell necrosis for skin were stage III lesions. Stage III lesions were identified more frequent for lake batu effulent site than lake hawassa effulent site and stage III lesions were nor identified for reference sites of both lakes. Based on microscopic lesions, haemorrhage, aneurysm and lamellar fusion in gill; aneurysm and hydropic degeneration in liver; haemorrhage, aneurism and separation of layers in skin, were stage II lesions. Stage II lesions were frequently identified in fish collected from effulent sites of both lakes and less frequent in reference sites of both lakes. Stage I lesions were lymphocytic infiltration, hyperplastic primary and secondary lamellae and goblet cell hyperplasia in gill; lymphocytic infiltration in liver; lymphocytic infiltration in skin. Stage I lesions were found in both reference and effluent sites of both lakes. It could be summarized that severe necrotic lesions (stage III) and degenerative and hemorrhagic lesion (stage II) swere more ferquent in fish collected from effulenet site than refrence site and this was also in parelell to chemical parameters of water quality. It could be concluded that the majority of stage III & stage II lesions were probably induced by contamination of lake waters by town sewerage, agricultural and industrial wastes. It also looks that lake Batu is more contaminated than lake Hawassa (based on lesion stages & water quality parameters), however, this need further detailed study. We think the result can be basics for further study and we recommend more detailed study.

Keywords: *Fish, Grospathology, Histopathology, Lake, Pollution, Water Quality*

1. INTRODUCTION

Now a days aquatic environments are at high risk of pollution from agriculture, industries, and urban activities. The discharge of waste from agriculture, industries, and urban activities into aquatic ecosystems results in the production of a wide variety of organic and inorganic pollutants deteriorating water quality of the lake and impose high risk to fish health (Rajamanickam and Narayanan, 2009). Industry is one of significant contributors of harmful pollutants and various toxic chemicals, organic and inorganic substances and these wastes released into aquatic ecosystems without adequate treatment will degrade aquatic environment (Chowdhary *et al.,* 2020). Agricultural activities will deteriorate quality of the water with nitrates, nitrogen fertilizers, phosphorus, pesticides, organic farm wastes, nutrient salts and pathogens (Parris, 2011). Exposure of fish to single chemical is rare case; instead are exposed to complex, fluctuating mixtures of contaminants that may act in various ways which could induce combination effects on physiological and pathological changes of fish (Thorpe *et al*., 2003, Zhou *et al*., 2008).

Fish production has been practiced in different parts of the world including Ethiopia. From the overall fish produced in Ethiopia, around 40 % is produced in the rift valley lakes in which Lake Ziway produces the highest portion (Abera *et al.*, 2018). The benefits gained from the development of fisheries are significant. Fish is a source of protein food supply for a rapidly increasing human population. It is also source of income generation and employment creation. The aquatic ecosystem should be maintained safe to benefit from fish (Mengesha and Belachew, 2017).

Morphological and histopathological lesions in fish tissue can be used as a tool to detect effects of water pollution and changes in water quality on target organs of fish (Van der Oost *et al.,* 2003; Lins *et al.,* 2010; Santos *et al.,* 2012). Pollutants from any sources can develop various pathologic lesions in different organs of fish impacting on the internal structure and functions, affecting the performance of vital organs (Naeemi *et al.,* 2013).

Fish exposed to environmental water pollutants can develop a variety of lesions and injuries to various organs, but the gills, liver and skin are three crucial target organs that can be examined histopathologically to check for tissue and cell damage (Rabitto *et al.,* 2005; Yancheva *et al.,* 2016, Bengu *et al.,* 2017; Mabika *et al.,* 2017). Knowledge on histopathological reponses of fish organs to varous pollutants can be used to assess the current health status of fish and water quality in an aquatic environment (Marchand *et al*., 2009, van Dyk *et al*., 2012).

Ethiopia is one of the tropical countries, which is gifted with a variety of aquatic ecosystems, especially a number of lakes that are of great scientific interest and economic importance. Lake Hawassa and Lake Batu are prominent rift valley Lakes in Ethiopia and they were affected by pollutants from point and non point sources released from industries, urban wastes, flouriculture and agricultural activities (Zinabu, 2002; Abayneh *et al.,* 2003). In addition investors prefer the rift valley for floriculture activities due to its water availability and closer location to the capital city of the country. The area around Lake Batu in Ethiopia is going through a major agricultural t ransformation with both small scale farmers and large horticultural companies using a gricultural chemicals such as pesticides at an increased rate (Teklu *et al*., 2018). Unre gulated release of pesticides and fertilizers and increased use of agricultural fertilizers used for growth and production of flowers into water bodies of rift valley areas have impacted in deterioration of lake water quality for fish health and biodiversity (Merga *et al.,* 2020; Mitike, 2015).

In Ethiopia, there are few information's on aquatic pollution and its impact on water quality (Yirgu, 2011, Gebremedhin and Berhanu, 2018; Mengesha and belachew, 2017; Gebresllassie et al., 2014; Merga *et al*., 2020; Yohannes *et al*., 2013; Worako, 2015; Birhanu *et al*., 2015), however, these studies did not cover pathologic lesions on fish organs that may be impacted by deteriorated water quality of Lake. We hypothesised that there is difference in occurrence and seveirty of pathologic lesions in fish inhabiting Lake Batu and Lake Hawassa as the two Lake were subjected to different sources of pollution.

1.1. Objectives of the Study

1.1.1. General objective

- The general purpose of this study was comparative evaluation of gross and histopathological lesions in fish collected from differnt effluent discharge sites in Lake Batu and Lake Hawassa.

1.1.2. Specific objectives

- To determine water quality of Lake Hawassa and Lake Batu for selected sampling site
- To comparatively characterize gross and histopathological lesions by effulent disch - rarge points and reference sites of study Lakes.

2. LITREATURE REVIEW

2.1. Distributions of fish in study area

Lake Batu is relatively one of the most studied lakes in Ethiopia endowed with different kinds of indigenous and exotic fish species. There are six indigenous fish species in the Lake Batu; comprising *Labeobarbus ethiopicus, Interomius paludinosus, Labeobarbus intermedius, Garra makiensis, Garra dembecha* and *Oreochromis niloticus* (Dejen *et al., 2010*; Mathewos, 2011). Common commercially important fish species in Lake Hawassa are Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*), Barbus species, Nile perch, *Lates niloticus*, *Cyprinus caprio* (Tilahun *et al.,* 2016). Among these species *nile tilapia* (locally named as "koroso" is commerceally important and dominantly harvested fish in Lake Batu (Lemma and Desta, 2016) and in Lake Hawassa (Tilahun *et al*., 2016*).*

2.2. Sources of water pollution in Lake Batu and Lake Hawassa

Water pollution is degradation of the quality of water which renders water unsuitable for its intended purpose. Anything which degrades the quality of water is termed as pollutant. Possible causes for deterioration of water quality can be generalized as anth ropogenic and natural causes (Chaterjee and Raziuddin, 2007). Agricultural practices, industrial wastes and urban runnoff are majior anthropogenic factors with significant impact for deterioration of water quality (Zinabu, 2002; Tamiru, 2006; Mengesha and Belachew, 2017). Numerous toxic chemicals, organic materials and inorganic substances may be released from industrial wastewater duscharges. These wastes will cause damage to aquatic environments if they are dumped into them without being properly treated (Zinabu, 2002).

Large scale flower industry in Batu together with small-scale farming in Meki and other nearby towns and villages, are believed to have the strongest effects on the Lake's water quality (Gudeta, 2012). Wastes from floriculture operations may contain high concentrations of nutrients, pesticides and other chemicals, such as disinfectants, glycerin solution or a silica gel (used as drying agents for preserved flowers), dyes (to colour dried flowers and floral preservatives), and a wetting agent (to prolong the vase life of the flower while in storage) (Kassa, 2017).

Lake Hawassa is well-known and among the most threatened rift valley Lakes due to rapid industrial growth and urbanization of Hawassa city (Zinabu, 2002). A study on Lake Hawassa tributary, the Tikur wuha river, revealed that a significant amount of effluents were discharged into the river and enter the lake without an effective controlling system (Desta *et al.,* 2013).

2.3. Effects of pollution on water quality

To understand the well being of aquatic life and fish health; it is crucial to evaluate the quality of water bodies (Algahwari, 2007). Any water body's quality can be assessed using total dissolved solids, nitrite, ammonia, alkalinity, silica, total phosphate and; total dissolved solid changes in the given water was indicator that effluents from factories contain significant amounts of organic and inorganic chemicals (Tesfahun, 2019).

2.3.1. Ammonia, Nitrate and Nitrite

Nutrients salts necessary for aquatic species' growth and metabolism, but when their concentration rises, fish's biological equilibrium is impacted (Buhl and Hamilton, 200 0). Domestic sewage, agricultural waste, industrial wastes or micro-organisms in anox ic waters can all cause ammonia pollution of Lakes (Hashem *et al*., 2020). The degrad ation of organic matter in the wastewater, where nitrosoman bacteria convert ammoni a to nitrite through denitrification, may be the cause of the high nitrite content (Saad *et al.,* 2017).

2.3.2. Total dissolved solids (TDS)

Total dissolved solid is a measure of the combined inorganic and organic substances dissolved in water. Changes in the amount of dissolved solids can be harmful to the fish because the density of TDS determines the flow of water in and out of an organism cell. Dissolved solid on the other hand includes those materials dissolved in the water, such as, bicarbonate, sulphate, phosphate, calcium, magnesium, sodium, and other ions. These ions are important in sustaining aquatic life. However, high concentrations can result to damage in organism’s cell (Muhammad and Ullah, 2022). Water quality of the Lake can be deteriorated and aquatic life negatively impac ted by the discharge of waste water with a high TDS level from various industries, inc luding the textile, ceramic and food processing industries, waste from hospitals and ag ricultural pesticides (Walakira and Okot-Okumu, 2011; Lwimbo *et al.*, 2019). Surface water's alkalinity is principally determined by its carbonate and hydroxide content, as well as by the contributions of borates, phosphates and other bases. Total alkalinity gauges how well water can balance acids. Increased quantity of salts carried from urban sewage and industry effulents, increases the level of chloride and subsequently the salinity of water, salinity inturn reduces the amount of soluble oxygen in the water, which is further responsible for increasing the osmotic pressure (Farkas *et al.*, 2000). International standards of water quality was mwntione in the table below.

