ADDIS ABABA UNIVERSITY

DEPARTMENT OF ZOOLOGICAL SCIENCES

THE WATER QUALITY OF FLORICULTURE EFFLUENT AND ITS EFFECT ON *Oreochromis niloticus* AND *Cyprinus carpio* IN AQUARIA: THE CASE OF LAKE ZIWAY, ETHIOPIA

MSc. Thesis Submitted to Graduate Program in Partial Fulfillment of the Requirement for Masters of Science in Zoological Sciences (Aquatic Sciences, Fisheries and Aquaculture)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS

ANOVA	Analysis of Variance
APHA	American Public Health Association
DO	Dissolved Oxygen
EC	Electrical Conductivity
FAO	Food and Agricultural organization of the United Nations
FEPA	Federal Environmental Protection Authority
Mg/l	milligram per liter
µS/cm	micro Siemens per centimeter
MPL	Maximum permissible limit
PANNA	Pesticide Action Network North America
USEPA	United States Environmental Protection Authority
WHO	World Health Organization

ABSTRACT

Floriculture industry is a booming sector in Ethiopia and is spreading throughout the country. It is believed to create pressure on biodiversity through the release of hazardous chemicals in the form of fertilizers, pesticides and disposal of untreated wastes which contaminates the water body and could be harmful to fishes and other aquatic organisms. Previous studies in Lake Ziway imply that floriculture industries are major sources of chemical substances resulting in deterioration of water quality that affect fish species in the lake. The present experimental study aimed at assessing the water quality of the floriculture effluent discharge that reaches Lake Ziway and its impact on the survival, behavior and histology of *Oreochromis niloticus* and *Cyprinus carpio* in aquaria with 0%, 25%, 50%, 75% and 100% concentrations of the effluent. Histological changes in gills, kidney and liver were examined by using light microscope to evaluate the health of *C. carpio* and *O. niloticus* subjected to the effluent, which lasted for 15 days. The results of all physicochemical analysis indicated that all parameters in the effluent and lake water were within the range of maximum permissible limits (MPL) set by Environmental Protection Authority (Ethiopia-EPA, 2003) and FAO, WHO guideline for the maintenance for fisheries and aquatic life. But, measured values of some parameters such as pH, conductivity, total phosphorus and soluble reactive phosphorus indicate the pollution status of Lake Ziway. There were no behavioral alterations and deaths observed in the exposed and control treatments of both fish species during the experimental period. In the result of the histology sections, the degree of lesions were comparatively increased with the increase in effluent concentration, while the alteration in the liver and kidney of *O. niloticus* was higher than *C. carpio,* this indicates that the floriculture effluent may be one of the problems for the decrease in catch size of *O. niloticus* than *C. carpio*. The gills, livers and kidneys of control groups exhibited a normal structure. Therefore, the effluent discharge from Sher Ethiopia flower industry has affected the water quality and ecology of the lake; Lake Ziway becoming less suitable for fishes. Actions should be taken before the once precious fresh water lake turns out to be seriously polluted and fishes and aquatic organisms are exposed to severe pollution.

1. INTRODUCTION

1.1. Background

At present, fruits, vegetables, flowers and ornamentals are growing not only within the home grounds, but also in large quantities on a commercial scale. Floriculture is a discipline of horticulture concerned with the cultivation of flowering and ornamental plants for gardens and for floristry, comprising the floral industry. It can also be defined as the segment of horticulture concerned with commercial production, marketing and sale of bedding plants, cut flowers, potted flowering plants, foliage plants, flower arrangements and noncommercial home gardening (Abayneh Tilahun, 2013).

Floriculture is one of the booming sectors in Ethiopia. The first private floriculture company, Meskel flower and Ethio–flora, started activities in Ethiopia around 1997 on a few hectares of land (Abayneh Tilahun, 2013). Ethiopia is a developing country, attempting to diversify its export base with a view to gaining new sources of income and foreign exchange and thus reducing its exposure to price volatility that typify international markets. Besides, the country is benefiting from this development through creating employment opportunity for unemployed citizens. But recently, there are a number of challenges that must be resolved to continue the development of the sector with present rapid speed. One of the challenges is over use of chemicals in greenhouses and their effects on worker health, allegedly causing “skin lesions and allergies, respiratory problems, fainting and headaches. However, the flower industry contributes to poverty alleviation, which is the major focus of the government (Samuel Gitonga, 2013).

