



**BACTERIAL PATHOGENS OF NILE TILAPIA FISH, *Oreochromis niloticus*
(LINNAEUS, 1758) WITH SPECIAL REFERENCE TO *Aeromonas hydrophila* IN
LAKE HAWASSA, ETHIOPIA AND MODULATION OF INFECTION USING
DIEATERY SUPPLIMENTATION WITH GINGER (*Zingiber Officinale*)**

PhD DISSERTATION

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HAWASSA UNIVERSITY, HAWASSA, ETHIOPIA

MARCH, 2024

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**A DISSERTATION SUBMITTED TO THE
DEPARTMENT OF AQUATIC SCIENCES, FISHERIES AND
AQUACULTURE,
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DEGREE OF
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(SPECIALIZATION: AQUATIC SCIENCES, FISHERIES AND AQUACULTURE)**

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DEDICATION

This dissertation is dedicated to my late mother **Mrs. Zena Shanko** and father **Mr. Ergena Keshamo**. I also dedicate this dissertation to my beloved wife, **Selam Pawulos** who encouraged me to advance my career and took full responsibility of caring for our children (Drusila Alazar and Obed Alazar) while I was doing my PhD.

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LISTS OF ACRONYMS AND ABBREVIATIONS

AEF- American Water Environmental Federation

AFS-American Fisheries Society

AG- Amora Gedel

ANOVA- Analysis of Variance

APHA- American Public Health Association

AWWA-American Water Works Association

BOD5- Biochemical Oxygen Demand

CFU- Colony Forming Unit

CLSI- Clinical and Laboratory Standards Institute

CRD- Completely Randomized Design

DO-Dissolved Oxygen

EC-Electrical Conductivity

EPA- Environmental Protection Agency

ESR- Erythrocyte Sedimentation Rate

FAO- Food and Agriculture Organization

FC-Faecal Coliform

FDA- Food and Drug Administration

FH -Fikr Hayiq

fl- Femtoliters

GW -Green Wood

Hb- Haemoglobin

HBC-Heterotrophic Bacteria Count

Hct- Haematocrit

KW- Kuyu Wata

LFDP- Lake Fisheries Development Project

LD50- Lethal Dose 50%

MAAIF- Ministry of Agriculture, Animal Industry and Fisheries

MAS- Motile Aeromonas Septicemia

MCV- Mean Corpuscular Volume

MCH- Mean Corpuscular Haemoglobin

MCHC - Mean Corpuscular Haemoglobin Concentration

μl – Micro liter

μS - Micro Siemens

NH_4^+ -Ammonium ion

NO_3^- – Nitrate

NO_2^- - Nitrite

NA-Nutrient Agar

NTUs-Nephelometric Turbidity Unit

PCV-Packed Cell Volume

PO_4^{-3} - Phosphate

RH- Referral Hospital

RBC- Red Blood Cell

SD Standard Deviation

SNNPR- South Nations Nationality and Peoples Region

SSA; Shigella-Salmonella Agar

TW- Tikur Wuhan

TSA- Tryptic Soy Agar

TSA- Thiosulphate Citrate Bile Salt Sucrose Agar

TC- Total Coliform

TDS -Total Dissolved Substances

Temp.-Temperature

Turb.-Turbidity

WB- World Bank

WBC-White Blood Cell

WHO- World Health Organization

WWF- World Wide Fund

WFP-World Food Programme

WWAP- World Water Assessment Programme

XLD- Xylose lysinedeoxycholate

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ABSTRACT

Fish is one of the known aquatic animals which serve as food for human beings. However, fish are constantly exposed to diseases and usually succumb to infections after being exposed to stress. Environmental factors such as variations in the physico-chemical parameters of water act as stressors and can predispose fish to diseases. This study was thus undertaken to evaluate the physico-chemical and bacteriological quality of Lake Hawassa, characterize bacterial pathogens with emphasis on *Aeromonas hydrophila* of *Oreochromis niloticus*, and assess the effect of Ginger (*Zingiber officinale*) powder on *Aeromonas hydrophila* infection. Seventy-two water samples and 360 fish samples were collected from Lake Hawassa for 12 months from January 2021 to December 2021. The physico-chemical and bacteriological quality of the water, isolation, characterization and identification of bacterial pathogens of fish and, haematological parameters of fish were assessed following standard procedures. To test the pathogenicity of *Aeromonas hydrophila* and their effects on haematology of fish, 270 fish were collected from the ponds located at Hawassa University and transported to the laboratory and fed a basal diet. After two weeks of acclimatization, the fish were divided into one control and eight treatments each in triplicate and injected with different concentrations of *Aeromonas hydrophila* (1×10^1 to 1×10^8 CFU/ml). To determine the effect of Ginger powder on the haematology of *Oreochromis niloticus* and *Aeromonas hydrophila* infection 300 fish were collected from the Fish ponds located in Hawassa University and transported to laboratory and fed a basal diet during two weeks of acclimatization. The fish were distributed into one control and four treatment groups and fed with different concentrations of Ginger powder (0.00, 1, 3, 5, 8 and 12 g ginger/kg) for eight weeks. At the end of eight weeks, the fish were injected with 0.2ml of *Aeromonas hydrophila* at the concentration of 1×10^7 CFU/ml. Blood samples were collected from the fish before and after the *Aeromonas hydrophila* injection. The study showed a significant variation in water quality parameters

among sites and seasons ($P < 0.05$). Seventy-two point seven percent (72.7%) of physico-chemical parameters of Lake Hawassa were not found within the critical limits for aquatic life, particularly fish. The study also indicated high bacterial loads in water samples of the lake. Sixty-four percent (64%) prevalence of bacterial infection was recorded for fish in the lake. The prevalence of infection in female fish was higher than in male fish. The highest prevalence was recorded within the smaller fish group (12-18 cm) than the larger ones (24-29 cm). Significantly high bacterial loads were recorded from the intestine of fish during the summer (wet) season ($P < 0.05$). Bacterial isolates such as *Aeromonas hydrophila*, *Escherichia coli*, *Edwardiella tarda*, *Vibrio*, *Bacillus*, *Staphylococcus*, *Salmonella*, *Flavobacterium*, *Pseudomonas* and *Shigella* species were isolated from *Oreochromis niloticus* of Lake Hawassa. A prevalence of 17.7% of *Aeromonas hydrophila* was recorded in Nile tilapia. All *Aeromonas* isolates were 100% resistance to amoxicillin, ampicillin and penicillin. The majority of *Aeromonas* isolates showed multiple antibiotic resistances. The value of red blood cells (RBCs), haemoglobin (Hb) and haematocrit (Hct) was significantly decreased ($P < 0.05$) in the blood of *Oreochromis niloticus* collected from more polluted sites of the lake compared to relatively less polluted site. In contrast, erythrocyte sedimentation rate (ESR) and white blood cells (WBCs) count showed significant ($P < 0.05$) elevation for more polluted sites than less polluted site. RBCs count, Hb, Hct and WBCs count showed a significant rising trend in their values with increasing size of the fish ($P < 0.05$). The male fish have significantly higher concentrations of haematological parameters, such as RBCs, Hb and Hct than the females ($P < 0.01$). Haematological parameters of infected fish were significantly different from control fish ($P < 0.01$). The haematological parameters of the fish such as RBC, Hct, Hb, MCH, MCHC and WBC were significantly increased to all concentrations of the Ginger powder-supplemented diet when compared to fish fed the control diet. There was no mortality of *Oreochromis niloticus* before injection

with *Aeromonas hydrophila*. Mortality in experimental fish was observed after 3 days of the bacterial challenge. The highest mortality rate (70%) was recorded for the control group followed by the 3 g/kg and 12 g/kg Ginger powder-supplemented diet. Supplementation of Ginger powder at the concentration of 5 g/kg diet provided better protection to *Oreochromis niloticus* against *Aeromonas hydrophila* infection. There is an urgent need for continuous monitoring of Lake Hawassa to detect changes in the water quality. The recovery of potentially pathogenic bacteria of humans, in the fish, suggests that if the fish are improperly handled, undercooked, or consumed raw may cause diseases in susceptible consumers. The use of 5 g/kg dietary Ginger powder diet is recommended for the control of *Aeromonas hydrophila* infection in Nile tilapia, *Oreochromis niloticus*.

Key words: *Aeromonas hydrophila*, haematology, Lake Hawassa, *Oreochromis niloticus*, prevalence, water quality, *Zingiber officinale*

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background of the study

Fish are highly diversified aquatic animals that provide food to the world population. They are highly nutritious, so even small quantities can improve people's diets (FAO, 2020). Moreover, fisheries provide employment and generate income for millions of people living in underdeveloped and developing countries (FAO, 2018).

Fish can be obtained come from capture fishery and culture fishery. Capture fishery is the method of obtaining fish from natural resources with the help of fishing crafts and gears while culture fishery is the method of rearing the desired varieties of fish in specific water bodies and harvested according to the need. Total fisheries and aquaculture production reached a record 214 million tonnes in 2020, comprising 178 million tonnes of aquatic animals (FAO, 2022). Of the total production, 63% was harvested from marine waters with 70% originating from capture fisheries and 30% coming from aquaculture. On the other hand, 37% of the total production came from inland waters with 83% coming from aquaculture and 17% coming from capture fisheries (FAO, 2022). However, fishery resources continue to decline due massive changes in land use, pollution of waters with effluent, and the spread of disease (Toni *et al.*, 2017) and also fish health has become a top concern for consumers (Zhou *et al.*, 2020).

With the progress of fish culture and the advancement of science and technology, there is an increasing demand for the information on diseases of fish and their control. It is in this context that, the main focus should be to identify the factors that limit fish production, and attention should be given to solve such constraints. Although several factors have been identified as factors responsible to limit fish production, diseases of aquatic organisms have been identified as the primary constraint to the growth of many aquaculture species, and are

now responsible for severely impeding both economic and socio-economic development in many countries of the world (Behera *et al.*, 2018). Thus, addressing fish health issues becomes the need of the hour and primary requirement to sustain fish production. The major factors include water quality deterioration and onset of infections in fish which often resulted in production loss.

The physical, chemical and biological factors of water will serve as an environmental indicator of water quality resources. These factors either individually or collectively influence fish health. The physical stressors that influence water quality and fish health include temperature, color, turbidity, total dissolved solids, electrical conductivity and light; the chemical stressors are dissolved oxygen, carbon dioxide, pH and total alkalinity, ammonia, nitrite, hydrogen sulfide, phosphate, while the biological factors include feeds and feeding, fish density, pathogens and parasites (Spellman, 2017).

Water quality deterioration in aquatic ecosystem is mainly due to human induced factors such as effluent discharge from industries, agricultural operation, influx of sewage from domestic and municipal which often produces stress affecting the physiological activities and survival of fish species (Ekubo and Abowel, 2011; Bhat *et al.*, 2017). The outstanding changes that occur in water bodies due to anthropogenic disturbances include eutrophication, increase in mineralization and turbidity, acidification and accumulation of toxic materials (Vatukuru, 2005; Mateo-Sagasta *et al.*, 2017). The reports also indicate that these disturbances severely alter the physico-chemical and biological characteristics of water bodies that fish species living in such stressful environment (Huicab-Pech *et al.*, 2016).

Most often fish species exposed to environmental stress are subjected to infection by diverse groups of parasites, pathogens and diseases of different kinds. When the fish are stressed by adverse or subnormal environmental conditions, the saprophytic microorganisms on the skin

and gills and in the alimentary tract may become pathogenic (Brown and Gratzek, 1980; Baumgartner *et al.*, 2017). Stress is known to reduce the immunity of fish and increases susceptibility to parasite and microbial infection.

Diseases of fish constitute one of the most important problems in fisheries and aquaculture. Significant changes in disease prevalence are ecologically relevant warning sign, since diseases may affect growth, reproduction and survival of infected individuals and may therefore have implication at the population level with possible implication for fisheries. Austin and Austin (1993) suggested that, several biological agents including bacteria, fungi, viruses and protozoa parasites have been implicated to cause disease in wild and cultured fish population. Protozoa damage and reduced growth of the host fish favoring secondary bacterial infections (XU *et al.*, 2012) and mortality, all leading to constraints in global fish production. As aquatic ecosystems are conducive to parasitic organisms to live and reproduce, the fish that live in such ecosystems harbor different types of parasites and pathogens (Baumgartner *et al.*, 2017). Reports also clearly demonstrate that factors such as age, sex, feeding behavior and migratory pattern of fish influence onset of infections in fish (Akoll and Mwanja, 2012). Further, host immunological processes, stage of development, genetic history, biochemical and haematological processes affect fish and produce diseases (Poulin and Morand, 2000).

Pathogenic bacteria are found in all aquatic environments and fish live in water bodies are constantly exposed to them (Sandeep *et al.*, 2016). Bacterial pathogens do affect fish species and produce diseases that cause mortality to fish, and incur severe economic loss to fishing and farming industry (Behera *et al.*, 2018). Bacterial responsible for disease outbreaks in different fish species are *Aeromonas*, *Edwardsiella*, *Vibrio*, *Pseudomonas*, *Vibrio*, *Streptococcus*, *Pseudomonas*, *Streptococcus*, *Salmonellas*, *Salmonellas* and *Shigella* species

among others (Menanteau-Ledouble *et al.*, 2016; Fadel *et al.*, 2018; Pandey *et al.*, 2021; Yang *et al.*, 2021; Colussi *et al.*, 2022).. These are all clearly spelled the need for controlling fish diseases due to bacterial infection.

Haematological changes in blood are important indicators used in monitoring status of fish health (Satheeskumar *et al.*, 2011). Haematological parameters are carried out on fish to ascertain the normal range of blood parameter in relation to size and sex, and to determine the effects of disease condition in fish (Svobodova *et al.*, 1996). Haematologic disorders are marked by aberrations in structure or function of the blood cells or the mechanisms of coagulation (Fazio *et al.*, 2019). Although many other diseases may be reflected by the blood and its constituents, the abnormalities of erythrocytes, leukocytes, thrombocytes, and clotting factors are considered primary blood disorders (Clauss *et al.*, 2008). Therefore analysis of haematological indices in the blood of fish is good for identifying the health status of the fish.

Different methods were employed to control bacterial infections, which include chemical and plant based drugs (Heuer *et al.*, 2009; Devi *et al.*, 2016). Antibiotics are chemicals used to control and treat fish diseases in aquaculture. However, the use of antibiotics has limitations such as microbial resistance to antibiotics and unacceptable residues in aquaculture products and the environment (Heuer *et al.*, 2009). Medicinal plants can be used as an alternative to antibiotic to prevent bacterial diseases of fish. They produce secondary metabolites which have antibacterial, antifungal, anti-inflammatory and antioxidant effects on fish (Nile and Park, 2015; Van Hai, 2015).

Bacterial diseases of fish have been reported by different authors from different lakes of Ethiopia (Eshetu Yimer 2000; Nuru Anwar, *et al.*, 2012; Abayneh Tekele *et al.*, 2017; Tesfaye Shimels *et al.*, 2018; Guta Dissasa *et al.*, 2022). Even though Abayneh Tekele *et al.* (2017) and Guta Dissasa *et al.* (2022) reported different bacterial species from fish of Lake

Hawassa, they did not include how to control bacterial infection. In this regard the present study focused on bacterial pathogens and their effects on *Oreochromis niloticus* in Lake Hawassa and to develop plant based drugs to control bacterial infection.

1.2. Statement of the problem

Food security is the major problem of Ethiopia. About 25% of the population which is equivalent to around 25 million people have food insecurity issues, 31% of the population are undernourished (WFP, 2020). Fisheries remain important sources of food, nutrition, income, and livelihoods for hundreds of millions of people around the world (FAO, 2016). However, there is huge loss of fish due to diseases. Bacterial diseases are common and where the studies have been conducted, *Aeromonas hydrophila* (*A. hydrophila*) infection is reported as one of the most devastating bacterial infections, accounting for millions of dollars losses in the global freshwater aquaculture sector (Hossain *et al.*, 2014; Sebastião *et al.*, 2015; Peterman and Posadas, 2019). However, there is limited information about the status of *A. hydrophila* infection in fish of Ethiopia, particularly in Lake Hawassa. The occurrence of this bacterial infection elsewhere has been influenced by poor water quality. Untreated or partially treated wastes from industries and urban sources are directly or indirectly discharged in to Lake Hawassa (Zinabu Gebre-Mariam and Zerihun Desta, 2002). This causes variation in the physical and chemical parameters of water which can greatly alter the normal physiology of fish and induce stress. Stress weakens fish immune systems, and increases susceptibility to infectious disease (Romero *et al.*, 2012). These can reduce fish production in the lake and make the fish unsuitable for human consumption. Therefore, there is a need to have closer investigation of the water quality of the lake and disease conditions of fish and their potential pathogens.

1.3. Significance of the study

Ethiopia is one of the developing countries where food security is not ensured yet and there is a need to increase fish production and provide healthy fish particularly among rural areas where there is high shortage of protein rich foods. Thus such studies on increasing the yield to meet the high demand of fish are important in the country. Understanding the epidemiology of the disease and characteristics of pathogenic bacteria would enable development of protection measures against the disease and sustain or accelerate fish production in the country. The output of these findings is also expected to give base line information on water quality of Lake Hawassa and bacterial disease of fish for further research.

1.4. Objectives

1.4.1. General objective

The general objective of this study was to isolate, characterize and identify potential bacterial pathogens from naturally infected Nile tilapia, *O. niloticus*, in Lake Hawassa and to assess the effect of ginger on control of bacterial infection.

1.4.2. Specific objectives

The specific objectives of the present study were to:

- assess water quality of Lake Hawassa,
- isolate, characterize and identify pathogenic bacteria of Nile tilapia, *O. niloticus* in Lake Hawassa.
- determine effect of water quality and *A. hydrophila* on the haematology of Nile tilapia, *O. niloticus*,
- evaluate effect of Ginger powder on the haematology of Nile tilapia, *O. niloticus* and in the control of *A. hydrophila* infection.

CHAPTER TWO

2. REVIEW OF LITERATURE

2.1. Water quality characteristics

Aquatic ecosystems, particularly the freshwater ecosystems, are exposed to supplementary contamination than other environments, as water is used in various industrial practices as well as release of discharges commencing from industry and urban growths (Fernandesa *et al.* 2007). Water pollution is a worldwide task that has augmented in both advanced and emerging nations (Mateo-Sagasta *et al.*, 2017). Universally, 80% of municipal wastewater is discharged into water bodies untreated, and industry is responsible for dumping millions of tons of heavy metals (Mateo-Sagasta *et al.*, 2017), solvents, toxic sludge and other wastes into water bodies each year (WWAP, 2017). Agriculture exploits 70% of water globally and plays a key part in water contamination. Huge amounts of agrochemicals, organic matter, drug residues, sediments, and saline drainage from agricultural lands are released into water bodies and hence pose significant threats to aquatic environments, human health and productive activities (UNEP, 2016; Mateo-Sagasta *et al.*, 2017).

Lakes are important reserves of available freshwater and are often controlled for provisioning environmental services, which include consuming water, irrigation, hydropower technology, and fisheries (La Notte *et al.*, 2017). However, lakes are the most poorly controlled useful resource of the world (Fakayode, 2005). Maximum of the sewage is discharged into lakes without treatment and is accountable for the pollutants of lakes (WWAP, 2017). The proportion of polluted water is constantly growing as a result of changes in the modes of industrial activities, agricultural production and growing urbanization (Pestle, 2000).

Expansion of agricultural activities in the Ethiopian Rift Valley has rapidly increased pressure on its natural resources (Ayenew Tenalem and Legesse Dagnachew, 2007). The conversion of large parts of the natural vegetation to cropland during the last decades and the sharp increase in inorganic fertilizer application to improve food security has resulted in enhanced soil erosion and increased nutrient run-off (Hengsdijk and Jansen, 2006). For instance, phosphate and nitrate concentrations of Lake Ziway have reportedly increased in recent years (Ayenew Tenalem and Legesse Dagnachew, 2007). Moreover, the expansion of small-scale farms and large-scale investment projects, mainly floriculture industries and irrigation farms, and the subsequent intensive and unintended applications of agrochemicals, untreated waste discharge, and poor watershed management practices are deteriorating the water quality of the lake (Hayal Desta *et al.*, 2017).

Intense algal blooms and the occurrence of cyanobacteria toxin (microcystin) have been reported in multiple Rift Valley Lakes, including in Koka, Chamo, Langanu and Ziway Lake. The microcystin concentration in Lake Koka is the highest reported in African lakes to date ($45\text{--}51\ \mu\text{g L}^{-1}$) and exceeded the levels that represent health risks for humans, livestock, and wildlife (Willén *et al.*, 2011).

Deforestation, which may occur due to the need for fuel wood, land for livestock production is another common threat to water quality of Rift Valley Lakes of Ethiopia. It leads to increased sedimentation or the filling of water bodies with sediment from surrounding areas which affect water quality (Kloos and Legesse Worku, 2011). Lake Hawassa has been exposed to environmental and ecological modifications due to population growth, urbanization and agricultural activities around the watershed of the lake (Abebe Yonas *et al.*, 2018). Based on the report on Lake Hawassa, COD (3 times higher), TDS (19 times higher); and PO_4^{3-} (39 times higher) concentrations of textile factory effluent are higher than

the maximum permissible limit (EPA, 2003). Also, PO_4^{3-} (1.31 mg/L), and BOD5 (68.7 mg/L) exceeded WHO standard in lake Hawassa (Zigde Haile and Tsegaye Malaku, 2019). Moreover, the municipal effluents can affect fish stocks in Lake Hawassa (Fetahia Tadesse and Mengistu Seyoum, 2007).

Heavy metals are also another cause for low water quality of Ethiopian Rift valley Lakes. As heavy metals cannot be degraded, they are continuously being deposited and incorporated in water, thus causing heavy metal pollution in water bodies. According to Samuel Bekele *et al.* (2020a) soils with in the Hawassa Industrial Zone are highly contaminated with heavy metals and further downstream in to Lake Hawassa. Samuel Bekele *et al.* (2020b) reported the presence of heavy metals such as Zn, Fe, Se, Cu, As, Hg, Cr, Ni, Pb and Co in the muscle tissues of fish from Lake Hawassa. Earlier, Dsikowitzky *et al.* (2013) reported heavy metals such as Cd, Hg, Cr, Pb, Se and As from Lake Hawassa and Lake Koka. Tigst Ashagre *et al.* (2014) also reported heavy metals such as Hg, Cr, Zn Cd and Pb from Lake Hawassa. Zerihun Desta *et al.* (2006) noted that African big barb *Labeobarbus intermedius* whose catch is mainly from the Rift Valley Lakes like Lake Hawassa and Lake Koka is found to be unsafe for human consumption due to its high mercury concentration, and the authors are of the opinion that higher Hg burdens of these heavy metals significantly cause neurotoxin affecting human health, and this is a result of poor water quality caused by the effluents of textile, ceramic and soft drink factories near to Lake Hawassa.

Water quality of lake can be evaluated by applying the methods, such as Physical assessment, Chemical assessment and Bacteriological assessment. The Physical assessment of water is carried out by studying physical parameters like Turbidity, Total dissolved solids (TDS), Electric Conductivity and Temperature. The chemical assessment is carried out by studying the presence of chemical pollutants in the water bodies, following chemical parameters such

as pH, dissolved oxygen (DO), Nitrate (NO_3^-), Nitrite (NO_2^-) and Phosphate (PO_4^{3-}) (Fouzia and Amir, 2013). Moreover, the bacteriological assessment of any water body is highly essential because of the presence of pathogenic bacteria in water which can create several water-borne diseases in both humans and animals. The bacteriological assessment of water includes mainly faecal coliform and total coliform.

Poor water quality places stress on fish which will increase their vulnerability to diseases (Romero *et al.*, 2012). Furthermore, disease outbreaks substantially reduce production potential, increase farm inputs from treating illnesses, and render fish unsuitable for human consumption (Akoll and Mwanja, 2012).

2.2. Microbial diseases

A disease can be infectious or non-infectious. Pathogenic microorganisms cause diseases to cultivable species of fish causing fish kill in many occasions. Several factors influence the intensity of infections by pathogenic organisms besides this; diverse environmental parameters particularly environmental contaminants reduce host resistance to pathogens (Teffer *et al.*, 2019). Infectious diseases have already been implicated in the decline of some wild fish populations (Steinbach *et al.* 2017).

The risk of pathogen exposure and disease is especially pronounced in aquatic systems (Sterk *et al.*, 2013) where animals are continually exposed to potential pathogens. The sources and modes of infection among fish are variable, as fish disease is a simple association between pathogen, a host fish and an environmental problem. Other stressors, such as poor water quality often contribute to the outbreak of disease and the complexity of the challenge. Snieszko (1973) indicated that in most cases an infectious disease outbreak occurs when a virulent pathogen, a susceptible host, and an unfavourable environment are all concurrently present (Figure 2.1). Often, an infectious disease can be avoided if one of these components is

missing. However, even under the best conditions, a highly virulent pathogen may cause disease in a susceptible host.

While many pathogens are either normal inhabitants in or on fish or saprophytes, majority of infections are stress related (OlivaTeles, 2012). The transmission of infection to fish occurs through direct and indirect exposure of fish to pathogens. In the wild, fish are chronically exposed to a heterogeneous composition of infectious agents and are rarely burdened with a single pathogen at a given time, which can directly mediate host–pathogen dynamics (Hellard *et al.*, 2015). For infection to occur a fish, fish must be exposed to pathogen for a sufficient amount of time (Scott and Smith, 1994). For secondary infections to occur, susceptible hosts must come into close contact with infected fish for transmission to occur (Scott and Smith, 1994). The numerous disease agents, their modes of transmission, and the number of different species of fish affected by them make difficult to assess the impact of the disease in wild fish. The occurrence and severity of disease is dependent on the status of the host and the pathogen which are affected by a number of factors including their environment. Fish populations face extraordinary threats to their health and survival from climate alternate, water shortages, habitat alteration, invasive species, and environmental contaminants. These environmental stressors can immediately affect the prevalence and severity of disease in fish populations (Akoll and Mwanja, 2012).

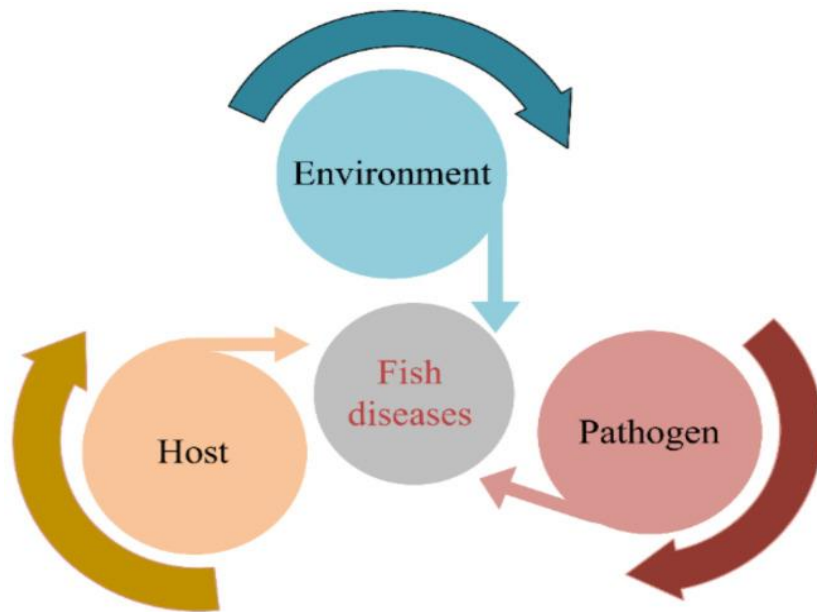


Figure 2.1: Host, Pathogen and Environment Interaction

Fish are exposed to local and worldwide environmental stressors mainly adverse chemical conditions of the water. Although pollutants are common environmental stressors, extreme conditions or changes in water quality parameters such as dissolved oxygen, ammonia, hardness, pH, gas content and partial pressures, temperature etc. can unduly stress fish (Hamed *et al.*, 2018). Exposure of fish to stressors may result in a series of biochemical and physiological modifications and plays a key function within the outbreaks of fish disorder because of opportunistic micro-organism (OlivaTeles,2012), which are abundant in aquatic environments (Buller, 2014). A stressed fish population is vulnerable to pathogen either from the surroundings or a carrier fish and in the long run succumbs to the infection (Romero *et al.*, 2012). The percentage of infectious disease outbreaks of fish caused by major causative agents is presented in Figure 2.2.

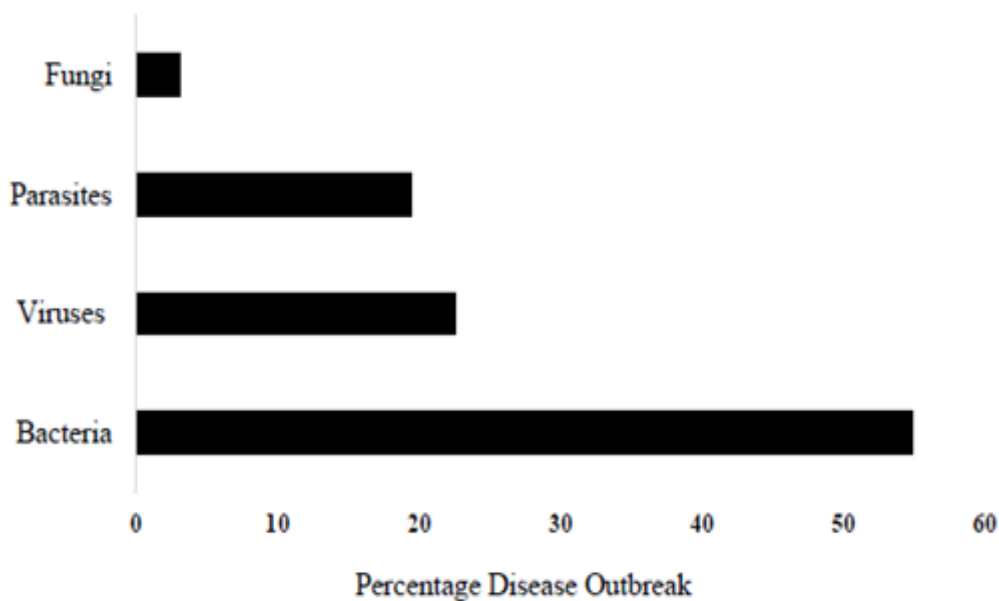


Figure 2.2: Percentage of infectious disease outbreaks in fish caused by different causative agents (Dhar *et al.*, 2014)

2.2.1. Bacterial pathogens

Bacterial diseases caused by bacterial pathogens are one of the important diseases both in capture and culture fisheries affecting the fish in the wild and in captivity (Elgendy *et al.*, 2021). Bacteria are usual pathogens of fish, and when outbreaks occur, they cause great losses to aquaculture in addition to mortalities in wild populations of fish (Buller, 2004). While some represent primary pathogens, many are opportunistic pathogens. Opportunistic bacteria under unfavorable environmental conditions or when fishes are injured or under malnutrition, become pathogenic and cause diseases to fish populations (Austin and Austin, 2007). With the increased interest in aquaculture, there has been a steady increase in the study of bacterial diseases in fish and as a result, several new bacterial pathogens have been described in the literature (Austin and Austin, 2007). The most common bacterial species from farmed and wild fish are listed in Table 2.1.

Table 2.1: Common bacterial pathogens of fishes

Agents	Disease	Host Fish Targets	References
<i>A. hydrophila</i>	Motile Aeromonas septicemia (MAS), hemorrhagic septicemia, red-sore disease, ulcer disease, epizootic ulcerative syndrome (EUS)	Tilapia, catfish, striped salmonid, non-salmonid fish, sturgeon, bass and eel	Menanteau-Ledouble <i>et al.</i> , 2016
<i>Aeromonas salmonicida</i>	Furunculosis	Trout, salmon, goldfish, koi carp, and a wide range of fish species	Alghabshi <i>et al.</i> , 2018
<i>Edwardsiella ictaluri</i>	Enteric septicaemia	Catfish and tilapia	Dong <i>et al.</i> , 2019
<i>Edwardsiella tarda</i>	Edwardsiellosis	Salmon, carps, tilapia, catfish, striped bass, flounder and yellowtail	Pandey <i>et al.</i> , 2021
<i>Yersinia ruckeri</i>	Enteric redmouth	Salmonids, eel, minnows, sturgeon and crustaceans	Jozwick <i>et al.</i> , 2019
<i>Piscirickettsia salmonis</i>	Piscirickettsiosis	Salmonids	Jozwick <i>et al.</i> , 2019
<i>Flavobacterium psychrophilum</i>	Coldwater disease	Salmonids, carp, eel, tench, perch and ayu	Viel <i>et al.</i> , 2021
<i>Flavobacterium columnare</i>	Columnaris disease	Cyprinids, salmonids, silurids, eel and sturgeon	Viel <i>et al.</i> , 2021

Table 2.1: continued

Agents	Disease	Host Fish Targets	References
<i>Vibrio anguillarum</i>	Vibriosis	Salmonids, turbot, sea bass, striped bass, eel, ayu and cod	Yang <i>et al.</i> , 2021
<i>Vibrio salmonicida</i>	Vibriosis	Atlantic salmon and cod	Bjelland <i>et al.</i> , 2012
<i>Pseudomonas anguilliseptica</i>	Pseudomonadiosis, winter disease	Sea bream, eel, turbot, and ayu	Fadel <i>et al.</i> , 2018
<i>Mycobacterium marinum</i>	Mycobacteriosis	Sea bass, turbot, and Atlantic salmon	Davidovich <i>et al.</i> , 2020
<i>Lactococcus garvieae</i>	Streptococcosis	Yellowtail, rainbow trout and eel	Torres-Corral <i>et al.</i> , 2021
<i>Streptococcus iniae</i>	Streptococcosis	Adriatic sturgeon and rainbow trout	Colussi <i>et al.</i> , 2022

2.2.1.1. *A. hydrophila*

Among the fish pathogens, *A. hydrophila* is very important as they produce devastating diseases and cause great economic loss to fishing and farming industry (Amin *et al.*, 1985). *A. hydrophila* is a non-spore-forming, Gram-negative, pleomorphic bacillus, motile by polar flagellum. It has the capability to grow at cold temperatures, as low as -0.1 °C for some strains (Daskalov, 2006). It shows good growth at 28 °C however, it can grow within extensive temperature ranges of 4 °C to 37 °C in aerobic and anaerobic environments (Rasmussen-Ivey *et al.*, 2016).

A. hydrophila is a ubiquitous bacterium located in a selection of aquatic environments worldwide, such as wild waters, aquaculture, sewage and public water systems (Ünüvar, 2018). It is found in the water column, associated with algae, biofilms, organic matter, sediments, and zooplankton, and is a natural component of fish and some aquatic amphibians, reptiles and invertebrates (Batra *et al.*, 2016) known to cause diseases in animals (Hossain *et al.*, 2013). It is a virulent pathogen with a number of strains produce cytotoxicity in fish (Beaz-Hidalgo and Figueras, 2013).

A. hydrophila causes infections in fish, concomitantly with different infectious agents, which include viruses, fungi, and parasitic agents (Austin, 2019). Coinfections in fish arise as two or greater genetically specific microbial pathogens infect the same fish simultaneously, both as a primary or secondary concurrent infection (Abdel-Latif and Khafaga, 2020). Coinfection can exchange the fish's susceptibility to particular pathogens. It could substantially affect the dynamics of fish produce disorder or outbreaks by means of amplifying the severity, period, and progression of infection. In addition, coinfection can have an effect on the clinical results and pathological results in the affected host (Elgendy *et al.*, 2016). The effect of concurrent infectious pathogens on every other may want to have synergistic or adversarial results (Bradley and Jackson, 2008). In synergistic coinfection, the primary pathogen suppresses the infected host's immune system, permitting the secondary pathogen to upsurge disorder severity and fish mortalities (Dong *et al.*, 2016). As an example, *A. hydrophila* acts synergistically with Tilapia Lake Virus (TilV) to boost mortality and worsen the disease severity in tilapia (Nicholson *et al.*, 2020). On the other hand, antagonistic coinfection occurs when the primary pathogen modifies the immunological reaction and consequently delays the secondary pathogen (Kotob *et al.*, 2016).