Parameter	WHO standrands
Total dissolved solid	500 mg/l
Nitrate	10 mg/l
Nitrite	1 mg/l
Ammonia	1.5
Phosphate	0.3 mg/l
Alkalinity	200 mg/l

2.4. Pathological lesions of fish from deteriorated water quality

2.4.1. Gross pathologic lesions of fish

Gross external pathological lesions are frequently used as indicators of anthropogenic influence in aquatic environment (Capkin *et al*., 2009; Velmurugan *et al.*, 2009). A colour change is one of the first gross pathological alteration associated with skin and gills of fish due to pollution (Antychowicz and Matras, 2008). Gills showed congested filaments, sometimes dark in color and the gill chamber filled with turbid mucous *(*Zaki *et al*., 2020). Healthy gills are bright red; but with anaemia they become pale pink and paleness with haemorrhage most often indicates pathologic change to gills. Paleness of this organ detected in fish immediately after capture is a sign of anaemia of the whole body and is an excellent indicator of health status in fish. When the gills are brown and pale brownish, methaemoglobinaemia associated with nitrite poisoning is the cause (Antychowicz and Matras, 2008).

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Gross changes such as discouloration of skin, discouloration of liver and pale internal organs of fish impacted by industrial and agricultural discharges were identified by (Zaki *et al*., 2014). Postmortem changes; pale discoloration and necrosis of the liver due to damage to liver cells; blood filled abdominal cavity and darkened and congested gills were also reported from exposure to pollution (Blazer *et al.*, 2018). Variety of external raised skin lesions including small, discrete white spots, larger white areas, slightly raised mucoid lesions and multilobed raised areas were observed on the body surface of fish from polluted stream as mentioned by Blazer *et al.*, (2018). Discoloration of the skin, congestion of gill chambers and gill lamellae were observed in *Oreochromis niloticus* fish as exposed to pollution as mentioned by (Zaki *et al*., 2020).

2.4.2. Histopathologic lesions of fish

Microscopic lesions in fish tissue can be used as a tool to detect effects of water pollution or changes in water quality on target organs of fish (Mitrovic-Tutundzic, 1994; Lins *et al.,* 2010; Santos *et al.*, 2012). Fish histopathology has been applied as an integrative method for environmental health assessment in monitoring programmes (Salamat and Zarie, 2016, Briaudeau, 2020). Biological responses from exposure to environmental pollutants or stressors identified by cellular bio-markers (i.e. oxidative stress, neurotoxicity, altered endo-lysosomal system) may progress to autophagy and apoptosis (Moore *et al*., 2006; Chiarelli *et al.*, 2016) and potentially generate tissue-level alterations (Köhler *et al.*, 2004).

Histopathological lesions are considered indicators of biological effects of exposures to environmental pollutants (Feist *et al.*, 2015; Briaudeau, 2020). They are considered non specific biological responses as they may originate from exposure to various environmental stressors such as mixtures of contaminants (Yancheva *et al.*, 2015). His topathology involves the microscopic examination of cells and tissues of an organism and quantitative determination of histological abnormalities (Costa *et al*., 2010). Gills, liver and skin are the most frequently used organs for assessing environmental impact on health status of fish (van Dyk *et al.*, 2009; Marchand *et al.,* 2009; McHugh *et al.,* 2013).

Histopathology of Gills: Fish gill comprise a multifunctional organ and constitute over 50 percent of the total surface area of the animal that makes it sensitive to chemicals in water. Variety of pollutants that causes various gill lesions are actually non-specific and are not correlated with the kind of toxicant or fish species (Au, 2004). Histopathological changes of gills such as hyperplasia and hypertrophy, epithelial uplifting, aneurysm, fusion of gill lamella and increase in mucus secretion have been observed after the exposure of fish to a variety of pollutants (Marchand *et al.*, 2009; Reddy and Rawat, 2013).

Filament cell proliferation, lamellar fusion, lamellar cell hyperplasia and epithelial lifting were observed in tilapia fish exposed to herbicides (Ayoola, 2008). Gill histopatholoy lesions; epithelial lifting, hyperplasia and hypertrophy of the epithelial cells and partial fusion of some secondary lamellae are examples of defence mechanisms. This Pathologic responses results in the increase of the distance between the external environment and the blood and thus serve as a barrier tothe entrance of c ontaminants. In ordered to increase the epithelial area for diffusion and thus reduce th e absorption of pollutants in to the blood, epithelial hyperplasia is considered as a pro tective response to toxics, irritants and environmental stressors (Baskar., 2014). Lamel lar aneurism, on the other hand, represents a lesion that can result from the rupture of pillar cells, and this corresponds to the deleterious effect of xenobiotics on branchial tissue (Martinez *et al.*, 2004).

In general; all observed microscopic gill lesions of fish in this study were because; gill was an important indicator of waterborne toxicants by which any pollutant comes into contact and is very sensitive to changes in the composition of the environment. This is due to its multifunctional properties such as respiration, osmo-regulation, acid–base balance and nitrogenous waste excretion. Gill structure provides a large surface area for direct and indirect contact with water pollutants. Thus, this organ is too sensitive to chemicals in the water and is considered as the primary target organ to the contaminants (Camargo and Martinez, 2007).

Histopathology of Liver: Liver is a detoxification organ and it is essential for both the metabolism and the excretion of toxic substances in the fish body. Due to its large blood supply which causes noticeable toxicant exposure is primary organ for bioaccumulation and thus has been extensively studied in regards to the toxic effects of different xenobiotics (Simonato *et al*., 2008). Necrotic areas scattered throughout the hematopo ietic tissues of fish as a result of changes in water was found by (Capkin *et al., 2006).* The sort of histological alterations observed depends on individual exposition time to pollutants, as well as on pollutant type and concentration (Pinto *et al*., 2010).

It was revealed that histopathological lesions of liver are not specific to pollutants and there are several studies at different times. Exposure to industrial and agricultural pollutants increases the presence of liver lesions such as foci of cellular alteration , hepatocellular nuclear polymorphism, hydropic vacuolation, non-neoplastic proliferativ e lesions and non-specific necr-otic lesions (Collier *et al.,* 2013). Irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position were observed in fish exposed to agricultural chemicals (Fanta *et al*., 2003; Figueiredo-Fernandes *et al*., 2007). Signs of degeneration (cytoplasmic and nuclear vacuolation) and the focal necrosis in the liver parenchyma were observed in fishes exposed to the industrial effluents (Reddy and Baghel, 2012).

Histopathology of Skin: Fish skin is considered as an important organ because it is the interface between the external and internal environment of fish. It forms the first line of defense which is direct contact with waterborne toxic chemicals. Normally fish skin is hydrated, unkeratinized and totally covered by a layer of slimy mucus and due this nature fish skin is very susceptible to waterborne chemicals (Eleyele *et al*., 2007). Histopathologic lesions of skin including architectural abnormalities in the surface of epidermal cells; abundant mucus secretion; loss of shape, size and structure of epidermal cells and goblet cells were observed in those fish exposed to environmental contaminants (Salamat and Zarie, 2012).

3. MATERIALS AND METHOD

3.1. Study areas

Lake Batu (Figure. 2) is located in the central rift valley lakes basin of Ethiopia with a lake surface area covering 450 km^2. It is located between 38.2° and 39.25°E longitudes and latitudes of 7.43° to 8.58°N. Meki and Katar rivers supply the majority of Lake Batu's water. Water from Lake Batu is released into the Bulbula River, which then empties into Lake Abiyata. The typical annual temperature in Lake Batu ranges from 13 to 27.5°C, making it a sub-humid to humid climate. The range of the average annual rainfall is 454 to 995 mm and mean annual rainfall varing from 454 to 995 mm. Between Lake Batu and the major highway, at elevations between 1600 and 1700 meters above sea level, are the horticultural businesses (Sahle and Potting, 2013).

As part of the central Ethiopian rift valley basin, Lake Hawassa is situated between 38º23′17.8″ and 38º28′52.9″ E longitude and 6o59′3.91″ to 7o7′42.24″ N latitude in the Southern Nations, Nationality and Peoples Regional State and Oromia Regional State (Figure 2). The caldera's borders reach a height of 2995 masl in the south-east, while Lake Hawassa is located at 1680 (masl) (Abebe *et al.*, 2018). Lake Hawassa is the source for commercial fishing, recreational site and tourist destination. Moreover, it is the main source for domestic, livestock and irrigation water for the community (Abebe *et al.*, 2018).

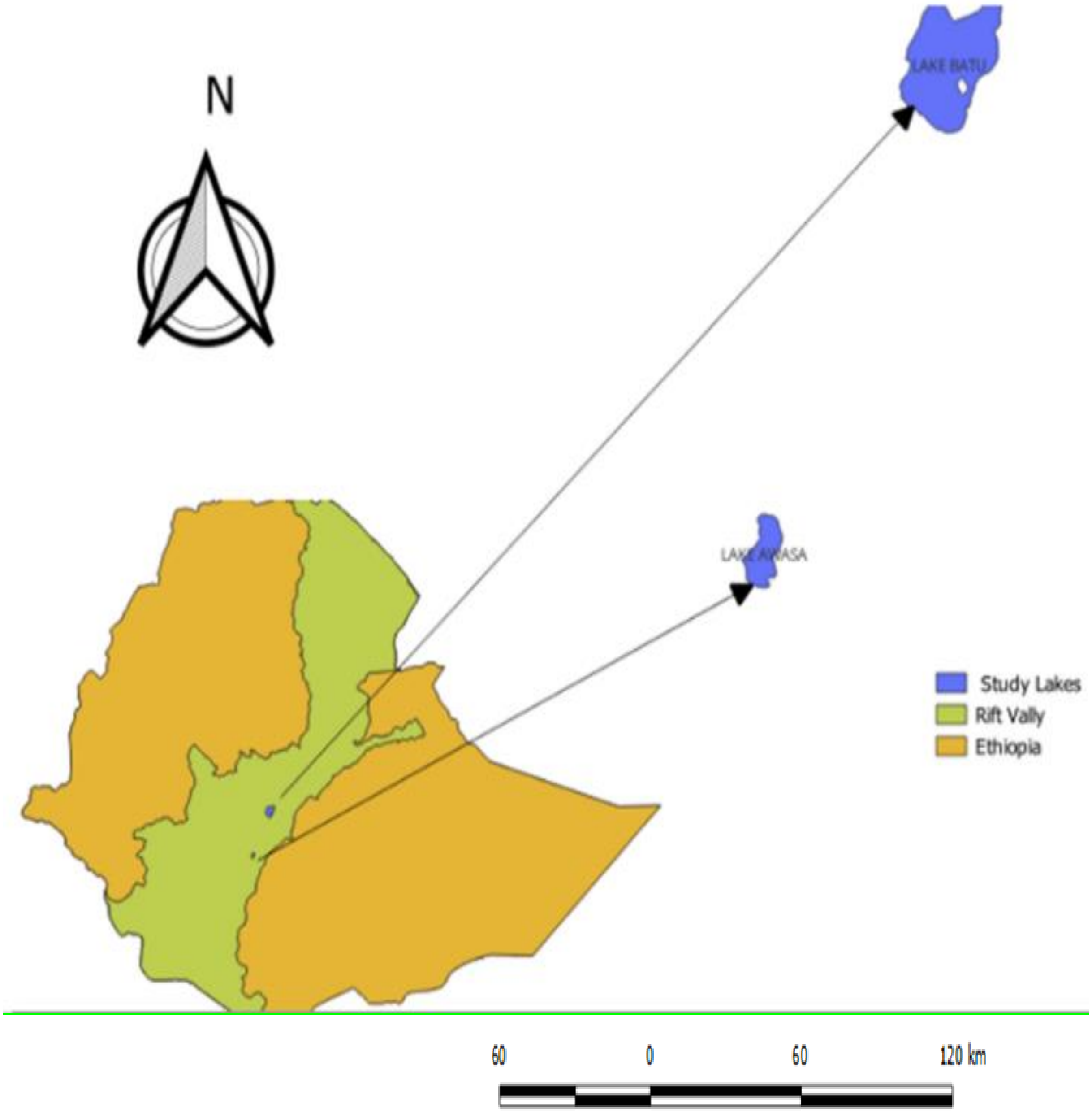


Figure 1. Map showing study areas

3.2. Selection of sampling sites

Before directly executing water and fish sampling for both lakes; general information regarding point sources of discharges in too the lake was obtained from fishermens , boat sailors and residents of Hawassa and Batu together with personal observation. Thus representative locations were slected purposively. Those sites of the lake highly impacted and receiving wastes from visible point-sources of pollution were taken as “effulent site of the lake” where as with no visible-point sources pollution and relatively less impacted site was taken as reference site of the lake.