One of the major features of flower farm is that it consumes high amount of water. For example, one hectare of a flower farm consumes over 60,000 litter/ ha/ day. Some studies show that about 90% of a flower is made up of water. This means that exporting flower is like exporting water (Degytnu Tilahun, 2012). The major challenges of the flower industry are its environmental impacts, which can create pressure on the sustainability of the industry and market acceptability of the products. In Ethiopia, the environmental risks of the flower industry are use of fertilizers, pesticides and disposal of wastes (Mulugeta Getu, 2009). Fertilizers like nitrates and phosphate can be washed away from fields by rain or irrigation eventually finding their way to water bodies and soil. Water pollution, soil and water quality degradation, human and cattle health effects, air pollution, risk on aquatic life, as well as water logging and salinization are only a few of the undesired impacts. Pesticides which include herbicides, insecticides, fungicides and more can pollute soil, water, organisms and other vegetation. It is estimated that less than 0.1 percent of the applied pesticide reaches the target pest, leaving 99.9 percent as a pollutant in the environment, including the soil, air, and water, or on nearby vegetation (Pimentel, 1995; Mulugeta Getu, 2009).

The adverse effect of pesticide use includes degrading water and soil quality, effect on non-targeted lives like soil organisms, aquatic life, human beings, insects, cattle, air pollution and increasing of pesticide resistance by targeted pests (Abayneh Tilahun 2013). There are around 120 chemicals that enter the country for the floriculture industry which are found on negative pesticide list of the WHO, while environmentalists have characterized some of these chemicals as having carcinogenic potential, such hazardous chemicals are used in the flower farming sector in Ethiopia. In the future, these problems could have a large impact on the country's environmental health as well as develop in to long-term health concerns for workers (PANNA, 2002; Malefia Tadele, 2009).

Flower farms mostly use those hazardous chemicals in the form of fertilizers or pesticides which can be easily washed off from the ground and enter into water bodies (Mesay Adugna, 2017). Moreover, excessive usage of inorganic chemicals in the farms which later produce nitrate soon after will get into water bodies which can be washed away from the farms by rain or some other means can cause serious problem like eutrophication. Solid wastes and toxic chemicals that contaminated water body can develop water borne diseases (Degytnu Tilahun, 2012).

Water is the basic element of the life support system of the planet and a constituent of both plant and animal tissues. When pesticides contaminate water, they can be harmful to the fish and other marine or freshwater animals that live there. Lake Naivasha in Kenya floriculture farms were accused of polluting the lake water through extensive use of chemicals, which has had a heavy effect on the lake's biodiversity. Fishermen highly blame that fish stocks are declining and the lake is being polluted by chemicals. Because of this there was frequent conflict raised between the community and the flower farms and some of them are feeling insecurity to produce comfortably (Becht *et al.*, 2006; Malefia Tadele, 2009). As a result, aquatic life is in danger with floriculture industries effluent of wastewater (Abayneh Tilahun, 2013).

The environmental impact from the expansion of large-scale flower farms in Ethiopia in general and around Lake Ziway area in particular is the major concern for many environmentalists (Mulugeta Getu, 2009). Floriculture industries are the major sources of the chemical substances responding for deteriorating water quality of Lake Ziway (Hayal Desta *et al*., 2015). The abundance of fish species in the lake were probably affected by changes in physico-chemical properties of the lake (Lema Abera, 2016).

The ecological impacts of the effluent discharge of many industries and the recently emerged floriculture which are directly released into the nearby water bodies have not been studied yet and nobody really knows what is going on. Until now, there is no recorded evidence of fish species loss in Lake Ziway. But, change in catch size and composition which is currently dominated by introduced species and decrease in catch per unit effort are already observed. Sedimentation, eutrophication and the subsequent mass fish kills have taken place in the Ethiopian Rift Valley lakes (Gashaw Tesfaye, 2010). The type and amount of pesticides and agro-chemical products being used, the manner of their usage and their disposal are creating concerns. This experimental study mainly aimed at assessing the potential effects of floriculture effluent on Nile tilapia and Common carp and to measure the water quality of the effluent as compared to the water quality of Lake Ziway.