A. hydrophila produces a wide range of diseases, including motile aeromonad septicaemia (MAS) in carp, tilapia, perch, catfish, and salmon; pink sore disease in bass and carp; and epizootic ulcerative syndrome in catfish, cod, carp, and goby (Holmes *et al.*, 1996). The MAS is the term used to describe disorder caused by infection with *A. hydrophila* and different aeromonad microorganisms such as *A. sobria* and *A. caviae*. The disease is associated with lesions resulting from this bacterium which is found in vital organs and ulcers on the body of fish. They are also associated with great economic losses in aquaculture (Stratev and Odeyemi, 2017). It has been reported in many ornamental, cultured, and wild fish populations globally (Sarkar and Rashid, 2012).

Unfavorable environmental factors inclusive of crowding, low-dissolved oxygen, and higher organic material, industrial pollutants, temperature fluctuation, bodily injuries, and physiological situations like spawning can also pave the way for *A. hydrophila* infection (Austin and Austin, 1993). Fish infected with *A. hydrophila* may have many exclusive signs and symptoms. The affected fish show a number of symptoms such as lack of appetite, swimming abnormalities, pale gills, bloated appearance, pores and skin ulcerations. The skin ulcers may also occur at any site at the fish and frequently are surrounded by means of a brilliant rim of red tissue. Other organs commonly affected by this disease consist of the gills, kidneys, liver, spleen, pancreas, and skeletal muscle. Ulcerous dermatitis in fish resulting from *A. hydrophila* is one of the useful biomarkers of pollution (Austin and Austin, 1993).

The occurrence of *A. hydrophila* in fish varies by season, species of fish, sampling stages and fish fillets. Wang and Silva (1999) stated that the best prevalence of *A. hydrophila* in catfish fillets is in summer. Rodrigues *et al.* (2019) reported 3.33% to 46.66% of the prevalence of *A. hydrophila*. Jimoh and Jatau (2010) detected 47%, while Balaji *et al.* (2004) detected

41.7% *A. hydrophila* in *O. niloticus*. A Malaysian survey study reported that, *A. hydrophila* affects 10 to 16% of tilapia (*Tilapia mossambica*), keli (*Clarias batrachus*), and puyu (*Anabas testudineus*) (Radu *et al.*, 2003).

The incidence of *A. hydrophila* in different parts of fish is also fish-species based. For instance, *A. hydrophila* was isolated from nine viscera samples (6.3%), but no muscle samples from 144 Mugil samples collected from retail shops in Egypt (Ahmed *et al.*, 2018). The study made in India, suggested that the incidence of *A. hydrophilla* was more in swab samples from the gills of fish from retail markets (44%) and less from the farms (28%) (Rather *et al.*, 2019).

A. hydrophila has also been reported to cause zoonotic diseases leading to intestinal and extra-intestinal diseases in humans such as septic arthritis, diarrhoea, gastroenteritis, skin and wound infections, meningitis, and fulminating septicaemia (Salunke *et al.*, 2015). People who may be immunodeficient or immunoincompetent such as the very young, the elderly, or those with other disease problems are at the highest risk. Primary routes for transmission include direct contact with mucus and tissues from infected or carrier fish, and contaminated water or equipment (Daskalov, 2006).

A. hydrophila has been recognized as potential foodborne pathogens. It has been isolated from a huge range of foods, together with finfish, seafood, meat, rooster, milk and dairy products, veggies, and ready- to- eat food products (Pal, 2018). *A. hydrophila* associated with foodborne illness are stable at -80 C, capable to grow at refrigeration temperatures (1.7 °C to 5 °C), and brings heat-stable enterotoxins (Goharrizi *et al.*, 2015). It is also listed on contaminant candidate list of rising water-borne pathogens because of its capability to persist in chlorinated water (Pal, 2018).

Aeromonas species in fish of Ethiopia were reported by Nuru Anwar *et al.* (2012) and Tesfaye *et al.* (2018) from fish of Lake Tana and Lake Hayike, respectively. Bacteria isolated from fish in Ethiopia are presented in Table 2.2.

Table 2.2; Bacteria isolated from fishes in Ethiopia

Bacteria isolates	Lake	Fish	References
<i>Edwardsiella tarda</i> , <i>Escherichia coli</i> , <i>Shigella</i> spp.	Lake Ziway	Nile tilapia and <i>Carp</i> spp.	Eshetu Yimar, 2000
<i>Edwardsiella tarda</i> , <i>Escherichia coli</i> , <i>Aeromonas</i> spp. <i>Vibrio</i> spp. <i>Shigella</i> spp. <i>Yersinia</i> spp.	Lake Tana	Barbs species <i>Oreocromis niloticus</i> , <i>Clarias gariepinus</i> <i>Varicorhinus beso</i> , <i>Garra</i> species	Nuru Anwar <i>et al.</i> , 2012
<i>Edwardsiella tarda</i>	Lake Hawasa and Lakes Bishoftu	Nile tilapia and catfish	Abayneh Tekele <i>et al.</i> , 2017,
<i>Salmonella</i> spp	Lake Abaya and Chamo	Nile tilapia	Teka Wendwesen <i>et al.</i> , 2017
<i>Edwardsiella tarda</i> , <i>Escherichia coli</i> , <i>Vibrio</i> spp. <i>Aeromonas</i> spp.	Lake Hayike,	Nile tilapia and catfish	Tesfaye Shimels <i>et al.</i> , 2018,
<i>Salmonella</i> spp.	Lake Haramaya	Nile tilapia and catfish.	Sufiyan Abdulahi <i>et al.</i> , 2022

2.2.2. Effect of bacterial diseases on haematology of fish

Haematological assays provide an index of the physiological status of fish. Leucocytes or White blood cells (WBCs), Red blood cells(RBCs), hematocrit (Hct), and hemoglobin (Hb) are especially endorsed as tests that might be carried out on a routine basis in fish farms to display the health status of fish when subjected to unfavorable conditions (Munkittrick and Leatherland, 1983). Examining morphology, figuring out the Hct values, and acquiring general erythrocyte counts and red blood cell indices, consisting of mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH); all can be useful in diagnosing diseases of fish. The Hct varies within

and between species and appears to correlate with the everyday activity stage of the fish. For instance, actively swimming species, such as tuna and other pelagic species normally possess higher Hct than sedentary bottom species such as flatfish, Elasmobranchs etc. As with other haematologic parameters, Hct values can also range with age, gender, water quality, photoperiod, diet, and season (Hrubec *et al.*, 2000).

Experimentally infected fish showed decreased Hct, Hb, erythrocyte, lymphocyte, and thrombocyte counts and increased neutrophil, monocyte, and neutrophils indicating strong phagocytosis; lymphocytes and RBCs (Lehmann *et al.*, 1989). Fish infected with *A. hydrophila* showed an elevated plasma glucose level, and increased WBC counts. *Cyprinus carpio* infected with *A. hydrophila* had a marked decrease in RBC counts, Hct and Hb, and plasma protein with resultant anemia (Takahashi, 1984). Fish with Hct of 45% or more are taken into consideration to have a relative polycythemia because of dehydration (Fänge, 1992). Anemias are well-documented in fish with less than 20% Hct. The Hct of some sharks varies among blood collection sites and this possibility must be taken into consideration while diagnosing anemia (Mylniczenko *et al.*, 2006).

There are 3 primary types of anemia: hemorrhagic (blood loss), hemolytic (erythrocyte destruction), and hypoplastic (poor erythropoiesis). The basic descriptive terminology used for anemias in different animals applies similarly to fishes and can refer to cell size (microcytic, normocytic, or macrocytic), Hb concentration (hypochromic or normochromic), cell loss (hemolytic or hemorrhagic), and hemopoietic status (regenerative or nonregenerative). Causes of non-regenerative anemias consist of anti-inflammatory disease, nutritional disorders, pollution, and renal or splenic ailment with disruption or destruction of hematopoietic tissues (Tonya *et al.*, 2008).

Higher WBC and overall RBC counts are found in fish from production systems with high densities, marginal water quality, and often increased bacterial load (Hrubec *et al.*, 2000). *Aeromonas* spp, *Pseudomonas* ssp, and *Vibrio anguillarum* produce hemolysins and are the most common reasons of hemolytic anemia in fish (Groff and Zinkl, 1999). Excessive hemorrhagic anemia in cold-water vibriosis was documented in juvenile Atlantic salmon (*Salmo salar*) (Waagbo *et al.*, 1988). Brook trout (*Salvelinus fontinalis*) affected by columnaris sickness (*Flavobacterium columnare*) developed macrocytic hypochromic anemia with evidence of fragmented erythrocytes (Rehulka and Minarik, 2007).

2.3. Disease control methods

Prevention through proper control practice reduces diseases and fish kills. Good control methods maintain desirable water quality, reduce stress during handling, provide excellent nutrition, and maintain sanitation in culture systems. The use of antibiotics and chemicals are popular disease control methods (Stoskopf, 1993).

Antibiotics are broadly used in aquaculture to control microorganisms from causing disease outbreaks. They are used not only as drugs to control diseases but also as growth promoters. Globally, the most commonly used antibiotics in aquaculture are oxytetracycline and chloramphenicol, which belong to the antibiotic category of tetracycline, as well as oxolinic acid from the quinolones category (Sapkota *et al.*, 2008). For instance, in 1988, the total intake of antimicrobial retailers in Norway was 670 kg, the lively elements being oxolinic acid (421 kg), florfenicol (128 kg), flumequine (117 kg), and oxytetracycline (4 kg). In 1992, 27.5 metric tons of antibacterial agents had been used in Norwegian fish farms, and in 1993 it was approximately 6.1 metric tons (Grave *et al.*, 1999). With the improvement of commercial aquaculture, several antimicrobial agents have been used to treat infectious fish diseases. Antibiotics are generally administered to fish through feeds (Cabello *et al.*, 2003).

Big problems related to any antibiotic therapy consist of inadequate dosage degrees, overdosing, drug resistance microorganisms, and transmission of resistant microorganisms from aquaculture environments to human beings (Pal, 2018). Also using antibiotics cause the emergence of drug-resistant bacterial strains (Aruna and Chandran, 1996; Wooley *et al.*, 2004). For example, *A. hydrophila* is resistant to Ampicilline, Cephalothin, Flumegquine, Gentamycin, Nitrofurantoin, Oxytetracycline, Penicillin, Streptomycin, Tetracycline, Trimethoprim and Trimethoprim-sulfamethoxazole (Taylor, 2003). Tendencia and Pena (2002) reported the resistance ability of furazolidone at 1.6%, oxolinic acid at 1%, and chloramphenicol at 0.66% in shrimps against the pathogens.

Disease prevention using vaccination and immunostimulation has been proven to be extremely powerful in fish disease management. Fish vaccines can significantly reduce fish mortality compared to antibiotic use. The study confirmed that at 6 and 12 weeks of the post-vaccination period, the control fish had a high mortality while the fish vaccinated with the vaccine accomplished good protection (Bakopoulos *et al.*, 2003). Fish vaccines are high quality over antibiotics due to the fact they are herbal organic substances that leave no residue inside the product or environment, and therefore will not result in a resistant stress of the disease organism. However, larvae and early fry are too small to be vaccinated and are very vulnerable to death because of the proliferation of pathogens (Gudding *et al.*, 1999). Moreover, specific vaccines are not readily available for farmed fish species. Due to the cited limitation of antibiotics and vaccines, there is a need for an alternative to control fish diseases which is the use of medicinal plants.

2.3.1. Medicinal plants

The application of medicinal herbs as immunostimulants can raise the innate protection mechanisms of fish against pathogens. Medicinal herbs are rich in a wide variety of secondary metabolites of phytochemical ingredients which include tannins, alkaloids, and

flavonoids, which act in opposition to specific diseases (Anjusha *et al.*, 2019). They function as essential raw materials for the production of medicine and can act as immune stimulants, reduce stress, and function as anti-parasitic, antibacterial, or anti-viral agents (Reverter, 2017). Further, they stimulate the production of phagocytic cells in addition to complement lysozyme and antibodies against pathogens in fish species (Anjusha *et al.*, 2019). They are very powerful, non-poisonous, and safe for consumers as well as eco-friendly. Also, phytomedicines are cheaper and show greater accuracy than chemotherapeutic agents in fish (Abasali and Mohamad, 2010).

In aquaculture systems, the use of plant extracts for the control of infections is very common. The inclusion of plant extracts with feed resulted in high growth of fish due to increased appetite, enhancement of the immune system, improvements in haematological picture, decrease in mortality, inhibition of bacteria, and increased antioxidant enzyme activity. Medicinal plants used in aquaculture consisted of Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Oats (*Avena sativa*), Beetroot (*Beta vulgaris*), Moringa (*Moringa oleifera*), Green tea (*Camellia sinensis*), Rosemary (*Rosmarinus officinalis*), Nettle (*Viscum album*), and Cumin (*Nigella sativa*). (Dörücü *et al.*, 2009; Abdel Tawwab *et al.*, 2010; Apines-Amar *et al.*, 2012; El-Sayed *et al.*, 2014; Khafagy *et al.*, 2014; Nile and Park, 2015; Devi *et al.*, 2016).

Herbs have been used to control diseases of fish and shrimps in Mexico, India, China, Thailand, and Japan (Yin *et al.*, 2008). The non-specific immune system of fish is considered to be the first line of defense against invading pathogens (Ahilan *et al.*, 2010). Many plant compounds were found to have non-specific immunostimulating effects in humans and animals (Pandey *et al.*, 2010) of which more than a dozen were evaluated in fish and shrimp.

Zingiber officinale (*Z. officinale*), one of the oldest recognized sources of natural therapy, contains flavonoids, steroids, alkaloids, shogaols, gingerols, zingeron, nutrients, carotenoids,

and polyphenols. Ginger also has antifungal, antiviral, antibacterial, and antioxidant effects in humans and fish, and contains enormous immunostimulants that degrades free radicals (Nya and Austin, 2009). Apines-Amar *et al.* (2012) suggested that the use of ginger and comparable immunomodulators can increase the viability and reduce the mortality of fish. Khafagy *et al.* (2014) have reported that ginger would enhance the immune system in catfish. It is effective in the control of bacterial, viral, fungal, and parasitic diseases (Agrawal *et al.*, 2001). El-Sayed *et al.* (2014) noticed an increase in growth performance at a ratio of 5% of the body weight in tilapia fed with ginger (1%) 4 times a day. He *et al.* (2015) reported that the addition of ginger at 2% of the body weight enhanced haematological parameters and production of liver enzymes compared to a 6% feeding ratio in *O. niloticus*.

Yin *et al.* (2008) mentioned that oral administration of ginger extract increases the phagocytic functionality of cells in rainbow trout. Ginger incorporates many medicinal properties for treating diseases (Jabran *et al.*, 2015). Galina *et al.* (2009) suggested that *Z. officinale* extract effectively increased phagocytosis and respiratory burst activity of leukocyte cells. Research confirmed that supplementation of *Z. officinale* into fish feed progressed the resistance of fish against *A. hydrophila* infection (Payung *et al.*, 2017). The study done by Haghghi *et al.* (2013) indicated that rainbow trout fed with a diet containing 1% powdered ginger rhizome for 12 weekshad elevated Hct, Hb, RBC, MCH, MCHC, WBC values, and neutrophils percentage in comparison to the control group ($P < 0.05$).

The effectiveness of medicinal plants in aquaculture depends substantially on factors such as plant part, method of preparation and application, dosage in relation to species, and age. The percentage of medicinal plant parts used in aquaculture is depicted in Figure 2.3.

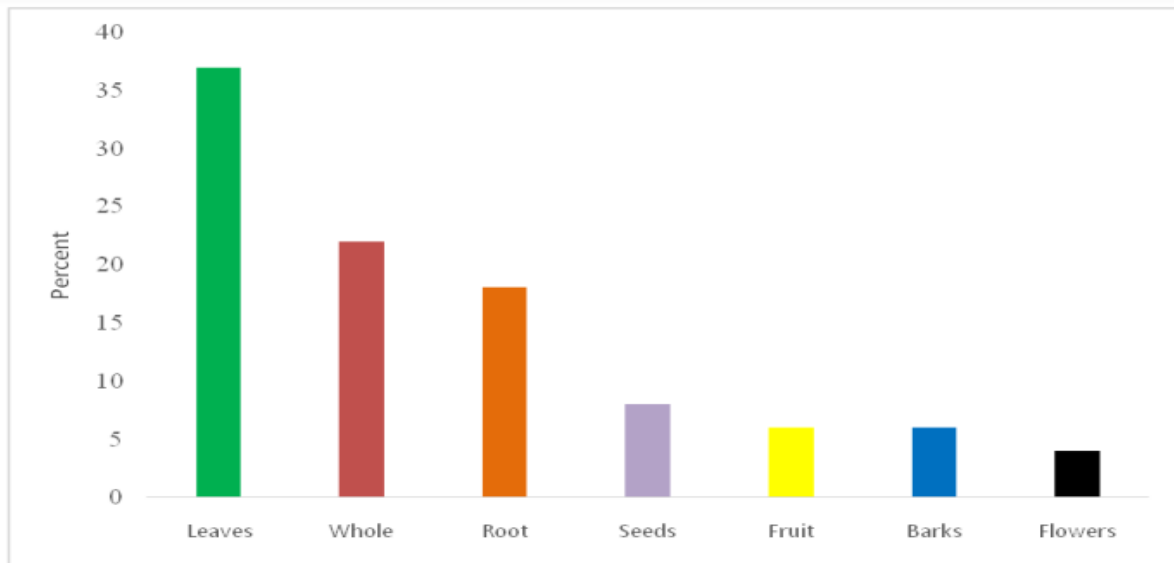


Figure 2.3: Medicinal plant parts used in aquaculture. Source: Reverter *et al.* (2017)

The highest percentage of bioactivity shown by medicinal plant is antibacterial (36%), after that antiparasitic (17%), then immune-stimulant (16%), antiviral (14%), followed by growth promoter (13%) and antifungal (4%) (Figure 2.4).

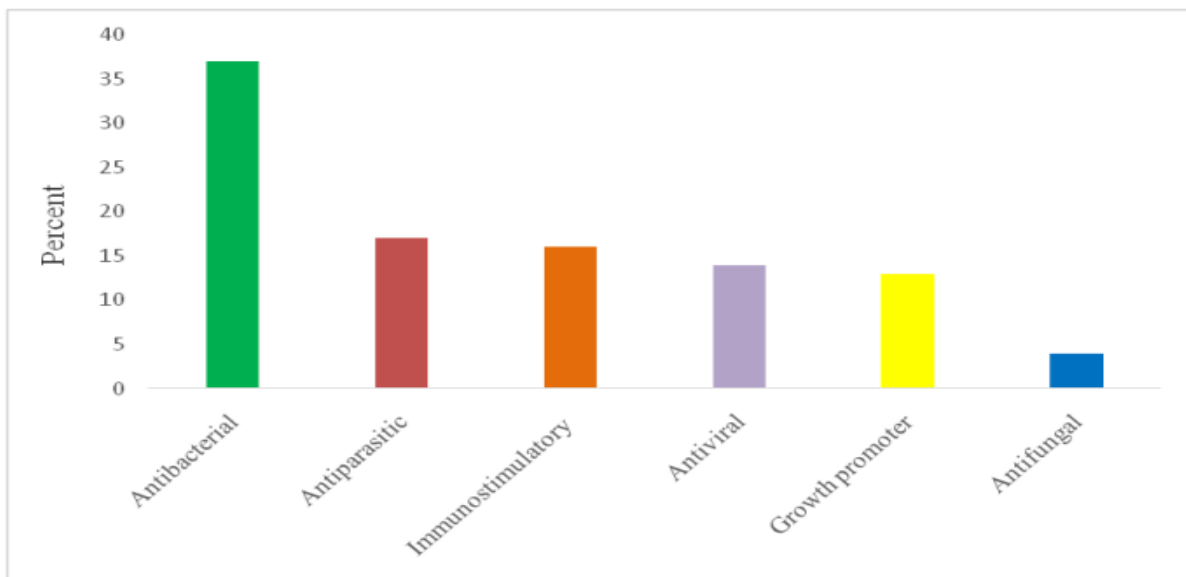


Figure 2.4: Mode of action of medicinal plants in aquaculture. Source: Reverter *et al.* (2017)

CHAPTER THREE

PHYSICOCHEMICAL AND BACTERIOLOGICAL QUALITY OF LAKE HAWASSA, ETHIOPIA

3.1. Introduction

Freshwater is a renewable natural resource that provides a variety of services for the human population, including water for drinking and irrigation, recreational opportunities, and habitat for economically important fishes (La Notte *et al.*, 2017). Freshwater is stored in rivers, lakes, reservoirs, and streams. However, freshwater quality is affected by anthropogenic activities such as chemical discharges from industries, untreated domestic and municipal sewage, and deforestation as a result, the aquatic organisms particularly the fish species living in it are affected (Bukola *et al.*, 2015). Freshwater quality is commonly characterized by physical, chemical, and biological characteristics (Chapman, 1996). Therefore, monitoring of these characteristics would show a good picture of water quality

Lakes, one of the freshwater ecosystems, found adjacent to one or more urban areas are widely used by farmers and other resource users surrounding the lake (Nontji, 2017). Thus there is no surprise that anthropogenic forces threaten lake water quality. Rift Valley Lakes of Ethiopia are highly vulnerable as urbanization is increasing steadily which impedes the quality of water in lakes. Lake Hawassa, one of the Rift Valley Lakes of Ethiopia, has been subjected to a series of changes for more than three decades. The deleterious anthropogenic activities such as the construction of irrigation and drainage systems, clearing of forests, building of factories and use of fertilizers, herbicides and pesticides, which have been observed in the lake catchment, contribute a significant effect towards the damage of the lake. A study conducted by Zerihun Desta (2003) showed that a huge amount of effluents are discharged especially from Hawassa Textile Factory into the Tikur Wuha River and entered

the lake without any proper treatment. Zinabu Gebre-Mariam and Zerihun Desta (2002) also observed that the effluent treatment ponds of the factory do not efficiently reduce the chemicals of the discharge which adversely affects the lake ecology. A recent study by Samuel Bekele *et al.* (2020a) showed that wastewater discharged from factories in the Hawassa Industrial Zone contained high concentrations of heavy metals. Also, the same authors reported that the soils within the Hawassa Industrial Zone are highly contaminated with heavy metals and further downstream into Lake Hawassa.

In addition, stormwater canals, runoff, turbidity, nutrient discharge, ionic compositions, and temperature of the lake are affecting the water quality of the lake. According to Nigatu Wondrade *et al.* (2014), the transformation of Lake Cheleleka to a grass-covered swamp, and ever-green vegetation on cultivated lands and urban areas caused adverse effects on the lake. Besides this, the wastes from the surrounding hotels and resorts, and traditional fisheries activities deteriorate water quality and severely affect the inhabitants of the lake. Furthermore, the aesthetic quality and socio-economic functions of the lake are also affected (WHO, 2003). Thus, water quality evaluation of the lake becomes important. Hence, the present study was designed to investigate the water quality of Lake Hawassa by using physicochemical and bacteriological parameters.

3.2. Objectives of the study

3.2.1. General objective

The general objective of this study was to investigate the water quality of Lake Hawassa.

3.2.2. Specific objectives

The specific objectives of the present study were to:

- evaluate the physicochemical parameters of Lake Hawassa;

- assess bacterial loads of Lake Hawassa;
- determine the relationship of water quality parameters of Lake Hawassa by using Pearson correlation matrix.

3.3. Materials and Methods

3.3.1. Description of the study area

The study was carried out in Lake Hawassa which is the smallest Rift Valley Lake in Ethiopia (Figure 3.1). Lake Hawassa lies 275 km south of Addis Ababa, in the Main Ethiopian Rift, has a surface elevation of 1,680 m above sea level with coordinates of 6°33' - 7°33' N and 38°22' - 38°29' E (Welcome, 1972). The surface area of the lake is about 90 km², 16 km long, 8 km width, 1.3 billion m³ volume and mean depth about 11 m. Its mean monthly maximum atmospheric temperature varies between 24.5 and 30°C, while the mean minimum monthly air temperature ranges from 10.3 to 14.5 °C and an annual rainfall of 1028 mm ((Elizabeth Kebede *et al.*, 1994; Girma Tilahun and Ahlgren, 2010).

Tikur Wuha is the only perennial tributary river, which comes from the eastern side of the catchment, contributes most of the water input to the lake. However, it has no visible outlet but there may be groundwater flow away from the lake on the south west and north sides, which may account for its relatively low alkalinity, compared with either Lake Shala or Lake Abiyata, both of which are also terminal lakes (Makin *et al.*, 1975).

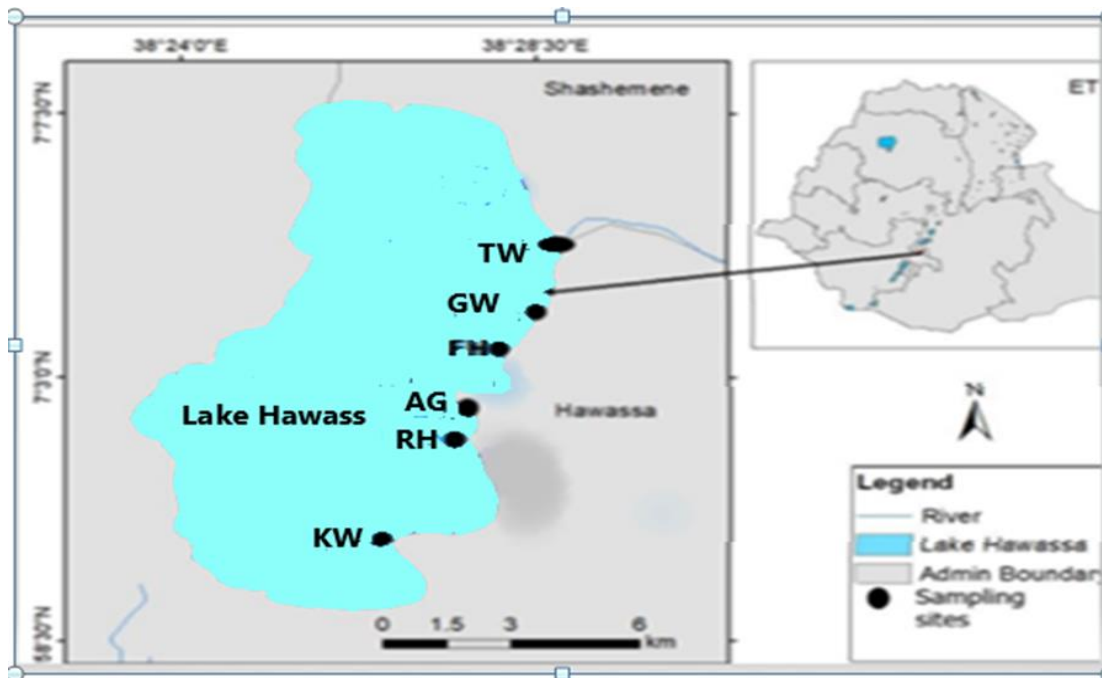


Figure 3.1: Location Map of sampling site in Lake Hawassa (modified after Zigde Haile and Tsegaye Endale, 2019).

3.3.2. Data collection

Water samples were collected every month from January 2021 to December 2021 from six sampling sites of Lake Hawassa (Figure 3.1; Appendix figure 1). A total of 72 water samples were collected aseptically in triplicate in sterile bottles of 200 ml. The bottle was opened to collect the samples, allowed to fill up with water, and then closed while still under water (Greenberg, 1985). This was labelled, kept in an ice box, and taken to the Biology laboratory at Hawassa University for bacteriological and water quality analysis.

The sampling sites were selected based on exposure of the lake to different anthropogenic activities. Tikur Wuhan (TW) site is situated at the junction point of the Tikur Wuha River and the lake. The River carries sediments, contaminants from the degraded catchment and industrial effluents to the lake. It also acts as the landing site through which fish harvesting, processing and marketing are practised. Green wood (GW) site is contaminated by washing clothes, bathing and dropping plastic cups. Fikr Hayiq (FH) site is used for recreational

activities, place for dropping plastic bottles, organic wastes and restaurant wastes. Amora Gedel (AG) is the main fish landing and processing site also forms a source of different contaminants. Referral Hospital (RH) is exposed to medical effluents from Hawassa Referral Hospital and a site for sewage and other waste disposal. Kuyu Wata (KW) site is the site where there are few inhabitants around the area, and the catchment area has been protected from human and animal presence.

3.3.3. Physicochemical and bacteriological examination of water samples

The various physico chemical and biological parameters analysed include temperature, pH, dissolved oxygen (DO), turbidity, biochemical oxygen demand (BOD₅), electric conductivity (EC), total dissolved solids (TDS), ammonium (NH₄⁺), nitrite (NO₂⁻), Nitrate (NO₃⁻), phosphate (PO₄⁻³), heterotrophic bacteria count (HBC), total coliform (TC) and fecal coliform (FC) count. Temperature, pH, EC, and DO were measured with a combined portable HQ40D Multimeter. Turbidity was measured by OAKTON Turbid meter (T-100). NO₂⁻, NO₃⁻, NH₄⁺, and PO₄⁻³ were analyzed using a spectrophotometer (DR/2010 HACH, Loveland, USA) according to HACH instructions. TDS was measured by a Portable multi-parameter analyser, Zoto, Germany. BOD₅ was also measured by a Manometric, BOD sensor according to the standard methods (APHA, 2005). HBC, TC and FC were determined by colony forming unit per ml (CFU/ml).

3.3.4. Water sample preparation and enumeration of bacteria

Each water sample was successively diluted, using sterile physiological saline solution. Briefly, 1 mL of the water sample was added to a tube containing 9 mL of sterile physiological saline (0.85% wt./vol NaCl) and this resulted in a dilution of 10⁻¹. Using separate sterile pipettes, decimal dilutions of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ and 10⁻⁶ were prepared by transferring 1 mL of the previous dilutions to 9 mL of diluents.

For bacteria count, 15- 18 mL of the molten sterilized agar medium (cooled to 44 °C – 47 °C) was poured into each Petridish and solidified. Then 0.1 ml of samples from the serial dilution was inoculated by spread plate method and incubated in an inverted position to prevent condensation at 37 °C for 24 hrs. Nutrient Agar, M- ENDO Media and M-FC media were used for total heterotrophic bacteria, total coliform bacteria and faecal coliform respectively.

The M- ENDO Media used for total coliform bacteria was prepared by using Peptone 3.7 g, Tryptose 7.5 g, Yeast extract 1.2 g, Lactose 9.4 g, Sodium Chloride 3.7 g, Dipotassium Hydrogen Phosphate 3.30 g, Potassium Dihydrogen Phosphate 1.0 g, Sodium Lauryl Sulphate 0.05 g, Sodium Deoxycholate 0.10 g, Sodium Sulphite 1.6 g, Basic Fuchsin 0.80 g, Agar 15.0 g and 1000 mL of water (APHA, 1998). The M-FC Media used for faecal coliform bacteria was prepared by using Tryptose 10.0 g, Proteose peptone 5.0g, Yeast extract 3.0 g, Sodium Chloride 5.0 g, Lactose 12.5 g, Bile Salts mixture 3 1.5 g, Aniline blue 0.1 g, Agar 15.0 g and 1000 mL of water (Rice *et al.*, 1987).

3.3.5. Statistical analysis

All the collected raw data were recorded in Microsoft Excel and transferred to SPSS version 20 for data analysis. One-way ANOVA (Tukey) test was used to evaluate the significant difference in parameters among sites and seasons. A probability at $P < 0.05$ was considered significant. The mean, range, and standard deviation of each parameter were determined. The parameters were correlated against each other to determine their relationship using Pearson's correlation.

3.4. Results and Discussion

3.4.1. Physicochemical parameters and bacterial loads

Physicochemical parameters and bacterial loads in all study sites and seasons are presented in Figure 3.2-3.5 and Table 3.1, respectively. Water quality parameters of Lake Hawassa showed significant variation among sites and seasons ($P < 0.05$).

The water temperature of Lake Hawassa was 25.06 ± 0.11 , 25.03 ± 0.05 , 23.84 ± 0.08 , and 24.91 ± 0.01 °C during winter (December, January and February), spring (September, October and Nove), summer (June, July and August), and autumn (March, April and May), respectively with a mean value of 24.71 ± 0.53 °C (Table 3.1). The mean water temperature records of Lake Hawassa, in the present study, were higher than the values reported in the previous studies by Girma Tilahun and Ahlgren (2010) and Adimasu Woldesenbet (2015), who reported 23.5 ± 1.8 °C and 21.23 °C, respectively for Lake.Hawassa The high temperature of the lake in the present study might be due to, the effect of land use changes like urban expansion and deforestation during the present study period as compared to the last decade. The suitable temperature for warm water fish culture ranges from 20 to 32 °C (Boyd, 1982; Bhatnagar and Devi, 2013). Ngugi *et al.* (2007) also recommended a temperature range of 20 to 35 °C for Nile tilapia. This indicates that the temperature values of Lake Hawassa were within the recommended limits. However, the same temperature range is also sufficient for the proliferation of most pathogenic bacteria (Cunningham *et al.*, 2014).

The pH value of Lake Hawassa in the present study was 9.63 ± 0.06 , 9.44 ± 0.05 , 8.44 ± 0.07 , and 8.53 ± 0.05 during winter, spring, summer and autumn, respectively with a mean value of 9.01 ± 0.55 (Table 3.1). The lake water was generally alkaline in all the sites and seasons (Figure 3.2 and Table 3.2). These results agreed with Zinabu Gebramariam *et al.* (2002), Girma Tilahun and Ahlgren (2010), and Zigde Haile and Tsegaye Malaku (2019) who

indicated that pH values of Lake Hawassa lie in the alkaline side. The responsible factors for the alkaline value of the pH might be agro-industrial activities found in the surrounding areas of the lake. According to El-Sherif and El-Feky (2009), the optimal water pH for Nile tilapia is 7 to 8 implying that the pH value of water recorded during this research was not within the limit for Nile tilapia, especially during winter and spring (Table 3.1). This might be due to bacterial decomposition of domestic wastes and agricultural activities near the lake.

In this study, DO value of the lake varied between 4.36 and 6.20 mg L⁻¹ with a mean value of 4.90 ± 0.64 mg L⁻¹ (Table 3.1). The mean DO value of the lake was below the acceptable limit of EPA (1998) (>5 mg L⁻¹), indicating the impairment of the water body for aquatic life (EPA, 1998). The highest DO was recorded at the KW site, while the lowest was recorded at TW (Figure 3.2). The low DO value at the TW site could be the result of the decomposition of excess nutrients and biodegradable organic matter by decomposing organisms like bacteria. These organic matters come through the influx of dissolved solutes from industrial wastes, surrounding urban areas, and agricultural fields. The lower DO is mainly caused by the consumption of DO by microorganisms in decomposition of organic matter. Boyd *et al.* (1978) found that, in tropical waters, the rate of DO consumption by decaying organic matter is greater.