Lake Hawassa and lake Batu were most threatened lakes in the main rift valley with different levels of anthropogenic activities (Wondrade *et al.,* 2014; Abebe *et al.,* 2018). So, it is hard to find sites on the shoreline of the lake free of human intervention to take as reference sites. But, according to Ontario Benthos Biomonitoring Network (OBBN) prot ocol it is recommended to use minimally disturbed sites as reference sampling sites (J one *et al.,* 2007).

Lake Hawassa effulent sites were entry of the tikur wuha river and Haile resort area. Tikur wuha is an area where the river inputs are high to the lake from small scale agricultural activities and factories which release their effluent into the river and then the river finally discharges their input into lake hawassa. Haile resort area is site where lake Hawassa receives point discharge of effulents from Hawassa industrial park. Haile resort area and Tikur wuha river were near to each other and considered as single sampling site. Lake Hawassa reference site was lake near to “Dorebafana”. Dorebafana is immediate opposite to Amora Gedel. The site name is after the local village that is found near to lake Hawassa “Doriebafana” and this site of Lake Hawassa does not have point source of pollution; but there may be non-point source of pollution from the agricultural land and soil of the area.

Lake Batu effulent site was near sher Ethiopia flouriculture effulent site where by the lake was receiving agricultural wastes released from sher Ethiopia flower production. Lake Batu reference site was “Deset zuria” . This area has no visible point sources of pollution except activities such as boat washing and cloth washing by the island community.

3.2. Study population

Study population was fish. Fish species namely; Nile tilapia (*Oreochromis niloticus*), its local name called “koroso” was selected ; because they form an integral part of the commercial fisheries in both Lakes and their abundance has gradually declined (Tilahun *et al.,* 2016; Lemm and Desta 2016). Fisherman, boat sailors and residients of Lake Hawassa and Batu were parts of convenience interview which was input for sampling site selection.

3.3. Study design

A cross-sectional study design was carried out between November 2022 and April 2023 with the objective; comparatively evaluation of pathologic lesions in fish collected from different effulent sites. Both fish and water sample collection sites were selected purposively.

3.4. Sample size determination

The desired sample size for the study was calculated using the formula given by Thrusfield (2018) using 50% prevalence

$$n = \frac{(1.96)^2 \text{ x Pexp } (1-\text{Pexp})}{d^2}$$

Where; n = required sample size; exp = expected occurrence of pathological lesions; 50% = 0.5, d = desired absolute precision with 95% confidence interval and 5% absolute precision. Accordingly, the calculated sample size was 384 fish; but 400 fish were included in the study to increase level of precision.

3.5. Sample collection and sampling strategies

3.5.1. Water sampling

The equipment used for water sample collection was one liter polyethylene bottles after cleaning each bottle with detergent. Then, bottles were rinsed with lake water three times before taking the samples at each sampling sites and took one liter water samples from each site. This was performed from 50 cm depth in order to excluded the dust materials as well as oily liquids suspended above the Lake to the container. The same procedure was applied for all 0sampling sites. For Lake Hawassa effulent site; one liter of water sample was taken from lake near to inlet of Tikur wuha river and another one liter from Haile resort area and then mixed properly and one liter of the mixture was considered as representative sample for “Lake Hawassa effulent site”. Water samples were collected from all selected sites of both lakes.The sample were placed in a deep freez container. All collected water samples were placed in a labeled sample container and stored in a refrigerator at 4°C until laboratory anlaysis.

3.5.2. Fish sampling

From the same location where water samples were taken, a total of four hundred fish (one hunderend fish from each site) were gathered for examination. Professional fishermen assisted fish collection was done for all sampling sites using seine netting and fishing boats. Collected fish were examined immediately for presence of any visible gross lesions. Fish alive were dorsoventrally severed for postmortem examination. Fish with gross pathological lesions were recorded in post-mortem examination sheet and representative samples were collected into 10% neutral buffered formalin for histopatholgic examination.

3.6. Laboratory analysis

3.6.1. Water sample analysis

All collected water samples were transported to the limenology laboratory at Addis Ababa University's College of Natural and Computional Science. Water samples were analyzed for nitrite, nitrate, ammonia, silica, total alkalinity and total dissolved solids according to the requirements for the analysis of water and waste water described by American Public Health Association's (APHA) (2002) in limnology laboratory.

3.6.2. Gross and microscopic examinations of Fish

Gross examination of fish were done according to descriptive guide to observe fish gross lesions (Kane, 2005). All fish samples collected were examined for any vissible gross pathologic lesions. Lesions on skin surface and gills were observed and well documented. Gill opercula were opened to check for gill deformities. Abdominal cavity was opened using a scissors by cutting from anal area to expose liver. Observed abnormalities on fish were carefully documented in gross examination sheet and photograph was taken, if possible. Tissues with any gross visible pathological alterations were immediately sampled and and fixed in 10% neutral buffered formalin.

Briefly (Appendix 3 and 4); formalin fixed tissues were trimmed to 2-3mm, dehydrated in increasing alcohol concentration, cleared in three passes of xylene and impregnated with molten paraffin. Tissue block were made and then, the tissues were sectioned at

5μm thickness and the sectioned tissue (ribbon) was straighten on water bath. Then, the ribbon was adhered on frost ended and clear slide which is labeled and put in an incubator. The slides were deparaffinized in 3 changes of xylene, hydrated in decreasing alcohol concentration, placed in hematoxylin, rinsed in tap water, decolorized in acid and checked for differentiation of nucleus and cytoplasm. Then,rinsed again in tap water and stained in Eosin. Dehydrate again in increased alcohol concentration, clear in three changes of xylene and mount cover slide with Dipex. Finally, the slides were read under a microscope (Talukder, 2007).

Tissue samples were transported to animal health institute for histopathological processing. Histopathological technique was according to histopathology procedures mentioned in (Talukder, 2007). Details of histopathogical procedures were mentioned in (Appendix 5). Slides will be examined under microscope and photographed. Tissue sections were scanned at 10x, 40x and 100x magnification.

The presence and severity of the lesions in each gill, skin, and liver were graded according to Poleksic and Mitrovic-Tutundzic (1994). Gills, skin and liver lesions were progressively classified in three stages of tissue damage: Lesions, which were less sever and did not impair normal functioning of organ were classified as stage I; lesions which were more severe and induced reparable or reversible damage of organ were classified as stage II lesions. Lesions, which were very severe and induced irreparable damage to organ were classified as stage III lesions.

3.7. Data Management and analysis

The field collected data was entered, classified, filtered and coded using Microsoft Excel Spread Sheet (2016) and analyzed using the software package STATA 14 (Stata Corp, 2013). ANOVA and mean comparission t-test were used for comparission of mean for water quality parameters. Descriptive statistics were used to describe and su mmarize gross pathological lesions. Logistic regression and chi -square association (χ 2) test were used to evaluae statistical association of risk factors and gross pathologic al lesions. Statistical significance was considered at $P < 0.05$ and 95% confidence interval . In cases of estimating the effect of the strength of association of risk factors with gross lesion was estimated by odds ratio (OR).

3.8. Ethical consideration

An ethical certificate with a Reference number VM/ERC/20/03/15/2023 was received form Animal Research Ethics and Review Commite of Addis Ababa University, College of Vetrinary Medicine and Agriculture (Appendix 6).

4. RESULTS

4.1. Water quality

The results for analysis of chemical parameters of water was summarized in the table 1. Mean (±SD) of water quality parameters were statistically different for ammonia, ni trate, nitrite, silica, TDS and alkalinity by sampling sites (P < 0.05).

Table 1: Summary of water quality (mean±SD) sampling sites from Lake Batu and Lake Hawassa (ANOVA).

Parametr s	**Sampling Sites**	**Mean**	**SD**	**Min.**	**Max.**	**Prob > F**
Ammonia (*, ***)	HR	43	4.286	38.714	47.286	0.00
	HE	61.571	1.428	60.143	63	
	BR	130.143	1.428	128.714	131.571	
	BE	186.333	7.190	178.714	193	
Ntrate (*, ***)	HR	46.762	.825	46.286	47.714	0.00
	HE	44.381	1.649	43.428	46.286	
	BR	38.190	.825	37.714	39.143	
	BE	8.667	2.974	56.286	62	
Nitrite (*, ***)	HR	113.427	1.428	112	114.857	0.00
	HE	285.809	.825	284.857	286.286	
	BR	511.048	6.751	503.429	516.286	
	BE	885.333	.825	884.857	886.286	
Silica (*, ***)	HR	16.784	.099	16.893	16.893	0.00
	HE	14.148	.113	14.082	14.278	
	BR	11.142	.113	11.076	11.272	
	BE	5.281	.165	5.128	5.455	
TDS (**, ***)	HR	206.667	11.547	200	220	0.00
	HE	403.333	5.774	400	410	
	BR	343.333	5.774	340	350	
	BE	423.333	5.773	420	430	

Total alkalinity(**, ***)	HR	3751.67	2.687	3750	3755	0.00
	HE	4501.67	2.887	4500	4505	
	BR	4200.67	1.155	4200	4202	
	BE	4500.33	.577	4500	4501	

* = Unit(Ug/L=Microgram/liter), ** = Unit(Mg/L= Milligram/Liter), ***=(Three observations for each site), HR= Lake Hawassa reference site, HE = Lake Hawasaa effulent site, BE=Lake Batu reference site, BE = Lake Batu effulent site, SD.=Standered deviation, Min = Minimum, Max = Maximum, Prob > F = Significant values with mean comparission difference with in and between groups.