1.2. Statement of the problem

The floriculture industry at the shore of Lake Ziway is impacting the lake through discharging effluent directly into the water. Effluents from the farm are causing algal blooms around the outlet of the farm which is driven by excessive nitrate and phosphate residues. Besides, pesticides from the effluent can migrate via water into the food chain as well, which may lead to deterioration of the water quality and aquatic life (Malefia Tadele, 2009). Lake Ziway has shown some undesirable changes in terms of some physicochemical factors and shift in species composition of fishes (Lemma Abera, 2016).

The water pollution and the ecological impact of the lake due to the intensive and toxic chemical usage and waste disposal system of the flower industry is an area of interest which needs an immediate solution. Inappropriate discharge of effluent or chemicals and fertilizers from the flower farm has negative impacts on the water quality and aquatic organisms through formation of algal bloom, creating anoxic condition on the lake and bioaccumulation of toxic chemicals in fishes. The change in catch size and composition of the lake is currently dominated by introduced species (Catfish and Carp species) and decrease in catch per unit effort of *Oreochromis niloticus* and *Barbus* species are already observed (Gashaw Tesfaye, 2010).

The impact of floriculture effluent which is directly discharged into the lake with particular reference to the abundance of fishes and water quality of the lake has not been currently studied. Specially, the cause for the decrease in catch size of Nile tilapia and the increase of Common carp has not been studied yet before. This means, the floriculture effluent which is directly discharged into the lake containing different chemicals, pesticides and fertilizers may be one of the problems for the decrease in catch size of Nile tilapia than Common carp. Because of this an experimental study was carried out in aquaria to assess the potential effect of the floriculture effluent on the two fish species.

1.3. Research questions

- ✓ Is the water quality of the floriculture effluent different from that of the lake?
- ✓ Does the level of toxicity of the effluent affect the survival, behavior and histology of *Oreochromis niloticus* and *Cyprinus carpio*?
- ✓ Would the result obtained be within the MPL range for effluent discharge and guidelines for the maintenance of fisheries and aquatic life?

1.4. Objectives of the study

1.4.1. General objective

- ➢ To assess the impact of effluents of floriculture industry on the health of *Oreochromis niloticus* and *Cyprinus carpio* in Lake Ziway.

1.4.2. Specific objectives

- ✓ To determine the water quality of the effluent and Lake Ziway.
- ✓ To assess the concentration of effluent on the survival, behavior and histology of the two fish species (*O. niloticus* and *C. carpio*) in aquarium.
- ✓ To compare results obtained with the MPL range for effluent discharge and guidelines for the maintenance of fisheries and aquatic life.

2. LITRATURE REVIEW

2.1. Environmental impacts of floriculture industry

Environmental implication of floriculture industry involves the intensive use of water as well as soil, land cover change, water and air pollution because of its intensive and toxic chemical usage and waste disposal system of the industries. Here are main environmental impacts of the cut flower industries:

2.1.1. Intensive water use

One of the major environmental issues in which floriculture industry has been accused of is the intensive use of water. There are water shortages in the towns and villages in most floriculture areas; because flower production requires large quantities of water (USEPA, 200). The consumption of water for the production of cut flowers reached 60,000 litter/ ha/ day. For example, after intensive water use by floriculture, the water table has dropped under the savanna surrounding Bogota (David, 2002). One of the cases which cause conflict between the floriculture farms of Ecuador and the neighboring community is depletion of water in the surrounding (Frank & Cruz, 2001).

Fishermen and local residents living on the shores of Lake Naivasha in Kenya have recently made alarming announcements that the water levels of the lake have been dropping for the past several years, fish stocks are declining and the lake is being polluted by chemicals. The blame for this situation has been put on the flower industry around the lake. Approximately 30 large flower farms, mainly producing cut flowers for the European markets, are situated around the lake. The flower industry is using water from the lake for irrigation purposes; Lake Naivasha is the only fresh water ecosystem in the Eastern Rift Valley with area between 114 and 991 square kilometers; depending on the rainfall (Becht *et al.*, 2006).