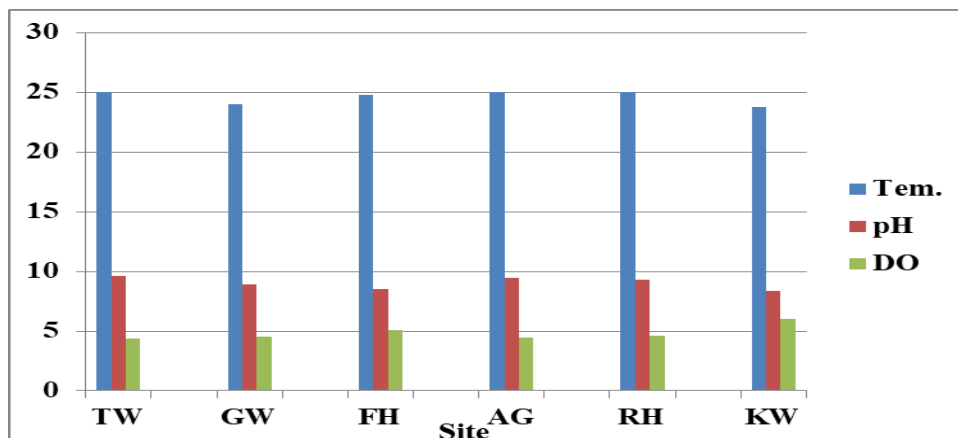


Figure 3.2: Mean temperature (Temp.) (°C), pH and DO (mg/l) of the lake

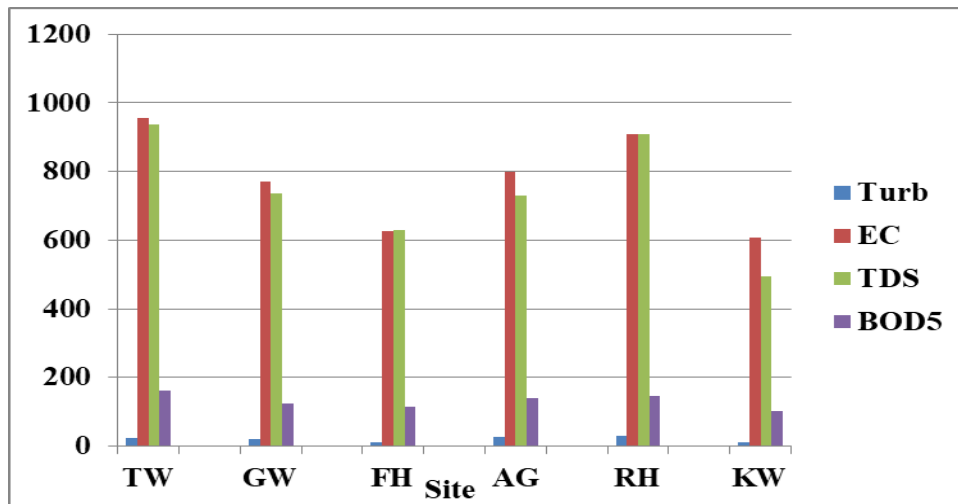


Figure 3.3: Mean turbidity (Turb) (NTUs), EC ($\mu\text{S}/\text{cm}$), TDS (mg/l) and BOD5 (mg/l) of the lake

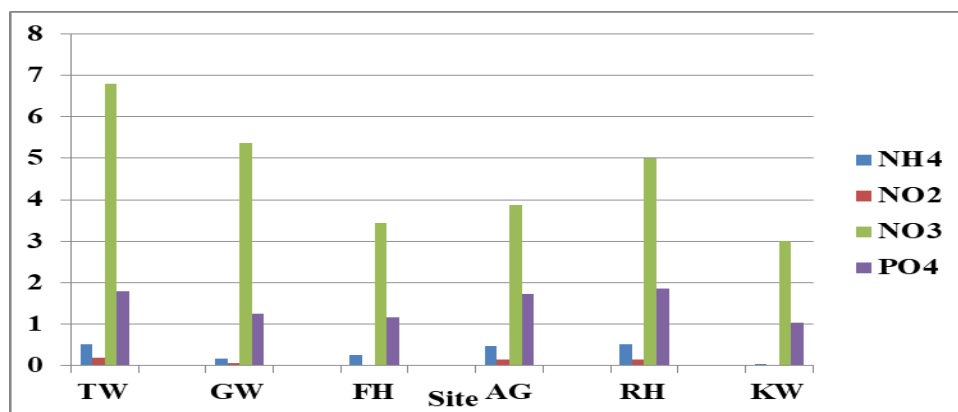


Figure 3.4: Mean NH_4^+ (mg/l), NO_2^- (mg/l), NO_3^- (mg/l) and PO_4^{+3} (mg/l) of the lake

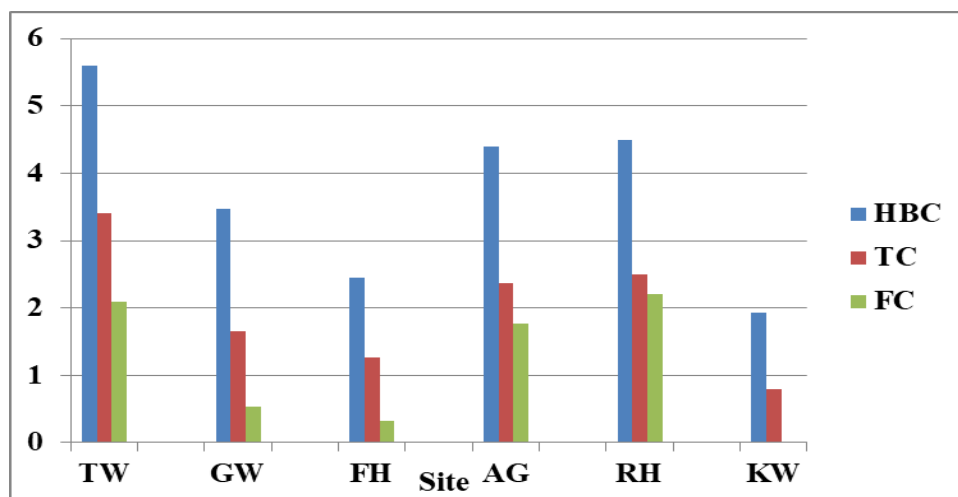


Figure 3.5: Mean HBC (CFU/ml), TC (CFU/ml) and FC (CFU/ml) of the lake

Table 3.1: Physicochemical parameters and bacterial loads of Lake Hawassa among seasons (mean \pm SD, n=216).

Seasons	Parameters													
	Temperature	pH	DO	Turbidity	EC	TDS	BOD5	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	PO ₄ ⁻³	HTB	TC	FC
Winter	25.06 \pm 0.11 ^b	9.63 \pm 0.06 ^c	4.37 \pm 0.02 ^a	9.85 \pm 0.41 ^a	778.8 \pm 18.34 ^b	750.45 \pm 25.59 ^b	124.89 \pm 4.23 ^b	0.09 \pm 0.05 ^a	0.05 \pm 0.01 ^a	5.37 \pm 0.54 ^b	1.26 \pm 0.02 ^b	3.43 \pm 0.10 ^b	1.6 \pm 0.13 ^b	0.53 \pm 0.11 ^a
Spring	25.03 \pm 0.05 ^b	9.44 \pm 0.05 ^b	4.48 \pm 0.07 ^a	20.44 \pm 0.30 ^b	612.55 \pm 10.9 ^a	538.53 \pm 40.91 ^a	105.64 \pm 4.99 ^a	0.25 \pm 0.04 ^b	0.03 \pm 0.01 ^a	3.34 \pm 0.62 ^a	1.08 \pm 0.07 ^a	2.07 \pm 0.23 ^a	0.91 \pm 0.35 ^a	0.10 \pm 0.1 ^a
Summer	23.84 \pm 0.08 ^a	8.44 \pm 0.07 ^a	5.7 \pm 0.50 ^a	29.07 \pm 0.47 ^d	957.33 \pm 3.3 ^c	928.75 \pm 9.75 ^c	157.56 \pm 8.26 ^b	0.53 \pm 07 ^d	0.19 \pm 0.00 ^b	6.80 \pm 0.17 ^c	1.83 \pm 0.05 ^c	5.60 \pm 0.16 ^d	3.40 \pm 0.08 ^d	2.10 \pm 0.08 ^b
Autumn	24.91 \pm 0.01 ^b	8.53 \pm 0.05 ^a	5.06 \pm 0.58 ^a	27.23 \pm 0.20 ^c	772.22 \pm 45.22 ^b	735.00 \pm 4.33 ^b	135.33 \pm 13.27 ^b	0.48 \pm 0.01 ^c	0.14 \pm 0.02 ^b	3.69 \pm 0.31 ^a	1.72 \pm 0.06 ^c	4.44 \pm 0.08 ^c	2.43 \pm 0.16 ^c	1.90 \pm 0.74 ^b
Total	24.71 \pm 0.53	9.01 \pm 0.55	4.90 \pm 0.64	21.65 \pm 7.87	780.23 \pm 129.2	738.18 \pm 145.81	130.86 \pm 20.86	0.34 \pm 0.19	0.10 \pm 0.07	4.80 \pm 1.49	1.47 \pm 0.32	3.80 \pm 1.36	2.10 \pm 0.98	1.15 \pm 0.95
Range	23.75- 25.20	8.35- 9.70	4.36- 6.20	9.40- 29.62	600- 960	491.30- 940.00	100.00- 165.93	0.03- 0.59	0.02- 0.20	2.80- 7.00	1.00- 1.90	1.80- 5.80	0.50- 3.50	0.00- 2.34

Within the same column mean value with the different superscript is significantly different at $P < 0.05$ based on the ANOVA (Tukey) test. Units of parameters are given by mg L^{-1} except, Temperature ($^{\circ}\text{C}$) Turbidity (NTUs), EC ($\mu\text{S}/\text{cm}$), pH with no scale, HBC (CFU/ml, 10^5), TC (CFU/ml, 10^4) and FC (CFU/ml, 10^2). HBC-heterotrophic bacteria count, NH₄⁺ -ammonium ion, BOD5- five-day biochemical oxygen demand, DO-dissolved oxygen concentration, EC-electrical conductivity, FC-faecal coliform, NO₃⁻ – nitrate, NO₂⁻ - nitrite, pH -hydrogen ion concentration, PO₄⁻³ - phosphate, Temp.-temperature, TC- total coliform, TDS -total dissolved substances, Turb-turbidity

The turbidity value of Lake Hawassa was 9.85 ± 0.41 , 20.44 ± 0.30 , 27.23 ± 0.20 , and 29.07 ± 0.47 NTUs during winter, spring, autumn, and summer, respectively with a mean value of 21.65 ± 7.87 NTUs (Table 3.1). The high turbidity value measured during summer might be attributed to agricultural and urban runoff from the catchment area. The highest turbidity value was recorded at the RH site (Figure 3.3), this might be due to the presence of highly biodegradable organic matter that comes from wastes of surrounding urban and discharged from the Referral Hospital. The turbidity of the Lake Hawassa water is higher than the permissible limit of < 5 NTU for aquatic life (WHO, 1984).

The EC value of Lake Hawassa varied between 600 and 960 $\mu\text{S}/\text{cm}$ with a mean value of $780.23 \pm 129.20\mu\text{S}/\text{cm}$ (Table 3.1). According to Russell *et al.* (2011), water conductivity between 150 and 500 $\mu\text{S}/\text{cm}$ is ideal for fish culture. This indicated that the EC values recorded in the present study were above the optimum ranges for fish culture. This might be due to the solutions of most inorganic compounds and more abundant ions resulting from industries and agricultural drainage that have high conductivity (APHA, 2005).

The TDS of Lake Hawassa varied between 491.30 and 940 mg L^{-1}), with a mean value of $738.18 \pm 145.81 \text{ mg L}^{-1}$. Statistical analysis showed that the mean differences of TDS recorded in the Lake were significantly different among seasons and sites ($P < 0.05$). The TDS values recorded in this study were higher than the value recommended $<500 \text{ mg L}^{-1}$ for fish farming by Charkhabi and Sakizadeh (2006).

The BOD₅ of Lake Hawassa varied between 100 and 165.93 mg L^{-1} , with a mean value of $130.86 \pm 20.86 \text{ mg L}^{-1}$ (Table 3.1). The result found in this study was higher than the previous result reported by Zigde Haile and Tsegaye Endebe (2019) $68.7 \pm 7.50 \text{ mg L}^{-1}$ for BOD₅ of the lake. This may be associated with lower dissolved oxygen levels in the current study compared to the previous study. Bhatnagar and Devi (2013) recommended a desirable

BOD of 1.2 mg L⁻¹ for aquaculture water quality and greater than 10 mg L⁻¹ causes stress. The results of BOD₅ recorded from Lake Hawassas exceeded the maximum permissible limits for fish culture; which indicates the lake is highly polluted by organic matter. These might be due to the continued release of wastes from residents, hotels, industries, the agricultural field, and fish markets nearer to the lake, and other human activities that could produce organic wastes. Favorable environmental conditions for microbiological activities at higher temperatures increase BOD (Tamot *et al.*, 2008).

The values of NH₄⁺ ranged between 0.03 and 0.59 mg L⁻¹ with a mean value of 0.34±0.19 mg L⁻¹ (Table 3.1) in the lake during the study periods. The highest NH₄⁺ value was recorded at the TW site (Figure 3.4). The highest NH₄⁺ recorded at the TW site might be due to microbial degradation of organic matter containing nitrogen (Wetzel, 2001). The value of NH₄⁺ in the present study was higher than the value (118±59 µg L⁻¹) reported by Girma Tilahun and Ahlgren (2010) in Lake Hawassa.

The NO₃⁻ value of Lake Hawassa ranged between 2.80 and 7.00 mg/L with a mean value of 4.80 ± 1.49 mg L⁻¹ (Table 3.1). The value recorded for NO₃⁻ in this study exceeded the value (2.507 ± 2.15 µg L⁻¹) reported by Girma Tilahun and Ahlgren (2010). This might be due to the input of fertilizer application by agricultural land. The fertilizers used on farms through surface runoff enter into the lakes and contribute to the high levels of nitrate in the lakes (Rao, 2011). Also, the high rate of microbial decomposition of organic compounds increases the nitrogen content of the lakes (Abu Khatit *et al.*, 2017).

In this study, NO₂⁻ ranged between 0.02 and 0.20 mg L⁻¹ with the mean value of 0.10 ± 0.07 mg L⁻¹. Boyd (1998) suggested the desired concentration of nitrite in the water is less than 0.3mg/l in aquaculture; this indicated that the values recorded in the present study were within the acceptable range of NO₂⁻ for fish culture.

The PO_4^{-3} value of Lake Hawassa ranged between 1.00 and 1.90 mg L⁻¹ with a mean value of 1.47 ± 0.32 mg L⁻¹ (Table 3.1). The concentration of PO_4^{-3} in this study was higher than the values (16.9 and 15.47 ± 3.9 $\mu\text{g L}^{-1}$) recorded in the same lake by Zinabu Geberamariam *et al.* (2002) and, Girma Tilahun and Ahlgren (2010), respectively. This might be due to high agricultural fertilizers and industrial discharges. The desired concentration of phosphate in the water is in the range of 0.005 to 0.2 mg/l (Boyd, 1998). This indicates that the result of this study was higher than the limit of the optimum and productive PO_4^{-3} values for fish farming.

The HBC of Lake Hawassa ranged between 1.80×10^5 and 5.80×10^5 CFU/ml with a mean value of $3.80 \pm 1.36 \times 10^5$ CFU/ml (Table 3.1). TW, AG, and RH sites, located next to areas with high anthropogenic activities, showed higher concentrations of bacterial loads (Figure 3.5). It has been suggested that the number of heterotrophic aerobic bacteria in freshwater ecosystems is directly proportional to the degree of organic pollution (Hiraishi *et al.*, 1984). Lakhmanan *et al.* (1984) reported HBC above 10^5 is considered poor quality. The present results give indicators that Hawassa Lake is highly polluted and the results thus agree with the results of Abdel-Hamid (2017) who reported increased pollution in Manzala Lake with increased bacterial load.

The TC count of the present study ranged between 0.50×10^4 and 3.50×10^4 CFU/ml (Table 3.1). TW site was found to have the maximum value while the minimum value was recorded at the KW site (Figure 3.5). According to Fricker (2003), the sewage polluted surface water contains greater numbers of bacteria than unpolluted waters. Considering all study sites, the mean value ($2.00 \pm 0.89 \times 10^4$ CFU/100 mL) obtained from all sites (Appendix Table 1) was not by the standard limits recommended by World Wide Fund-Pakistan (WWF-Pakistan, 2007) for drinking less than or equal to 20 CFU in 100 mL and recreational water less than or

equal to 1000 CFU in 100 mL. TC concentrations showed a strong seasonal dynamic, with significantly higher concentrations measured in the rainy season, summer ($3.40 \pm 0.08 \times 10^4$ CFU/ml) compared to the dry season, winter ($1.65 \pm 0.13 \times 10^4$ CFU/ml) ($P < 0.05$; Table 3.1). Similar to the present results in Lake Victoria, higher concentrations of TC in the wet season were recorded (Ouma *et al.*, 2016). The presence of high coliform during summer was most likely due to diffuse runoff pollution, human activities and settlements, household sewage, livestock manure, and open defecation. During the rainfall period, the lake increasingly received in sewage and waste effluents from the surrounding areas. The presence of coliform bacteria in surface water should at least be seen as a probable threat or indicator of deterioration of microbiological water quality (Ndlovu *et al.*, 2015) and implies that enteric pathogens may also be present (Perkins *et al.*, 2014). Discharge of effluents from wastewater treatment elevated coliform concentrations (Sibanda *et al.*, 2013).

Faecal coliform (FC) ranged between 0.00 and 2.34×10^2 CFU/ml with a mean value of $1.12 \pm 0.95 \times 10^2$ CFU/ml (Table 3.1). According to WHO (2003), the permissive level of FC for drinking water should be 0 CFU/ml. This indicates that the FC of Lake Hawassa exceeded the permissible values mentioned by WHO (2003) for drinking water.

3.3.2. Correlation matrix

A Pearson correlation matrix of fourteen (14) parameters of the lake, namely, temperature, pH, DO, turbidity, EC, TDS, BOD5, NH_4^+ , NO_2^- , NO_3^- , PO_4^{-3} , HBC, TC, and FC was constructed, and is shown in Table 3.2. There was a high positive-to-negative correlation among these parameters. The HBC of the lake water had positive correlation ($P < 0.01$) with temperature where ($r = 0.715$, $P = 0.001$), moreover it had significant and strong positive correlation with pH, turbidity, EC, TDS, BOD5, NH_4^+ , NO_2^- , NO_3^- , PO_4^{-3} TC and FC where ($r = 0.973$, 0.958 , 0.966 , 0.928 , 0.960 , 0.870 , 0.921 , 0.825 , 0.917 , 0.982 and 0.852 ,

respectively). It had a significant negative correlation ($P < 0.01$) with DO where ($r = -0.777$, $p = 0.000$; Table 3.2). An increase in heterotrophic bacteria results in the depletion of oxygen due to the high decomposition of organic matter in the lake. In line with these findings, Sugumar and Anandharaj (2016) revealed a positive correlation between HBC and nutrients. The TC count of the lake water had a positive significant ($P < 0.01$) correlation with temperature, pH, turbidity, EC, TDS, BOD₅, NH_4^+ , NO_2^- , NO_3^- and PO_4^{-3} where ($r = 0.754$, 0.939 , 0.918 , 0.947 , 0.920 , 0.955 , 0.884 , 0.908 , 0.813 and 0.898), respectively. It had negative significant ($P < 0.01$) correlation with dissolved oxygen where ($r = -0.748$; Table 3.2). Abdel-Hamid (2017) suggested that DO decrease was suitable for the coliform group which considers facultative anaerobic bacteria. A comparison of the nutrient levels revealed that the TC was high when the nutrient levels were also high. For example, concentrations of FC are coincident with the increased phosphorus and nitrogen concentrations of the lake water for the same period (Table 3.1). This observation is in agreement with a study reporting that nitrogen and phosphorus concentration correlated well with the number of bacteria (Matsumoto and Omura, 1980). Romero (1999) also indicated that concentrations of coliform bacteria are coincident with the increased phosphorus and nitrogen concentrations of lake water.

The FC of the lake water had a significant and positive correlation ($P < 0.01$) with temperature, pH, turbidity, EC, TDS, BOD₅, NH_4^+ , NO_2^- and PO_4^{-3} where ($r = 0.765$, 0.900 , 0.882 , 0.840 , 0.799 , 0.888 , 0.884 , 0.905 and 0.917), respectively. It had a significant and negative ($P < 0.01$) correlation with DO where ($r = -0.682$; Table 3.2). Similar to the present result, Spietz *et al.* (2015) found a strong negative association between bacterial richness and DO. In contrast to the current result, some authors reported an inverse relationship between FC and water temperature (Matsumoto and Omura, 1980). The pH of water influences the survival of FC. Wahyuni (2015) reported the highest survival of FC at a neutral pH and an

alkaline environment. This indicated that the pH of Lake Hawassa is suitable for the survival of FC during the study period. In the present study, FC showed a significantly positive correlation with BOD which was similar to the results of Hiraishi *et al.* (1984) who observed a positive correlation between BOD and, TC and FC in a river ecosystem highly polluted by industrial pollutants. The result of correlation analysis also indicated that water temperature had significant and a positive correlation ($P < 0.01$) with pH, Turbidity, EC, TDS, BOD5, NH_4^+ , NO_2^- and PO_4^{3-} ($r = 0.712, 0.660, 0.630, 0.705, 0.737, 0.907, 0.682$ and 0.786), respectively and it had negative correlation with DO ($r = -0.649$). In line to this study, Sharma *et al.* (2008) found that temperature has a negative correlation with DO ($r = -0.9$) and a positive correlation with pH, TDS, BOD5, PO_4^{3-} and NO_3^- in Narmada River, India. The result also revealed that EC had a positively strong correlation with turbidity and TDS ($r = 0.957$ and 0.969), respectively (Table 3.2), which supports the results obtained by Toufeek and Korium (2009).

Table 3.2 Pearson correlation matrix of Physicochemical and bacteriological parameters in Lake Hawassa

	Temperature	pH	DO	Turbidity	EC	TDS	BOD5	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	P ₀₄ ⁻³	HBC	TC	FC
Temperature	1													
pH	.712**	1												
DO	-.649**	-.812**	1											
Turbidity	.660**	.978**	-.787**	1										
EC	.630**	.936**	-.747**	.957**	1									
TDS	.705**	.882**	-.791**	.901**	.969**	1								
BOD5	.737**	.938**	-.767**	.923**	.937**	.915**	1							
NH ₄ ⁺	.907**	.874**	-.716**	.868**	.840**	.851**	.897**	1						
NO ₂ ⁻	.682**	.922**	-.630**	.913**	.920**	.856**	.886**	.870**	1					
NO ₃ ⁻	.373	.726**	-.686**	.723**	.853**	.852**	.782**	.544*	.680**	1				
PO ₄ ⁻³	.786**	.938**	-.703**	.946**	.915**	.883**	.879**	.920**	.938**	.603**	1			
HBC	.715**	.973**	-.777**	.958**	.966**	.928**	.960**	.870**	.921**	.825**	.917**	1		
TC	.754**	.939**	-.748**	.918**	.947**	.920**	.955**	.884**	.908**	.813**	.898**	.982**	1	
FC	.765**	.900**	-.682**	.882**	.840**	.799**	.888**	.884**	.905**	.563*	.917**	.862**	.851**	1

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

CHAPTER FOUR

ISOLATION AND CHARACTERIZATION OF BACTERIAL PATHOGENS FROM *Oreochromis niloticus* L. IN LAKE HAWASSA, ETHIOPIA

4. Introduction

Fish are aquatic animals that serve as food for humans. They provide more than 20 percent of the average per capita animal protein intake for 3.2 billion people (FAO, 2018). Sixty percent of the developing countries derive 30% of their annual protein from fish (Abisoye *et al.*, 2011). It is especially critical for rural populations, who often have less diverse diets and lower food security rates (Thompson and Amoroso, 2014). However, disease outbreaks become a major constraint in fish production and economic development throughout the world (El-Adawy *et al.*, 2020).

Fish production sectors lose about fifty percent (50%) due to diseases which ever more severe in developing countries (Leung and Bates, 2013). The world loses up to 6 billion dollars annually due to fish diseases. For instance, fish diseases are estimated to contribute to more than 30% of the overall production loss in China, India, and Vietnam, (Mohd-Aris *et al.*, 2019). Infectious salmon anemia alone costs 2 billion dollars and causes 20,000 workers to lose their jobs in Chile (Leung and Bates, 2013). Sea lice infections of salmon in Norway led to a loss estimated at >US\$ 400 million in 2011 alone (Abolofia *et al.*, 2017). Due to these considerable economic risks, disease outbreaks represent one of the main obstacles to the sustainable growth of aquaculture (Subasinghe *et al.*, 2019).

Microorganisms that cause diseases in fish include bacteria, fungi, algae, protozoa, and viruses. They are found in aquatic ecosystems (Ajayi and Okoh, 2014). Bacteria are the most disease-causing organism in wild and farm fish (Elgendy *et al.*, 2017; El-Adawy *et al.*, 2020). They are usually present in the fish's environment, in the surrounding water, on the fish's

surface, or in their internal organs (Brown and Gratzek, 1980). Different species of bacteria can cause different symptoms of disease in fish. Common symptoms include swimming abnormally, bloody scales at the base of the fins, open sores on the side of the body and near the fins, and reddened and frayed fins (Cipriano and Holt, 2005).

Fish are usually susceptible to bacterial disease in unfavorable conditions such as poor water quality, parasitic infections, poor nutrition, or temperature extremes (Snieszko, 1975). Stress decreases the immunity of fish and leads to decreased resistance to diseases as a result opportunistic bacteria that are found in aquatic environments cause outbreaks of fish diseases (Wedemeyer *et al.*, 1976; OlivaTeles, 2012; Buller, 2014).

Bacterial species responsible for disease outbreaks in different fish species are *Aeromonas*, *Edwardsiella*, *Vibrio*, *Pseudomonas*, *Streptococcus*, *Salmonellas* and *Shigella* species among others (Menanteau-Ledouble *et al.*, 2016; Fadel *et al.*, 2018; Pandey *et al.*, 2021; Yang *et al.*, 2021; Colussi *et al.*, 2022). In Ethiopian water bodies including lakes the only group of pathogens of fish that have been relatively well-studied are helminths (Eshetu Yimer and Mulualem Eneyew 2003; Yewubdar Gulelat *et al.*, 2013), however less attention has been given to bacterial pathogens of fish. Therefore, this research was focused on the isolation, characterization, and identification of common pathogenic bacterial species of Nile tilapia, *O. niloticus*, in Lake Hawassa.

4.2.Objective of the study

4.2.1. General objective

The general objective of the current study was to isolate and characterize hetrotrophic pathogenic bacteria of Nile tilapia, *O. niloticus* in Lake Hawassa.

4.2.2. Specific objectives

The specific objectives of the study were to:

- assess the occurrence of bacteria in Nile tilapia, *O. niloticus* in Lake Hawassa;
- characterize and identify pathogenic bacteria of Nile tilapia, *O. niloticus*, in Lake Hawassa;
- assess bacteria loads of Nile tilapia, *O. niloticus* in Lake Hawassa;
- evaluate the antibiotic susceptibility of *A. hydrophila* of Nile tilapia, *O. niloticus* in Lake Hawassa.

4.3. Materials and Methods

3.3.1. Study population and design

A total of 360 *O. niloticus*, every month 30 fish, were collected from six sampling sites of Lake Hawassa (Figure 3.1). The fish were placed in an icebox containing ice and immediately transported to Biology Department laboratory at Hawassa University for analyses. In the laboratory, before dissection, each fish was observed macroscopically for the identification of clinical signs. Individual fish weight was recorded using digital weighing balance, and their lengths were determined using a measuring board to record infection in relation to length and weight of fish.

4.3.2. Total heterotrophic bacteria count

Out of 30 fish samples collected every month, ten fish samples were randomly selected and dissected aseptically and 1 gm of sample from skin, gill, intestine, liver and kidney each was collected and homogenized in sterile saline solution using a sterile mortar and pestle (Plumb *et al.*, 1982). The body surface of the fish was disinfected using 70% ethanol before dissecting to remove organs of the fish. Appropriate serial dilutions from 10^{-1} to 10^{-6} were made with sterile saline solution (0.85% w/v). Then 0.1 ml aliquot of each dilution (10^{-2} to

10^{-5}) was inoculated by on nutrient agar plate, in triplicate using the spread plate technique for enumeration of total heterotrophic bacteria count. All plates were incubated in an inverted position to prevent condensation at 37 °C for 24hrs under aerobic condition. The heterotrophic bacterial load was counted colony forming unit per ml for different organs of fish (Ayeloja *et al.*, 2018).

CFU/ml⁻¹ =No of colonies X Dilution factor/volume of sample plate

4.3.3. Isolation and identification of bacteria

Samples from skin, gill, intestine, liver and kidney of fish were cultured by streak plate method on different media such as Aeromonas medium base (AMB), Nutrient Agar (NA), Salmonella Shigella Agar (SSA), Thiosulfate–citrate–bile salts–sucrose agar (TCBS), Trypticase Soy Agar (TSA), Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37 °C for 24 to 48 hours. The morphological characteristics of bacterial colonies including colony shape, size, color, elevation, edge and opacity were investigated (Holt, 1982). The representative pure colonies were taken for identification of bacteria by gram staining and biochemical tests that included catalase test, Oxidase test, indole and hydrogen sulfide production, Coagulase test, methyl red, Simmons citrate test, and urease according to Bergey's Manual of Systematic Bacteriology (Bergey and Holt, 2000; Austin and Austin, 2016).

4.3.4. Antimicrobial susceptibility testing

In present study, antimicrobial susceptibility test was carried for the bacterial pathogen, *A. hydrophila*. It was performed using the agar diffusion method described by CLSI (2020). In total, 10 commercial antibiotics were tested and they are amoxicillin (30 µg), ampicillin (10 µg), azithromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), streptomycin (10 µg), tetracycline (30 µg) and penicillin (10

µg). The selection of antibiotics was based on the importance and common use in veterinary and human medicines for preventing and treating the infections. *A. hydrophila* isolates were inoculated into Mueller–Hinton agar and specific antibiotic discs were impregnated on the surface of the inoculated plates in three replications. Then the plates were incubated at 28 °C for 18 to 24 h. The diameter of the inhibition zone was measured in millimetres using a meter stick and antimicrobial resistance was classified as sensitive, intermediate, or resistant on the basis of the zone of inhibition (CLSI, 2020). As no inhibition zone was formed for some of the antibiotics tested, they could only be classified as resistant.

4.3.5. Statistical analysis

All the collected raw data were recorded in Microsoft excel and transferred to SPSS version 20 for descriptive analysis. The mean values were compared by using one-way ANOVA test. A probability of $P < 0.05$ was considered significant.

4.4. Results

4.4.1. Morphometric parameters of *Oriochromis niloticus*

The results of the means of morphometric parameters of fish samples are shown in Table 4.1. The weight of *O. niloticus* ranged between 50 and 285 g with mean weight of 154.72 ± 41.57 g, while the total length of *O. niloticus* varied between 12 and 29 cm with mean length of 19.83 ± 2.43 cm. The minimum and maximum weight varied from 70 g to 285 g for male fish with mean value of 182.88 ± 23.84 g and 50 g to 168 g for female fish with mean value of 115.74 ± 26.74 g. The minimum and maximum length varied from 16 cm to 29 cm for male and 12 cm to 21 cm for female fish.

Table 4.1: Morphometric data of *O. niloticus* in Lake Hawassa

	Sex	Number of fish	Mean \pm SD	Minimum	Maximum
Weight (g)	Female	151	115.74 \pm 26.74	50.00	168.00
	Male	209	182.88 \pm 23.84	70.00	285.00
	Total	360	154.72 \pm 41.57	50.00	285.00
Length(cm)	Female	151	17.73 \pm 1.93	12.00	21.00
	Male	209	21.35 \pm 1.42	16.00	29.00
	Total	360	19.83 \pm 2.43	12.00	29.00

cm; centemeter, g; gram, SD: Standard deviation

4.4.2. Prevalence of infection

Results for Prevalence of infection in *O. niloticus* between sexes are shown in Table 4.2. Out of 360 fish collected 121 females were infeced (80 and males 110 (52.6) with the overall prevalence of 64.2%.

Table 4.2: Prevalence of infection between sexes of *O. niloticus* in Lake Hawassa

Sex	No. fish examined	Number of fish infected	Percentage of infection (%)
Female	151	121	80
Male	209	110	52.6
Total	360	231	64.2

The results of length-weight infection showed that 90% within 12-18 cm and 99 g average weight group, 50.6% within 19-21 cm and 162.1 g average weight group, 60% within 22-23 cm and 195.9 g average weight group, and 42.8% within 24-29 cm and 232.5 g average weight group were infected with bacteria (Table 4.3).

Table 4.3: Infection in relation to Length–weight of *O. niloticus* in Lake Hawassa

Length range (cm)	Weight average (g.)	No. within the group	No. of infected fish	Prevalence (%) within the group
12-18	99	101	91	90
19-21	162.1	162	82	50.6
22-23	195.9	85	51	60
24-29	232.5	12	7	42.8
Total		360	231	64.2

cm; centimeter, g; gram

The prevalence of infection of Nile tilapia, *O. niloticus* among months is shown in Figure 4.1. The highest prevalence rate (12.55%) was recorded during July, following by August (11.7%) and September (10.4%), while the least (5.6%) was obtained during December and January.

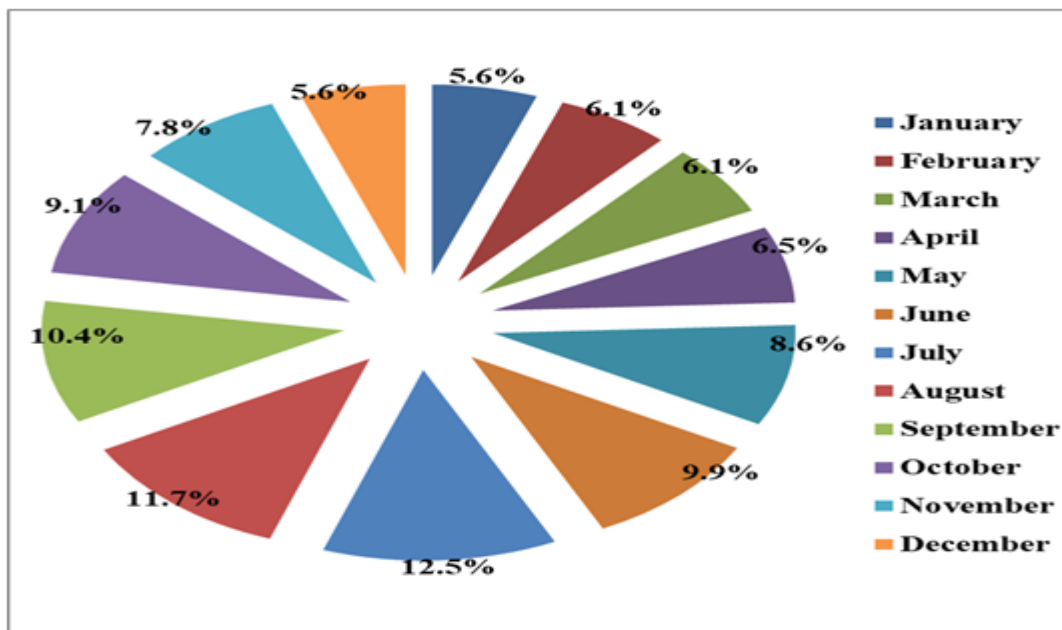


Figure 4.1: Prevalence of bacterial infection of Nile tilapia, *O. niloticus* among months

4.4.3. Symptoms of infection

A total of 360 fish were collected and clinically examined. Naturally infected fish showed different signs such as dark skin, bleeding skin, lesion on the skin, frayed fins, and body ulceration (Figure 4.2).