The results of chemical analysis of the water by mean comparision t-test was summarized in table 2 and table 3. It had showed; means of analysed water quality parameters such as ammonia, nitrite, silica and alkalinity were found significantly higher ($P < 0.05$) in Lake Batu water samples compared to Lake Hawassa. Regarding effulent and reference sites of Lake batu and Lake Hawassa ; means of ammonia, nitrite, nitrate total alkalinity, TDS and silica were found significantly higher ($P < 0.05$) in effluent sites compared to reference sites and detailed in table 5.

Table 2: Summary of mean comparission of water quality parameters between Lake Batu and Lake Hawassa (t-test).

Parametrs (*,,***)**	**Sampling sites**	**Mean**	**P-value**
Ammonia(*, ***)	Lake Batu	158.24	0.0000
	Lake Hawassa	52.28	
Nitrate(*, ***)	Lake Batu	48.43	0.5570
	Lake Hawassa	45.57	
Nitrite(*, ***)	Lake Batu	698.19	0.0003
	Lake Hawassa	199.62	
Silica(*, ***)	Lake Batu	8.2108	0.0005
	Lake Hawassa	15.465	
TDS(**, ***)	Lake Batu	383	0.1311
	Lake Hawassa	305	
Total(**, ***)	Lake Batu	4350.5	0.2435
Alkalinity	Lake Hawassa	4126.667	

* =Unit(Ug/L=Microgram/liter), ** = Unit (Mg/L=Milligram/Liter), *** = Six obserbations for each lakes); P-value = significant values with independent mean comparission t- test

Table 3: Summary of mean comparission of water quality parameters between effulent and refrerence sampling sites of Lake Batu and Lake Hawassa (t-test).

Parametr (*,,***)**	**Sampling Sites**	**Lake Hawassa**		**Lake Batu**	
		Mean	**P-value**	**Mean**	**P-value**
Ammonia**(*,***)**	Reference site	61.571	0.0021	130.143	0.0002
	Effluent site	43		186.333	
Nitrate **(*,***)**	Reference site	44.381	0.0890	58.667	0.0003
	Effluent site	46.762		38.190	
Nitrite **(*,***)**	Reference site	0.089	0.0000	511.048	0.0000
	Effluent site	113.429		885.333	
Silica **(*,***)**	Reference site	14.148	0.0000	5.281	0.0000
	Effluent site	16.784		11.142	
TDS**(**,***)**	Reference site	206.67	0.0000	423.333	0.0001
	Effluent site	403.33		343.333	
Total **(**,***)** Alkalinity	Reference site	164	0.0000	30.667	0.0000
	Effluent site	675.667		15.333	

* = Unit(Ug/L=Microgram/liter), ** = Unit (Mg/L = Milligram/Liter),*** = Six observations for each lake, P-value = Significant values with mean comparison t-test, Obs = Number of observations, TDS =Total Dissolved Solid

4.3. Gross pathologic findings

Statistical association of gross lesions by sampling sites were summarized table 4. Fish inhabiting Lake Batu were more likely to develop gross pathological lesions in gill (OR = 2.46, P = 0.001) and skin (OR = 1.8, P = 0.036) than fish inhabiting Lake Hawassa. The difference was found insignificant for liver (P = 0.09). Regarding effule nt and refrence sites, fish inhabiting effulent site of Lake Batu were mor likely positive to develop gross gill lesions (OR = 2.95, P = 0.002), gross liver lesions (OR = 4.67, P = 0.001) and gross skin lesions (OR = 2.98, P = 0.029) compared to Lake Batu reference site. For effulent and reference sites of Lake Hawassa, fish inhabiting effulent site was more positive to develop gross gill lesions (OR = 2.9, P = 0.023) and gross skin lesions (OR = 2.98, P=0.001) in effulent sites compared to reference sites.

Table 4: Summary for frequency and percentage of gross pathologic findings by sampling site.

Organ lesions	Sampling sites		Freq (%)	χ2	OR	P-value	CI
Gill	B/n lakes	Hawassa	25(12.5%)	11.93	Ref.		
		Batu	52(26 %)		2.46	0.001	1.455 4.156
	With in Lake Batu	Reference	16(16%)	10.6	Ref.		
		Effulent	36(36%)		2.95	0.002	1.507 5.787
	With in lake Hawassa	Reference	7(7%)	5.7	Ref.		
		Effulent	18(18)		2.9	0.023	1.159 7.334
Liver	B/n Lakes	Hawassa	18(9%)	2.9	Ref.		
		Batu	29(14.5%)		1.7	0.090	.919 3.201
	With in Lake Batu	Reference	6(6)	12.3	Ref.		
		Effulent	23(23)		4.67	0.001	1.814 12.07
	With in Lake Hawassa	Reference	7(7)		Ref.		
		Effulent	11(11)	0.98	1.64	0.327	.609 4.424
Skin	B/n Lakes	Hawassa	22(11%)	4.5	Ref.		
		Batu	37(18.5%)		1.8	0.036	1.04 3.24
	With in Lake Batu	Reference	9(9)	5.28	Ref.		
		Effulent	28(28)		2.98	0.029	1.12 7.98
	With in Lake Hwassa	Reference	6(6)	12.46	Ref.		
		Effulent	16(16)		3.9	0.001	1.75 8.86

B/n = Between; χ 2 =Chi-square; OR = Odds Ratio

Details of over all manifested lesons were summarized in table 5. In gill the most frequent gross lesion was congestion observed in 32 (41.55 %), in liver necrotic lesion with mottled appearance was most frequent lesion 16 (34.04 %), while in skin scale loss 19 (32.2 %) was frequent gross pathologic lesion.

Table 5: Over all frequency and percentages of gross pathological findings in fish or gans (N=183).

Gill (n=77)	**Freq (%)**	**Liver (n=47)**	**Freq (%)**	**Skin (n=59)**	**Freq (%)**
Excesiive mucus	18 (23.37)	Pale to yellowish	10(21.)	Dark Discouloration	17(28.8)
Pallor	8(10.38)	Congestion	15(31.9)	Scale loss	19(32.2)
Congestion	32(41.55)	Hemmorhge	2(4.25)	Reddened/hyperea mic lesions	13(22)
Hemorrhage	3 (3.89)	Necrosis and mottling	16(34.)	Hemmorhage	10(16.9)
Necrosis and mottling	16(20.78)	Cirrhosis	4(8.5)		

Freq (%) = Frequency (Percentage)

Figure 2. Gross pathologic findings of fish skin: (A) Apparently healthy Nile tilapia skin; (B) hypericmia on the pectoral body surface(arrows) with haemorrhagic pectoral fin; (C) hemorrhagic lateral body surface with minimal scale loss and raised white lesions; (D) scale loss and hemmorhagic lesions (arrow).

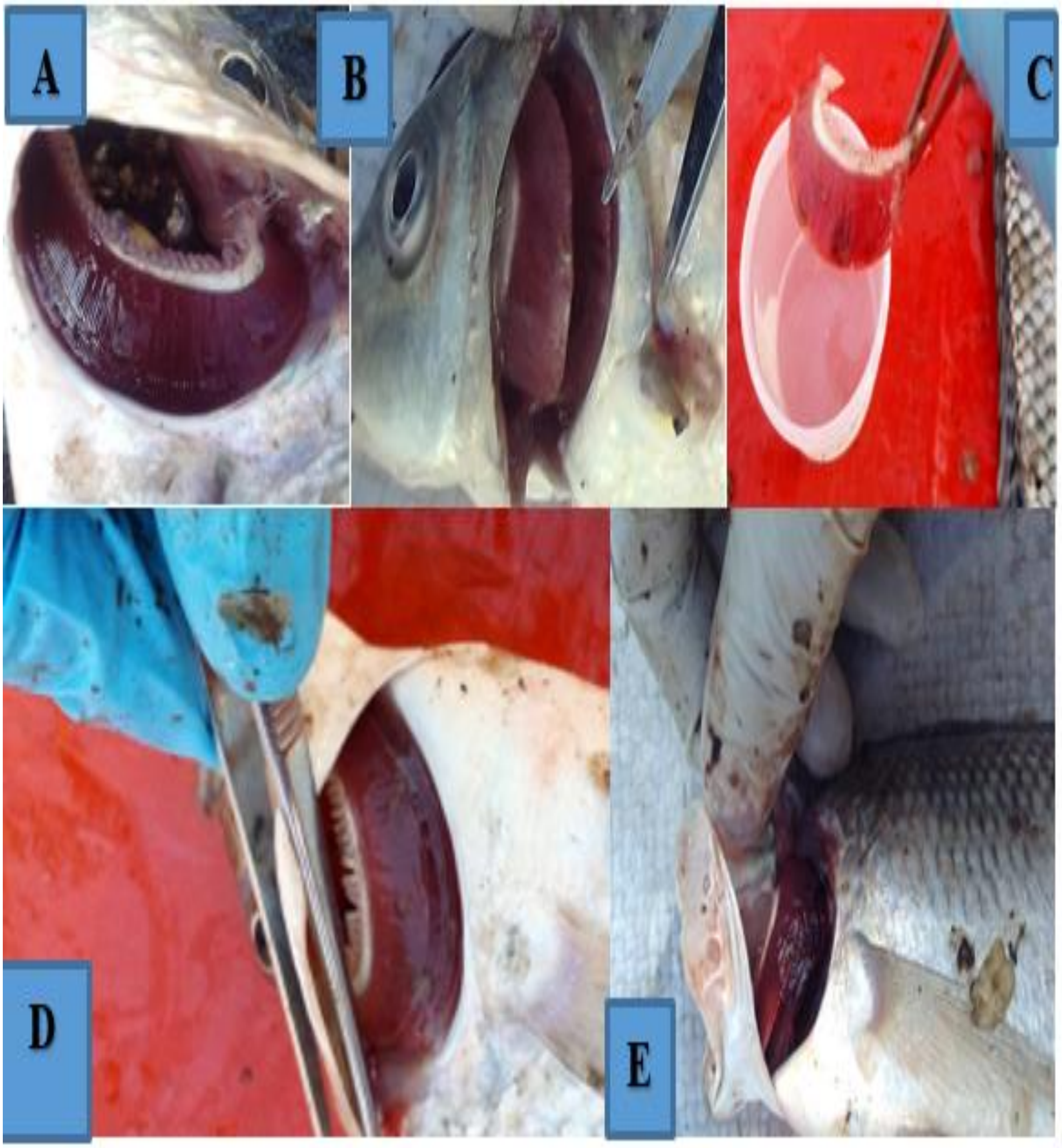


Figure 3. Gross pathological lesions of gill. (A) Apparently healthy gill; (B) pallor gill with excessive mucus; (C) Focal necrosis of gill; (D) Congested gill; (E) Necrosis of gill (mottled appearance)