2.1.2. Waste disposal system

Liquid wastes from floriculture activities may contain high levels of nutrients, pesticides and other chemicals like disinfectants (mild bleach solution used on knives, shears and harvesting equipment), glycerin solution or a silica gel (used as drying agents for preserved flowers), dyes (to color dried flowers and floral preservatives), and a wetting agent (to enhance the flower's vase life while in storage). Whereas, solid wastes are all packaging, washed chemical drums and bags should be recycled, or disposed of outside any sensitive environment at a local government approved waste disposal facility and inert waste material, such as clean soil or rock, should be reused or disposed of at an approved inert landfill facility (Mesay Adugna, 2017).

Hazardous wastes are those, which contain hazardous substance in a quantity liable to cause death, injury or impairment to living beings, or pollution on the environment if not properly treated, handled or disposed off. It is believed that toxic/hazardous wastes from floriculture activities have an adverse effect on the environment and human health. These include increased exposure to cancer and children born near toxic waste sites can be physically deformed or have developmental disabilities. Empty chemical containers (fertilizers, pesticides) and their washing waters and obsolete chemicals are the major spheres of concern in addition to which other agricultural wastes such as cutoff crop parts, unused soil, and wastewater are generated in the sector are some of major solid wastes (Abiy Tamirat, 2011).

Up to 500 tons of residues per hectare per year are generated from flower farms. Liquid waste that cannot be reused or recycled should be collected and kept in impermeable containers or solar evaporation ponds. The waste residue should be transported off-site for safe disposal at a local, council-approved waste disposal area. However, the flower farms in Ethiopia have been heavily criticized for not having adequate means of waste management systems (Abayneh Tilahun, 2013).

2.1.3. Impact on water bodies

Most flower industries (greenhouses) are built in close proximity to water, which has negatively impacted the water resources of Ethiopia (Hengsdijk & Jansen, 2006). Use of excessive amounts or improper application of fertilizers and pesticides by floriculture industries, may result in harmful chemical contamination of groundwater. In Costa Rica, pesticide residues of floriculture industries are directly discharged into waterways, pesticide equipment are washed into streams and rivers, and runoff is allowed to enter aquifer recharge areas (David, 2002). Contamination of the main sources of water supply for human consumption, the intensive use and disposal of chemicals, freely released into the natural water basins is common problem in Colombia with floriculture industries. In Ecuador also the disposal of contaminated water into natural water ways creates conflict with neighboring communities (Cathrine and Kristina, 2003).

Many of the lakes in the Great Rift Valley are endorheic, meaning they are end points of watersheds that do not drain (Ramsar, 1996; Graichen, 2011). These lakes are highly susceptible to damage from the floriculture industry because of the agricultural residue discharges that regularly flow from the greenhouses into the lakes (Mulugeta Getu, 2009; Jansen & Harmsen, 2011). Rivers around the flower farms in Debrezeit are highly exposed to direct effluents of fertilizer wastes from flower farms and the water quality of the river is changed and the eutrophication process takes place which finally brings severe consequence to ecology of the river Wedecha (Sisay Misganaw, 2007; Mesay Adugna, 2017).

2.1.4. Impact on aquatic organisms

The industrial effluent generally contains dissolved and suspended solids, organic and inorganic chemicals that cause deleterious effects on the freshwater fauna when discharged in to water bodies. The disposal of wastewater from floriculture industries to Wedecha River in Debrezeit has a negative impact on the aquatic environment through macro-invertebrate's depletion or degradation of biodiversity (species abundance and diversity), the disappearance of sensitive taxa in the downstream stretches, food chain of aquatic organisms and pollution of water for consumption by the local communities (Sisay Misganaw, 2007; Mesay Adugna, 2017). Aquatic life is in danger with floriculture industries effluent of wastewater. As a result of the Second North Sea Conference in 1987, a number of countries agreed to reduce discharges of certain chemicals into water systems (Megara and John, 1999; Abayneh Tilahun, 2013).

Indiscriminate discharge of compounds that contain mixtures of heavy metals such as herbicide, pesticides etc, into natural waterways have harmful effects on the fish population and other forms of aquatic life and may couse long term effects in the environment. The fishes are quite sensitive to the contaminated water since the pollutants significantly damage their physiological and biochemical processes (Nemesok *et al.,* 1987). Due to the harmful effect of pollutants in aquatic environments, the histological responses of fishes is an important step to determine a variety of xenobiotics (Hinton and Lauren, 1990; Hassaninezhad *et al*., 2014). Nowadays, histology is known as an important instrument to assess the effects of pollutants in vital processes, detecting early changes in cells, tissues and organs of fishes. Such a study also offers opportunity to locate the effect of pollutants in various organs and systems of animals (Oliveira *et. al*., 2006).