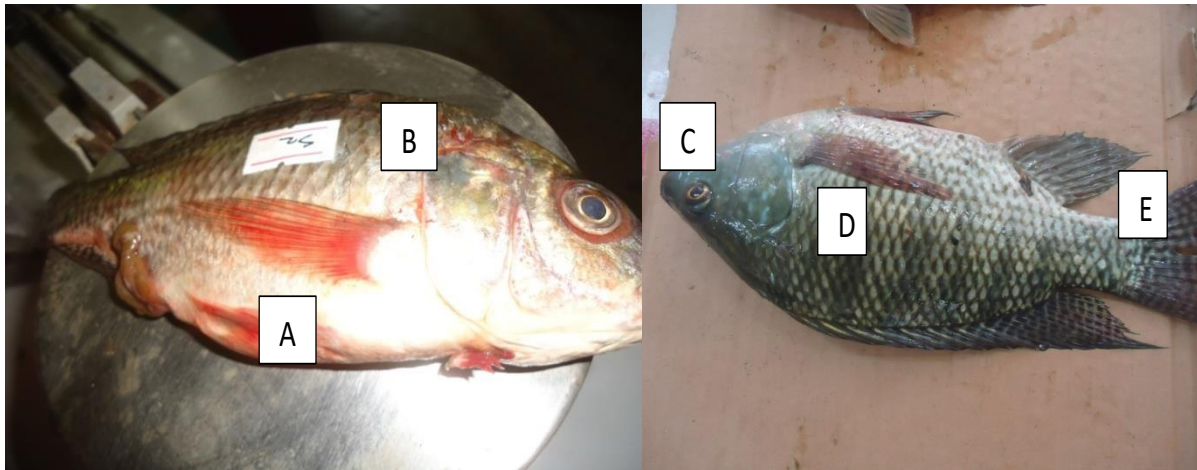


Figure 4.2: Symptoms of naturally infected Nile tilapia, *O. niloticus* showed Hyperemia at the bases of the Pelvic fin (A), Ulcerations (B), corneal opacity (C), darkness in skin (D) and frayed fin (E)

4.4.4. Bacterial population

The total viable bacterial counts recorded in the form of number of colonies in the various tissues of *O. niloticus* are shown in Table 4.4. Each number is the average value of viable colonies which grew in triplicate agar plates to each sample. The results showed that, colonies in the skin ranged from 4.20×10^5 CFU/ml and 5.42×10^5 CFU/ml with average of $4.72 \pm 0.48 \times 10^5$ CFU/ml. The highest colonies were recorded in intestine for summer ($5.81 \pm 0.01 \times 10^5$ CFU/ml), while the lowest value was recorded in kidney of Nile tilapia for spring ($2.23 \pm 0.00 \times 10^5$ CFU/ml). Bacteria loads of the liver during winter and spring was not significantly different ($P > 0.05$).

Table 4:4 Total viable bacteria count of *O. niloticus* in Lake Hawassa (10^5 CFU/ml), \pm SD

Season	Sample source				
	Skin	Gill	Intestine	Liver	Kidney
Summer	5.41 \pm 0.01 ^d	4.91 \pm 0.01 ^d	5.81 \pm 0.01 ^d	3.71 \pm 0.01 ^c	3.11 \pm 0.01 ^d
Autumn	4.91 \pm 0.01 ^c	4.21 \pm 0.01 ^c	4.51 \pm 0.00 ^b	3.10 \pm 0.00 ^a	2.70 \pm 0.00 ^c
Winter	4.34 \pm 0.10 ^b	4.03 \pm 0.01 ^b	4.71 \pm 0.01 ^c	3.50 \pm 0.00 ^b	2.50 \pm 0.00 ^b
Spring	4.21 \pm 0.01 ^a	3.43 \pm 0.01 ^a	4.41 \pm 0.00 ^a	3.50 \pm 0.01 ^b	2.23 \pm 0.00 ^a
Average	4.72 \pm 0.48	4.14 \pm 0.53	4.86 \pm 0.56	3.45 \pm 0.23	2.63 \pm 0.32
Range	4.20-5.42	3.41-4.92	4.40-5.82	3.10-3.72	2.23-3.12

Within the same column mean value with the different superscript letter is significantly different at $P < 0.05$ based on the ANOVA (Tukey's) test. SD; standard deviation

4.4.5. Bacteriological findings

Based on morphological characteristics (Appendix table 4) and conventional biochemical test (Appendix table 5) different groups of bacteria isolates that belonged to 10 species including *Escherichia coli* (*E. coli*), *A. hydrophila*, *Edwardsiella tarda* (*E. tarda*), *Pseudomonas*, *Vibrio*, *Bacillus*, *Staphylococcus*, *Flavobacterium*, *Salmonella* and *Shigella* species were identified and their prevalence is presented in Table 4.5. The highest prevalence was recorded for *E. coli* (19.3%), followed by *A. hydrophila* (17.8%), *Pseudomonas* species (5.6%) and *Vibrio* species (3.3%). The highest number of bacteria occurrence was recorded from intestine (95%) followed by skin (92.5%) and gills (90%). The lowest occurrence was recorded from live (16.7%). All isolates frequently occurred in intestine compared to other fish organs, except *Flavobacterium* spp. which is absent in intestine. The results also showed high occurrence of *A. hydrophila* and *E. coli* in all fish organs examined. *E. tarda* and *Shigella* species were occurred only in skin and intestine of Nile tilapia. The most frequently isolated bacterium from intestine of Nile tilapia was *E. coli* (31.7%), followed by *A. hydrophila* (27.5%).

Table 4.5: Occurrence of bacterial isolates from tissues of Nile tilapia in Lake Hawassa

Isolates	Skin n=120	Gill n=120	Intestine n=120	Liver n=120	Kidney n=120	Total N=600
<i>E. coli</i>	28(23.3)	25(20.8)	38(31.7)	8(6.7)	17(14.2)	116(19.3)
<i>A. hydrophila</i>	30(25)	28(23.3)	33(27.5)	8(6.7)	8(6.7)	107(17.8)
<i>Pseudomonas</i> spp.	8(6.7)	10(8.3)	6(5)	0	10(8.3)	34(5.6)
<i>Vibrio</i> Spp.	6(5)	9(7.5)	5(4.2)	0	0	20(3.3)
<i>Bacillus</i> spp.	4(3.3)	3(2.5)	6(5)	0	6(5)	19(3.2)
<i>Staphylococcus</i> spp.	4(3.3)	4(3.3)	6(5)	4(3.3)	0	18(3)
<i>Salmonella</i> spp.	5(4.2)	3(2.5)	8(6.7)	0	2(1.7)	18(3)
<i>Flavobacterium</i> sp	7(5.8)	8(6.7)	0	0	1(0.8)	16(2.7)
<i>Shigella</i> spp.	6(5)	0	10(8.3)	0	0	16(2.7)
<i>E. tarda</i>	13(10.8)	0	2(1.7)	0	0	15(2.5)
Total	111(92.5)	90(75)	114(95)	20(16.7)	44(36.7)	379(63.2)

Values in parantheses indicate percentage of occurrence

4.4.6. Antibiogram test

The results of antibiogram test of *A. hydrophila* isolates are presented in Table 4.6. High levels of *A. hydrophila* resistance to various antibiotics were found in this study, especially to amoxicillin, ampicillin and penicillin (100%, n=14), erythromycin (57.2%, n=8) azithromycin (50%, n=7) and streptomycin (42.8%, n=6). In contrast the level of resistance to tetracycline, gentamycin, ciprofloxacin and chloramphenicol were zero.

Multiple antibiotics resistant (MAR) of *A. hydrophila* isolates of selected antibiotics are presented in Table 4.7. The results showed that MAR of the isolates was between values of 0.4 and 0.6.

Table 4.6: Antimicrobial susceptibility of *A. hydrophila* isolates (n = 14)

Antibiotics	Resistant		Intermediate		Sensitive	
	N	%	N	%	N	%
Amoxicillin(30 µg)	14	100	0	0.0	0	0.0
Ampicillin (10 µg)	14	100	0	0.0	0	0.0
Azithromycin (15 µg)	7	50	7	50	0	0.0
Chloramphenicol (30 µg)	0	0.0	0	0.0	14	100
Ciprofloxacin (5 µg)	0	0.0	0	0.0	14	100
Erythromycin (15 µg)	8	57.2	6	42.8	0	0.0
Gentamicin (10 µg)	0	0.0	0	0.0	14	100
Streptomycin(10 µg)	6	42.8	8	57.2	0	0.0
Tetracycline (30 µg)	0	0.0	0	0.0	14	100
Penicillin (10 µg)	14	100	0	0.0	0	0.0

N: number of isolates, %: susceptibility index

Table 4.7: Multiple antibiotic resistance indices of *A. hydrophila* isolates

Bacterial isolates	Number of antibiotics resistant (a)	Number of antibiotics used	MAR index (a/b)
LH2	6	10	0.6
LH8,	4	10	0.4
LH12	5	10	0.5
LH22	4	10	0.4
LH30	5	10	0.5
LH34	4	10	0.4
LH37	4	10	0.4
LH47	4	10	0.4
LH51	5	10	0.5
LH55	5	10	0.5
LH60	5	10	0.5
LH65	4	10	0.4
LH72	4	10	0.4
LH78	4	10	0.4
Average MAR = 0.45			

LH; Lake Hawassa, MAR; Multiple antibiotic resistance

4.5. Discussion

In the current study, the mean of morphometric parameters of the fish showed that male fish were higher in average body weight and total length compared to female fish. This might be due to the difference in growth pattern as male Nile tilapia grow faster and bigger than the female fish. Toshiharu *et al.* (2012) reported that male and female fish growth had significant differences ($P < 0.001$) in body weight and total length. Also, the same author reported that the growth rate of female fish decreased earlier than male and this suggests that increasing sexual maturation inhibits the size and growth rate of female fish.

The present study also showed that the prevalence of infection in female fish was higher (80%) than the male fish (52.6%). Similar to this finding, Rediet Wolde *et al.* (2022) reported that 34.6% of the female fish and 17.65% of the male fish were positive for *E. tarda* in Lake Bishophtu and Babogaya. Also, Fonkwa *et al.* (2022) reported that the females were more infected (67.07%) than the males (58.94%). This indicates that female fishes are more susceptible than male fishes. This might be due to of stronger immunity of male than females. More of the energy in female fish is devoted to reproduction than for immunity (Wootton, 1985).

The results of length-weight in relation to infestation showed that the highest prevalence (90%) was recorded in 12-18 cm length group and 99 g average body weight. It shows that as the size of fish increases the infection decreases. This indicates that the smaller the fish greater the infection. In line with these findings, Begonesh *et al.* (2019) reported that smaller Nile tilapia were more sensitive than larger ones. Also, Mzula *et al.* (2019) reported that younger fishes are more vulnerable to infections than older ones. This might be the ability of fish to withstand infections at later ages. For example, the immunoglobulin concentration in serum increases with increasing age. In some species of fish, this increase continues

throughout the lifetime while in other species maximum level is reached at sexual maturation (Magnadottir *et al.*, 1999).

In the current study, it was observed that the percentage of prevalence increased gradually from January onwards and reached a peak in July. The prevalence remained almost the same for January, February, March, and December. The outbreaks of fish diseases are seasonally based and highly experienced in summer (Ibrahim *et al.*, 2008). Similar findings have been observed in the current study where high occurrences of bacteria were recorded during July (12.5%) and August (11.7%). The highest prevalence record for the months of summer (July and August) compared with the other months in the present study might be due to favorable temperature as well as the flow of flood that contains wastewater to the lake as these months are found in the rainy season in the study area. Similar results were reported by Mzula *et al.* (2019) who reported the highest occurrence of infection during May and August.

In the present study on careful examination, it was found that naturally infected Nile tilapia showed ulceration, corneal opacity, mucus secretion, frayed fin, and hyperemia at the bases of fin, lesion, and darkness in the skin. Consistent with the current study, Aishi-Hamom *et al.* (2020) reported clinical signs of disease such as skin lesions, corneal opacity, excess mucus secretion, hemorrhages, and frayed fins. Thune *et al.* (1993) also reported hemorrhagic septicemia in fish due to bacterial infection, especially when fish are under stress. The present result is also in conformity with the findings of Kaleeswaran *et al.* (2012) and Noor El-Deen *et al.* (2014).

Callinan *et al.* (1995) reported the seasonal occurrence of diseases in fishes due to climatic variations in aquatic ecosystems. In the present study, bacteria loads of lake water (Table 3.1) and fish tissues (Table 4.4) showed considerable variation among seasons during the study period. The bacteria loads were high throughout the period of study. The distributions of

heterotrophic bacteria were higher during summer compared to winter and spring (September, October and November). The aquatic environment that harbours fish may be the source of contaminants due to animal excreta and other environmental wastes, especially during the rainy season. The highest bacterial load detected during the summer season might be attributed to discharges of wastewater to the lake through flood as this is found within the rainy season. Ali *et al.* (2011) reported that the richness of the effluents in organic carbon exerted a specific enrichment effect on the microbial population. A similar finding was noticed by Wondimu Lakew *et al.* (2015) in Koka Reservoir. The results also showed that the bacteria load in the intestine of fish was higher than in other organs. This result was similar to the previous findings where higher bacteria loads in the intestine of fish than in other organs were reported (Shankar *et al.*, 2009; Nebyu Kassa and Marshet Adugna, 2021). The highest bacteria in intestine of the fish might be due voracious feeding behavior of Nile tilapia and poor water quality (Beveridge *et al.*, 1988).

The highest and significantly different bacteria load ($5.81 \pm 0.01 \times 10^5$ CFU/ml) was recorded during summer from the intestine of the fish ($P < 0.05$), while the lowest bacterial loads ($4.41 \pm 0.00 \times 10^5$ CFU/ml) was recorded during spring. In opposite to the present finding, El-Fadaly *et al.* (2019) reported the highest bacterial loads (4.62×10^7 CFU/g) of the fish intestine in spring and the lowest value in autumn from Manzala Lake in Egypt and this contradictory results to the season of bacterial loads suggest that other factors may be intrinsic (parasite and host) or extrinsic (water physico-chemical characteristics) regulate the bacterial population. Pathogenic bacteria in the intestine of fish may lead to food safety and health risks in humans.

Similarly, the highest ($5.41 \pm 0.01 \times 10^5$ CFU/ml) and lowest ($4.21 \pm 0.01 \times 10^5$ CFU/ml) bacteria loads from fish skin were recorded during summer and spring ($P < 0.05$). The highest

bacterial load in the skin of fish could be owing to constant exposure of fish skin to contaminated water (Apun *et al.*, 1999). Also, the scale of Nile tilapia can harbour feed matter suitable for the survival of bacteria. The higher load of bacterial isolates in gills was also recorded in the present study due to the fact that the gill mucus is in constant contact with the environment, providing a habitat for heterotrophic bacteria and a portal of entry for several pathogens. Mwege *et al.* (2019) stated that the high proportion of infection in gills is due to the exposed nature of the organ to microbiota. A higher number of bacteria on the surface of the skin and gills indicate the level of lake pollution.

In the current study bacteria species such as *A. hydrophila*, *E. coli*, *E. tarda*, *Pseudomonas*, *Vibrio*, *Bacillus*, *Staphylococcus*, *Flavobacterium*, *Salmonella* and *Shigella* spp. were isolated from Nile tilapia in Lake Hawassa. The phenotypic and biochemical characteristics of all isolates recorded in the present study were in line with those reported in Bergey's manual of determinative bacteriology (Bergey and Holt, 2000; Austin and Austin, 2016).

A. hydrophila is one of the most opportunistic pathogens for freshwater fish and the main etiological agent in disease outbreaks and mortality of fish (Das and Mukhyec, 1999). Austin and Austin (2016) and Tooba *et al.* (2022) reported *A. hydrophila* from naturally infected Nile tilapia. In the present study, *A. hydrophila* showed a high frequency of occurrence of (17.8%). The prevalence of *A. hydrophila* in the present study was in conformity with Abd-El-Malek (2017), who reported 16% of the occurrence of *A. hydrophila* from wild Nile tilapia in Egypt. However, Balaji *et al.* (2004), Jimoh and Jatau (2010) and Rodrigues *et al.* (2019) reported a higher prevalence of 41.7%, 47%, and 46.66% for *A. hydrophila* from fish of India, Nigeria, and Brazil, respectively. The highest prevalence (6.3%) of *A. hydrophila* was observed in the intestine of Nile tilapia. Al-Harbi *et al.* (2005) indicated that the presence of bacteria in fish's digestive flora is normal. However, the outbreak of disease is related to the

existence of a stress factor based on the interaction between fish, pathogens, and the aquatic environment as a natural habitat of the organism, as well as poor water quality or excess of organic matter factors which allows the incidence of disease to be greater. Also, Khalil and Mansour (1997) added that stress is often considered to be a contributing factor in the outbreaks of disease caused by these bacteria.

Antimicrobial agents are used not only in human and veterinary medicine but also in livestock production for disease prevention or as growth promoters in animal husbandry. In the present study, *Aeromonas* isolates of Nile tilapia in Lake Hawassa were 100% resistant to amoxicillin, ampicillin and penicillin. Similar results were reported by Yano *et al.* (2015) from Thailand. In the present study, none of the *Aeromonas* isolates showed resistance to chloramphenicol, ciprofloxacin, tetracycline and gentamycin. The high resistance of *Aeromonas* isolates to these antibiotics may be due to prolonged exposure of the organism to these drugs. The results on the sensitivity of *Aeromonas* isolate to gentamycin are consistent with the results obtained by Belèm-Costa and Cyrino (2006) from tilapia that showed 100% of *Aeromonas* isolates susceptible to gentamycin. In the present study majority of *Aeromonas* isolates were resistant to most of the antibiotics used. This indicates their ability to detoxify antibacterial organic compounds. The development of multiple antibiotic resistances by *A. hydrophila* from aquatic environments is a major problem in many parts of the world (Igbiosa *et al.*, 2012).

The present study showed the highest frequency of occurrence of *E. coli* (19.3%) from Nile tilapia in Lake Hawassa. Aynadis Tilahun and Aweke Engdawork (2020) reported a 24% occurrence of *E. coli* from Nile tilapia in Lake Hawassa. Also, Adanech Haile and Temesgen Getahun (2018) reported 12% of the occurrence of *E. coli* from fish in Lake Zeway. The presence of *E. coli* in the tissues of fish indicates faecal contamination of water from which

the fish were harvested (Gerokomou *et al.*, 2011). The occurrence of *E. coli* in the intestine of Nile tilapia was higher than in the other organs during the study period. This may be attributed to the existence of bacterium as normal intestinal microflora of fish and may result in clinical infection if it gets spread to other organs (Hanson *et al.*, 2008). Bacteria often occur on the body or internal parts of fish indicating the extent of pollution of the aquatic ecosystem (Ibemenuga and Okeke, 2014), thus the occurrence of *E. coli* in the intestine of Nile tilapia in the current study reflects the water quality of the lake.

Vibrio spp. showed the occurrence at the rate of 3.3% which was lower than the occurrence of *Vibrio* spp. of 17% as reported by Traoré *et al.* (2014) from Nile tilapia of reservoirs in Ouagadougou, Burkina Faso and Nabiyu Kassa and Mareshet Adugana (2021) at a rate of 6.6%, . The highest occurrence of *Vibrio* spp. found in the gill of Nile tilapia (7.5%). Runfit *et al.* (2014) found that the bacterium attached to the fish intestinal epithelium and formed micro-colonies. Similar to the present findings, Tesfaye Shimels *et al.* (2018) and Nur Anwar *et al.* (2012) reported *Vibrio* species from the Nile tilapia of Lake Hayqe and Lake Tana, respectively. The presence of *Vibrio* species in the tissues of fish indicates that the bacterium is a potential threat to public health.

E. tarda infects both cultured and wild fish with worldwide distribution as well as an opportunistic pathogen in other animals including humans. Marked variation in the occurrence rate of this bacterium from different species of fish has been recorded by different authors (Eissa and Yassine, 1994; Rashid *et al.*, 1994; Nur Anwar *et al.*, 2012; Abayneh Tekele *et al.*, 2017). Based on percentage frequency occurrence, *E. tarda* showed the lowest frequency of occurrence of 2.5% which was lower than the result reported (7.6%) by Abayneh Tekele *et al.* (2017) for Lake Hawassa. Similar to the present results, Nur Anwar *et al.* (2012) and Adanech Haile and Temesgen Getahun (2018) reported 4% and 2% of *E. tarda*

from Lake Tana and Lake Ziway, respectively. A similar result was reported by Galal *et al.* (2005) from Egypt, which was 3.5%. In the present study, *E. tarda* was recorded only from the skin and intestine. Bedaso Kebede and Habtamu Tilahun (2016) reported *E. tarda* most frequently from the liver (6.5%) followed by the intestine (2.4%) and kidney (0.8%) with significant differences among organs from Lake Zeway and Lake Langano. Mohanty and Sahoo (2007) stated that *Edwardsiella* is a Gram-negative bacterium that could be found in the intestine of healthy fish. The variation in the occurrence of *E. tarda* in fish may be due to the stress of the fish and water quality.

Salmonella species are among the most important causes of human gastrointestinal disease worldwide. The presence of *Salmonella* species indicates faecal contamination of water from which the fishes were harvested. In the present study, *Salmonella* species were recorded from the skin, gill, intestine and kidney of *O. niloticus*. The occurrence of *Salmonella* species was 3%. The result was lower than the report by Ertas *et al.* (2015) and Esther (2022) who reported a 5% and 24% prevalence of *Salmonella* species in Nile tilapia from Turkey and Tanzania, respectively. Adams and Tobaias (1999) have demonstrated that fish and fish products are only occasionally associated with *Salmonella* and that filter-feeding shellfish harvested from polluted water have been identified as higher-risk products.

The presence of *Staphylococcus*, *Shigella* and *Salmonella* species in fish indicates faecal and environmental pollution (Sichewo *et al.*, 2013; Gufe, 2019). Therefore they are most likely to cause food-borne diseases for fish consumers (Sichewo *et al.*, 2013). The frequency occurrence of *Shigella* species in the present study was 2.7%. The result was lower than the report by Onyango *et al.* (2009) who reported 14.3% the frequency occurrence of *Shigella* species in fish from the Winam Gulf of Lake Victoria, Kenya. The percentage frequency of occurrence of *Pseudomonas* species, in the present study was 5.6%, which was lower than the

report by Abd-El-Naser *et al.* (2021) who reported 23.3% of occurrence of *Pseudomonas* species from Fish Marketed in Sohag Governorate, Egypt. The occurrence, of *Staphylococcus* species showed a frequency of occurrence of 3%. The result was higher than the report by Ali (2017) who reported 1.75% occurrences of *Staphylococcus* species in fresh Indian mackerel (*Rastrelliger kanagurta*) in Unguja Island.

Bacillus species have great potential to be used as disease inhibitors (Kesacordi-Watson *et al.*, 2008). They are used as a dietary supplement for the improvement of human and animal health (Lee *et al.*, 2019). However, some *Bacillus* species are also known producers of toxins in their human and animal hosts (Elshagabee *et al.*, 2017). The occurrence of *Bacillus* species in the current study was 3.2%. The result was higher than the result reported by Nebiyu Kassa and Mareshet Aduga (2021) at a rate of 2% from Ethiopia, but much lower than the findings that reported 80% of occurrence of *Bacillus* species from pond water fish in Trinidad, South America (Newaj-Fyzu *et al.*, 2008).

CHAPTER FIVE

EFFECTS OF WATER QUALITY AND *Aeromonas hydrophila* ON HAEMATOLOGICAL PARAMETERS OF Nile tilapia, *Oreochromis niloticus* L.

5.1. Introduction

Haematological changes are important indicators used in monitoring physiological and pathological changes in fish (Satheeskumar *et al.*, 2011). Bahmani *et al.* (2001) reported that analysis of haematological indices in the blood of fish is good for identifying the health status of fish as they provide reliable information on metabolic disorders and deficiencies. Banerjee *et al.* (2002) reported that blood composition is moderately constant under normal condition with little variation. However, the composition of blood can be changed by dietary treatment, malnutrition and disease condition (Feist *et al.*, 2000). Ferreira *et al.* (2007) reported that biochemical parameters provide early warning of potentially harmful changes in stressed organisms. Bello-Olusoji *et al.* (2006) further explained that changes in haematology of fish in response to stressing agents are indicators of the stressful stage of fish producing useful information to curb any unfavourable condition that may affect the fish health.

Haematological parameters are non-specific in their responses towards chemical stressors. Nevertheless, they may provide important information in assessment studies, by providing an indication as to the general physiology and health status of the organism under investigation (Ayoola *et al.*, 2014). The growing attention to fish haematology is also due to the relationship between haematological parameters and aquatic environmental pollution (Fazio *et al.*, 2019; Sayed *et al.*, 2019). Several researchers have investigated the toxicity, uptake and tissues distribution and haematological changes of pollutants in fish (Akinrotimi *et al.*, 2007; Chindah *et al.*, 2008), and the use of haematological techniques in fisheries research is growing rapidly, as it is very important in toxicological research which results in monitoring

and predicting health conditions of the fish (Akinrotimi *et al.*, 2009; Ayoola *et al.*, 2014). Since fish are so intimately associated with the aqueous environment, the blood will reveal measurable physiological changes in the fish more rapidly than any physiological assessment parameters (Ezeri *et al.*, 2004)

Various exogenous factors such as environmental conditions, water pollution, and stress (Nikoo *et al.*, 2010; Gabriel *et al.*, 2011), and endogenous factors such as age, sex and fish species (Svetina *et al.*, 2002; Nikoo *et al.*, 2012) influence haematological parameters in fish values. Fish blood characteristics change in response to environmental conditions, thus the variation of haematological features could serve as a biomarker of sub-lethal environmental stress. Haematology is used as an index of fish health status in many fish species to detect physiological changes following different stress conditions such as exposure to pollutants, diseases, heavy metals, hypoxia etc. According to Svobodova *et al.* (1996), haematological parameters are carried out on fish to ascertain the normal range of blood parameters in relation to age and sex to determine the effects of disease conditions in fish.

Haematology must be analysed when fish are exposed to environmental changes due to pollutants, stress and infections (Martins *et al.*, 2004). Basic haematological parameters are hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte (RBC) and leukocyte count (WBC). Additional derived red blood parameters can also be calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) using Ht, Hb, and RBC values and appropriate formulas. Therefore, this study aimed to evaluate the effect of water quality of Lake Hawassa and pathogenic bacteria on the haematological parameters of *O. niloticus*.

5.2. Objective of the study

5.2.1. General objective

General objective of present study was to investigate the effect of water quality and *Aeromonas hydrophila* on haematological indices of Nile tilapia, *O. niloticus*

5.2.2. Specific objectives

The specific objectives of the present study were to;

- assess haematological parameters of *O. niloticus* in Lake Hawassa
- determine the effect of *A. hydrophila* on haematological parameters of *O. niloticus*.
- determine mortality rate of *O. niloticus* infected with different concentration of *A. hydrophila*
- evaluate the contribution of sampling sites, size and sex of fish on haematological parameters of *O. niloticus*

5.3. Materials and Methods

5.3.1. Collection of fish and haematological examination

For haematology studies 72 Nile tilapia of length ranged between 12 and 29 cm, and weight ranged between 50 and 285 g were taken from 360 fish collected from different sampling sites such as Tikur Wuha, Green Wood, Fiqir Hayik, Amora Gedel, Refereal Hospital and Kukyu Wata of Lake Hawassa (Figure 3.1). Individual fish sex, weight and length were recorded to determine their relation to haematological parameters of fish. The haematological parameters were also determined in relation to sampling sites. Fish were anesthetized with 50 mg l⁻¹ of tricaine methanesulfonate (MS222, Sigma Chemical Co. St. Louis, MO, USA) and then blood samples were collected from the caudal peduncle with the use of a 5 ml syringe and needle that has been treated with heparin to prevent clotting and transferred to sampling bottles that contain ethylene diamine tetra-acetic-acid (EDTA). After the collection, the blood

samples were taken to the Veterinary laboratory, where the haematological analysis was carried out. Blood was analysed with routine methods adopted in fish haematology (Blaxhall and Daisley, 1973). The total erythrocyte count ($\text{RBC} \times 10^6/\mu\text{l}$) and total leukocyte count ($\text{WBC} \times 10^3/\mu\text{l}$) were determined manually using a Neubauer's haemocytometer with Hayem solution as a diluent. The Hct percentage was determined in duplicate by using microhaematocrit-heparinized capillary tubes of 75 μl volume and a microhaematocrit centrifuge at 15000 g for 5 min. For the determination of Hb concentrations Sahli's method was used. The values of Hb were expressed as g/dl. The values of RBC indices of MCV (fl), MCH (pg) and MCHC (g/dl) were calculated.

$$\text{MCV} = \text{Hct} * 10 / \text{RBCs}$$

$$\text{MCH} = \text{Hb} * 10 / \text{RBCs}$$

$$\text{MCHC} = \text{Hb} / \text{Hct}$$

5.3.2. Preparation of different concentrations of *A. hydrophila* isolates

A. hydrophila LH2 isolate used in this study was isolated from naturally infected Nile tilapia in Lake Hawassa. The isolate was selected based on the ability of multiple antibiotic resistance. The isolates were streaked onto TSA (Oxoid, Hampshire, UK) and further sub-cultured onto ASA (Oxoid) and incubated at 37 °C for 18 hrs. For preparation of different concentration of *A. hydrophila*, five colonies of *A. hydrophila* grown on ASA were randomly picked and inoculated into 1 L of TSB, and incubated for 18 h at 37 °C. Serial dilutions were performed up to tenfold and 0.1 mL of TSB from each dilution was plated on TSA in triplicate, and further incubated at 37 °C for 24 h. The colony forming units per millilitre (CFU/mL) were determined using standard plate count.

5.3.3. Pathogenicity test

Two hundred seventy Nile tilapia of 20 ± 1.00 g body weight and 11.06 ± 0.08 cm lengths were taken from the Aquaculture Research Centre, Hawassa University and transported to laboratory and kept in a tank for 2 weeks. The fish fed commercial feed three times daily during acclimatization and experimental period until apparent satiation. No sign of disease and mortality was observed during the acclimatization period. Five fish were randomly sampled and examined for the presence of parasites and bacteria. Before a challenge test, the fish were distributed into one control group (C) and eight treatment groups (T1 to T8) of 10 fish. The experiment was conducted in triplicates. The fish in each of the eight treatment groups were IP injected with 0.2 mL of *A. hydrophila* at concentrations 10^1 to 10^8 CFU/mL, respectively. The fish in the control group were injected with sterile 0.2ml TSB. All fish were observed for clinical signs and mortalities for a period of 240 h post-infection (hpi), and the LD₅₀ value was calculated (Plumb and Bowser, 1983). The bacteria were re-isolated from moribund fish, cultured onto ASA, incubated at 28°C for 24 h, and reconfirmed whether or not the infection was due to *A. hydrophila*.

5.3.4. Statistical analysis

The data were analysed with a one-way analysis of variance (ANOVA) Tukey's post hoc test, using SPSS version 22. Differences at $P < 0.05$ were regarded as statistically significant.

5.4. Results

5.4.1. Haematology

5.4.1.1. Haematological parameters in relation to sampling sites

The results of haematological parameters of Nile tilapia in Lake Hawassa among sampling sites are presented in Table 5.1. The statistical analysis of the parameters exhibited a significant difference among sites ($P < 0.05$). While comparing the sites, TW site recorded significantly the lowest RBCs, Hb, and Hct ($P < 0.05$). Conversely, KW site recorded significantly highest RBCs, Hb and Hct ($P < 0.05$). Significantly highest ESR (6.97 ± 0.01

mm/hr) recorded at TW site followed by AG (6.13 ± 0.01 mm/hr) and RH (5.68 ± 0.01 mm/hr) sites ($P < 0.05$). There is no significant difference between AG and RH for MCV value ($P > 0.05$).

Table 5.1: Mean values of haematological parameters of *O. niloticus* in Lake Hawassa (mean \pm SD, n=72).

Site	RBCs ($10^6/\text{mm}^3$)	Hb (g/dl)	Hcr (%)	ESR (mm/hr)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($10^3/\text{mm}^3$)
TW	1.54 \pm 0.01 ^a	6.14 \pm 0.01 ^a	16.65 \pm 0.01 ^a	6.97 \pm 0.01 ^f	107.93 \pm 1.24 ^c	39.78 \pm 0.40 ^e	36.87 \pm 0.03 ^f	50.34 \pm 0.00 ^f
GW	1.79 \pm 0.01 ^d	6.84 \pm 0.01 ^d	20.68 \pm 0.01 ^e	5.05 \pm 0.01 ^b	115.53 \pm 0.53 ^c	38.41 \pm 0.18 ^d	33.17 \pm 0.02 ^b	47.24 \pm 0.01 ^b
FH	1.89 \pm 0.00 ^e	7.13 \pm 0.01 ^e	20.07 \pm 0.01 ^d	5.14 \pm 0.01 ^c	106.19 \pm 0.43 ^b	37.72 \pm 0.12 ^c	35.52 \pm 0.02 ^d	47.87 \pm 0.01 ^c
AG	1.68 \pm 0.00 ^b	6.79 \pm 0.01 ^c	18.68 \pm 0.01 ^b	6.13 \pm 0.01 ^e	111.19 \pm 0.51 ^d	40.41 \pm 0.15 ^f	36.34 \pm 0.03 ^e	49.36 \pm 0.01 ^e
RH	1.73 \pm 0.00 ^c	6.35 \pm 0.01 ^b	19.24 \pm 0.01 ^c	5.68 \pm 0.01 ^d	111.21 \pm 0.52 ^d	36.74 \pm 0.20 ^b	33.01 \pm 0.05 ^a	48.37 \pm 0.01 ^d
KU	2.35 \pm 0.01 ^f	7.78 \pm 0.00 ^f	23.14 \pm 0.02 ^f	4.54 \pm 0.02 ^a	98.48 \pm 0.27 ^a	33.11 \pm 0.14 ^a	33.63 \pm 0.05 ^c	44.4 \pm 0.10 ^a
Total	1.83 \pm 0.25	6.84 \pm 0.53	19.74 \pm 1.99	5.58 \pm 0.80	108.42 \pm 5.39	37.69 \pm 2.42	34.75 \pm 1.56	47.93 \pm 1.88

Values within a column with different superscripts differ significantly at $P < 0.05$. RBCs; Red blood cells, Hb; Hemoglobin, Hcr; Hematocrit, ESR; Erythrocyte sedimentation rate, MCV; Mean corpuscular volume, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, WBCs, White blood cells

5.4.1.2. Length-weight related variations in the haematological parameters of

Nile tilapia

The values of haematological parameters of Nile tilapia in relation to length-weight are presented in Table 5.2. The results showed statistically significant difference among length-weight groups ($P < 0.05$). The fish belonging to 12-18 cm length and 99 g average weight group showed significantly low value of RBCs, Hb, Hct and WBCs ($P < 0.05$). Contrary, significantly highest value of RBCs, Hb, Hct and WBCs ($P < 0.05$) were recorded for the fish belonging to 24-29 cm length and 232.5 g average weight group. The fish belonging to 12-18 cm and 99 g average weight group recorded significantly highest ESR (6.97 ± 0.01 mm/hr), while 24-29 cm length and 232.5 g average weight group recorded significantly lowest value (4.54 ± 0.01 mm/hr) for ESR ($P < 0.05$).