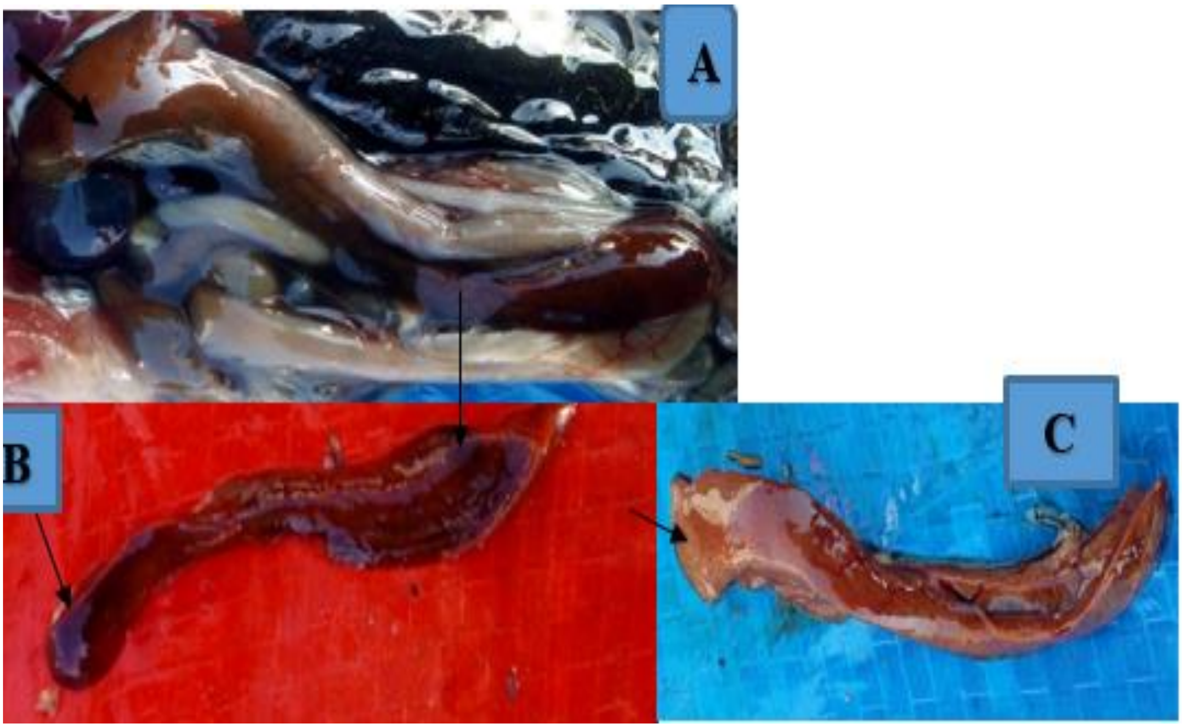


Figure 4. Gross lesion of liver. (A) Congested liver with necrotic margins; (B) Congestion of liver with localized necrotic foci (arrows); (C) Necrotic liver (paleness to yellowish areas)

4.5. Histopathological findings

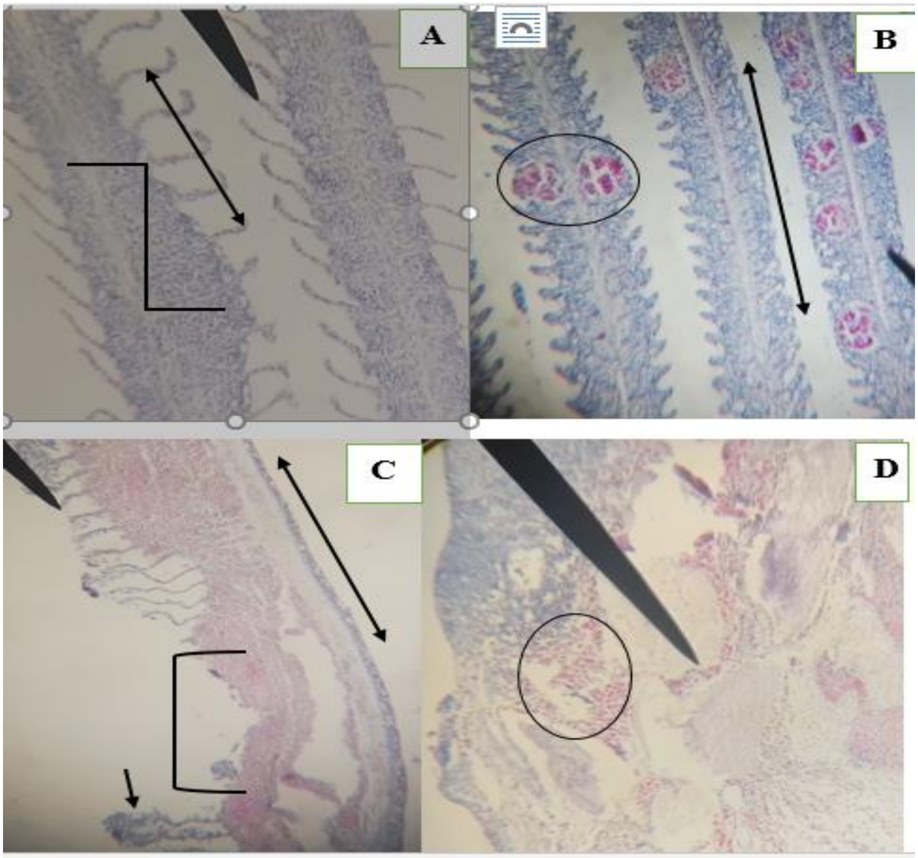


Figure 5. Microscopic lesions of gill. (A) Relatively normal secondary and primary la mellae (double head arrow) on slightly thickened primary lamellae (zigzag) (from refe rence site/Hawassa); (B) on the left side of this gill, primary and secondary lamellae in position but lamellar capillaries dilated (aneurism) and filled with RBCs (arrow), on the right two primary lamellae with total loss of secondary lamellae (double arrow); (C) Necrosis of primary gill filament (double arrow), necrosis and sloughing of secondary lamellae (bracket), fusion of secondary lamellae (arrow); (D) total necrosis of cartilage of primary lamellae and hemorrhage (circle); (40X) H&E.

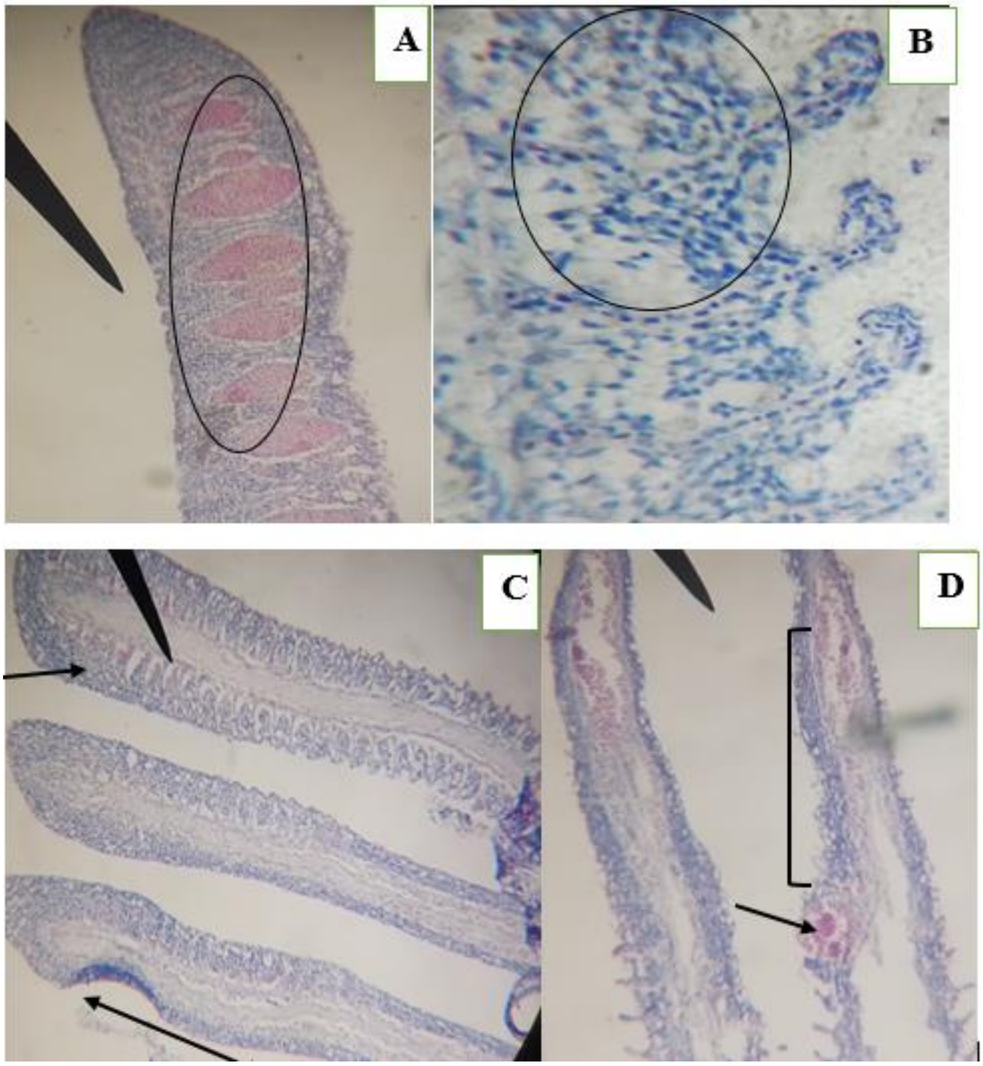


Figure 6. Microscopic lesion of gills. (A) from lake Batu reference site; aneurysm (by thin arrow); (B) heavy lymphocytic infiltration (circular inset) at the base of secondary lamellae and into primary lamellar filament. Hyperplasia of secondary lamellae (from effluent site/Hawassa); (C) Hyperplasia and fusion of secondary lamellae (thick arrow), loss of secondary lamellae (double head arrow) (effluent site/Batu); (D) loss of secondary lamellae, fusion of secondary lamillae (bracket) lamellar capillary dilation (arrow) (effluent site/Hawassa) (H &E) (10X and 40x).