In addition, behavioral study also gives a direct response of the animals to the pollutant. The behavioral activities of an organism represent the final integrated results of a diversified biochemical and physiological processes (Warner *et al*., 1966; Sivakumar *et al.*, 2014).

The impact of floriculture effluent on Lake Ziway

Lake Ziway is one of the rift valley fresh water lakes in Ethiopia. The lake water is an important water source for the community. There are a lot of assumptions about floriculture industries located at the shore discharging untreated effluent directly into the lake. The excessive fertilizer and pesticide residue from the farm is deteriorating the water quality as well as the aquatic life. The Flower farm is situated between Lake Ziway and the main highway with altitude ranging between 1600–1700 m above sea level (Malefia Tadele, 2009).

Plate.2.1. Sher Ethiopia Flower industry located between Lake Ziway and the main highway

Effluents from the flower farm are causing algal blooms formation around the outlet of the farm which is caused by excessive nitrate and phosphate residues. This means that, pesticides from the effluent can flow via water into the food chain and cause corrosion of the water quality and aquatic life (Malefia Tadele, 2009).

2.2. Distribution and composition of fishes in Lake Ziway

Lake Ziway is relatively one of the most studied lakes in Ethiopia among several diverse aquatic ecosystems of great importance in the country which is endowed with different kinds of indigenous and exotic fish species. There are six indigenous fish species in the lake comprising *Labeobarbus ethiopicus*, *Interomius paludinosus, Labeobarbus intermedius*, *Garra makiensis*, *Garra dembecha* and *Oreochromis niloticus* (Eshete Dejen *et al*., 2010; Mathewos Hailu, 2011). The lake also harbors five exotic fish species (*Coptondon zillii, Cyprinus carpio, Carassius carassius* and *Carassius auratus)* which were introduced to enhance its production and *Clarias gariepinus* that shipped into the lake accidentally. Among these species *O. niloticus*, and *C. carpio* are the two commercially important ones (Lemma Abera, 2016).

2.3. *Oreochromis niloticus* and *Cyprinus carpio* in Lake Ziway

Nile tilapia (*Oreochromis niloticus*), is one of the most commercially important fish species in Lake Ziway. It was the major species of the catch accounting to 94 % in previous studies during 1984 however; the abundance of the fish has gradually declined to 89.3 % of total catch in 1994, 50.9 % in 2010, 42 % in 2013, 31 % in 2012 and 27.88 % in 2016. Although, *O. niloticus* catch decreased through the years, it is still the most dominant and the most preferred species in Lake Ziway (Lemma Abera 2016). However, Cyprinidae is a clearly dominating fish composition of Lake Ziway (Lemma Abera, 2016**).** From this family, the catch of *Cyprinus carpio* (common carp) and *Carassius carassius* (crucian carp) is increasing and this condition may be due to a high tolerance of these fishes to turbidity variation of the lake (Lemma Abera, 2016). The exotic carps (crucian carp and common carp) are increasing in the catch composition. Particularly, the common carp is appearing to be an important commercial catch in the major landing sites since the last two years (Megersa Endebu *et. al.*, 2015). That is why this study was initiated and it hopes to answer the main reason for the decrease in catch size of Nile tilapia than Common carp with particular reference the effect of floriculture effluent discharge into the lake.

3. MATERIALS AND METHODS

3.1. Description of study area

The study was conducted from June to September to determine the impact of floriculture effluent on the survival, behavior and histology of *O. niloticus* and *C. carpio* on aquaria under greenhouse condition located at Addis Ababa University, College of Natural & Computational Sciences at 9^0 02' N and 38 ^{0} 45' E and 2434m above sea level.

3.2. Field study

The fish, effluent and water samples were collected for the required tests and insitu measurements like temperature, pH, conductivity and dissolved oxygen were measured at the sampling sites.