Table 5.2: Values of haematological parameters of fish in accordance to length-weight group

Length (cm)	Weight Av.(g)	RBCs ($10^6/\text{mm}^3$)	Hb (g/dl)	Hcr (%)	ESR (mm/hr)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($10^3/\text{mm}^3$)
12-18	99	1.54 ± 0.17^a	6.14 ± 0.01^a	16.6 ± 0.01^a	6.97 ± 0.01^d	107.89 ± 1.17^b	39.78 ± 0.40^d	36.87 ± 0.03^d	44.40 ± 0.01^a
19-21	162.1	1.71 ± 0.01^b	6.57 ± 0.00^b	18.96 ± 0.0^b	5.91 ± 0.01^c	110.30 ± 0.29^c	37.77 ± 0.03^b	34.31 ± 0.05^b	47.55 ± 0.01^b
22-23	195.9	1.84 ± 0.00^c	6.98 ± 0.00^c	20.3 ± 0.01^c	5.10 ± 0.01^b	110.46 ± 0.59^c	38.14 ± 0.25^c	34.66 ± 0.03^c	48.86 ± 0.01^c
24-29	232.5	2.35 ± 0.01^d	7.78 ± 0.00^d	23 ± 0.2^d	4.54 ± 0.01^a	98.48 ± 0.27^a	33.12 ± 0.13^a	33.62 ± 0.04^a	50.34 ± 0.00^d
Total		1.86 ± 0.30	6.87 ± 0.61	$19. \pm 2.36$	5.62 ± 0.92	106.78 ± 4.98	37.21 ± 2.51	34.86 ± 1.22	47.79 ± 2.20

Values within a column with different superscripts differ significantly at $P < 0.05$. ESR; Erythrocyte sedimentation rate, Hb; Hemoglobin, Hcr; Hematocrit, MCV; Mean corpuscular volume, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, RBCs; Red blood cells, WBCs, White blood cells

5.4.1.3. Sex related variations in the haematological parameters of Nile tilapia

The values of haematological parameters of Nile tilapia between sexes are shown in Table 5.3. The results indicated that female Nile tilapia recorded significantly low RBCs, Hb and Hct compared to male fish ($P < 0.01$). Conversely, female fish recorded significantly higher value of ESR, MCV, MCH, MCHC and WBCs than male fish ($P < 0.01$).

Table 5.3: Mean values of haematological parameters of *O. niloticus* in accordance to sex

Sex group	RBCs ($10^6/\text{mm}^3$)	Hb (g/dl)	Hcr (%)	ESR (mm/hr)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($10^3/\text{mm}^3$)
Male	1.84± 0.00 ^b	6.98± 0.00 ^b	20.37± 0.01 ^b	5.10± 0.01 ^a	110.73 ± 0.04 ^a	37.98± 0.01 ^a	34.32± 0.01 ^a	47.55± 0.00 ^a
Female	1.71± 0.01 ^a	6.57± 0.00 ^a	18.98± 0.00 ^a	5.91± 0.01 ^b	119.12 ± 0.04 ^b	38.42± 0.01 ^b	34.65± 0.01 ^b	48.86± 0.01 ^b
Total	1.78± 0.06	6.78± 0.20	19.66± 0.71	5.50± 0.41	114.93 ± 4.22	38.20± 0.21	34.48± 0.16	48.21± 0.65

Values within a column with different superscripts differ significantly at $P < 0.01$. ESR; Erythrocyte sedimentation rate, Hb; Hemoglobin, Hcr; Hematocrit, MCV; Mean corpuscular volume, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, RBCs; Red blood cells, WBCs; White blood cells

5.4.1.4. Pathogenicity test of *A. hydrophila*

The rates of mortality following infection by different concentrations of *A. hydrophila* were presented in Figure 5.1. Mortalities were observed as early as 36 h post-challenge in 10^7 CFU/ml and 10^8 CFU/ml groups and lasted for 192 h. The highest cumulative mortality (100%) was observed in the group infected with 10^8 CFU/ml followed by group infected with 10^7 CFU/ml (66.66%), 10^6 CFU/ml (30%), 10^5 CFU/ml (26.66%) and 10^4 CFU/ml (23.33%). No mortality was observed in 10^3 CFU/ml, 10^2 CFU/ml, 10^1 CFU/ml of *A. hydrophila*

concentrations, and control group injected with sterile TSB. In the present study, the LD₅₀ - 240h of *A. hydrophila* was 1×10^7 CFU/ml.

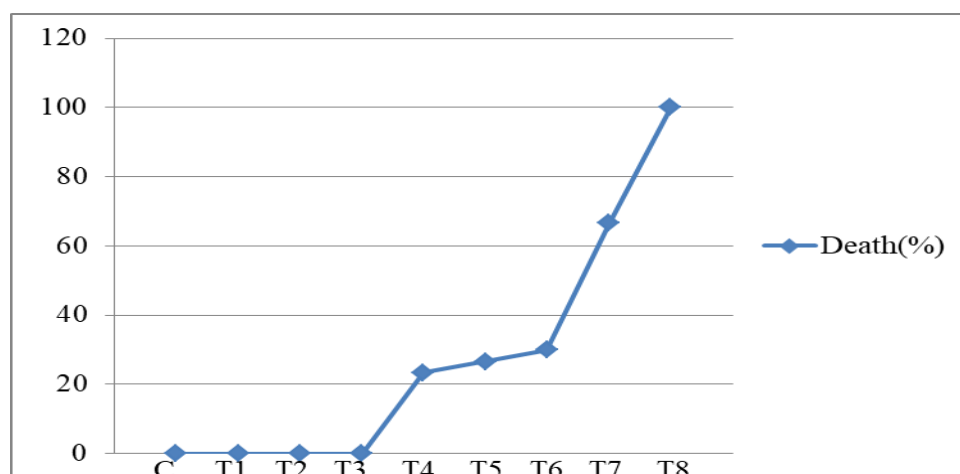


Figure 5.1: Mortality rate of Nile tilapia at different concentrations of *A. hydrophila* C; Control(TBS), T1; 10^1 CFU/ml, T2; 10^2 CFU/ml, T3; 10^3 CFU/ml, T4; 10^4 CFU/ml, T5; 10^5 CFU/ml, T6; 10^6 CFU/ml, T7; 10^7 CFU/ml, T8; 10^8 CFU/ml

5.4.1.5. Effects of *A. hydrophila* on haematological parameters of Nile tilapia

The values of haematological parameters for uninfected and infected fish with *A. hydrophila* are shown in Tables 5.4. The results showed significant difference of haematological parameters between uninfected and infected fish ($P < 0.01$). Significantly lower RBC (1.12 ± 0.99), Hb (3.34 ± 0.08), Hct (19.64 ± 0.61) and MCHC (17.00 ± 0.80), and higher ESR (7.71 ± 0.53), MCV (176.58 ± 20.32), MCH (29.67 ± 3.47) and WBC (43.19 ± 0.39) were recorded in infected fish.

Table 5.4: Mean values of haematological parameters of uninfected and infected *O. niloticus* with *A. hydrophila*

	RBCs ($10^6/\text{mm}^3$)	Hb (g/dl)	Hcr (%)	ESR (mm/hr)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBCs ($10^3/\text{mm}^3$)
uninfected	2.96 ± 0.22^b	7.51 ± 0.19^b	31.52 ± 0.85^b	4.92 ± 0.15^a	106.94 ± 6.21^a	23.36 ± 1.53^a	23.85 ± 0.83^b	31.31 ± 0.82^a
Infected	1.12 ± 0.99^a	3.34 ± 0.08^a	19.64 ± 0.61^a	7.71 ± 0.53^b	176.58 ± 20.32^b	29.67 ± 3.47^b	17.00 ± 0.80^a	43.19 ± 0.32^b

Values within a column with different superscripts differ significantly at $P < 0.01$. ESR; Erythrocyte sedimentation rate, Hb; Hemoglobin, Hcr; Hematocrit, MCV; Mean corpuscular volume, MCH; Mean corpuscular hemoglobin, MCHC; Mean corpuscular hemoglobin concentration, RBCs; Red blood cells, WBCs, White blood cells

5.5. Discussion

Blood is the most accessible tissue of the fish body which can be frequently examined to assess the health status of fish. In fish, exposure to pollutants can induce either an increase or decrease in haematological levels. In the present study, haematological parameters of Nile tilapia in Lake Hawassa showed statistically significant differences among sites ($P < 0.05$). The value of RBCs, Hb and Hct was significantly decreased in the blood of *O. niloticus* from polluted sites, compared to relatively less polluted site (KW). The decrease of RBCs, Hb and Hct as well as the WBCs enhancement in more polluted sites than less polluted site might be attributed to the exposure of the fish to stress and bacterial pathogens which damage mature RBCs or inhibit erythropoiesis. One more possible explanation for the decrease in Hb concentration might be due to pollutants-induced production of reactive oxygen species (ROS) which might have caused the destruction of the cell membrane of erythrocytes and its function which consecutively induced anaemic condition in fish (Kumar *et al.*, 2019). Pachego and Sanos (2002) observed that the structure of red blood cells of fish exposed to environmental pollution had deformed. Similar to the present findings, Olufemi *et al.* (2019) reported that *C. gariepinus* exposed to different concentrations of municipal waste significantly decreased ($P < 0.05$) the levels of RBC, Hb, Hct and MCHC compared with those in the control. Esther *et al.* (2017) reported lower RBC, Hb and Hct in fish from polluted environment compared to the control one. Low MCHC value was recorded from RH site. The MCHC is a good indicator of red blood cell swelling (Wepener *et al.*, 1992). The significant decreased in the MCHC from fish is probably an indication of red blood cell swelling and/ or a decrease in hemoglobin synthesis (Bhagwant and Bhikajee, 2000). Yonar *et al.* (2012) found a decrease in RBC, Hb, Ht and MCHC in *Cyprinus carpio* subjected to organophosphate insecticide and explained anemia with possible impairment of erythropoietic activity, osmoregulatory disturbances, or accelerated eryptosis in the

hematopoietic tissue. Javed *et al.* (2016) reported that RBC, Hb and Hct declined significantly ($P < 0.01$) in fish exposed to thermal power plant effluent as compared to reference fish (control). Chris *et al.* (2022) observed decreased RBCs, HB, and Hct in *Clarias gariepinus* when exposed to different doses of xylene. The WBC count increased in the order of $KW < GW < FH < RH < AG < TW$. Fish from polluted sites showed significant ($P < 0.05$) elevation of WBCs, this might be due to a protective response to stress and tissue damage by pathogenic bacteria.

In the present study, the mean values of the observed haematological parameters such as RBCs, Hb, Hct and WBCs clearly reveal a significant rising trend in their values with increasing size of the fish ($P < 0.05$). An increase of RBC and Hb simply suggests that it helps to meet the increasing oxygen demand of the fish for metabolism. Their size-related increase gets clearly authenticated by an increase in the Hct values also. It means that as RBC increases, Hb increases and so does the Hct i.e. the packed cell volume of the fish. An increase in Hct values may also relates with the reproductive potential of the fish. As the fish becomes sexually mature the energy requirement now gets diversified towards the reproductive preparation of the fish. Jawad *et al.* (2004) states that the rise in Hct values with the advancement of size and age of the fish is due to physiological factor evoked by high energy demand during the breeding period of fish. In agreement with the present findings, Esther *et al.* (2017) reported that shorter fish had lower RBC, Hb, Hct and MCHC. Gupta *et al.* (2012) reported an increase in RBC and Hb as the size of the fish increased. Gabriel *et al.* (2011) reported that there is a relationship between blood Hct and body length: longer fish have higher Hct in *C. carpio*. This implies that rapid increases in mean body weight and length and blood volume are accompanied by adequate erythropoiesis (Svetina *et al.*, 2002). Contrary to present findings, Joshi and Tandon (1977) also reported that with the increase in length and weight in *Clarias batrachus*, there is a corresponding increase in the RBC and Hb

but only up to a certain age till they attain their peaks. They further added that thereafter these values tend to either fall or may almost become constant. Elaborating further, they stated that as fishes grow older they become less active and their metabolic rate decreases and so do their blood values. WBC exhibited an increase in fish with the advancing size. An increase in WBCs in fish means the strengthening of immune system and preparing them to cope up or resist the various stressors (both natural as well as anthropogenic) which they may encounter in their natural environment.

Gabriel *et al.* (2004) revealed that the source of fish (wild or pond) and sex may exert some degree of influence on some haematological characteristics of fish. The male fish recorded significantly higher concentrations of haematological parameters, except ESR, MCV, MCH and MCHC than the females ($P < 0.01$). Kulkarni (2017) observed significantly higher haematocrit and haemoglobin levels in male fish than in female fish, *Notopterus notopterus*. These differences in haematology with regard to males and females may be related to differential oxygen demand by sex, which in turn may be related to reproductive activity. Rainza–Paiva *et al.* (2000) reported higher MCH and MCHC in male fish than female fish *Salminus maxillosus*.

In the present study, infected Nile tilapia exhibited respiratory problems, erratic swimming, loss of equilibrium, darkened skin abdominal distension, hemorrhages on their body surface, symptoms of fin rot and abdominal dropsy. The observed results were similar to those observed in affected catfish and Nile tilapia in natural outbreaks of *A. hydrophila* infection (Laith and Najiah, 2013; El Deen *et al.*, 2014). *Aeromonas* species infect the fins, integument and abdomen causing ulcerative lesions on the integumentary surface (Samayanpaulraj *et al.*, 2019). Darkened skin, hemorrhages on their body surface, symptoms of fins rot and

abdominal dropsy could be attributed to the intensity of bacteria and its concurrent interaction with the tissues of the fish (Younes *et al.*, 2016).

The pathogenicity test of *A. hydrophila* in this study revealed that Nile tilapia was susceptible to *A. hydrophila* infection. The survival of Nile tilapia infected with different concentration of *A. hydrophila* decreased as concentrations increased. This might be due to a release of more toxins that damage the internal organs of the fish (Dong *et al.*, 2017). The cumulative mortality ranged from 23.33% to 100%. The previous experimental studies on *A. hydrophila* infection in Red Hybrid Tilapia (*Oreochromis niloticus* × *O. mossambicus*) mortality ranged from 15% to 100% (Rozi, *et al.*, 2018; Samayanpaulraj *et al.*, 2019; Azzam-Sayuti *et al.*, 2021), which indicates a wide range of fish host susceptible to this pathogen. In this study, the LD₅₀ was determined at 1.0×10⁷CFU/mL. In previous studies on the experimental infection of *A. hydrophila* using IP routes, the LD₅₀ was 4.53×10⁶ CFU/mL in gourami, *Osphronemus goramy* (Rozi *et al.*, 2018), 4.1×10⁸ CFU/mL in snakehead fish (Samayanpaulraj *et al.*, 2019) and 1.0×10⁵ in Red Hybrid Tilapia (Azzam-Sayuti *et al.*, 2021). Bacteria with an LD₅₀ value between 10⁴ and 10⁵ CFU/mL are very virulent and those with an LD₅₀ value between 10⁵ and 10⁷ CFU/mL were lethal; and those with an LD₅₀ value >10⁷ CFU/mL were avirulent (Turnip *et al.*, 2017). The results of this study indicated that the *A. hydrophila* bacteria used were lethal, as their LD₅₀ values were between 10⁵ and 10⁷ CFU/mL.

In the present study, RBC, Hb and Hct values were significantly decreased in Nile tilapia infected with *A. hydrophila* (P < 0.01) which is similar to the report of Pathiratne and Rajapakshe (1998). Decreased RBC counts and hematocrit indicate that erythrocytes are being affected or destroyed by the infection. WBC showed a significant increase in infected fish (P < 0.01). Contrary to this, Rafiq *et al.* (2001) did not observe any alteration in the

differential counts of white blood cells in tilapia challenged with *A. hydrophila*. A similar trend was reported by Harikrishnan *et al.* (2003) who observed increased WBC in experimentally infected carp by *A. hydrophila*.

CHAPTER SIX

DETERMINATION OF SOME HAEMATOLOGICAL PARAMETERS AND DISEASE RESISTANCE CAPACITY OF *Aeromonas hydrophila* INFECTION IN NILE TILAPIA, *Oreochromis niloticus* L. FED WITH DIETARY SUPPLEMENTATION OF GINGER (*Zingiber Officinale* Roscoe)

6. Introduction

Rapid population growth, urbanization, and rise in income increase the demand for food (FAO, 2019). Fish play a significant role in food security and good nutrition (Beveridge *et al.*, 2013). Fish provide 17% of the global population's intake of animal protein (FAO, 2014). In freshwater aquaculture, tilapias are the most farmed tropical species of fish in the world after carp because of their suitability for aquaculture, good marketability, and stable prices (Wang and Lu, 2016). Nile tilapia, *O. niloticus*, can be easily cultivated in freshwater (FAO, 2015). However, the production of aquaculture in general and tilapia culture, in particular, is hampered by diseases-causing microorganisms (Van Hai, 2015).

Bacterial infection is one of the most significant threats to successful Nile tilapia production. *Aeromonas* species has been identified as the major disease causative bacteria of Nile tilapia (Baumgartner *et al.*, 2017). Pathogenicity of *Aeromonas* species correlated with the virulence genes in them (Li *et al.*, 2011). The pathogenicity of *Aeromonas* spp. is multifactorial and complex, and *Aeromonas* isolates could carry more virulence factors that play an important role in the development of disease (Galindo *et al.*, 2015). *Aeromonas* species are widespread in untreated and treated water. The occurrence of diseases by *Aeromonas* species is related to stress conditions such as overcrowding and poor water quality. *A. hydrophila*, one of *Aeromonas* species is a major fish pathogen known to infect a variety of fishes, predominantly present in freshwaters. *A. hydrophila* infection in *O. niloticus* has caused

severe disease outbreaks causing 60% mortality thereby affecting fish production (Hardi *et al.*, 2017), protein supply of aquaculture and affecting the economy of the world (Nugroho *et al.*, 2017). Stress is frequently considered one of the factors contributing to disease outbreaks by these bacteria (Laleh *et al.*, 2015). Skin darkness, ulcers, swelling of tissues, haemorrhage, and necrosis of the visceral organs are the major symptoms of infected Nile tilapia by *A. hydrophila* (Yardimci and Aydin, 2011).

Antibiotics are chemicals used to control and treat fish diseases in aquaculture. However, the use of antibiotics has limitations such as microbial resistance to antibiotics and unacceptable residues in aquaculture products and the environment. The resistant bacterial strains could hurt the therapy of fish diseases or human diseases and the environment of fish farms increases the accumulation of chemicals in fish, which is not safe for a human being who is the final consumer (Heuer *et al.*, 2009). Therefore, there is a need to find an alternative to antibiotics to control bacterial diseases of fish including Nile tilapia.

Medicinal plants in aquaculture can be used as an alternative to antibiotics. Out of fifteen medicinal plants tested, ten (62.5%) species showed an antibacterial effect against *A. hydrophila* (Chowdhury and Rahman, 2008). They can be given to fish through fish feed or intraperitoneal injection to improve their health status of fish and protect against infectious diseases (Bulfon *et al.*, 2015; Awad and Awad, 2017; Reverter *et al.*, 2017).

Medicinal plants such as Ginger (*Zingiber officinale*), garlic (*Allium sativum*), oats (*Avena sativa*), beetroot (*Beta vulgaris*), and Moringa (*Moringa oleifera*) among others have been used as an alternative to antibiotics (Bichi *et al.*, 2012; Bilen *et al.*, 2016; Devi *et al.*, 2016; Skariyachan *et al.*, 2016). They produce secondary metabolites (Van Hai, 2015). Ginger (*Zingiber officinale*) for instance produces secondary metabolites such as alkaloids, steroids, flavonoids, gingerols, zingerone, carotenoids, vitamins, and polyphenols, which have

antibacterial, antifungal, anti-inflammatory, and antioxidant effects on fish (Nile and Park, 2015). Ginger is also effective as an immunomodulatory agent in fish and helps to reduce the loss in aquaculture by diseases (Apines-Amar *et al.*, 2012). However, ginger added to fish feed is often made through its ethanolic extract, which may lead to a reduction of some chemical compounds at the filtration stage. Also, the powdering process is considered less resource demanding than essential oil extraction. Thus, this research aimed to evaluate the effect of Ginger powder (*Z. officinale*) on haematology and resistance of Nile tilapia (*Oreochromis niloticus*) challenged with *A. hydrophila*. *A. hydrophila* was chosen since it is considered the most common and important opportunistic pathogen in freshwater fish, accounting for millions of dollars in losses in the global freshwater aquaculture sector (Hossain *et al.*, 2014; Sebastião *et al.*, 2015; Peterman and Posadas, 2019).

6.1. Objective of the study

6.1.1. General objective

The general objective of the study was to investigate the effect of Ginger powder on the haematology of Nile tilapia and control of *A. hydrophila* infection.

6.1.2. Specific objectives

The specific objectives of the present study were to;

- evaluate haematological indices of *O. niloticus* fed on Ginger powder supplemented diets,
- assess the haematological changes of *O. niloticus* experimentally infected with *A. hydrophila*,
- evaluate disease resistance of *O. niloticus* fed on Ginger powder supplemented diets.

6.2. Materials and Methods

6.2.1 Experimental fish and design

A completely randomized design (CRD) was used for the experiment. A total of fifteen experimental glass aquaria (65 × 40 × 50 cm) were used for the experiment. The water level was maintained at a volume of 40 liters throughout the study period. A total of 300 healthy live Nile tilapia of 20 ± 1.00 g body weight and 11.06 ± 0.08 cm lengths were taken from the Research centre, Hawassa University, Ethiopia and randomly assigned to five treatment glass aquaria in the biotechnology laboratory, Hawassa University. Each treatment contained 20 fish in triplicates. The fish were acclimatized before the experiment and fed basal diet for two weeks three times daily until apparent satiation. At the end of two weeks, fish in each group were fed different concentrations of Ginger powder three times at a level of 3% of body weight daily for 10 weeks (through experimental periods). Control diet fishmeal was free from Ginger powder. Settled fish wastes of aquaria water were siphoned daily. Siphoned water was replaced by clean and aerated water from the storage tank. Water temperature ($25.0 \pm 2.0^\circ\text{C}$), dissolved oxygen concentration (5.6 ± 0.15 mg l⁻¹), and pH level (6.8 ± 0.2) were monitored once a week.

6.2.2. Preparation of feed

The fresh Ginger rhizome used for the feeding trial was purchased from the local market in Hawassa, Ethiopia. It was dried under shade for one week. The dried Ginger was ground to become powder, homogenized, and sieved using a hand sieve. Then different concentrations of Ginger powder (3, 5, 8, and 12 g ginger/kg) and fish meal content were transformed into pellet form by a food grinder and stored at -3°C before feeding.

6.2.3. Challenge test

A. hydrophila LH2 that had previously been isolated from skin of Nile tilapia of Lake Hawassa was grown in TSB at 28°C for 18 hrs. The bacteria suspension was adjusted to 1×10^7 CFU/ml in phosphate buffered saline using the McFarland scale. This concentration was obtained in a previous LD50 trial. At the end of 8 weeks feeding trial, fish were challenged

with pathogenic *A. hydrophila*. A 0.2 ml of *A. hydrophila* culture containing 1.0×10^7 CFU/ml was given by intraperitoneal (IP) injection using a 21/gauge sterile needle (Schaperclaus *et al.*, 1992). Twenty-four hours after injection, fish were fed the same experimental diet as in the feeding trial for 15 days of the challenge period and any clinical symptoms were recorded. The dead fish were removed daily and mortality was confirmed by re-isolating the microorganism from the internal organs of the dead fish. Survival rates of Nile tilapia were computed in percentages.

$$\text{Survival rate (\%)} = \text{Nf} \times 100/\text{Ni}$$

Nf = Number of cultured fish alive at the end of the experiment

Ni = Number of cultured fish stocked at the beginning of the experiment

6.3.4. Blood collection and haematological examination

Blood samples were collected in the early morning hours before and after bacterial injection of the fish for haematological analysis. Three fish from each replicate were sampled and anesthetized with 50 mg l⁻¹ of tricaine methanesulfonate (MS222, Sigma Chemical Co. St. Louis, MO, USA) and then blood samples were collected from the caudal peduncle with the use of a 5 ml syringe and needle that has been treated with heparin to prevent clotting and transferred to sampling bottles that contain ethylene diamine tetra-acetic-acid (EDTA). After the collection, the blood samples were taken to the Veterinary laboratory, at Hawassa University where the haematological analysis was carried out. Blood was analysed with routine methods adopted in fish haematology (Blaxhall and Daisley, 1973). The total erythrocyte count ($\text{RBC} \times 10^6/\text{mm}^3$) and total leukocyte count ($\text{WBC} \times 10^3/\text{mm}^3$) were determined manually using a Neubauer's haemocytometer with Hayem solution as a diluent. The haematocrit percentage was determined by using microhaematocrit-heparinized capillary tubes of 75 μl volume and a microhaematocrit centrifuge at 15000 g for 5 min. For the determination of haemoglobin concentrations Sahli's method was used. The values of

haemoglobin were expressed as g/dl. The values of mean corpuscular volume (MCV fl) mean corpuscular haemoglobin (MCH pg) and mean corpuscular haemoglobin concentration (MCHC g/dl), were calculated. Neutrophils, lymphocytes, and monocytes counts were performed by the diluent/dye direct method in a Neubauer chamber at a dilution of 1:100.

6.2.5. Statistical analysis

Data obtained were expressed as mean \pm standard error of the mean. The results were analysed with a one-way analysis of variance (ANOVA), using SPSS version 22. Differences at $P < 0.05$ were regarded as statistically significant. Data were presented as mean \pm SD before and after the challenge test.

6.3. Results

6.3.1. Clinical signs

After 36 hrs of injection with *A. hydrophila*, Nile tilapia showed clinical symptoms like exophthalmia, fin rot and swelling of tissues, darkness of skin and haemorrhage (Figure 6.1). The dead fish showed severe congestion in the abdominal area and a protruded vent due to accumulation of fluid in peritoneal cavity.

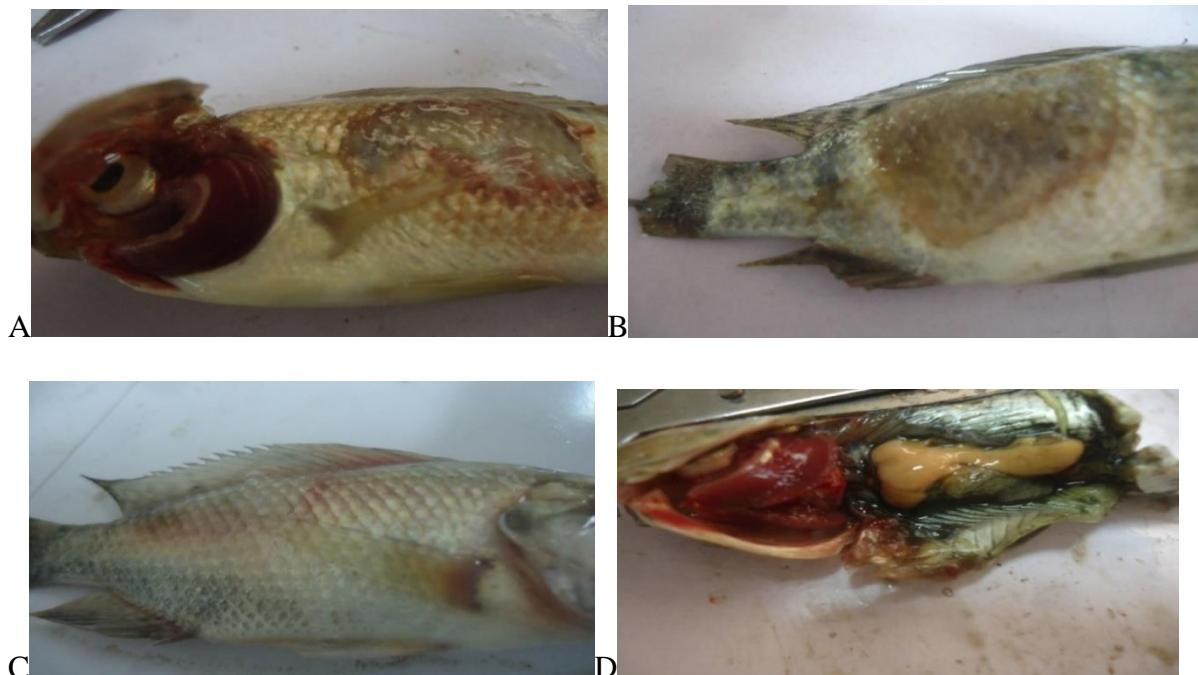


Figure 6.1: Symptoms of Nile tilapia after infection with *A. hydrophila*; (A) exophthalmia (B) fin rot and swelling of tissues, (C) darkness of skin (D) haemorrhage

6.3.2. The effect of ginger powder on haematological parameters of

O. niloticus

The haematological parameters of *O. niloticus* are shown in Tables 6.1A and 6.1B. Blood parameters of *O. niloticus* in all Ginger concentrations were significantly different ($P < 0.05$), except MCV, MCH, and MCHC ($P > 0.05$) from the control diet before and after injection with *A. hydrophila*. *O. niloticus* fed a diet at a concentration of 5 g Ginger powder/kg feed had significantly highest RBC counts, haematocrit, haemoglobin, WBC counts and lymphocytes value, while the Ginger powder at a concentration of 12 g feed had significantly lowest WBC, lymphocytes, neutrophils and monocytes before and after injection with *A. hydrophilus*. ESR and MCV of *O. niloticus* showed increment, while MCH and MCHC decreased after injection with *A. hydrophila* (Table 6.1A)

WBC, lymphocytes, neutrophils and monocytes of *O. niloticus* were significantly higher than that of the control group before injection of *O. niloticus* with *A. hydrophila* and their results are presented in Table 6.1B. WBC was significantly different in all treatments both before and after injection ($P < 0.05$). WBC was dominated by lymphocytes (23.18 ± 0.16 - $27.73 \pm 0.15 \times 10^3/\mu\text{l}$), followed by neutrophils (5.22 ± 0.47 - $7.06 \pm 0.23 \times 10^3/\mu\text{l}$) and monocytes (4.26 ± 0.16 - $6.88 \pm 0.08 \times 10^3/\mu\text{l}$) after injection of *O. niloticus* with *A. hydrophila* (Figure 6.2 and Table 6.1B).

Table 6.1A: Mean values of haematological parameters of experimental fish {red blood cells (RBCs), haematocrit value, haemoglobin (Hb) content, erythrocyte sedimentation rate (ESR), mean corpuscle volume (MCV), Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC)} of Nile tilapia, *O. niloticus*, fed different concentration of Ginger powder (0.0, 3, 5, 8, and 12 g *Z. officinale*/kg feed).

Variables	Experimental diet	Before injection	After injection
RBC ($10^6/\text{mm}^3$)	0.0 g <i>Z. officinale</i> /kg feed (control)	3.0±0.26 ^{bB}	1.27±0.06 ^{aA}
	3 g <i>Z. officinale</i> /kg feed	3.04±0.00 ^{cB}	1.43±0.1 ^{bA}
	5 g <i>Z. officinale</i> /kg feed	3.87±0.15 ^{eB}	3.55±0.11 ^{eA}
	8 g <i>Z. officinale</i> /kg feed	3.11±0.06 ^{dB}	2.77±0.12 ^{dA}
	12 g <i>Z. officinale</i> /kg feed	2.92±0.05 ^{aB}	2.10±0.93 ^{cA}
Haematocrit (%)	0.0 g <i>Z. officinale</i> /kg feed (control)	36.71±0.22 ^{bB}	20.52±0.53 ^{aA}
	3 g <i>Z. officinale</i> /kg feed	37.68±0.16 ^{cB}	21.77±0.77 ^{cA}
	5 g <i>Z. officinale</i> /kg feed	40.70±0.2 ^{eB}	37.59±0.21 ^{eA}
	8 g <i>Z. officinale</i> /kg feed	38.14±0.08 ^{dB}	31.57±0.06 ^{dA}
	12 g <i>Z. officinale</i> /kg feed	36.15±0.08 ^{aB}	20.96±0.56 ^{bA}
Haemoglobin (g/dl)	0.0 g <i>Z. officinale</i> /kg feed (control)	7.59±0.17 ^{bB}	3.69±0.21 ^{bA}
	3 g <i>Z. officinale</i> /kg feed	7.64±0.2 ^{cB}	4.21±0.49 ^{cA}
	5 g <i>Z. officinale</i> /kg feed	9.12±0.1 ^{eB}	7.29±0.53 ^{eA}
	8 g <i>Z. officinale</i> /kg feed	8.05±0.03 ^{dB}	5.66±0.11 ^{dA}
	12 g <i>Z. officinale</i> /kg feed	7.50±0.04 ^{aB}	3.58±0.07 ^{aA}

Table 6.1A (continued)

ESR (mm/hr)	0.0 g <i>Z. officinale</i> /kg feed (control)	5.61±0.12 ^{dA}	8.68±0.05 ^{dB}
	3 g <i>Z. officinale</i> /kg feed	5.19±0.07 ^{bA}	8.72±0.13 ^{eB}
	5 g <i>Z. officinale</i> /kg feed	4.68±0.08 ^{aA}	5.55±0.22 ^{aB}
	8 g <i>Z. officinale</i> /kg feed	5.85±0.09 ^{eA}	7.33±0.10 ^{bB}
	12 g <i>Z. officinale</i> /kg feed	5.41±0.12 ^{cA}	8.65±0.21 ^{cB}
MCV(fl)	0.0 g <i>Z. officinale</i> /kg feed (control)	121.18±0.98 ^{cA}	133.44±7.41 ^{aA}
	3 g <i>Z. officinale</i> /kg feed	123.69±1.15 ^{dA}	130.48±4.39 ^{aA}
	5 g <i>Z. officinale</i> /kg feed	105.1±4.52 ^{aA}	119.26±1.13 ^{aA}
	8 g <i>Z. officinale</i> /kg feed	117±8.26 ^{bA}	121.14±4.67 ^{aA}
	12 g <i>Z. officinale</i> /kg feed	123.85±2.18 ^{eA}	134.29±13.05 ^{aA}
MCH(pg/dl)	0.0 g <i>Z. officinale</i> /kg feed (control)	22.97±1.67 ^{aA}	25.06±0.78 ^{aA}
	3 g <i>Z. officinale</i> /kg feed	24.25±0.47 ^{dA}	25.09±0.81 ^{aA}
	5 g <i>Z. officinale</i> /kg feed	25.52±1.14 ^{eA}	23.54±0.67 ^{aA}
	8 g <i>Z. officinale</i> /kg feed	23.14±0.64 ^{cA}	25.91±0.59 ^{aB}
	12 g <i>Z. officinale</i> /kg feed	23.79±0.61 ^{bA}	25.69±0.55 ^{aA}
MCHC (g/dl)	0.0 g <i>Z. officinale</i> /kg feed (control)	20.68±0.57 ^{bB}	16.84±0.05 ^{aA}
	3 g <i>Z. officinale</i> /kg feed	20.30±0.46 ^{aB}	18.93±0.64 ^{bA}
	5 g <i>Z. officinale</i> /kg feed	22.41±0.34 ^{eA}	21.36±0.52 ^{eA}
	8 g <i>Z. officinale</i> /kg feed	21.11±0.08 ^{dB}	19.04±0.57 ^{cA}
	12 g <i>Z. officinale</i> /kg feed	20.74±0.13 ^{cA}	19.46±0.62 ^{dA}

Mean ± standard deviation followed by different superscript letters (a, b, c, d, e) in the same column in each treatment trial showed significantly different at $P < 0.05$. Different superscript letters (A, B) in the same row indicated significant difference at $P < 0.05$ before and after injection of Nile tilapia with *A. hydrophila*.