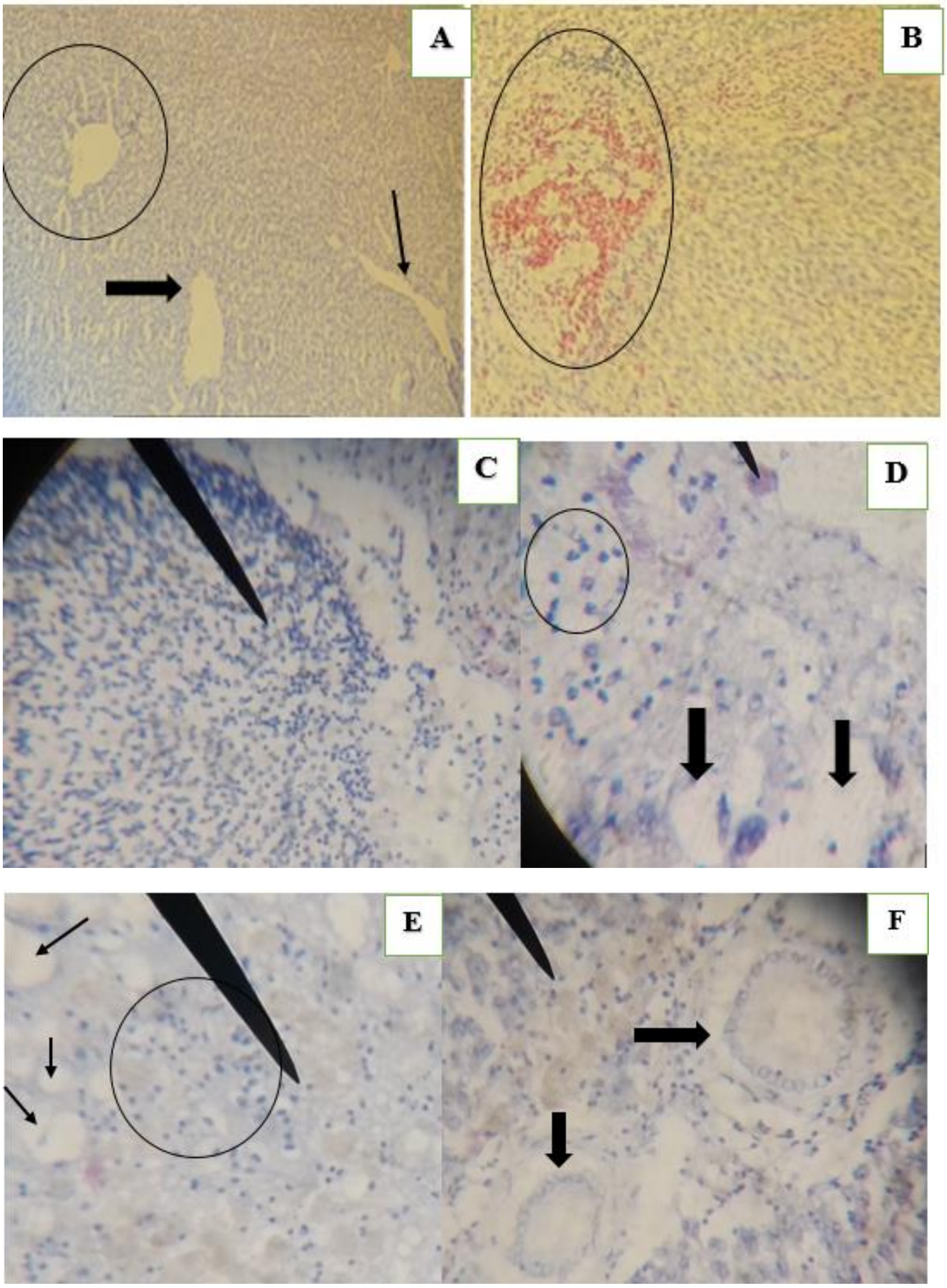
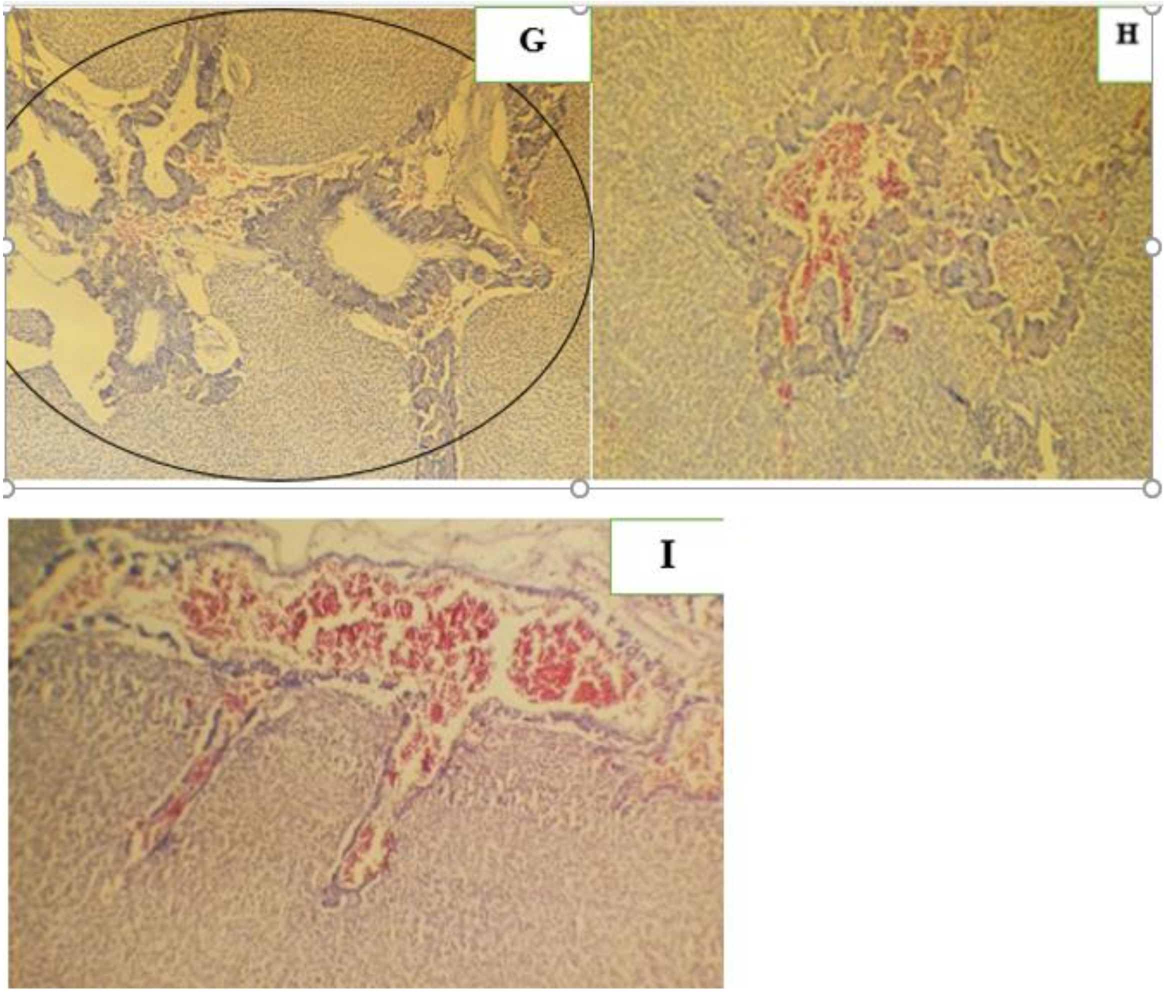


Figure 7. Microscopic lesion of liver: (A) Relatively normal hepatocytes, central vein (circle); hepatic vein (thick arrow); sinusoid (thin arrow) (reference site/Hawassa; (B) from Batu effluent site: hemorrhage (circle); (C) The central vein is dilated and contain excessive lymphocytic infiltrations; (D) total hepatic necrosis (thick arrow) and dark brown pigments (hemosiderin) (thin arrow); (E) Hydropic degeneration and hepatocel lular necrosis (thin arrow) (effluent site/Hawassa); (F) Degeneration and necrosis of b ile duct epithelium (thin arrows) (Effluent site/Hawassa).



Continued from (Figure 7); (G) Hepatobiliary adenoma (circle); (H) Hepatocellular ad enoma and accompanied blood vessels , hepatocytes are enlarged, cytoplasm are prom inently dark, nuclei are vesicular and nucleoli are prominent. In some figure’s(I) angio -genesis from the accompanied blood vessels were clearly seen (effluent site /Hawassa). (H &E) (10X &40x).

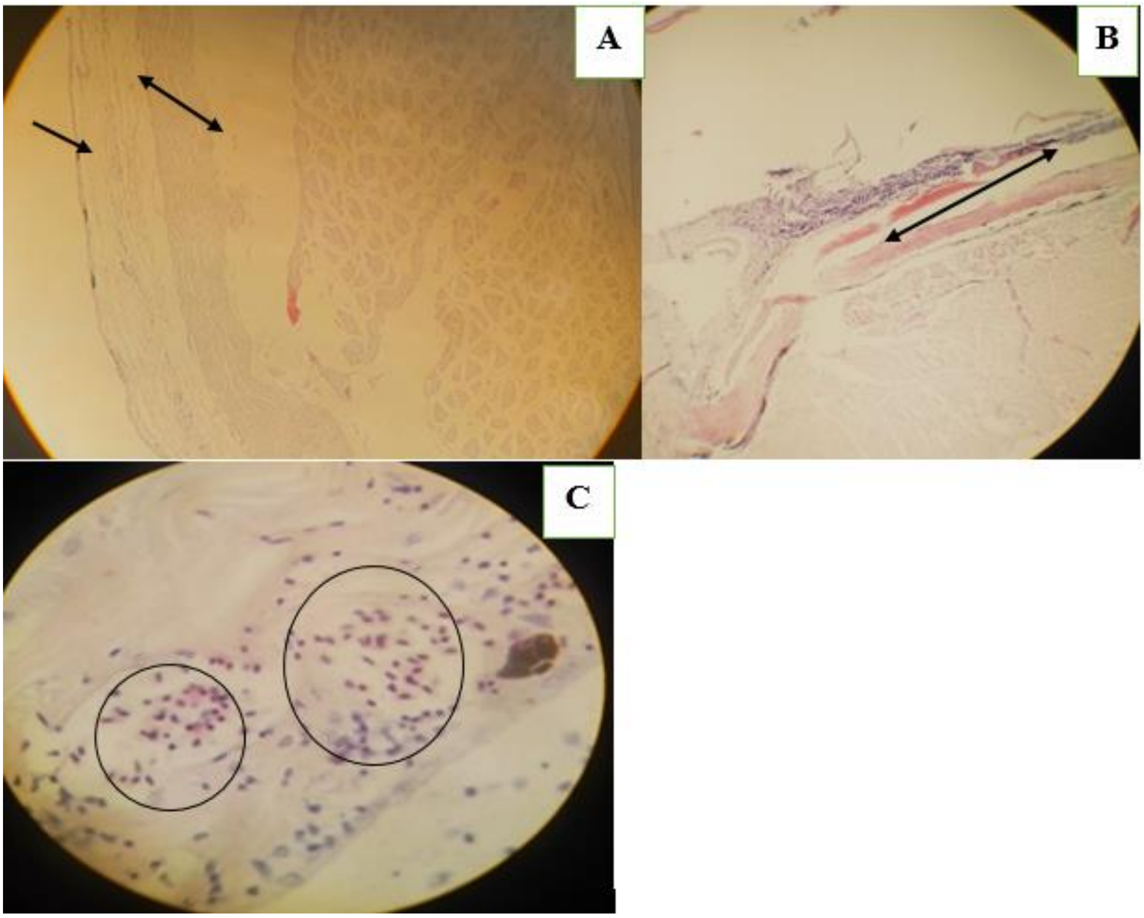


Figure 8. Microscopic lesion of skin.(A) from Hawassa reference site, skin layers (epi dermis(arrow) and dermis (double arrow) (at 10X (arrow); (B) from Batu effluent site, sever necrosis of epidermal cells and separation of epidermis from dermis (double arrow); (C) from Hawassa effluent site; heavy lymphocytic infiltration in to dermis (at 40X).

Table 6: Summary of types, severity stages and frequency of microscopic lesios by sa mpling site.

Gill microscopic lesions	Stages	Site
Necrosis of secondary lamellae	III	A (+++), C(++)
Heavy hemorrhage in primary lamellae	II	A(++), C(+)
Fusion of lamellae (primary or secondary)	II	A(+++), C(++), B(+), D(+)
Hyperplastic secondary lamellae	I	A(+++), B(+), C(+++), (D+)
Heavy lymphocytic infiltration	I	A(++), B(+), C(++),
Aneurysm (dilation of blood vessels &full of RBC)	II	A(++), C(++), B(+)
Goblet cell hyperplasia	I	A(++), C(++), B(+)

Liver microscopic lesions		
Total hepatic necrosis	III	A(+++), C(+)
Necrosis of biliary epithelum	III	A(++)
Hydropic degeneration	II	A(++), B(+), C(++)
Aneurism(dilation of HA and HV)	II	A(++), C(+)
lymphocytic infiltration	I	A(++), C(++), D(+)
Hemosiderin	I	C+
Hepatocellular and hepatobiliary adenoma	II	C++
Congestion of bile duct	I	A++ C++
Hemorrhages	II	C+
Skin microscopic lesions		
Epidermal necrosis	III	A(+++)
Hemorrhage	II	A(+), C(+)
Lymphocytic infiltration	I	A(++), C(+)
Dilated capillaries &full of RBC	II	A(++), C(++), B(+)

HP= Histopathological lesion, site; A=Lake Batu effluent site, B=Lake Batu reference site, C=Lake Hawassa effluent site, D=Lake Hawassa reference site.

Frequency of observed microscopic lesions were described as follows; + = Rare (if les ion was observed in single fish specimen); ++ = Less frequent (if lesion was observed in 2-3 specimens), +++ = Frequent (if lesion observed in more than 3 specimens).

5. DISSCUSION

The quality of water and the well-being of fishes are interconnected and directly proportional as fluctuations of any of the parameters strongly affect fish health (Greig, 2005). Elevated levels of water quality parameters implicated pollution as the source of alteration in water quality (Gaber, 2013).

(Mean±SD) difference was found statistically significant between sampling sites for analysed water quality parameters ($P < 0.05$) refered from (table 1). Regarding mean diference between Lakes, Lake Batu was observed with significantly higher ammonia, nitrite and silica ($P < 0.05$) than Lake Hawassa (table 2). This may be due to increased large scale agricultural activities (sher Ethiopia floriculture) and from surrounding sm all-scale agricultural activities in and around Batu releasing fertilizer together with other agricultural wastes into Lake water system. There was buffer zone in sher Ethiopia flouriculture but it was very close to lake around 100 feeets (personal observation) and this maight increase chance of waste water discharge in to the Lake with out adequate buffering. Both small scale and large-scale floriculture activities around the Lake has increased the demand for fertilizers due to the year-round production of flowers. However, the crops do not absorb all applied fertilizers and excess fertilizers together with other agricultural wastes runoff into Lake water system. Instead, the residue of these fertilizers and wastes may cause deterioration of Lake water from increased nitrate, nitrite and ammonia concentration. Related finding to present finding was reported by Mahmoud, (2002); Abdel-Satar *et al*., (2010) and Gaber (2013).

We expected higher TDS and alkalinity in Lake Hawassa than Lake Batu because of industrial discharges in to the Lake; but this was not the case. This could be because the industrial park contains a waste treatment facility, which may have decreased the quantity of TDS entering the Lake. Additionally, some alkaline pollutants in the Lake could likely neutralize the organic load.

Regarding effulent and reference sites; ammonia , nitrite, silicate, TDS and alkalinity were found significantly higher for effulent site compared to reference site ($P < 0.05$) (table 3). This could be as a result of inputs from the Tikur Wuha River and effluents from the Hawassa Industrial Park having an effect on the water quality of Lake

Hawassa. For Lake Batu mean values of all water quality parameters were found significantly higher for Lake Batu effulent site compared to Lake Batu reference site ($P < 0.05$) (table 3). This may be because sher Ethiopia flouriculture had deteriorated the water quality of lake batu at this site than reference site. Sher Ethiopia flouriculture effulent discharge which maight contain excess fertilizers and pesticides released to the lake after certain buffering sytem. There was buffer zone in sher Ethiopia flouriculture but it was very close to lake around 100 feeets (personal observation) and this might increase probability of agricultural waste discharge with out adequate filtering. Evaluation of morphological lesions is also a useful tool for assessing the impact of chemical contaminants on the health status of fish (Capkin *et al*., 2009; Velmurugan *et al.*,2009).

Regarding difference in gross pathologic gill, skin and liver lesions by site; Statistically significant difference was existed for gill and skin between Lake Hawassa and Lake Batu. Fish inhabiting Lake Batu were 2.46 times more likely to develop gross pathologic lesions in gill ($P = 0.001$) and 1.8 times likely to develop gross pathologic lesions in skin ($P = 0.036$) than fish inhabiting Lake Hawassa. Existed difference for gill and skin was in parallel with water quality findings because; mean consentrations of ammonia, nitrite and silicate were found significantly higher ($P < 0.005$) for Lake Batu compared to Lake Hawassa (table 1).

Regarding effulent and refrence sites; fish inhabiting effulent sites were 2.95 times mor likely to develop gross gill lesions ($P = 0.002$); 4.67 times more likely to develop gross liver lesions ($P = 0.001$) and 2.98 times more likely to develop gross skin lesions ($P = 0.029$) in Lake Batu effulent site compared to Lake Batu reference site. This may be because of more detriorated lake water and cumulative effect of elevated water quality parameters had impacted on fish health at Lake Batu effulent site; hence mean concentrations of ammonia, nitrite, nitrate, silicates, TDS and total alkalinity were found significantly higher for effulent site than reference sites ($P < 0.05$) (table 3). This may be the case; because fish in this area may have been impacted by the effluent from the Sher Ethiopian flouriculture, which may have extra fertilizers and pesticides that were released into the lake after a particular buffering system.

Regarding effulent and refrence sites of Lake Hawassa; fish inhabiting effulent site were 2.9 times more likely to develop gross gill lesions ($P= 0.023$) and 2.98 times more likely to develop gross skin lesions (P = 0.001) in effulent sites compared to reference sites. The difference may have been seen because; fish inhabiting effulent site of the Lake Hawassa were more affected by significantly higher levels of ammonia, nitrite, TDS, alkalinity and silica (P < 0.05) (table 3).

Both for Lake Hawassa and Lake Batu effulent sites were found more likely to have gross pathologic lesions compared to effulent sites and this showed us detected lesions were inparallel or directly proportional to changes in water quality.

Regarding types of observed gross lesions in gill; pale discolouration of gill was indication of anaemia may be due to decreased dissolved oxygen. this finding was related with findings of Antychowicz and Matras (2008) who found colour change as the first pathologic lesion associated with water pollution. Gill congestion observed was an attempt to increase blood flow to damaged gills in order to improve oxygen uptake and supply to internal organs. This finding was related findings of Doherty *et al*. (2013). Gill congestion and excessive mucus production observed in this study were consistent with findings of Antychowicz and Matras (2008), Yacoub *et al.* (2008), Ibrahim *et al.* (2009) and Zaki *et al.* (2020) who reported similar findings in gills of tilapia fish inhabiting polluted water.

Pallor gill, increased mucus production and hemmorhage immediately after capture observed in this study were related to findings of Lease *et al.* (2003), Svobodova *et al*. (2005), Capkin *et al*. (2009) who reported the same finding associated with nitrite poisoning and irritation of the gills by toxins. Excessive ammonia mentioned to induce mucus production (Roberts, 2001; Abbas, 2006). Necrosis was indicated the gills prolonged or chronic exposure to irritants. The current finding agrees with findings of Rodger (2007). Necrosis is the final stage. In the most advanced cases, necrosis may result in complete atrophy of the soft tissue covering the gill filaments and, consequently, in uncovering of the cartilaginous elements (Strzyzewska *et al*., 2016).

Post mortem findings of liver such as pale to yellowish discolouration, enlarged liver and necrosis or mottled appearance of liver observed in this study were also found by EI-Ghamdi, *et al.(*2014) and Blazer *et al*. (2018).

Normally fish skin is hydrated, unkeratinized and totally covered by a layer of slimy mucus and due this nature fish skin is very susceptible to waterborne chemicals (Eleyele *et al*., 2017). Findings of Kaur and Dua (2012) strongly suggest that fish scale loss can be successfully employed as indicators of wastewater pollution. scale loss was found frequently observed gross lesion in present study and was related finding was reported in Nile tilapia fish inhabititng polluted lake (Ahmed *et al.,* 2007 and Saad *et al.,* 2022). Dark and necrosis discoloration of skin found in present study was related with findings of Antychowicz and Matras (2008) and sun *et al*.(2009).

The presence and severity of the microscopic lesions in each gill, skin and liver described according to Poleksic and Mitrovic-Tutundzic(1994). In present study, observed microscopic lesions were graded in to stage I, stage II and stage III.

Regarding severity and incidence of microscopic lesions; Lamellar necrosis was stage III lesions found frequent in Lake Batu effulent site, less frequent in Lake Hawassa effulent site and abscent for reference sites of both Lakes. Observed difference for stage III lesions indicated as fish in Lake Batu effulent site was threatened from sever and irreparable damages. Observed difference was in parallel with water quality finding; ammonia, nitrite and silica greater for for Lake Batu effluent site than others ($P < 0.05$) (tabel 1). Related finding to present study were reported by Yacoub *et al.(*2008), Ibrahim *et al.(*2009), Mohammed (2009), Abou ElGheit *et al. (*2012), Radh akrishnan and Hemalatha (2010) who revealed necrosis and degeneration in gill due t o increased ammonia in water polluted by sewage and agricultural discharge in lake Qarun.