Table 6.1B: Mean values of haematological parameters of experimental fish [White blood cells (WBCs), lymphocytes, neutrophils, and monocytes] of Nile tilapia, *O. niloticus*, fed different concentration of Ginger powder (0.0, 3, 5, 8 and 12 g *Z. officinale* /kg feed)

Variables	Experimental diet	Before infection	After infection
WBC($10^3/\text{mm}^3$)	0.0 g <i>Z. officinale</i> /kg feed (control)	33.72±0.23 ^{aA}	44.37±0.12 ^{bB}
	3 g <i>Z. officinale</i> /kg feed	40.30±0.12 ^{cA}	45.91±0.75 ^{dB}
	5 g <i>Z. officinale</i> /kg feed	43.38±0.13 ^{eA}	45.51±0.12 ^{cB}
	8 g <i>Z. officinale</i> /kg feed	41.37±0.09 ^{dA}	53.49±0.06 ^{eB}
	12 g <i>Z. officinale</i> /kg feed	38.75±0.07 ^{bA}	44.29±0.30 ^{aB}
Lymphocyte ($10^3/\mu\text{l}$)	0.0 g <i>Z. officinale</i> /kg feed (control)	27.23±0.17 ^{bB}	23.34±0.90 ^{bA}
	3 g <i>Z. officinale</i> /kg feed	27.63±0.09 ^{cB}	24.56±0.23 ^{cA}
	5 g <i>Z. officinale</i> /kg feed	30.19±0.13 ^{eB}	27.73±0.15 ^{eA}
	8 g <i>Z. officinale</i> /kg feed	28.37±0.04 ^{dB}	25.21±0.48 ^{dA}
	12 g <i>Z. officinale</i> /kg feed	25.09±0.06 ^{aB}	23.18±0.16 ^{aA}
Neutrophil($10^3/\mu\text{l}$)	0.0 g <i>Z. officinale</i> /kg feed (control)	3.44±0.08 ^{cA}	5.21±0.08 ^{bB}
	3 g <i>Z. officinale</i> /kg feed	3.33±0.07 ^{bA}	5.48±0.05 ^{cB}
	5 g <i>Z. officinale</i> /kg feed	4.54±0.09 ^{eA}	7.06±0.23 ^{eB}
	8 g <i>Z. officinale</i> /kg feed	4.22±0.10 ^{dA}	5.81±0.14 ^{dB}
	12 g <i>Z. officinale</i> /kg feed	3.30±0.12 ^{aA}	5.22±0.47 ^{aB}
Monocytes($10^3/\mu\text{l}$)	0.0 g <i>Z. officinale</i> /kg feed (control)	1.38±0.03 ^{aA}	4.69±0.21 ^{aB}
	3 g <i>Z. officinale</i> /kg feed	2.86±0.05 ^{bA}	5.58±0.05 ^{dB}
	5 g <i>Z. officinale</i> /kg feed	3.61±0.04 ^{dA}	6.88±0.08 ^{cB}
	8 g <i>Z. officinale</i> /kg feed	3.05±0.05 ^{cA}	5.66±0.21 ^{eB}
	12 g <i>Z. officinale</i> /kg feed	2.85±0.06 ^{bA}	4.26±0.16 ^{bB}

Mean ± standard deviation followed by different superscript letters (a, b, c, d, e) in the same column in each treatment or prevention trial showed significantly different at $P < 0.05$. Different superscript letters (A, B) in the same row indicated significant difference at $P < 0.05$ before and after injection of Nile tilapia with *A. hydrophila*

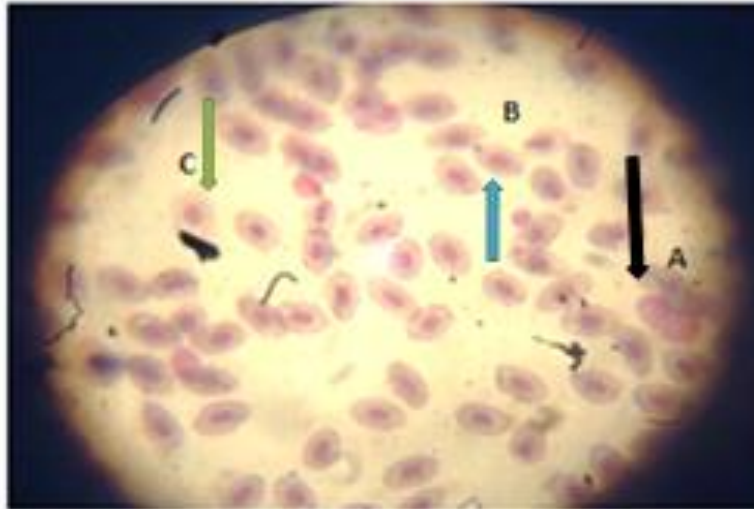


Figure 6.2: Different types of white blood cell (a) lymphocyte, (b) neutrophils, and (c) Monocyte

6.3.3. Diseases resistance

Survival and mortality rate of Nile tilapia challenged with *A. hydrophila* are presented in Figure 6. 3. The fish in the treatment trial with 5 g/kg Ginger powder had the highest survival rate (81.66%) compared to the other Ginger powder concentration. The highest mortality was recorded in the control group (70%), followed by the 3 g Ginger powder diet (66.7%) and 12 g Ginger powder diet (55%).

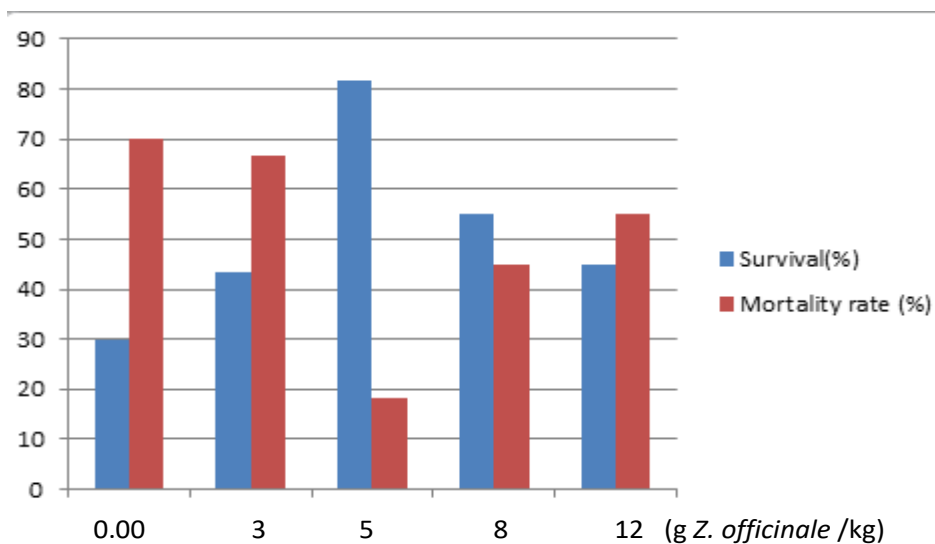


Figure 6.3: Disease resistance of *O. niloticus* after challenged test with *A. hydrophila*

6.4. Discussion

Medicinal plants have been used for disease control in fish due to the active phytochemicals such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils (Reverter *et al.*, 2014). In the present study, the effect of *Z. officinale* powder on the haematological parameters of Nile tilapia and its control to *A. hydrophila* infection was determined. It was observed that all concentrations of the Ginger powder-supplemented diet exhibited significantly higher values of RBC when compared to fish fed the control diet. The study by Ferri-Lagneau *et al.* (2012) demonstrated that ginger extract stimulated haematopoiesis in fish, which could explain the present result. Ginger also contains antioxidant that protects the RBC against haemolysis by free radicals (Mohammed *et al.*, 2020). The result also showed that the RBC of Nile tilapia infected with *A. hydrophila* in the control diet and all Ginger powder-supplemented diets decreased significantly ($P < 0.05$). Similar to the present study, Talpur *et al.* (2013) reported that the RBC was decreased in tilapia infected with *A. hydrophila*. Decreased RBC counts indicate that erythrocytes are being affected or destroyed by the infection. The changes in fish haematology are to be expected for fish infected with diseases (Hrube and Smith, 2010).

Haemoglobin content is directly related to the oxygen-binding capacity of the blood. Therefore it is crucial for the survival of fish. The results of the current study indicated that haemoglobin contents increased significantly in Ginger powder-supplemented Nile tilapia compared to the control diet and these findings are in accordance with the results of Haghghi and Rohani (2013) and Chelladuria *et al.* (2014). Ginger powder showed a significant difference in haemoglobin before and after injection of the fish ($P < 0.05$). There was a significant decrease in haemoglobin after injection of fish with *A. hydrophila*. This might be due to an increased rate of breakdown of RBC by pathogenic bacteria and/or a reduction in

the rate of formation of RBC (Ayotunde *et al.*, 2011). According to Lie *et al.* (1989), the haemoglobin content decreases due to RBC swelling and poor haemoglobin mobilization of the spleen and other haematopoiesis organs. In the present study, the significant reduction of haemoglobin after injection of fish might be the result of severe anaemia. The anaemic response could be a result of disruption in erythrocyte production (Omoriegie, 1995), and destruction of intestinal cells involved in the production of vitamin B12 used in the production of the haemoglobin portion of the red cells (Gardner and Yevich, 1970). The present result disagrees with the result of Brum *et al.* (2017) who reported no significant effect of ginger oil on the haemoglobin of Nile tilapia.

Haematocrit value (or packed cell volume) is the simplest measure of erythrocyte content in blood as a percentage of erythrocytes in blood volume. In the current study, the percentage of haematocrit value significantly declined after Nile tilapia was injected with *A. hydrophila* ($P < 0.05$). The decrease in haematocrit value might be due to an anaemic state. Brum *et al.* (2017) described anaemia for Nile tilapia fed diets supplemented with 10 g/kg of ginger.

The erythrocyte sedimentation rate (ESR) is a common haematology test that may indicate and monitor an increase in inflammatory activity within the body caused by one or more conditions such as autoimmune disease, infections, or tumours. The current study showed that the ESR of *O. niloticus* was significantly increased after the fish were injected with *A. hydrophila*. Blaxhall and Daisley (1973) stated that the values are usually raised with increased tissue destruction as in acute infection and heavy metal poisoning among others.

In the current study, the maximum value of MCV was recorded for a 12 g/kg Ginger powder supplemented diet. The results also showed that the application of Ginger powder at varying concentrations significantly increased MCV and MCH ($P < 0.05$), while significantly

decreasing the value of MCHC after injection of Nile tilapia with *A. hydrophila*, except 5 g/kg and 12 g/kg Ginger powder-supplemented diet. Similar to this study, Haniffa and Mydeen (2010) demonstrated that catfish exhibited a decrease in MCHC during *A. hydrophila* infection. But contrarily to the present study, Stanley *et al.* (2017) reported no significant difference ($P > 0.05$) for MCV, MCH, and MCHC at varying concentrations of powdered ginger. Significant increases or decreases in red cell indices may indicate macrocytic or microcytic anemia.

WBC plays a crucial role in the protection from diseases caused by pathogenic organisms (Harikrishnan and Balasundaram, 2005). WBC counts of Nile tilapia fed different concentrations of Ginger powder were significantly higher compared to the control group ($P < 0.05$). Similar to the present study Haghghi and Rohani (2013) reported that a fish fed ginger-supplemented diet showed a significant immune-stimulatory effect and increased RBC, Hct value, and WBC values when compared to the control ($P < 0.05$). An increase in WBC was believed to be caused by the migration of WBC from the spleen to the blood circulation (Puisford *et al.*, 1994). According to Sutili *et al.* (2018) feeding fish with ginger-supplemented diets produces immunomodulatory effects. The results also indicated that WBC increased significantly in Nile tilapia after injection with *A. hydrophila* ($p < 0.05$). This result is similar to those of a previous study, which stated that the WBC increased to tackle the infection in tilapia injected with *A. hydrophila* (Hardi *et al.*, 2016).

The value of neutrophils was significantly increased in the Ginger powder-supplemented diet compared to the control diet ($P < 0.05$). The maximum value of neutrophils $7.06 \pm 0.23 \times 10^3/\mu\text{l}$ was recorded at the concentration of 5 g *Z. officinalis*/kg diet after injection of Nile tilapia with *A. hydrophila*. These results are in line with the results of a previous study, which found that fish treated with immunostimulants usually show enhanced phagocytic cell activities

(Sakai, 1999). The results were also supported by Talpur *et al.* (2013) who reported a beneficial effect of ginger which improves the immune system of fish.

The lymphocyte was the most common leukocyte observed in the current study. The number of lymphocytes in fish injected with *A. hydrophila* was significantly lower than those which are not injected ($P < 0.05$). Decreases in lymphocytes after infection of Nile tilapia with *A. hydrophila* were associated with re-trafficking of cells to lymphoid tissues which consequently leads to clearance of these cells from the bloodstream (Harris and Bird, 2000).

Blood monocytes contribute to tissue-resident macrophage populations during inflammatory conditions and the depletion of resident macrophages in their environment (Hashimoto *et al.*, 2013). In the current study, the number of monocytes was significantly increased in the Nile tilapia fed Ginger powder-supplemented diet after injection with *A. hydrophila* ($P < 0.05$), this might be due to intensification of the cell defence mechanism (Tavares-Dias and Faustino, 1998). Also, it was observed that the monocyte value of Nile tilapia fed 5 g/kg ginger was higher than that fed 8 and 12 g/kg. According to Citarasu (2010), the choice of herbs, their dose, and time of application were very important for obtaining higher efficiency.

In present study no mortality was observed in Nile tilapia fed with different concentrations ginger powder before injection with *A. hydrophila*. From this result, it can be suggested that Ginger powder has no harmful effect on fish. Hence, it can be considered safe for use in fish feed. However, during 15 days of the bacterial challenge, mortality was observed. Fish mortality was recorded as early as 36 hrs of the experiment for the control group. No mortality was recorded until the 3rd day of the experimental period for a fish fed with diet containing different concentrations of Ginger powder. In all Ginger powder-supplemented diet groups, mortality began to occur on day three post-challenged and continued until day twelve. The highest mortality rate (70%) was recorded for the control group followed by 3

g/kg (66.66%) and 12 g/kg (55%) Ginger powder-supplemented diet, respectively. In line with this study, Payung *et al.* (2017) reported the highest mortality in the control diet followed by a 3 g/kg Ginger powder-supplemented diet and the lowest mortality in the 5 g/kg Ginger powder-supplemented diet. The increasing survival rate is related to the increased immune function of the fish. Increased fish immunity will result in increased fish resistance to pathogens. The addition of ginger (*Z. officinale*) extract in fish feed increases the resistance of fish to pathogens because ginger contains ingredients that can improve the immune system of the fish (Payung *et al.*, 2017). According to Nugroho *et al.* (2017), traditional herbs improve the immune response of fish by increasing granulocytes, macrophages, monocytes, and neutrophils. Maqsood *et al.* (2011) also reported that immunostimulants enhanced the general defence system and decreased the mortality against pathogens and increased the viability rate. The results also revealed that the Nile tilapia treated with 5 and 8 g/kg Ginger powder showed a decrease in mortality rates compared to the control diet after *A. hydrophila* injection. However, as the concentration of *Z. officinale* in fish feed increases above 8g/kg survival appeared to decrease. This indicates that excessive doses will have an immunosuppression effect that suppresses the immune system of the fish (Sakai, 1999).

In the present study, before the bacterial challenge, Nile tilapia presented health characteristics within the normal parameters for the species. But after two days of injection, the fish showed different clinical symptoms such as erratic swimming behaviour, darkness of the skin, darkness on their dorsal body part, fluid accumulation in the scale of the pockets, swelling of tissues, ulcers, and haemorrhage, necrosis and exophthalmia. Similar to the present result Noor El-Deen *et al.* (2013) stated that the bacteria caused acute mortality among infected fishes in which the most visible clinical signs included exophthalmia. The present results also agree with the findings of Kaleeswaran *et al.* (2012) and Noor El-Deen *et al.* (2014).

CHAPTER SEVEN

GENERAL CONCLUSION AND RECOMMENDATIONS

7.1. Conclusion

In this study, from a total of 11 physicochemical parameters, 8 parameters (72.7%) such as pH, DO, turbidity, EC, TDS, BOD, NO_3^- and PO_4^{3-} were found above the permissible limits for aquatic life particularly for fish. The minimum DO value was recorded at the TW site. All the sites except FH and KW recorded significantly lower DO values than the recommended for fish ($P < 0.05$).

The bacterial population in water samples of Lake Hawassa was significantly higher at TW, AG and RH mainly during summer ($P < 0.05$). Bacteria loads of the water showed a strong seasonal dynamic with higher concentrations measured in the rainy season (summer). The highest bacterial population recorded during the summer season may be attributed to flooding due to heavy rains. While observing the water samples of the collected sites, it was noticed that the bacterial loads in the water samples were higher in highly polluted sites (RH, AG and TW) of the lake. Coliform bacteria were recorded from all studied sites of the lake which is a good indicator of deterioration of microbiological water quality of the lake. The correlation matrix results showed a significant positive correlation between bacterial loads and nutrients, and a significant negative correlation ($P < 0.01$) with DO. Bacterial loads also showed a significant positive correlation with temperature and BOD ($P < 0.01$).

In aquatic environments, fish are commonly exposed to microbial infection. Sixty-four percent (64%) of the prevalence of bacterial infection was recorded from fish in Lake Hawassa. Female fish were more infected than the male fish. Small-sized fish were highly sensitive to bacterial infection compared to larger-sized fish. The highest prevalence was

recorded during summer. Significantly high bacterial loads were recorded during the summer in both water and organs of fish ($P < 0.05$).

The fish collected from Lake Hawassa showed many abnormalities. Upon external observation, body lesions, corneal opacity, mucus secretion, frayed fin, hyperemia at the bases of the fin and darkness in the skin were noted from naturally infected Nile tilapia.

Nile tilapia found in Lake Hawassa were infected with bacterial species such as *E. coli*, *A. hydrophila*, *E. tarda*, *Staphylococcus*, *Pseudomonas*, *Vibrio*, *Bacillus*, *Flavobacterium*, *Salmonella* and *Shigella* species. The percentage composition of isolates of Nile tilapia indicates that the predominant group was *E. coli* followed by *A. hydrophila*, *Pseudomonas* and *Vibrio* species. The highest occurrence of bacteria was from the intestine followed by skin and gills. All isolates frequently occurred in the intestine compared to the other fish organs, except *Flavobacterium* species which is absent in intestine. *A. hydrophila* were isolated from all fish organs examined. The presence of *E. coli*, *Vibrio*, *Shigella*, *Salmonella*, *Pseudomonas* and *Staphylococcus* species in fish indicates faecal contamination of Lake Hawassa which reflects the water quality of the lake. *Aeromonas* isolates of Nile tilapia in Lake Hawassa were 100% resistant to amoxicillin, ampicillin and penicillin. Moreover, the majority of the isolates showed multiple antibiotic resistance.

The value of RBCs, Hb and Hct was significantly decreased ($P < 0.05$) in the blood of *O. niloticus* from more polluted sites (TW, AG and RH), compared to relatively less polluted site (KW). In contrast, ESR and WBC showed significant ($P < 0.05$) elevation over fish of KW. This might be due to the presence of more pressure of stress and bacterial pathogens in polluted sites. Haematological parameters such as RBCs, Hb, Hct and WBCs showed a significant rising trend in their values with increasing size of the fish ($P < 0.05$). The male

fish have significantly higher concentrations of haematological parameters such as RBCs, Hb and Hct than the females ($P < 0.01$).

In the present study, experimentally infected Nile tilapia exhibited respiratory problems, erratic swimming, loss of equilibrium, darkened skin, abdominal distension, hemorrhages on their body surface, symptoms of fin rot and abdominal dropsy. The survival of Nile tilapia infected with different concentrations of *A. hydrophila* was decreased as concentration increased. The blood parameters of infected fish were significantly different from uninfected fish ($P < 0.01$).

In the present study, the effect of Ginger powder on the haematological parameters of Nile tilapia and its control to *A. hydrophila* infection was determined. Significantly higher values of RBC, Hct, Hb, MCH, MCHC, WBC and monocytes were recorded to all concentrations of the Ginger powder-supplemented diet when compared to fish fed the control diet ($P < 0.05$). The efficiency generally followed the order, 5>8>3>12 g/kg Ginger powder-supplemented feed. This clearly indicates that the dose of Ginger powder was very important for obtaining higher efficiency. There was no mortality to Nile tilapia before injection with *A. hydrophila* which suggested that Ginger powder caused no harmful effect on fish. Hence, it can be considered safe for use in fish feed. The highest mortality rate was recorded for the control group followed by a 3 g/kg and 12 g/kg Ginger powder-supplemented diet. This indicates that supplementation of Ginger powder in the fish diet had a significant additive benefit on the immune status and increases the resistance of Nile tilapia to *A. hydrophila*. As the concentration of Ginger powder in fish feed increased, survival appears to decrease. This indicates that excessive doses will have an immunosuppression effect that suppresses the immune system of the fish. Supplementation of Ginger powder at the concentration of 5 g/kg diet provided better protection to Nile tilapia against *A. hydrophila* infection.

7.2. Recommendations

Based on the findings of the present study, the following general recommendations are suggested;

- ❖ Frequent monitoring of the water parameters of Lake Hawassa is fundamental to avoid water quality deterioration in the lake.
- ❖ Creating public awareness about the impact of pollution on the lake ecosystems can help improve water quality of the lake.
- ❖ As fish in Hawassa Lake are infected by bacterial pathogens, there is an imperative need to cook the fish before eating.
- ❖ Molecular characterization is recommended to identify virulence genes/factors in *A. hydrophila*
- ❖ Supplementation of Ginger powder in fish feed is recommended to boost the immunity of *O. niloticus*.
- ❖ Supplementation of 5g/kg dietary Ginger powder in *O. niloticus* diet is recommended to control *A. hydrophila* infection.

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AAPPENDICES

Appendix figure 1: Sampling site of Lake Hawassa



Amora Gedel



Tikur wuha

Referal Hospital



Fikr hayiq



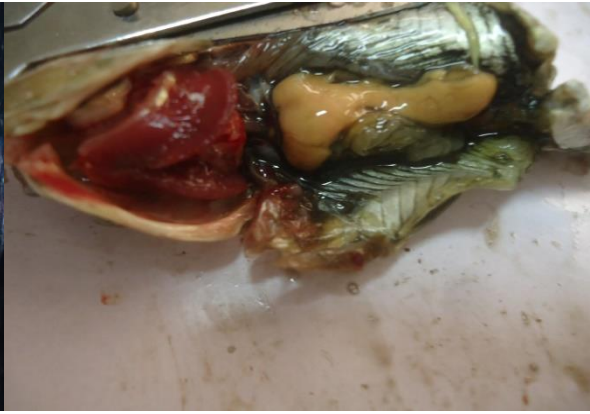
Green wood

Kuyu wata

Appendix figure 2: Photographs of field and laboratory experimental works



Fish collected from Lake Hawassa



Fish dissection



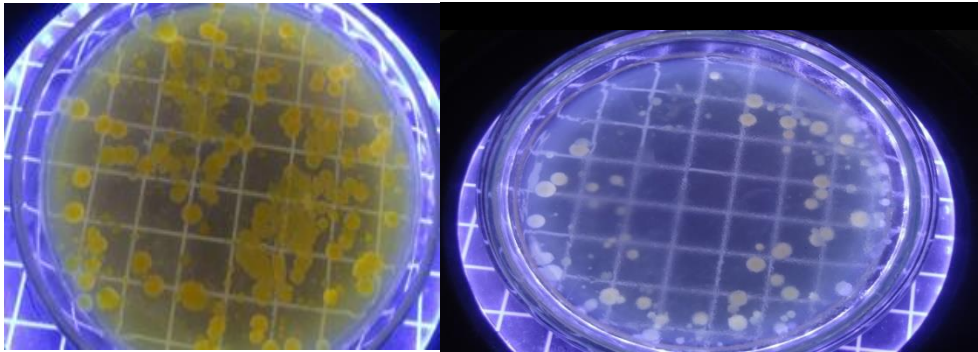
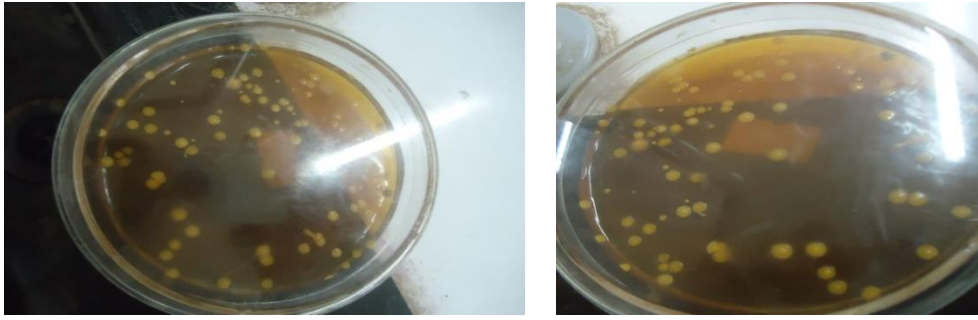
Media preparation



Bacteria inoculation



Water quality analysis



Bacterial colonies



Experimental Nile tilapia collection from HU pond



Feeding fish and Psiphoning of wastewater from glass aquaria



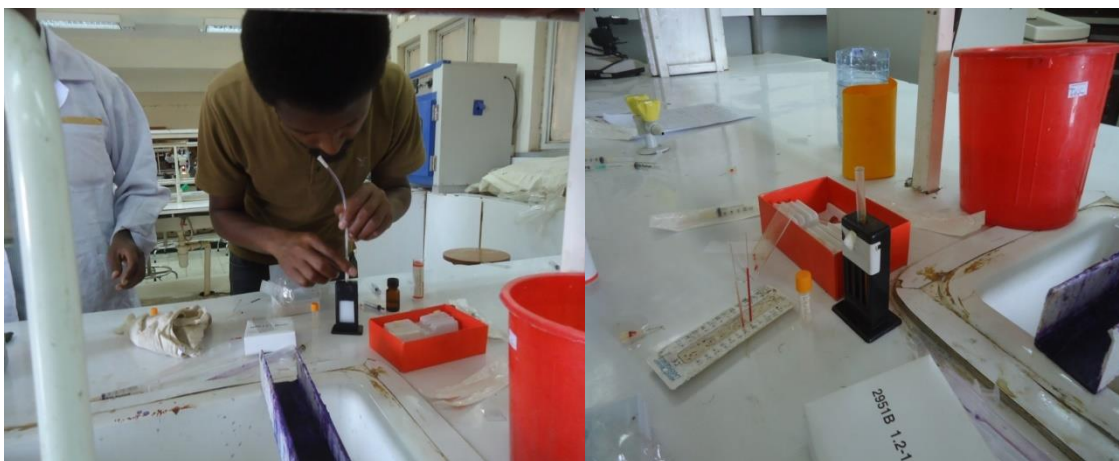
Injection of fish with *A. hydrophila*



Clinical symptoms of fish after infection with *A. hydrophila*



Blood sampling for haematological analysis



Haematological parameters analysis

Appendix table 1: Physicochemical and bacteriological parameters of Lake Hawassa among sites

Site	Parameters													
	Tem	pH	DO	Turb.	EC	TDS	BOD5	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	PO ₄ ⁻³	HBC	TC	FC
1	25.0± 0.11 ^c	9.63 ± 0.05 ^e	4.37 ± 0.02 ^a	24.5± 0.30 ^d	957.33± 2.30 ^f	935.67 ± 3.75 ^e	160.62± 9.19 ^b	0.52 ± 0.10 ^c	0.19 ± 0.00 ^b	6.80± 0.17 ^c	1.80 ± 0.08 ^b	5.60 ± 0.16 ^e	3.40± 0.08 ^d	2.10± 0.08 ^b
2	24.03± 0.05 ^b	8.95± 0.04 ^b	4.54± 0.05 ^a	20.48± 0.24 ^c	772.15± 6.80 ^c	737.11 ± 2.49 ^c	124.8 ±4.23 ^a	0.18± 0.10 ^b	0.06 ± 0.01 ^a	5.37± 0.54 ^d	1.26± 0.02 ^a	3.47± 0.01 ^c	1.65 ± 0.13 ^b	0.53 ± 0.11 ^a
3	24.82± 0.01 ^c	8.53± 0.05 ^a	5.06± 0.58 ^a	10.41 ± 0.18 ^b	626.00 ±3.46 ^b	629.60 ± 3.11 ^b	113.25± 2.81 ^a	0.25± 0.04 ^b	0.03± 0.01 ^a	3.44± 0.38 ^b	1.16 ± 0.03 ^a	2.45± 0.21 ^b	1.27 ± 0.06 ^b	0.33 ± 0.02 ^a
4	25.03± 0.05 ^c	9.44± 0.05 ^c	4.48 ± 0.06 ^a	27.2± 0.20 ^e	798.89± 0.96 ^d	728.33 ± 7.21 ^c	140.00± 5.19 ^a	0.48± 0.01 ^c	0.14 ± 0.03 ^b	3.88± 0.24 ^b	1.72 ± 0.06 ^b	4.39± 0.17 ^d	2.37 ± 0.15 ^c	1.76 ± 0.57 ^b
5	25.00± 0.00 ^c	9.33± 0.05 ^c	4.62± 0.10 ^a	28.32± 0.28 ^f	907.78± 6.73 ^e	910.33 ± 2.88 ^d	144.19± 7.95	0.52± 0.01 ^c	0.16± 0.02 ^b	4.99± 0.00 ^d	1.87± 0.02 ^b	4.49± 0.00 ^d	2.50 ± 0.18 ^c	2.21± 0.12 ^b
6	23.75± 0.00 ^a	8.40± 0.08 ^a	6.02± 0.15 ^b	9.63± 0.20 ^a	607.54± 6.52 ^a	494.17 ± 5.05 ^a	102.78± 2.40 ^a	0.04± 0.02 ^a	0.028± 0.02 ^a	3.00± 0.17 ^b	1.04± 0.04 ^a	1.93± 0.11 ^a	0.79± 0.25 ^a	0.00± 0.00 ^a
Total	24.61± 0.54	9.04± 0.47	4.85 ± 0.62	20.92± 8.46	778.28± 133.9	739.20± 157.2	130.95± 20.66	0.33± 0.19	0.10 ± 0.06	4.58± 1.35	1.47± 0.34	3.71± 1.29	2.00 ± 0.89	1.15 ± 0.94
Range	23.75- 25.20	8.35- 9.70	4.36- 6.20	9.40- 29.62	600.00- 960.00	491.25- 940.00	100.00- 165.93	0.03- 0.59	0.02- 0.20	2.80- 7.00	1.00- 1.90	1.80- 5.80	0.50- 3.50	0.00-2.34

Within the same column mean value with the different superscript is significantly different at $P < 0.05$ based on the ANOVA (Tukey) test. Units of parameters are given by mg L^{-1} except, Temperature ($^{\circ}\text{C}$) Turbidity (NTUs), EC ($\mu\text{S}/\text{cm}$), pH with no scale, HBC (CFU/ml, 10^5), TC (CFU/ml, 10^4) and FC (CFU/ml, 10^2). HBC-heterotrophic bacteria count, NH_4^+ -ammonium ion, BOD5- five-day biochemical oxygen demand, DO-dissolved oxygen concentration, EC-electrical conductivity, FC-faecal coliform, NO_3^- - nitrate, NO_2^- - nitrite, pH -hydrogen ion concentration, PO_4^{-3} - phosphate, Temp.-temperature, TC- total coliform, TDS -total dissolved substances, Turb-turbidity

Appendix table 2: ANOVA Multiple Comparisons table for physicochemical and bacteriological parameters of Lake Hawassa among sites

Dependent Variable	(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Temp	1	2	1.03333*	.04749	.000	.8738	1.1929
		3	.24667*	.04749	.002	.0871	.4062
		4	.03333	.04749	.978	-.1262	.1929
		5	.06667	.04749	.725	-.0929	.2262
		6	1.31667*	.04749	.000	1.1571	1.4762
	2	1	-1.03333*	.04749	.000	-1.1929	-.8738
		3	-.78667*	.04749	.000	-.9462	-.6271
		4	-1.00000*	.04749	.000	-1.1595	-.8405
		5	-.96667*	.04749	.000	-1.1262	-.8071
		6	.28333*	.04749	.001	.1238	.4429
	3	1	-.24667*	.04749	.002	-.4062	-.0871
		2	.78667*	.04749	.000	.6271	.9462
		4	-.21333*	.04749	.007	-.3729	-.0538
		5	-.18000*	.04749	.024	-.3395	-.0205
		6	1.07000*	.04749	.000	.9105	1.2295
	4	1	-.03333	.04749	.978	-.1929	.1262
		2	1.00000*	.04749	.000	.8405	1.1595
		3	.21333*	.04749	.007	.0538	.3729
		5	.03333	.04749	.978	-.1262	.1929
		6	1.28333*	.04749	.000	1.1238	1.4429
5	1	-.06667	.04749	.725	-.2262	.0929	
	2	.96667*	.04749	.000	.8071	1.1262	
	3	.18000*	.04749	.024	.0205	.3395	
	4	-.03333	.04749	.978	-.1929	.1262	

Appendix Table 2: continued

		6	1.25000*	.04749	.000	1.0905	1.4095
	6	1	-1.31667*	.04749	.000	-1.4762	-1.1571
		2	-.28333*	.04749	.001	-.4429	-.1238
		3	-1.07000*	.04749	.000	-1.2295	-.9105
		4	-1.28333*	.04749	.000	-1.4429	-1.1238
		5	-1.25000*	.04749	.000	-1.4095	-1.0905
pH	1	2	.68000*	.04981	.000	.5127	.8473
		3	1.10000*	.04981	.000	.9327	1.2673
		4	.19333*	.04981	.021	.0260	.3607
		5	.30000*	.04981	.001	.1327	.4673
		6	1.23333*	.04981	.000	1.0660	1.4007
	2	1	-.68000*	.04981	.000	-.8473	-.5127
		3	.42000*	.04981	.000	.2527	.5873
		4	-.48667*	.04981	.000	-.6540	-.3193
		5	-.38000*	.04981	.000	-.5473	-.2127
		6	.55333*	.04981	.000	.3860	.7207
	3	1	-1.10000*	.04981	.000	-1.2673	-.9327
		2	-.42000*	.04981	.000	-.5873	-.2527
		4	-.90667*	.04981	.000	-1.0740	-.7393
		5	-.80000*	.04981	.000	-.9673	-.6327
		6	.13333	.04981	.152	-.0340	.3007
	4	1	-.19333*	.04981	.021	-.3607	-.0260
		2	.48667*	.04981	.000	.3193	.6540
		3	.90667*	.04981	.000	.7393	1.0740
		5	.10667	.04981	.330	-.0607	.2740

Appendix Table 2: continued

		6	1.04000*	.04981	.000	.8727	1.2073
	5	1	-.30000*	.04981	.001	-.4673	-.1327
		2	.38000*	.04981	.000	.2127	.5473
		3	.80000*	.04981	.000	.6327	.9673
		4	-.10667	.04981	.330	-.2740	.0607
		6	.93333*	.04981	.000	.7660	1.1007
	6	1	-1.23333*	.04981	.000	-1.4007	-1.0660
		2	-.55333*	.04981	.000	-.7207	-.3860
		3	-.13333	.04981	.152	-.3007	.0340
		4	-1.04000*	.04981	.000	-1.2073	-.8727
		5	-.93333*	.04981	.000	-1.1007	-.7660
DO	1	2	-.16667	.20722	.961	-.8627	.5294
		3	-.69333	.20722	.051	-1.3894	.0027
		4	-.10667	.20722	.995	-.8027	.5894
		5	-.24667	.20722	.833	-.9427	.4494
		6	-1.64667*	.20722	.000	-2.3427	-.9506
	2	1	.16667	.20722	.961	-.5294	.8627
		3	-.52667	.20722	.186	-1.2227	.1694
		4	.06000	.20722	1.000	-.6360	.7560
		5	-.08000	.20722	.999	-.7760	.6160
		6	-1.48000*	.20722	.000	-2.1760	-.7840
	3	1	.69333	.20722	.051	-.0027	1.3894
		2	.52667	.20722	.186	-.1694	1.2227
		4	.58667	.20722	.119	-.1094	1.2827
		5	.44667	.20722	.324	-.2494	1.1427
		6	-.95333*	.20722	.006	-1.6494	-.2573

Appendix Table 2: continued

	4	1	.10667	.20722	.995	-.5894	.8027
		2	-.06000	.20722	1.000	-.7560	.6360
		3	-.58667	.20722	.119	-1.2827	.1094
		5	-.14000	.20722	.981	-.8360	.5560
		6	-1.54000*	.20722	.000	-2.2360	-.8440
	5	1	.24667	.20722	.833	-.4494	.9427
		2	.08000	.20722	.999	-.6160	.7760
		3	-.44667	.20722	.324	-1.1427	.2494
		4	.14000	.20722	.981	-.5560	.8360
		6	-1.40000*	.20722	.000	-2.0960	-.7040
	6	1	1.64667*	.20722	.000	.9506	2.3427
		2	1.48000*	.20722	.000	.7840	2.1760
		3	.95333*	.20722	.006	.2573	1.6494
		4	1.54000*	.20722	.000	.8440	2.2360
		5	1.40000*	.20722	.000	.7040	2.0960
Turb	1	2	8.96667*	.19572	.000	8.3092	9.6241
		3	19.03333*	.19572	.000	18.3759	19.6908
		4	2.21333*	.19572	.000	1.5559	2.8708
		5	1.12000*	.19572	.001	.4626	1.7774
		6	19.81333*	.19572	.000	19.1559	20.4708
	2	1	-8.96667*	.19572	.000	-9.6241	-8.3092
		3	10.06667*	.19572	.000	9.4092	10.7241
		4	-6.75333*	.19572	.000	-7.4108	-6.0959
		5	-7.84667*	.19572	.000	-8.5041	-7.1892
		6	10.84667*	.19572	.000	10.1892	11.5041
	3	1	-19.03333*	.19572	.000	-19.6908	-18.3759

Appendix Table 2: continued

		2	-10.06667*	.19572	.000	-10.7241	-9.4092
		4	-16.82000*	.19572	.000	-17.4774	-16.1626
		5	-17.91333*	.19572	.000	-18.5708	-17.2559
		6	.78000*	.19572	.017	.1226	1.4374
	4	1	-2.21333*	.19572	.000	-2.8708	-1.5559
		2	6.75333*	.19572	.000	6.0959	7.4108
		3	16.82000*	.19572	.000	16.1626	17.4774
		5	-1.09333*	.19572	.001	-1.7508	-.4359
		6	17.60000*	.19572	.000	16.9426	18.2574
	5	1	-1.12000*	.19572	.001	-1.7774	-.4626
		2	7.84667*	.19572	.000	7.1892	8.5041
		3	17.91333*	.19572	.000	17.2559	18.5708
		4	1.09333*	.19572	.001	.4359	1.7508
		6	18.69333*	.19572	.000	18.0359	19.3508
	6	1	-19.81333*	.19572	.000	-20.4708	-19.1559
		2	-10.84667*	.19572	.000	-11.5041	-10.1892
		3	-.78000*	.19572	.017	-1.4374	-.1226
		4	-17.60000*	.19572	.000	-18.2574	-16.9426
		5	-18.69333*	.19572	.000	-19.3508	-18.0359
EC	1	2	185.18667*	4.11709	.000	171.3577	199.0157
		3	331.33333*	4.11709	.000	317.5043	345.1623
		4	158.44667*	4.11709	.000	144.6177	172.2757
		5	49.55333*	4.11709	.000	35.7243	63.3823
		6	349.79333*	4.11709	.000	335.9643	363.6223
	2	1	-185.18667*	4.11709	.000	-199.0157	-171.3577
		3	146.14667*	4.11709	.000	132.3177	159.9757

Appendix Table 2: continued

		4	-26.74000*	4.11709	.000	-40.5690	-12.9110
		5	-135.63333*	4.11709	.000	-149.4623	-121.8043
		6	164.60667*	4.11709	.000	150.7777	178.4357
	3	1	-331.33333*	4.11709	.000	-345.1623	-317.5043
		2	-146.14667*	4.11709	.000	-159.9757	-132.3177
		4	-172.88667*	4.11709	.000	-186.7157	-159.0577
		5	-281.78000*	4.11709	.000	-295.6090	-267.9510
		6	18.46000*	4.11709	.008	4.6310	32.2890
	4	1	-158.44667*	4.11709	.000	-172.2757	-144.6177
		2	26.74000*	4.11709	.000	12.9110	40.5690
		3	172.88667*	4.11709	.000	159.0577	186.7157
		5	-108.89333*	4.11709	.000	-122.7223	-95.0643
		6	191.34667*	4.11709	.000	177.5177	205.1757
	5	1	-49.55333*	4.11709	.000	-63.3823	-35.7243
		2	135.63333*	4.11709	.000	121.8043	149.4623
		3	281.78000*	4.11709	.000	267.9510	295.6090
		4	108.89333*	4.11709	.000	95.0643	122.7223
		6	300.24000*	4.11709	.000	286.4110	314.0690
	6	1	-349.79333*	4.11709	.000	-363.6223	-335.9643
		2	-164.60667*	4.11709	.000	-178.4357	-150.7777
		3	-18.46000*	4.11709	.008	-32.2890	-4.6310
		4	-191.34667*	4.11709	.000	-205.1757	-177.5177
		5	-300.24000*	4.11709	.000	-314.0690	-286.4110
TDS	1	2	198.55333*	3.58996	.000	186.4949	210.6117
		3	306.06667*	3.58996	.000	294.0083	318.1251
		4	207.33333*	3.58996	.000	195.2749	219.3917

Appendix Table 2: continued

	5	25.33333*	3.58996	.000	13.2749	37.3917
	6	441.50000*	3.58996	.000	429.4416	453.5584
2	1	-198.55333*	3.58996	.000	-210.6117	-186.4949
	3	107.51333*	3.58996	.000	95.4549	119.5717
	4	8.78000	3.58996	.215	-3.2784	20.8384
	5	-173.22000*	3.58996	.000	-185.2784	-161.1616
	6	242.94667*	3.58996	.000	230.8883	255.0051
3	1	-306.06667*	3.58996	.000	-318.1251	-294.0083
	2	-107.51333*	3.58996	.000	-119.5717	-95.4549
	4	-98.73333*	3.58996	.000	-110.7917	-86.6749
	5	-280.73333*	3.58996	.000	-292.7917	-268.6749
	6	135.43333*	3.58996	.000	123.3749	147.4917
4	1	-207.33333*	3.58996	.000	-219.3917	-195.2749
	2	-8.78000	3.58996	.215	-20.8384	3.2784
	3	98.73333*	3.58996	.000	86.6749	110.7917
	5	-182.00000*	3.58996	.000	-194.0584	-169.9416
	6	234.16667*	3.58996	.000	222.1083	246.2251
5	1	-25.33333*	3.58996	.000	-37.3917	-13.2749
	2	173.22000*	3.58996	.000	161.1616	185.2784
	3	280.73333*	3.58996	.000	268.6749	292.7917
	4	182.00000*	3.58996	.000	169.9416	194.0584
	6	416.16667*	3.58996	.000	404.1083	428.2251
6	1	-441.50000*	3.58996	.000	-453.5584	-429.4416
	2	-242.94667*	3.58996	.000	-255.0051	-230.8883
	3	-135.43333*	3.58996	.000	-147.4917	-123.3749
	4	-234.16667*	3.58996	.000	-246.2251	-222.1083

Appendix Table 2: continued

		5	-416.16667*	3.58996	.000	-428.2251	-404.1083	
BOD	1	2	35.73333*	4.78999	.000	19.6441	51.8225	
		3	47.37333*	4.78999	.000	31.2841	63.4625	
		4	20.62000*	4.78999	.010	4.5308	36.7092	
		5	16.43333*	4.78999	.044	.3441	32.5225	
		6	57.84000*	4.78999	.000	41.7508	73.9292	
		2	1	-35.73333*	4.78999	.000	-51.8225	-19.6441
			3	11.64000	4.78999	.220	-4.4492	27.7292
			4	-15.11333	4.78999	.070	-31.2025	.9759
			5	-19.30000*	4.78999	.016	-35.3892	-3.2108
			6	22.10667*	4.78999	.006	6.0175	38.1959
		3	1	-47.37333*	4.78999	.000	-63.4625	-31.2841
			2	-11.64000	4.78999	.220	-27.7292	4.4492
			4	-26.75333*	4.78999	.001	-42.8425	-10.6641
			5	-30.94000*	4.78999	.000	-47.0292	-14.8508
			6	10.46667	4.78999	.311	-5.6225	26.5559
			4	1	-20.62000*	4.78999	.010	-36.7092
		2		15.11333	4.78999	.070	-.9759	31.2025
		3		26.75333*	4.78999	.001	10.6641	42.8425
		5		-4.18667	4.78999	.946	-20.2759	11.9025
		6		37.22000*	4.78999	.000	21.1308	53.3092
		5		1	-16.43333*	4.78999	.044	-32.5225
			2	19.30000*	4.78999	.016	3.2108	35.3892
			3	30.94000*	4.78999	.000	14.8508	47.0292
			4	4.18667	4.78999	.946	-11.9025	20.2759
	6		41.40667*	4.78999	.000	25.3175	57.4959	

Appendix Table 2: continued

	6	1	-57.84000*	4.78999	.000	-73.9292	-41.7508
		2	-22.10667*	4.78999	.006	-38.1959	-6.0175
		3	-10.46667	4.78999	.311	-26.5559	5.6225
		4	-37.22000*	4.78999	.000	-53.3092	-21.1308
		5	-41.40667*	4.78999	.000	-57.4959	-25.3175
NH ₄ ⁺	1	2	.34000*	.04114	.000	.2018	.4782
		3	.27333*	.04114	.000	.1351	.4115
		4	.04667	.04114	.858	-.0915	.1849
		5	.00667	.04114	1.000	-.1315	.1449
		6	.48000*	.04114	.000	.3418	.6182
		2	1	-.34000*	.04114	.000	-.4782
		3	-.06667	.04114	.602	-.2049	.0715
		4	-.29333*	.04114	.000	-.4315	-.1551
		5	-.33333*	.04114	.000	-.4715	-.1951
		6	.14000*	.04114	.046	.0018	.2782
	3	1	-.27333*	.04114	.000	-.4115	-.1351
		2	.06667	.04114	.602	-.0715	.2049
		4	-.22667*	.04114	.001	-.3649	-.0885
		5	-.26667*	.04114	.000	-.4049	-.1285
		6	.20667*	.04114	.003	.0685	.3449
		4	1	-.04667	.04114	.858	-.1849
	2		.29333*	.04114	.000	.1551	.4315
	3		.22667*	.04114	.001	.0885	.3649
5	-.04000		.04114	.918	-.1782	.0982	
6	.43333*		.04114	.000	.2951	.5715	
5	1		-.00667	.04114	1.000	-.1449	.1315

Appendix Table 2: continued

		2	.33333*	.04114	.000	.1951	.4715
		3	.26667*	.04114	.000	.1285	.4049
		4	.04000	.04114	.918	-.0982	.1782
		6	.47333*	.04114	.000	.3351	.6115
	6	1	-.48000*	.04114	.000	-.6182	-.3418
		2	-.14000*	.04114	.046	-.2782	-.0018
		3	-.20667*	.04114	.003	-.3449	-.0685
		4	-.43333*	.04114	.000	-.5715	-.2951
		5	-.47333*	.04114	.000	-.6115	-.3351
NO ₂ ⁻	1	2	.12667*	.01836	.000	.0650	.1883
		3	.16333*	.01836	.000	.1017	.2250
		4	.05333	.01836	.106	-.0083	.1150
		5	.03000	.01836	.594	-.0317	.0917
		6	.15667*	.01836	.000	.0950	.2183
		2	1	-.12667*	.01836	.000	-.1883
		3	.03667	.01836	.397	-.0250	.0983
		4	-.07333*	.01836	.017	-.1350	-.0117
		5	-.09667*	.01836	.002	-.1583	-.0350
		6	.03000	.01836	.594	-.0317	.0917
	3	1	-.16333*	.01836	.000	-.2250	-.1017
		2	-.03667	.01836	.397	-.0983	.0250
		4	-.11000*	.01836	.001	-.1717	-.0483
		5	-.13333*	.01836	.000	-.1950	-.0717
		6	-.00667	.01836	.999	-.0683	.0550
	4	1	-.05333	.01836	.106	-.1150	.0083
		2	.07333*	.01836	.017	.0117	.1350

Appendix Table 2: continued

		3	.11000*	.01836	.001	.0483	.1717
		5	-.02333	.01836	.795	-.0850	.0383
		6	.10333*	.01836	.001	.0417	.1650
	5	1	-.03000	.01836	.594	-.0917	.0317
		2	.09667*	.01836	.002	.0350	.1583
		3	.13333*	.01836	.000	.0717	.1950
		4	.02333	.01836	.795	-.0383	.0850
		6	.12667*	.01836	.000	.0650	.1883
	6	1	-.15667*	.01836	.000	-.2183	-.0950
		2	-.03000	.01836	.594	-.0917	.0317
		3	.00667	.01836	.999	-.0550	.0683
		4	-.10333*	.01836	.001	-.1650	-.0417
		5	-.12667*	.01836	.000	-.1883	-.0650
NO ₃ ⁻	1	2	1.42667*	.25056	.001	.5850	2.2683
		3	3.35333*	.25056	.000	2.5117	4.1950
		4	2.92000*	.25056	.000	2.0784	3.7616
		5	1.80667*	.25056	.000	.9650	2.6483
		6	3.79333*	.25056	.000	2.9517	4.6350
	2	1	-1.42667*	.25056	.001	-2.2683	-.5850
		3	1.92667*	.25056	.000	1.0850	2.7683
		4	1.49333*	.25056	.001	.6517	2.3350
		5	.38000	.25056	.661	-.4616	1.2216
		6	2.36667*	.25056	.000	1.5250	3.2083
	3	1	-3.35333*	.25056	.000	-4.1950	-2.5117
		2	-1.92667*	.25056	.000	-2.7683	-1.0850
		4	-.43333	.25056	.539	-1.2750	.4083

Appendix Table 2: continued

		5	-1.54667*	.25056	.001	-2.3883	-.7050
		6	.44000	.25056	.525	-.4016	1.2816
	4	1	-2.92000*	.25056	.000	-3.7616	-2.0784
		2	-1.49333*	.25056	.001	-2.3350	-.6517
		3	.43333	.25056	.539	-.4083	1.2750
		5	-1.11333*	.25056	.008	-1.9550	-.2717
		6	.87333*	.25056	.040	.0317	1.7150
	5	1	-1.80667*	.25056	.000	-2.6483	-.9650
		2	-.38000	.25056	.661	-1.2216	.4616
		3	1.54667*	.25056	.001	.7050	2.3883
		4	1.11333*	.25056	.008	.2717	1.9550
		6	1.98667*	.25056	.000	1.1450	2.8283
	6	1	-3.79333*	.25056	.000	-4.6350	-2.9517
		2	-2.36667*	.25056	.000	-3.2083	-1.5250
		3	-.44000	.25056	.525	-1.2816	.4016
		4	-.87333*	.25056	.040	-1.7150	-.0317
		5	-1.98667*	.25056	.000	-2.8283	-1.1450
PO ₄ ⁻³	1	2	.53333*	.04282	.000	.3895	.6772
		3	.64000*	.04282	.000	.4962	.7838
		4	.08000	.04282	.463	-.0638	.2238
		5	-.07333	.04282	.549	-.2172	.0705
		6	.75333*	.04282	.000	.6095	.8972
	2	1	-.53333*	.04282	.000	-.6772	-.3895
		3	.10667	.04282	.201	-.0372	.2505
		4	-.45333*	.04282	.000	-.5972	-.3095
		5	-.60667*	.04282	.000	-.7505	-.4628

Appendix Table 2: continued

		6	.22000*	.04282	.003	.0762	.3638
	3	1	-.64000*	.04282	.000	-.7838	-.4962
		2	-.10667	.04282	.201	-.2505	.0372
		4	-.56000*	.04282	.000	-.7038	-.4162
		5	-.71333*	.04282	.000	-.8572	-.5695
		6	.11333	.04282	.159	-.0305	.2572
	4	1	-.08000	.04282	.463	-.2238	.0638
		2	.45333*	.04282	.000	.3095	.5972
		3	.56000*	.04282	.000	.4162	.7038
		5	-.15333*	.04282	.034	-.2972	-.0095
		6	.67333*	.04282	.000	.5295	.8172
	5	1	.07333	.04282	.549	-.0705	.2172
		2	.60667*	.04282	.000	.4628	.7505
		3	.71333*	.04282	.000	.5695	.8572
		4	.15333*	.04282	.034	.0095	.2972
		6	.82667*	.04282	.000	.6828	.9705
	6	1	-.75333*	.04282	.000	-.8972	-.6095
		2	-.22000*	.04282	.003	-.3638	-.0762
		3	-.11333	.04282	.159	-.2572	.0305
		4	-.67333*	.04282	.000	-.8172	-.5295
		5	-.82667*	.04282	.000	-.9705	-.6828
TBC	1	2	2.13333*	.12069	.000	1.7279	2.5387
		3	3.15333*	.12069	.000	2.7479	3.5587
		4	1.21333*	.12069	.000	.8079	1.6187
		5	1.11333*	.12069	.000	.7079	1.5187
		6	3.67333*	.12069	.000	3.2679	4.0787

Appendix Table 2: continued

	2	1	-2.13333*	.12069	.000	-2.5387	-1.7279
		3	1.02000*	.12069	.000	.6146	1.4254
		4	-.92000*	.12069	.000	-1.3254	-.5146
		5	-1.02000*	.12069	.000	-1.4254	-.6146
		6	1.54000*	.12069	.000	1.1346	1.9454
	3	1	-3.15333*	.12069	.000	-3.5587	-2.7479
		2	-1.02000*	.12069	.000	-1.4254	-.6146
		4	-1.94000*	.12069	.000	-2.3454	-1.5346
		5	-2.04000*	.12069	.000	-2.4454	-1.6346
		6	.52000*	.12069	.010	.1146	.9254
	4	1	-1.21333*	.12069	.000	-1.6187	-.8079
		2	.92000*	.12069	.000	.5146	1.3254
		3	1.94000*	.12069	.000	1.5346	2.3454
		5	-.10000	.12069	.956	-.5054	.3054
		6	2.46000*	.12069	.000	2.0546	2.8654
	5	1	-1.11333*	.12069	.000	-1.5187	-.7079
		2	1.02000*	.12069	.000	.6146	1.4254
		3	2.04000*	.12069	.000	1.6346	2.4454
		4	.10000	.12069	.956	-.3054	.5054
		6	2.56000*	.12069	.000	2.1546	2.9654
	6	1	-3.67333*	.12069	.000	-4.0787	-3.2679
		2	-1.54000*	.12069	.000	-1.9454	-1.1346
		3	-.52000*	.12069	.010	-.9254	-.1146
		4	-2.46000*	.12069	.000	-2.8654	-2.0546
		5	-2.56000*	.12069	.000	-2.9654	-2.1546
TC	1	2	1.75333*	.12793	.000	1.3236	2.1830

Appendix Table 2: continued

	3	2.13333*	.12793	.000	1.7036	2.5630
	4	1.03333*	.12793	.000	.6036	1.4630
	5	.90000*	.12793	.000	.4703	1.3297
	6	2.61333*	.12793	.000	2.1836	3.0430
2	1	-1.75333*	.12793	.000	-2.1830	-1.3236
	3	.38000	.12793	.095	-.0497	.8097
	4	-.72000*	.12793	.001	-1.1497	-.2903
	5	-.85333*	.12793	.000	-1.2830	-.4236
	6	.86000*	.12793	.000	.4303	1.2897
3	1	-2.13333*	.12793	.000	-2.5630	-1.7036
	2	-.38000	.12793	.095	-.8097	.0497
	4	-1.10000*	.12793	.000	-1.5297	-.6703
	5	-1.23333*	.12793	.000	-1.6630	-.8036
	6	.48000*	.12793	.026	.0503	.9097
4	1	-1.03333*	.12793	.000	-1.4630	-.6036
	2	.72000*	.12793	.001	.2903	1.1497
	3	1.10000*	.12793	.000	.6703	1.5297
	5	-.13333	.12793	.894	-.5630	.2964
	6	1.58000*	.12793	.000	1.1503	2.0097
5	1	-.90000*	.12793	.000	-1.3297	-.4703
	2	.85333*	.12793	.000	.4236	1.2830
	3	1.23333*	.12793	.000	.8036	1.6630
	4	.13333	.12793	.894	-.2964	.5630
	6	1.71333*	.12793	.000	1.2836	2.1430
6	1	-2.61333*	.12793	.000	-3.0430	-2.1836
	2	-.86000*	.12793	.000	-1.2897	-.4303

Appendix Table 2: continued

		3	-.48000*	.12793	.026	-.9097	-.0503
		4	-1.58000*	.12793	.000	-2.0097	-1.1503
		5	-1.71333*	.12793	.000	-2.1430	-1.2836
FC	1	2	1.56667*	.20263	.000	.8860	2.2473
		3	1.76667*	.20263	.000	1.0860	2.4473
		4	.33333	.20263	.588	-.3473	1.0140
		5	-.11333	.20263	.992	-.7940	.5673
		6	2.10000*	.20263	.000	1.4194	2.7806
	2	1	-1.56667*	.20263	.000	-2.2473	-.8860
		3	.20000	.20263	.914	-.4806	.8806
		4	-1.23333*	.20263	.001	-1.9140	-.5527
		5	-1.68000*	.20263	.000	-2.3606	-.9994
		6	.53333	.20263	.162	-.1473	1.2140
	3	1	-1.76667*	.20263	.000	-2.4473	-1.0860
		2	-.20000	.20263	.914	-.8806	.4806
		4	-1.43333*	.20263	.000	-2.1140	-.7527
		5	-1.88000*	.20263	.000	-2.5606	-1.1994
		6	.33333	.20263	.588	-.3473	1.0140
	4	1	-.33333	.20263	.588	-1.0140	.3473
		2	1.23333*	.20263	.001	.5527	1.9140
		3	1.43333*	.20263	.000	.7527	2.1140
		5	-.44667	.20263	.303	-1.1273	.2340
		6	1.76667*	.20263	.000	1.0860	2.4473
	5	1	.11333	.20263	.992	-.5673	.7940
		2	1.68000*	.20263	.000	.9994	2.3606
		3	1.88000*	.20263	.000	1.1994	2.5606

Appendix Table 2: continued

	4	.44667	.20263	.303	-.2340	1.1273
	6	2.21333*	.20263	.000	1.5327	2.8940
6	1	-2.10000*	.20263	.000	-2.7806	-1.4194
	2	-.53333	.20263	.162	-1.2140	.1473
	3	-.33333	.20263	.588	-1.0140	.3473
	4	-1.76667*	.20263	.000	-2.4473	-1.0860
	5	-2.21333*	.20263	.000	-2.8940	-1.5327

*. The mean difference is significant at the 0.05 level.

Appendix table 3: ANOVA Multiple Comparisons table for physicochemical and bacteriological parameters among seasons

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Temp	1	2	1.22000*	.06377	.000	1.0158	1.4242
		3	.15667	.06377	.143	-.0475	.3609
		4	.03333	.06377	.951	-.1709	.2375
	2	1	-1.22000*	.06377	.000	-1.4242	-1.0158
		3	-1.06333*	.06377	.000	-1.2675	-.8591
		4	-1.18667*	.06377	.000	-1.3909	-.9825
	3	1	-.15667	.06377	.143	-.3609	.0475
		2	1.06333*	.06377	.000	.8591	1.2675
		4	-.12333	.06377	.287	-.3275	.0809
	4	1	-.03333	.06377	.951	-.2375	.1709
		2	1.18667*	.06377	.000	.9825	1.3909
		3	.12333	.06377	.287	-.0809	.3275
pH	1	2	1.19333*	.05110	.000	1.0297	1.3570
		3	1.10000*	.05110	.000	.9364	1.2636
		4	.19333*	.05110	.022	.0297	.3570
	2	1	-1.19333*	.05110	.000	-1.3570	-1.0297
		3	-.09333	.05110	.329	-.2570	.0703
		4	-1.00000*	.05110	.000	-1.1636	-.8364
	3	1	-1.10000*	.05110	.000	-1.2636	-.9364
		2	.09333	.05110	.329	-.0703	.2570
		4	-.90667*	.05110	.000	-1.0703	-.7430
	4	1	-.19333*	.05110	.022	-.3570	-.0297

Appendix table 3: Continued

		2	1.00000*	.05110	.000	.8364	1.1636
		3	.90667*	.05110	.000	.7430	1.0703
DO	1	2	-1.33000*	.31589	.013	-2.3416	-.3184
		3	-.69333	.31589	.204	-1.7049	.3182
		4	-.10667	.31589	.986	-1.1182	.9049
	2	1	1.33000*	.31589	.013	.3184	2.3416
		3	.63667	.31589	.259	-.3749	1.6482
		4	1.22333*	.31589	.020	.2118	2.2349
	3	1	.69333	.31589	.204	-.3182	1.7049
		2	-.63667	.31589	.259	-1.6482	.3749
		4	.58667	.31589	.317	-.4249	1.5982
	4	1	.10667	.31589	.986	-.9049	1.1182
		2	-1.22333*	.31589	.020	-2.2349	-.2118
		3	-.58667	.31589	.317	-1.5982	.4249
Turb	1	2	-1.84000*	.29576	.001	-2.7871	-.8929
		3	6.78667*	.29576	.000	5.8395	7.7338
		4	17.37667*	.29576	.000	16.4295	18.3238
	2	1	1.84000*	.29576	.001	.8929	2.7871
		3	8.62667*	.29576	.000	7.6795	9.5738
		4	19.21667*	.29576	.000	18.2695	20.1638
	3	1	-6.78667*	.29576	.000	-7.7338	-5.8395
		2	-8.62667*	.29576	.000	-9.5738	-7.6795
		4	10.59000*	.29576	.000	9.6429	11.5371
	4	1	-17.37667*	.29576	.000	-18.3238	-16.4295
		2	-19.21667*	.29576	.000	-20.1638	-18.2695
		3	-10.59000*	.29576	.000	-11.5371	-9.6429

Appendix table 3: Continued

EC	1	2	-178.52000*	20.43972	.000	-243.9752	-113.0648
		3	6.59333	20.43972	.988	-58.8618	72.0485
		4	166.26333*	20.43972	.000	100.8082	231.7185
	2	1	178.52000*	20.43972	.000	113.0648	243.9752
		3	185.11333*	20.43972	.000	119.6582	250.5685
		4	344.78333*	20.43972	.000	279.3282	410.2385
	3	1	-6.59333	20.43972	.988	-72.0485	58.8618
		2	-185.11333*	20.43972	.000	-250.5685	-119.6582
		4	159.67000*	20.43972	.000	94.2148	225.1252
	4	1	-166.26333*	20.43972	.000	-231.7185	-100.8082
		2	-344.78333*	20.43972	.000	-410.2385	-279.3282
		3	-159.67000*	20.43972	.000	-225.1252	-94.2148
TDS	1	2	-178.30333*	20.17765	.000	-242.9193	-113.6874
		3	15.44667	20.17765	.868	-49.1693	80.0626
		4	211.91333*	20.17765	.000	147.2974	276.5293
	2	1	178.30333*	20.17765	.000	113.6874	242.9193
		3	193.75000*	20.17765	.000	129.1341	258.3659
		4	390.21667*	20.17765	.000	325.6007	454.8326
	3	1	-15.44667	20.17765	.868	-80.0626	49.1693
		2	-193.75000*	20.17765	.000	-258.3659	-129.1341
		4	196.46667*	20.17765	.000	131.8507	261.0826
	4	1	-211.91333*	20.17765	.000	-276.5293	-147.2974
		2	-390.21667*	20.17765	.000	-454.8326	-325.6007
		3	-196.46667*	20.17765	.000	-261.0826	-131.8507

Appendix table 3: Continued

BOD	1	2	-32.67333*	6.92270	.007	-54.8423	-10.5044
		3	-10.44667	6.92270	.475	-32.6156	11.7223
		4	19.24333	6.92270	.091	-2.9256	41.4123
	2	1	32.67333*	6.92270	.007	10.5044	54.8423
		3	22.22667*	6.92270	.049	.0577	44.3956
		4	51.91667*	6.92270	.000	29.7477	74.0856
	3	1	10.44667	6.92270	.475	-11.7223	32.6156
		2	-22.22667*	6.92270	.049	-44.3956	-.0577
		4	29.69000*	6.92270	.011	7.5211	51.8589
	4	1	-19.24333	6.92270	.091	-41.4123	2.9256
		2	-51.91667*	6.92270	.000	-74.0856	-29.7477
		3	-29.69000*	6.92270	.011	-51.8589	-7.5211
NH ₄ ⁺	1	2	-.44333*	.04177	.000	-.5771	-.3096
		3	-.39000*	.04177	.000	-.5238	-.2562
		4	-.16333*	.04177	.019	-.2971	-.0296
	2	1	.44333*	.04177	.000	.3096	.5771
		3	.05333	.04177	.601	-.0804	.1871
		4	.28000*	.04177	.001	.1462	.4138
	3	1	.39000*	.04177	.000	.2562	.5238
		2	-.05333	.04177	.601	-.1871	.0804
		4	.22667*	.04177	.003	.0929	.3604
	4	1	.16333*	.04177	.019	.0296	.2971
		2	-.28000*	.04177	.001	-.4138	-.1462
		3	-.22667*	.04177	.003	-.3604	-.0929

Appendix table 3: Continued

NO ₂ ⁻	1	2	-.14333*	.01563	.000	-.1934	-.0933
		3	-.09667*	.01563	.001	-.1467	-.0466
		4	.02000	.01563	.599	-.0301	.0701
	2	1	.14333*	.01563	.000	.0933	.1934
		3	.04667	.01563	.068	-.0034	.0967
		4	.16333*	.01563	.000	.1133	.2134
	3	1	.09667*	.01563	.001	.0466	.1467
		2	-.04667	.01563	.068	-.0967	.0034
		4	.11667*	.01563	.000	.0666	.1667
	4	1	-.02000	.01563	.599	-.0701	.0301
		2	-.16333*	.01563	.000	-.2134	-.1133
		3	-.11667*	.01563	.000	-.1667	-.0666
NO ₃ ⁻	1	2	-1.42667*	.36696	.019	-2.6018	-.2515
		3	1.67667*	.36696	.008	.5015	2.8518
		4	2.03333*	.36696	.002	.8582	3.2085
	2	1	1.42667*	.36696	.019	.2515	2.6018
		3	3.10333*	.36696	.000	1.9282	4.2785
		4	3.46000*	.36696	.000	2.2849	4.6351
	3	1	-1.67667*	.36696	.008	-2.8518	-.5015
		2	-3.10333*	.36696	.000	-4.2785	-1.9282
		4	.35667	.36696	.769	-.8185	1.5318
	4	1	-2.03333*	.36696	.002	-3.2085	-.8582
		2	-3.46000*	.36696	.000	-4.6351	-2.2849
		3	-.35667	.36696	.769	-1.5318	.8185

Appendix table 3: Continued

PO ₄ ⁻³	1	2	-.56667*	.04859	.000	-.7223	-.4111
		3	-.45333*	.04859	.000	-.6089	-.2977
		4	.18667*	.04859	.021	.0311	.3423
	2	1	.56667*	.04859	.000	.4111	.7223
		3	.11333	.04859	.169	-.0423	.2689
		4	.75333*	.04859	.000	.5977	.9089
	3	1	.45333*	.04859	.000	.2977	.6089
		2	-.11333	.04859	.169	-.2689	.0423
		4	.64000*	.04859	.000	.4844	.7956
	4	1	-.18667*	.04859	.021	-.3423	-.0311
		2	-.75333*	.04859	.000	-.9089	-.5977
		3	-.64000*	.04859	.000	-.7956	-.4844
TBC	1	2	2.13333*	.13216	.000	1.7101	2.5566
		3	3.53667*	.13216	.000	3.1134	3.9599
		4	1.16000*	.13216	.000	.7368	1.5832
	2	1	-2.13333*	.13216	.000	-2.5566	-1.7101
		3	1.40333*	.13216	.000	.9801	1.8266
		4	-.97333*	.13216	.000	-1.3966	-.5501
	3	1	-3.53667*	.13216	.000	-3.9599	-3.1134
		2	-1.40333*	.13216	.000	-1.8266	-.9801
		4	-2.37667*	.13216	.000	-2.7999	-1.9534
	4	1	-1.16000*	.13216	.000	-1.5832	-.7368
		2	.97333*	.13216	.000	.5501	1.3966
		3	2.37667*	.13216	.000	1.9534	2.7999

Appendix table 3: Continued

TC	1	2	-1.75333*	.17245	.000	-2.3056	-1.2011
		3	-.78333*	.17245	.008	-1.3356	-.2311
		4	.74000*	.17245	.011	.1878	1.2922
	2	1	1.75333*	.17245	.000	1.2011	2.3056
		3	.97000*	.17245	.002	.4178	1.5222
		4	2.49333*	.17245	.000	1.9411	3.0456
	3	1	.78333*	.17245	.008	.2311	1.3356
		2	-.97000*	.17245	.002	-1.5222	-.4178
		4	1.52333*	.17245	.000	.9711	2.0756
	4	1	-.74000*	.17245	.011	-1.2922	-.1878
		2	-2.49333*	.17245	.000	-3.0456	-1.9411
		3	-1.52333*	.17245	.000	-2.0756	-.9711
FC	1	2	-1.56667*	.31410	.005	-2.5725	-.5608
		3	-1.36667*	.31410	.010	-2.3725	-.3608
		4	.43000	.31410	.550	-.5759	1.4359
	2	1	1.56667*	.31410	.005	.5608	2.5725
		3	.20000	.31410	.917	-.8059	1.2059
		4	1.99667*	.31410	.001	.9908	3.0025
	3	1	1.36667*	.31410	.010	.3608	2.3725
		2	-.20000	.31410	.917	-1.2059	.8059
		4	1.79667*	.31410	.002	.7908	2.8025
	4	1	-.43000	.31410	.550	-1.4359	.5759
		2	-1.99667*	.31410	.001	-3.0025	-.9908
		3	-1.79667*	.31410	.002	-2.8025	-.7908

*. The mean difference is significant at the 0.05 level.