Lamellar hemorrhages, aneurysm and lamellar fusion were stage II lesions. This lesions were observed less frequent to frequent for effulent sites of Lake Batu and Lake Hawassa. Where as this lesions were found rare and abscent for reference sites of both Lakes. This finding was indicated; as lesions were attributable to differences in levels of analysedparameters. This may be due to less affected and relatively good water quality of reference sites compared to effluent site (tabel 3). Occurrence of stage II lesion in reference site of lake batu indicates us overwhelming and mixing effect of the lake water as it was shallow lake.

Observed stage II gill microscopic lesions were the fastest and easiest adaptations to low water quality, and have the purpose of decreasing the respiratory surface and increasing diffusion, and result from exposure to a number of inorganic and organic pollutants (Fanta *et al.,* 2013). Haemorrhage and secondary lamellar fusion(stage II) lesions in this study were related to findings of (Lease *et al.*, 2003, Benli *et al.*, 2008 and van Dyk *et al.*, 2009) who reported similar alterations in ammonia induced nile tilapia (*Oreochromis niloticus*) fish from polluted streams.

Lymphocytic infiltration, hyperplastic secondary lamellae and goblet cell hyperplasia observed in gill were stage I lesions. This lesions were observed less frequent to frequent in effulent sites of both lakes where as; rare to less frequent in reference sites of both lakes. Observed stage I lesions in all sampling sites with different incidence has indicated as study lakes were more or less impacted by anthropogenic activities with relatively reference sites found minimally disturbed. In addition hyperplasia in present study was fastest and easiest adaptative response to low water quality and similar finding was reported by (Velasco-Santamaria and Cruz-Casallas, 2008). Hyperplasia of secondary lamella in chronic exposure of fish to gill irritants such as ammonia and nitrite was also reported by (Cengiz, 2006).

Multifocal hepatic necrosis and biliary epithelium necrosis were stage III liver lesions and this lesions were found frequent in Lake Batu effulent site; rare in Lake Hawassa effulent site and not found for specimens from reference sites. Observed multifocal hepato-necrosis only in batu effulent sites may be related higher levels of analysed parameters from ammonia, nitrite and TDS ($P < 0.05$) (table 1). This was in agreement with Authman and Abbas (2007) who stated detoxical role of endogenous waste products as well as externally derived toxins by liver. The cellular necrosis in the liver may be also due to oxygen deficiency because of the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2009).

Deteriorated water quality from high levels of ammonia, nitrite, silica, TDS and total alkalinity ($P < 0.05$) as detailed in (table 1) might be probable causes for existed difference in stages of lesion between sampling sites. Present finding was related to findings of Yacoub *et al.*(2021) who reported necrotic lesions in liver of fish inhabiting river Nile. Also related with findings of Osman *et al*. (2010) who reported hepatic necrosis in liver of fish from polluted streams. Atamanalp *et al.* (2008) and Velmurugan *et al.* (2007) had reported findings consistent to present liver findings.

Hydropic degeneration, haemorrhage, aneurism and hepatocellular and hepatobiliary adenoma in present study were stage II lesions which were found less frequent in effulent sites of both lakes and rare in reference sites. This was found in parallel with water quality as detailed in tabel 3. This maight be resulted in liver because of high detoxical role of externally derived chemicals. Similar findings were also observed by Malik (2020).

In present study observed stage I lesions of liver were biliary hyperplasia, hemosiderin and lymphocytic infiltration. Lymphocytic infiltration was an inflammatory change found less frequent in effulent sites of both lakes. Hemosiderin were found rare and only observed in hawassa effulent site and abscent for other sites. Accumulation of hemosiderin in liver cells may be due to rapid and continuous destruction of erythrocytes as stated by Ibrahim and Mahmoud (2005). All microscopic lesions observed in the liver indicated that those fishes were responding to the direct and indirect additive effects of contaminants.This would be because; liver was most associated with the detoxification and biotransformation process (Camargo and Martinez, 2007).

Epidermal necrosis was stage III skin lesions in this study. It was found frequent in Lake Batu effulent site. This lesion was not observed in reference sites of both lakes. Occurrence of stage III skin lesion in fish inhabiting Lake Batu effluent site was in support of water quality findings where by; means of most of chemical parameters were found significantly higher for Lake Batu effulent site (table 1). Aneyurism observed in this study is stage II lesion and was found rare to less frequent for effulent sites of both lakes. Lymphocytic infiltration observed in this study was stage I lesion. Lymphocytic infiltration was found less frequent for lake batu effluent site and rare for fish inhabiting lake hawassa effulent site and absent for other sites. Related findings were also bserved in fish from lake with detriorated lake water (Mahmoud, 2002; Saad *et al.,* 2022; Abou-El-Gheit *et al.,* 2012).

6. CONCLUSION AND RECOMMENDATIONS

In present study; mean values for water quality parameters were found significantly different and higher for effulent sites than reference sites. Higher ammonia, nitrite, TDS and total alkalinity at effulent sites looks created a suboptimal fish environment that is stressful for the fish in both lakes based on the lesions characterized. The higher frequency of gross and microscopic lesions some of which were irreversible or irreparable at effulent sites of both lakes; were inparallel with observed water quality. The majority of stage III & stage II lesions were probably induced by contamination of lake waters in effulent sites. Based on frequency of lesion occurrences it also looks that lake Batu is more contaminated than lake Hawassa.

Based on the above conclusion; the following recommendations are forwarded.

- The causal relationship of lesion and water contamination should be studied using fishes without pre-existing lesion and with detailed analys of water quality.
- Further detailed studies comprisisng of several sampling sites of the lake should be conducted to get more evidences on pathological impacts of various kinds of pollutants
- Microscopic characterizations of fish should be used as fundamental and biomonitoring tool to asses lake pollution.
- Responsible bodies should regularly assess and protect the lake water from contamination. Waste releasing industries, large scale flouriculture activities and small scale small agricultural activities should have to develop wastewater treatment plants and should not directly release wastes to lakes.

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8. APPENDICES

Appendix 1. Convenience interview questions for sampling site selection

1. Do you know what lake water pollution is ? A. Yes B. No

2. Do you think water pollution has negative impact on fish? A. Yes B. No

3. What do you feel about the present condition of pollution in Study Lake?

A. Better B. Good C. B. Poor

4. Are you aware of water pollution in this Lake area? A. Yes B. No

5. Which side of the Lake do you think are mostly impacted and immediate to pollution

Mention ? ---

6. Which side of the lake do you think is relatively less disturbed by pollution?

Mention it--

7. Is there any point source of waste discharge to the this lake ? A. Yes B. NO

8. If ye above mention it --

Appendix 2. Data collection sheets

A. Gross examination sheet

No.	Date	Sample code	Lake	Site	Species of Fish	Gross appearance & description of lesions	Tissue Sampled for histopathology	Remark
1								
2								

B. Histopathologic lesion record sheet

No.	Date	Sample code (code given on gross tissue sample container)	Description of histopathological findings	Remark
1				
2				

Appendix 3. Histopathological Technique Procedures (Takulder, 2007)

1. Preparation of formalin (10%) Buffered neutral formalin
   - ✓ 37-40 % formalin 1000ml
   - ✓ Distilled Water 900 ml
   - ✓ Sodium phosphate monobasic 4.0 gm
   - ✓ Sodium phosphate monobasic 6.5 gm
2. Trimming of tissue → tissue samples were trimmed and put into plastic tissue casset and processed using automatic tissue processor.
3. Fixation of tissue by 10% neutral buffered formaldehyde
4. Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).
5. Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohol concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.
6. Fixation by [Formalin-I 2hr →Formalin-II 2hr] --→ dehydrating by [70% Alcohol 1hr 95% Alcohol 1hr → 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs →100% Alcohol-III 2hrs] →clearing by [Xylene-I 1:30hrs →Xylene-II 1:30hrs →Xylene-III 1:30hrs] → impregnation with [Paraffin wax-I 2hrs →Paraffin wax-II 3hrs].
7. Embedding/Blocking of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60^{o}c) is poured and allowed to settle and solidify.
8. Sectioning: sectioning of tissue in 3-5 micron thickness and put on warm water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later
9. Label and put the slide in incubator at 60^{o}c overnight

Appendix 4.. Hematoxyline and Eosine stain Procedures (Talukder, 2007)

- Staining Procedure:

1. Deparaffinize slides in 3 changes of xylene (xylene-I, xylene-II and xylene-III) for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxylin for 8 - 15 minutes.
4. Rinse in tap water until water runs clear.
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.
6. Rinse in tap water until ripples disappear from slides.
7. Dip in bluing agent, 3 - 5 long dips. 8. Wash in lake-warm tap water for 5 minutes (37-40°C.)
8. Stain in Eosin for 30 seconds - 2 minutes.
9. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
10. Clear in 3 changes of xylene for 2 minutes each.
11. Mount cover glass with Canada balsam
13. Examination of the prepared slides under microscope at low to high magnification power (4x, 10x, 40x and 100x) and finally the photomicrographs taken for documentation of every histopathological lesions

Appendix 5. Image for effulent released from sher Ethiopia flouriculture.

- Effulents discharged from sher Ethiopia flouriculture to Lake Batu.

Sher Ethiopia flouriculture(long arrow) and effulent discharges from flouriculture(short arrow), lake is immediate opposite to this(thin arrow)

Appendix 6. Ethical certificate


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ADDIS ABABA UNIVERSITY
College of Veterinary Medicine
and Agriculture
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Animal Research Ethical Review Committee

Ethical clearance certificate

Certificate Ref. No: VM/ERC/20/03/15/2023

Name and affiliation of applicant: **Assaye Desta (DVM, MSc student)**
Department of Pathology and Parasitology, College of Veterinary Medicine and Agriculture, Addis Ababa University

Title of the project: *Comparative evaluation on pathological changes in fish collected from different effluent sites of Lake Hawassa and Lake Batu, Ethiopia*

Date of application: **December, 2022**
Nature of the project: **Filed investigation**
Target animal species: **Fish**
Number of animals involved: **400**
Study area: **Hawassa and Batu, Ethiopia**

Minutes No. and date of review: **VM/ERC/03/15/022, 25/01/2023**

The Animal Research Ethical Review Committee of the College of Veterinary Medicine and Agriculture of Addis Ababa University has reviewed the above research project and unanimously approved the application of Assaye Desta.

Professor Getachew Terefe (DVM, PhD)
Chairman

Signature

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