Appendix table 4: Morphological characteristics of bacterial isolates from *O. niloticus* in Lake Hawassa

Table 5: Morphological characteristics of bacterial isolates

Isolates	shape	Morphological characteristics of colony			Media	Suspected isolate
		colour	elevation	Optical feat		
1	Rod	whitish	flat	transparent	NA	<i>Escherichia coli</i>
2	Rod	yellowish	convex	Opaque	TSA	<i>Aeromonas</i> species
3	Rod	whitish	raised	Opaque	TSA	<i>Pseudomonas</i> species
4	coccus	white	raised	Opaque	NA	<i>Bacillus</i> species
5	Rod	collarless	raised	translucent	SSA	<i>Salmonella</i> species
6	Rod	colorless	convex	transparent	SSA	<i>Shigella</i> species
7	Rod	green	flat	Opaque	TCBS	<i>Vibrio</i> species
8	Rod	pinkish	raised	translucent	XLD	<i>Edwardsiella</i> species
9	Round	white	convex	S-translucent	NA	<i>Staphylococcus</i> species
10	Rod	yellow	flat	translucent	TSA	<i>Flavobacterium</i> species

NA; Nutrint agar, TSA; Thiosulphate citrate bile salt sucrose agar, SSA; Shigella-salmonella agar, S-translucent; semi-translucent, XLD; Xylose lysinedeoxycholate

Appendix table 5: Biochemical characterization of bacterial isolates of *O. niloticus* in Lake Hawassa

Isolate	GS	Biochemical characterization										
		CT	UT	Coa	OT	SCT	MR	VP test	MT	SIM Medium		
										IN	MT	H2S
<i>A. Hydrophila</i>	-	+	+	+	+	+	-	+	+	+	+	-
<i>E. coli</i>	-	+	-	-	-	-	+	-	+	+	+	+
<i>Vibrio spp.</i>	-	+	-	-	+	+	-	+	+	-	+	-
<i>Bacillus spp.</i>	+	+	-	-	-	-	-	+	-	-	+	-
<i>Pseudomonas spp.</i>	-	+	-	-	+	+	-	-	-	-	-	-
<i>Staphylococcus spp.</i>	+	+	+	+	-	+	+	+	+	-	-	-
<i>Salmonella spp.</i>	-	+	-	-	-	+	+	-	+	-	+	+
<i>Shigella spp.</i>	-	+	-	-	-	-	+	-	-	+	-	-
<i>E. tarda</i>	-	+	-	+	-	-	+	-	-	+	+	+
<i>Flavobacterium spp.</i>	-	+	-	+	-	-	-	-	-	+	+	-

GS; Gram stain, CT; Catalase test, UT; Urease test, Coa; Coagulase test, OT; Oxidase test, SCT; Simon's citrate test, MR; Methyl red test, VP; Voges-Proskauer test, MT; Manitol test, SIM; Sulphur indole motility, IT; Indole test, MT; Motility test, H2s; Hydrogen di sulphide test, A. *Hydrophila*; *Aeromonas hydrophila*, *E. coli*; *Escherichia coli*, *S. aureus*; *staphylococcus aureus*, *E. tarada*; *Edwardsiella tarada* (+); positive to the reaction, (-); negative

Appendix table 6: ANOVA Multiple Comparisons table for bacterial loads of fish in Lake Hawassa among seasons

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Skin	1	2	.50000*	.01353	.000	.4647	.5353
		3	1.06667*	.01353	.000	1.0314	1.1019
		4	1.20000*	.01353	.000	1.1647	1.2353
	2	1	-.50000*	.01353	.000	-.5353	-.4647
		3	.56667*	.01353	.000	.5314	.6019
		4	.70000*	.01353	.000	.6647	.7353
	3	1	-1.06667*	.01353	.000	-1.1019	-1.0314
		2	-.56667*	.01353	.000	-.6019	-.5314
		4	.13333*	.01353	.000	.0981	.1686
	4	1	-1.20000*	.01353	.000	-1.2353	-1.1647
		2	-.70000*	.01353	.000	-.7353	-.6647
		3	-.13333*	.01353	.000	-.1686	-.0981
Gills	1	2	.70000*	.00248	.000	.6935	.7065
		3	.87667*	.00248	.000	.8702	.8831
		4	1.48000*	.00248	.000	1.4735	1.4865
	2	1	-.70000*	.00248	.000	-.7065	-.6935
		3	.17667*	.00248	.000	.1702	.1831
		4	.78000*	.00248	.000	.7735	.7865
	3	1	-.87667*	.00248	.000	-.8831	-.8702
		2	-.17667*	.00248	.000	-.1831	-.1702
		4	.60333*	.00248	.000	.5969	.6098
	4	1	-1.48000*	.00248	.000	-1.4865	-1.4735
		2	-.78000*	.00248	.000	-.7865	-.7735

Appendix table 6: Continued

Intestine	1	3	-.60333 [*]	.00248	.000	-.6098	-.5969
		2	1.30000 [*]	.00248	.000	1.2935	1.3065
		3	1.09667 [*]	.00248	.000	1.0902	1.1031
		4	1.40000 [*]	.00248	.000	1.3935	1.4065
	2	1	-1.30000 [*]	.00248	.000	-1.3065	-1.2935
		3	-.20333 [*]	.00248	.000	-.2098	-.1969
		4	.10000 [*]	.00248	.000	.0935	.1065
	3	1	-1.09667 [*]	.00248	.000	-1.1031	-1.0902
		2	.20333 [*]	.00248	.000	.1969	.2098
		4	.30333 [*]	.00248	.000	.2969	.3098
	4	1	-1.40000 [*]	.00248	.000	-1.4065	-1.3935
		2	-.10000 [*]	.00248	.000	-.1065	-.0935
3		-.30333 [*]	.00248	.000	-.3098	-.2969	
Liver	1	2	.60333 [*]	.00186	.000	.5985	.6082
		3	.20333 [*]	.00186	.000	.1985	.2082
		4	.20333 [*]	.00186	.000	.1985	.2082
		1	-.60333 [*]	.00186	.000	-.6082	-.5985
	2	3	-.40000 [*]	.00186	.000	-.4048	-.3952
		4	-.40000 [*]	.00186	.000	-.4048	-.3952
		1	-.20333 [*]	.00186	.000	-.2082	-.1985
	3	2	.40000 [*]	.00186	.000	.3952	.4048
		4	.00000	.00186	1.000	-.0048	.0048
		1	-.20333 [*]	.00186	.000	-.2082	-.1985
	4	2	.40000 [*]	.00186	.000	.3952	.4048
		3	.00000	.00186	1.000	-.0048	.0048

Appendix table 6: Continued

Kidney	1	2	.40333*	.00152	.000	.3994	.4073
		3	.60667*	.00152	.000	.6027	.6106
		4	.87667*	.00152	.000	.8727	.8806
	2	1	-.40333*	.00152	.000	-.4073	-.3994
		3	.20333*	.00152	.000	.1994	.2073
		4	.47333*	.00152	.000	.4694	.4773
	3	1	-.60667*	.00152	.000	-.6106	-.6027
		2	-.20333*	.00152	.000	-.2073	-.1994
		4	.27000*	.00152	.000	.2660	.2740
	4	1	-.87667*	.00152	.000	-.8806	-.8727
		2	-.47333*	.00152	.000	-.4773	-.4694
		3	-.27000*	.00152	.000	-.2740	-.2660

*. The mean difference is significant at the 0.05 level.

Appendix table 7: Antibiotic sensitivity test

Isolates	Amp	Azi	Ery	Str	Tet	Pen	Amo	Gen	Chl	Cip
LH2	R	R	R	R	S	R	R	S	S	S
LH8	R	I	I	R	S	R	R	S	S	S
LH12	R	R	R	I	S	R	R	S	S	S
LH22	R	I	I	R	S	R	R	S	S	S
LH30	R	I	R	R	S	R	R	S	S	S
LH34	R	I	R	I	S	R	R	S	S	S
LH37	R	R	I	I	S	R	R	S	S	S
LH47	R	R	I	I	S	R	R	S	S	S
LH51	R	R	R	I	S	R	R	S	S	S
LH55	R	R	I	R	S	R	R	S	S	S
LH60	R	I	R	R	S	R	R	S	S	S
LH65	R	I	R	I	S	R	R	S	S	S
LH72	R	R	I	I	S	R	R	S	S	S
LH78	R	I	R	I	S	R	R	S	S	S

Amo: Amoxicillin, Amp; Ampicillin, Azi: Azithromycin, Chl: Chloramphenicol, Cip: ciprofloxacin Ery: Erythromycin, Gen; Gentamicin
 Pen: Penicillin, Str: Streptomycin Tet: Tetracycline

Appendix table 8: ANOVA Multiple Comparisons table for haematological parameters of fish in Lake Hawassa among sites

Dependent Variable	(I) Site	(J) Site	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
RBCs	1	2	-.24667*	.00434	.000	-.2594	-.2339
		3	-.34667*	.00434	.000	-.3594	-.3339
		4	-.13667*	.00434	.000	-.1494	-.1239
		5	-.18667*	.00434	.000	-.1994	-.1739
		6	-.80667*	.00434	.000	-.8194	-.7939
		2	.24667*	.00434	.000	.2339	.2594
	2	3	-.10000*	.00434	.000	-.1127	-.0873
		4	.11000*	.00434	.000	.0973	.1227
		5	.06000*	.00434	.000	.0473	.0727
		6	-.56000*	.00434	.000	-.5727	-.5473
		3	.34667*	.00434	.000	.3339	.3594
		2	.10000*	.00434	.000	.0873	.1127
	3	4	.21000*	.00434	.000	.1973	.2227
		5	.16000*	.00434	.000	.1473	.1727
		6	-.46000*	.00434	.000	-.4727	-.4473
		4	.13667*	.00434	.000	.1239	.1494
		2	-.11000*	.00434	.000	-.1227	-.0973
		3	-.21000*	.00434	.000	-.2227	-.1973
	4	5	-.05000*	.00434	.000	-.0627	-.0373
		6	-.67000*	.00434	.000	-.6827	-.6573
5		.18667*	.00434	.000	.1739	.1994	
2		-.06000*	.00434	.000	-.0727	-.0473	
3		-.16000*	.00434	.000	-.1727	-.1473	
4		.05000*	.00434	.000	.0373	.0627	
5	6	-.62000*	.00434	.000	-.6327	-.6073	

Appendix table 8: continued

	6	1	.80667 [*]	.00434	.000	.7939	.8194
		2	.56000 [*]	.00434	.000	.5473	.5727
		3	.46000 [*]	.00434	.000	.4473	.4727
		4	.67000 [*]	.00434	.000	.6573	.6827
		5	.62000 [*]	.00434	.000	.6073	.6327
Hb	1	2	-.72000 [*]	.00328	.000	-.7296	-.7104
		3	-.99000 [*]	.00328	.000	-.9996	-.9804
		4	-.65000 [*]	.00328	.000	-.6596	-.6404
		5	-.21000 [*]	.00328	.000	-.2196	-.2004
		6	-1.64333 [*]	.00328	.000	-1.6530	-1.6337
	2	1	.72000 [*]	.00328	.000	.7104	.7296
		3	-.27000 [*]	.00328	.000	-.2796	-.2604
		4	.07000 [*]	.00328	.000	.0604	.0796
		5	.51000 [*]	.00328	.000	.5004	.5196
		6	-.92333 [*]	.00328	.000	-.9330	-.9137
	3	1	.99000 [*]	.00328	.000	.9804	.9996
		2	.27000 [*]	.00328	.000	.2604	.2796
		4	.34000 [*]	.00328	.000	.3304	.3496
		5	.78000 [*]	.00328	.000	.7704	.7896
		6	-.65333 [*]	.00328	.000	-.6630	-.6437
	4	1	.65000 [*]	.00328	.000	.6404	.6596
		2	-.07000 [*]	.00328	.000	-.0796	-.0604
		3	-.34000 [*]	.00328	.000	-.3496	-.3304
		5	.44000 [*]	.00328	.000	.4304	.4496
		6	-.99333 [*]	.00328	.000	-1.0030	-.9837
	5	1	.21000 [*]	.00328	.000	.2004	.2196
		2	-.51000 [*]	.00328	.000	-.5196	-.5004

Appendix table 8: continued

		3	-.78000 [*]	.00328	.000	-.7896	-.7704
		4	-.44000 [*]	.00328	.000	-.4496	-.4304
		6	-1.43333 [*]	.00328	.000	-1.4430	-1.4237
	6	1	1.64333 [*]	.00328	.000	1.6337	1.6530
		2	.92333 [*]	.00328	.000	.9137	.9330
		3	.65333 [*]	.00328	.000	.6437	.6630
		4	.99333 [*]	.00328	.000	.9837	1.0030
		5	1.43333 [*]	.00328	.000	1.4237	1.4430
Hct	1	2	-4.03000 [*]	.00557	.000	-4.0463	-4.0137
		3	-3.42000 [*]	.00557	.000	-3.4363	-3.4037
		4	-2.03000 [*]	.00557	.000	-2.0463	-2.0137
		5	-2.59000 [*]	.00557	.000	-2.6063	-2.5737
		6	-6.49333 [*]	.00557	.000	-6.5097	-6.4770
	2	1	4.03000 [*]	.00557	.000	4.0137	4.0463
		3	.61000 [*]	.00557	.000	.5937	.6263
		4	2.00000 [*]	.00557	.000	1.9837	2.0163
		5	1.44000 [*]	.00557	.000	1.4237	1.4563
		6	-2.46333 [*]	.00557	.000	-2.4797	-2.4470
	3	1	3.42000 [*]	.00557	.000	3.4037	3.4363
		2	-.61000 [*]	.00557	.000	-.6263	-.5937
		4	1.39000 [*]	.00557	.000	1.3737	1.4063
		5	.83000 [*]	.00557	.000	.8137	.8463
		6	-3.07333 [*]	.00557	.000	-3.0897	-3.0570
	4	1	2.03000 [*]	.00557	.000	2.0137	2.0463
		2	-2.00000 [*]	.00557	.000	-2.0163	-1.9837
		3	-1.39000 [*]	.00557	.000	-1.4063	-1.3737
		5	-.56000 [*]	.00557	.000	-.5763	-.5437

Appendix table 8: continued

		6	-4.46333*	.00557	.000	-4.4797	-4.4470
	5	1	2.59000*	.00557	.000	2.5737	2.6063
		2	-1.44000*	.00557	.000	-1.4563	-1.4237
		3	-.83000*	.00557	.000	-.8463	-.8137
		4	.56000*	.00557	.000	.5437	.5763
		6	-3.90333*	.00557	.000	-3.9197	-3.8870
	6	1	6.49333*	.00557	.000	6.4770	6.5097
		2	2.46333*	.00557	.000	2.4470	2.4797
		3	3.07333*	.00557	.000	3.0570	3.0897
		4	4.46333*	.00557	.000	4.4470	4.4797
		5	3.90333*	.00557	.000	3.8870	3.9197
ESR	1	2	1.92000*	.00426	.000	1.9075	1.9325
		3	1.83000*	.00426	.000	1.8175	1.8425
		4	.84000*	.00426	.000	.8275	.8525
		5	1.29000*	.00426	.000	1.2775	1.3025
		6	2.43000*	.00426	.000	2.4175	2.4425
	2	1	-1.92000*	.00426	.000	-1.9325	-1.9075
		3	-.09000*	.00426	.000	-.1025	-.0775
		4	-1.08000*	.00426	.000	-1.0925	-1.0675
		5	-.63000*	.00426	.000	-.6425	-.6175
		6	.51000*	.00426	.000	.4975	.5225
	3	1	-1.83000*	.00426	.000	-1.8425	-1.8175
		2	.09000*	.00426	.000	.0775	.1025
		4	-.99000*	.00426	.000	-1.0025	-.9775
		5	-.54000*	.00426	.000	-.5525	-.5275
		6	.60000*	.00426	.000	.5875	.6125
	4	1	-.84000*	.00426	.000	-.8525	-.8275

Appendix table 8: continued

		2	1.08000*	.00426	.000	1.0675	1.0925
		3	.99000*	.00426	.000	.9775	1.0025
		5	.45000*	.00426	.000	.4375	.4625
		6	1.59000*	.00426	.000	1.5775	1.6025
	5	1	-1.29000*	.00426	.000	-1.3025	-1.2775
		2	.63000*	.00426	.000	.6175	.6425
		3	.54000*	.00426	.000	.5275	.5525
		4	-.45000*	.00426	.000	-.4625	-.4375
		6	1.14000*	.00426	.000	1.1275	1.1525
	6	1	-2.43000*	.00426	.000	-2.4425	-2.4175
		2	-.51000*	.00426	.000	-.5225	-.4975
		3	-.60000*	.00426	.000	-.6125	-.5875
		4	-1.59000*	.00426	.000	-1.6025	-1.5775
		5	-1.14000*	.00426	.000	-1.1525	-1.1275
MCV	1	2	-7.60333*	.27028	.000	-8.3966	-6.8100
		3	1.73667*	.27028	.000	.9434	2.5300
		4	-3.26333*	.27028	.000	-4.0566	-2.4700
		5	-3.28667*	.27028	.000	-4.0800	-2.4934
		6	9.44667*	.27028	.000	8.6534	10.2400
	2	1	7.60333*	.27028	.000	6.8100	8.3966
		3	9.34000*	.27028	.000	8.5467	10.1333
		4	4.34000*	.27028	.000	3.5467	5.1333
		5	4.31667*	.27028	.000	3.5234	5.1100
		6	17.05000*	.27028	.000	16.2567	17.8433
	3	1	-1.73667*	.27028	.000	-2.5300	-.9434

Appendix table 8: continued

		2	-9.34000*	.27028	.000	-10.1333	-8.5467
		4	-5.00000*	.27028	.000	-5.7933	-4.2067
		5	-5.02333*	.27028	.000	-5.8166	-4.2300
		6	7.71000*	.27028	.000	6.9167	8.5033
	4	1	3.26333*	.27028	.000	2.4700	4.0566
		2	-4.34000*	.27028	.000	-5.1333	-3.5467
		3	5.00000*	.27028	.000	4.2067	5.7933
		5	-.02333	.27028	1.000	-.8166	.7700
		6	12.71000*	.27028	.000	11.9167	13.5033
	5	1	3.28667*	.27028	.000	2.4934	4.0800
		2	-4.31667*	.27028	.000	-5.1100	-3.5234
		3	5.02333*	.27028	.000	4.2300	5.8166
		4	.02333	.27028	1.000	-.7700	.8166
		6	12.73333*	.27028	.000	11.9400	13.5266
	6	1	-9.44667*	.27028	.000	-10.2400	-8.6534
		2	-17.05000*	.27028	.000	-17.8433	-16.2567
		3	-7.71000*	.27028	.000	-8.5033	-6.9167
		4	-12.71000*	.27028	.000	-13.5033	-11.9167
		5	-12.73333*	.27028	.000	-13.5266	-11.9400
MCH	1	2	1.37333*	.09202	.000	1.1032	1.6434
		3	2.06333*	.09202	.000	1.7932	2.3334
		4	-.62667*	.09202	.000	-.8968	-.3566
		5	3.04667*	.09202	.000	2.7766	3.3168
		6	6.67000*	.09202	.000	6.3999	6.9401
	2	1	-1.37333*	.09202	.000	-1.6434	-1.1032
		3	.69000*	.09202	.000	.4199	.9601
		4	-2.00000*	.09202	.000	-2.2701	-1.7299

Appendix table 8: continued

		5	1.67333*	.09202	.000	1.4032	1.9434
		6	5.29667*	.09202	.000	5.0266	5.5668
	3	1	-2.06333*	.09202	.000	-2.3334	-1.7932
		2	-.69000*	.09202	.000	-.9601	-.4199
		4	-2.69000*	.09202	.000	-2.9601	-2.4199
		5	.98333*	.09202	.000	.7132	1.2534
		6	4.60667*	.09202	.000	4.3366	4.8768
	4	1	.62667*	.09202	.000	.3566	.8968
		2	2.00000*	.09202	.000	1.7299	2.2701
		3	2.69000*	.09202	.000	2.4199	2.9601
		5	3.67333*	.09202	.000	3.4032	3.9434
		6	7.29667*	.09202	.000	7.0266	7.5668
	5	1	-3.04667*	.09202	.000	-3.3168	-2.7766
		2	-1.67333*	.09202	.000	-1.9434	-1.4032
		3	-.98333*	.09202	.000	-1.2534	-.7132
		4	-3.67333*	.09202	.000	-3.9434	-3.4032
		6	3.62333*	.09202	.000	3.3532	3.8934
	6	1	-6.67000*	.09202	.000	-6.9401	-6.3999
		2	-5.29667*	.09202	.000	-5.5668	-5.0266
		3	-4.60667*	.09202	.000	-4.8768	-4.3366
		4	-7.29667*	.09202	.000	-7.5668	-7.0266
		5	-3.62333*	.09202	.000	-3.8934	-3.3532
MCHC	1	2	3.70333*	.01544	.000	3.6580	3.7487
		3	1.35333*	.01544	.000	1.3080	1.3987
		4	.52667*	.01544	.000	.4813	.5720
		5	3.86000*	.01544	.000	3.8147	3.9053
		6	3.24333*	.01544	.000	3.1980	3.2887

Appendix table 8: continued

2	1	-3.70333*	.01544	.000	-3.7487	-3.6580	
	3	-2.35000*	.01544	.000	-2.3953	-2.3047	
	4	-3.17667*	.01544	.000	-3.2220	-3.1313	
	5	.15667*	.01544	.000	.1113	.2020	
	6	-.46000*	.01544	.000	-.5053	-.4147	
	3	1	-1.35333*	.01544	.000	-1.3987	-1.3080
3	2	2.35000*	.01544	.000	2.3047	2.3953	
	4	-.82667*	.01544	.000	-.8720	-.7813	
	5	2.50667*	.01544	.000	2.4613	2.5520	
	6	1.89000*	.01544	.000	1.8447	1.9353	
	4	1	-.52667*	.01544	.000	-.5720	-.4813
4	2	3.17667*	.01544	.000	3.1313	3.2220	
	3	.82667*	.01544	.000	.7813	.8720	
	5	3.33333*	.01544	.000	3.2880	3.3787	
	6	2.71667*	.01544	.000	2.6713	2.7620	
	5	1	-3.86000*	.01544	.000	-3.9053	-3.8147
	2	-.15667*	.01544	.000	-.2020	-.1113	
5	3	-2.50667*	.01544	.000	-2.5520	-2.4613	
	4	-3.33333*	.01544	.000	-3.3787	-3.2880	
	6	-.61667*	.01544	.000	-.6620	-.5713	
	6	1	-3.24333*	.01544	.000	-3.2887	-3.1980
	2	.46000*	.01544	.000	.4147	.5053	
	3	-1.89000*	.01544	.000	-1.9353	-1.8447	
6	4	-2.71667*	.01544	.000	-2.7620	-2.6713	
	5	.61667*	.01544	.000	.5713	.6620	

Appendix table 8: continued

WBC	1	2	3.10000 [*]	.01713	.000	3.0497	3.1503	
		3	2.47000 [*]	.01713	.000	2.4197	2.5203	
		4	.98000 [*]	.01713	.000	.9297	1.0303	
		5	1.97000 [*]	.01713	.000	1.9197	2.0203	
		6	5.93667 [*]	.01713	.000	5.8864	5.9870	
		2	1	-3.10000 [*]	.01713	.000	-3.1503	-3.0497
	2	3	-.63000 [*]	.01713	.000	-.6803	-.5797	
		4	-2.12000 [*]	.01713	.000	-2.1703	-2.0697	
		5	-1.13000 [*]	.01713	.000	-1.1803	-1.0797	
		6	2.83667 [*]	.01713	.000	2.7864	2.8870	
		3	1	-2.47000 [*]	.01713	.000	-2.5203	-2.4197
		3	2	.63000 [*]	.01713	.000	.5797	.6803
	4		-1.49000 [*]	.01713	.000	-1.5403	-1.4397	
	5		-.50000 [*]	.01713	.000	-.5503	-.4497	
	6		3.46667 [*]	.01713	.000	3.4164	3.5170	
	4		1	-.98000 [*]	.01713	.000	-1.0303	-.9297
	4		2	2.12000 [*]	.01713	.000	2.0697	2.1703
		3	1.49000 [*]	.01713	.000	1.4397	1.5403	
		5	.99000 [*]	.01713	.000	.9397	1.0403	
		6	4.95667 [*]	.01713	.000	4.9064	5.0070	
		5	1	-1.97000 [*]	.01713	.000	-2.0203	-1.9197
		5	2	1.13000 [*]	.01713	.000	1.0797	1.1803
	3		.50000 [*]	.01713	.000	.4497	.5503	
	4		-.99000 [*]	.01713	.000	-1.0403	-.9397	
6	3.96667 [*]		.01713	.000	3.9164	4.0170		

6	1	-5.93667*	.01713	.000	-5.9870	-5.8864
	2	-2.83667*	.01713	.000	-2.8870	-2.7864
	3	-3.46667*	.01713	.000	-3.5170	-3.4164
	4	-4.95667*	.01713	.000	-5.0070	-4.9064
	5	-3.96667*	.01713	.000	-4.0170	-3.9164

*. The mean difference is significant at the 0.05 level.

Appendix table 9: ANOVA Multiple Comparisons table for Haematological parameters of Nile tilapia among size groups

Dependent Variable	(I) Size cm	(J) Size	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
RBC	1	2	-.30333*	.00370	.000	-.3131	-.2936
		3	-.17333*	.00370	.000	-.1831	-.1636
		4	-.80667*	.00370	.000	-.8164	-.7969
	2	1	.30333*	.00370	.000	.2936	.3131
		3	.13000*	.00370	.000	.1202	.1398
		4	-.50333*	.00370	.000	-.5131	-.4936
	3	1	.17333*	.00370	.000	.1636	.1831
		2	-.13000*	.00370	.000	-.1398	-.1202
		4	-.63333*	.00370	.000	-.6431	-.6236
	4	1	.80667*	.00370	.000	.7969	.8164
		2	.50333*	.00370	.000	.4936	.5131
		3	.63333*	.00370	.000	.6236	.6431
Hb	1	2	-.84667*	.00198	.000	-.8519	-.8415
		3	-.43333*	.00198	.000	-.4385	-.4281
		4	-1.64333*	.00198	.000	-1.6485	-1.6381
	2	1	.84667*	.00198	.000	.8415	.8519
		3	.41333*	.00198	.000	.4081	.4185
		4	-.79667*	.00198	.000	-.8019	-.7915
	3	1	.43333*	.00198	.000	.4281	.4385
		2	-.41333*	.00198	.000	-.4185	-.4081
		4	-1.21000*	.00198	.000	-1.2152	-1.2048
	4	1	1.64333*	.00198	.000	1.6381	1.6485
		2	.79667*	.00198	.000	.7915	.8019
		3	1.21000*	.00198	.000	1.2048	1.2152

Appendix table 9:

Hct	1	2	-3.72000 [*]	.00498	.000	-3.7331	-3.7069
		3	-2.31333 [*]	.00498	.000	-2.3265	-2.3002
		4	-6.49333 [*]	.00498	.000	-6.5065	-6.4802
	2	1	3.72000 [*]	.00498	.000	3.7069	3.7331
		3	1.40667 [*]	.00498	.000	1.3935	1.4198
		4	-2.77333 [*]	.00498	.000	-2.7865	-2.7602
	3	1	2.31333 [*]	.00498	.000	2.3002	2.3265
		2	-1.40667 [*]	.00498	.000	-1.4198	-1.3935
		4	-4.18000 [*]	.00498	.000	-4.1931	-4.1669
	4	1	6.49333 [*]	.00498	.000	6.4802	6.5065
		2	2.77333 [*]	.00498	.000	2.7602	2.7865
		3	4.18000 [*]	.00498	.000	4.1669	4.1931
ESR	1	2	1.88000 [*]	.00370	.000	1.8702	1.8898
		3	1.06000 [*]	.00370	.000	1.0502	1.0698
		4	2.43000 [*]	.00370	.000	2.4202	2.4398
	2	1	-1.88000 [*]	.00370	.000	-1.8898	-1.8702
		3	-.82000 [*]	.00370	.000	-.8298	-.8102
		4	.55000 [*]	.00370	.000	.5402	.5598
	3	1	-1.06000 [*]	.00370	.000	-1.0698	-1.0502
		2	.82000 [*]	.00370	.000	.8102	.8298
		4	1.37000 [*]	.00370	.000	1.3602	1.3798
	4	1	-2.43000 [*]	.00370	.000	-2.4398	-2.4202
		2	-.55000 [*]	.00370	.000	-.5598	-.5402
		3	-1.37000 [*]	.00370	.000	-1.3798	-1.3602
MCV	1	2	-2.41333 [*]	.22963	.000	-3.0181	-1.8086
		3	-2.57333 [*]	.22963	.000	-3.1781	-1.9686

Appendix table 9:

		4	9.41000 [*]	.22963	.000	8.8052	10.0148
	2	1	2.41333 [*]	.22963	.000	1.8086	3.0181
		3	-.16000	.22963	.898	-.7648	.4448
		4	11.82333 [*]	.22963	.000	11.2186	12.4281
	3	1	2.57333 [*]	.22963	.000	1.9686	3.1781
		2	.16000	.22963	.898	-.4448	.7648
		4	11.98333 [*]	.22963	.000	11.3786	12.5881
	4	1	-9.41000 [*]	.22963	.000	-10.0148	-8.8052
		2	-11.82333 [*]	.22963	.000	-12.4281	-11.2186
		3	-11.98333 [*]	.22963	.000	-12.5881	-11.3786
MCH	1	2	2.01000 [*]	.08283	.000	1.7918	2.2282
		3	1.64333 [*]	.08283	.000	1.4252	1.8615
		4	6.66667 [*]	.08283	.000	6.4485	6.8848
	2	1	-2.01000 [*]	.08283	.000	-2.2282	-1.7918
		2	-.36667 [*]	.08283	.000	-.5848	-.1485
		4	4.65667 [*]	.08283	.000	4.4385	4.8748
	3	1	-1.64333 [*]	.08283	.000	-1.8615	-1.4252
		2	.36667 [*]	.08283	.000	.1485	.5848
		4	5.02333 [*]	.08283	.000	4.8052	5.2415
	4	1	-6.66667 [*]	.08283	.000	-6.8848	-6.4485
		2	-4.65667 [*]	.08283	.000	-4.8748	-4.4385
		3	-5.02333 [*]	.08283	.000	-5.2415	-4.8052
MCHC	1	2	2.56333 [*]	.01370	.000	2.5273	2.5994
		3	2.21333 [*]	.01370	.000	2.1773	2.2494
		4	3.24667 [*]	.01370	.000	3.2106	3.2827
	2	1	-2.56333 [*]	.01370	.000	-2.5994	-2.5273

Appendix table 9:

		3	-.35000*	.01370	.000	-.3861	-.3139
		4	.68333*	.01370	.000	.6473	.7194
	3	1	-2.21333*	.01370	.000	-2.2494	-2.1773
		2	.35000*	.01370	.000	.3139	.3861
		4	1.03333*	.01370	.000	.9973	1.0694
	4	1	-3.24667*	.01370	.000	-3.2827	-3.2106
		2	-.68333*	.01370	.000	-.7194	-.6473
		3	-1.03333*	.01370	.000	-1.0694	-.9973
WBC	1	2	2.78333*	.01674	.000	2.7392	2.8274
		3	1.47667*	.01674	.000	1.4326	1.5208
		4	5.93667*	.01674	.000	5.8926	5.9808
	2	1	-2.78333*	.01674	.000	-2.8274	-2.7392
		2	-1.30667*	.01674	.000	-1.3508	-1.2626
		4	3.15333*	.01674	.000	3.1092	3.1974
	3	1	-1.47667*	.01674	.000	-1.5208	-1.4326
		2	1.30667*	.01674	.000	1.2626	1.3508
		4	4.46000*	.01674	.000	4.4159	4.5041
	4	1	-5.93667*	.01674	.000	-5.9808	-5.8926
		2	-3.15333*	.01674	.000	-3.1974	-3.1092
		3	-4.46000*	.01674	.000	-4.5041	-4.4159

*. The mean difference is significant at the 0.05 level.

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Appendix table 10: Composition of the basal diets used in the experiment

Ingredient	%
Fish meal	21.5
Soybean meal	24.00
Yellow corn	33.30
Corn gluten	5.50
Rice bran	10.00
Fish oil	2.90
Lysine	0.10
DL-methionine (98%)	0.20
Threonine	0.10
Di-calcium phosphate	1.20
* Vitamins and minerals premix	1.20
Total	100
Chemical analysis	
Digestible energy (kcal/kg)	2904
Crude protein, %	32.00
Ether extract, %	7.91
Nitrogen free extract, %	45.81
Calcium, %	0.90
Available phosphorus, %	0.45
Lysine, %	2.00
Methionine, %	0.88

* Vitamins and minerals/kg of product: 125 mg biotin, 200 mg folic acid, 28 mg cobalt, 5000 mg pantothenic acid, 2500 mg copper, 0.50 g antioxidant, 75 mg selenium, 17,500 mg zinc, 820 mg iron, 100 mg iodine, 3750 mg manganese, 5000 mg niacin, 1,000,000 IU vitamin A, 1250 mg vitamin B1, 2500 mg vitamin B2, 2485 mg vitamin B6, 3750 mg vitamin B12, 28,000 mg vitamin C, 500,000 IU vitamin D3, 20,000 IU vitamin E, and 500 mg vitamin K.

Appendix table 11: ANOVA table of Haematological parameters among uninfected and infected fish

		Sum of Squares	df	Mean Square	F	Sig.
RBC	Between Groups	.304	1	.304	133.14	.000
	Within Groups	.002	70	.000		
	Total	.306	71			
Hb	Between Groups	3.125	1	3.125	546.94	.000
	Within Groups	.004	70	.000		
	Total	3.129	71			
Hct	Between Groups	35.786	1	35.786	156.66	.000
	Within Groups	.002	70	.000		
	Total	35.787	71			
ESR	Between Groups	12.301	1	12.301	538.25	.000
	Within Groups	.002	70	.000		
	Total	12.302	71			
MCV	Between Groups	1267.058	1	1267.058	109.86	.000
	Within Groups	.081	70	.001		
	Total	1267.139	71			
MCH	Between Groups	3.432	1	3.432	150.25	.000
	Within Groups	.002	70	.000		
	Total	3.434	71			
MCHC	Between Groups	1.960	1	1.960	571.74	.000
	Within Groups	.002	70	.000		
	Total	1.963	71			
WBC	Between Groups	30.890	1	30.890	135.16	.000
	Within Groups	.002	70	.000		
	Total	30.891	71			

Appendix table 12: ANOVA table of Haematological parameters of *O. niloticus* among sex

		Sum of Squares	df	Mean Square	F	Sig.
RBC	Between Groups	60.720	1	60.720	196.53	.000
	Within Groups	2.163	70	.031		
	Total	62.883	71			
Hb	Between Groups	314.002	1	314.002	133.24	.000
	Within Groups	1.650	70	.024		
	Total	315.651	71			
Hct	Between Groups	2538.994	1	2538.994	459.53	.000
	Within Groups	38.680	70	.553		
	Total	2577.674	71			
ESR	Between Groups	139.779	1	139.779	893.990	.000
	Within Groups	10.945	70	.156		
	Total	150.724	71			
MCV	Between Groups	87303.490	1	87303.490	386.461	.000
	Within Groups	15813.361	70	225.905		
	Total	103116.851	71			
MCH	Between Groups	718.205	1	718.205	99.529	.000
	Within Groups	505.122	70	7.216		
	Total	1223.327	71			
MCHC	Between Groups	846.250	1	846.250	239.23	.000
	Within Groups	24.769	70	.354		
	Total	871.019	71			
WBC	Between Groups	2537.569	1	2537.569	648.53	.000
	Within Groups	27.392	70	.391		
	Total	2564.961	71			
	Between Groups	60.720	1	60.720	196.53	.000