3.2.1. Transportation of fish

A total of 500 fingerlings (5-15g) and juveniles (30-45g) healthy and active *O. niloticus* and *C. carpio* were collected from Sebeta National Fisheries and Aquaculture Research Center (NFLARC) and Koka Reservoir. The fish were oxygen packed in polythene bags and brought to the laboratory by bus with minimal stress during transit. Prior to the experiment fishes were released very carefully into the fish tanks and acclimatized for 30 days in 1000-L plastic tank for each of the two fish species under greenhouse (controlled) condition.

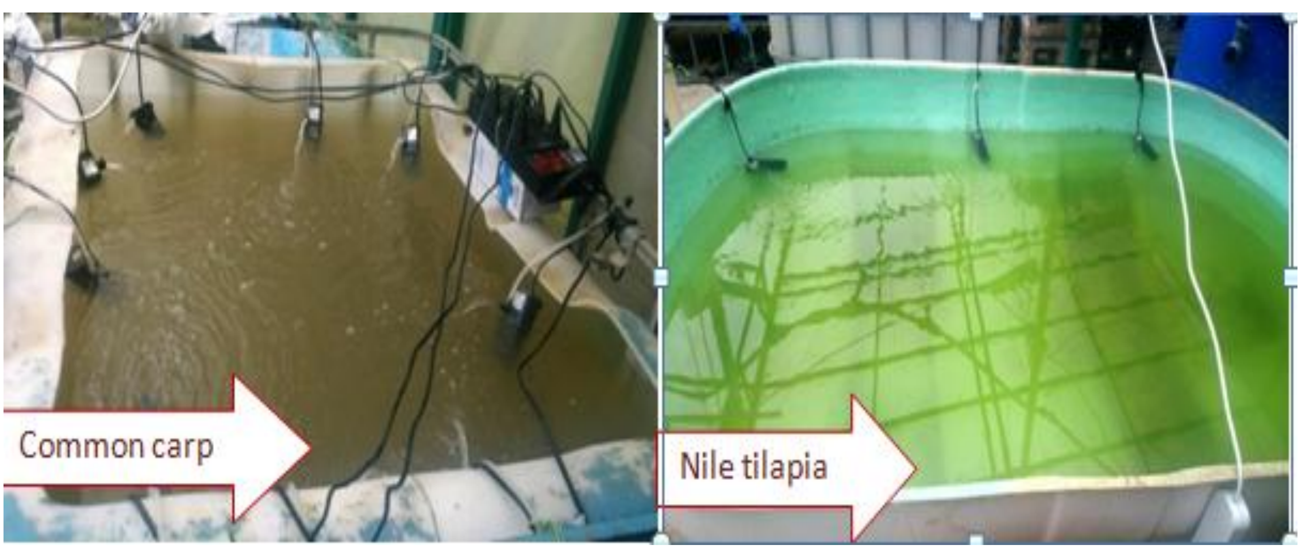

Plate.3.1. Acclimatization tanks for common carp and Nile tilpia

Continuous aeration was maintained in each tank using electric air pumping compressors. Fishes were fed daily on formulated fish diet at 6% of their body weight twice daily. The behavior of fishes was monitored daily and recorded and any dead fish was removed.

3.2.2. Collection of effluent

A total of 2000 liter (19 samples containing 60L) of effluent were collected two times from the discharge or out fall of floriculture farm which is located at the shore of Lake Ziway to perform the required tests.

Plate.3.2. Floriculture effluent sampling sites discharged in to Lake Ziway

Then the physicochemical parameters such as pH, temperature (Temp.), electrical conductivity (EC) and dissolved oxygen (DO) were measured at the sampling site by using a portable multimeter probe model (HQ40d); all the nineteen (19) sample containers were completely filled leaving no air space between the contents in order to minimize the loss of toxicity due to volatilization of toxic constituents and transported with a truck to the experiment place which is located in the greenhouse of Addis Ababa University.

3.2.3. Determination of water quality

Water sample from Lake Ziway was collected two times from five different sites namely Katar, Meki and Center of the lake, Flower farm site 1 and 2 (FF1 and FF2) to perform the required tests such as physicochemical parameters and nutrients. Then the physicochemical parameters such as pH, temperature (T), electrical conductivity (EC), and dissolved oxygen (DO) were measured with a portable multimeter probe (model HQ40d) at the sampling sites.

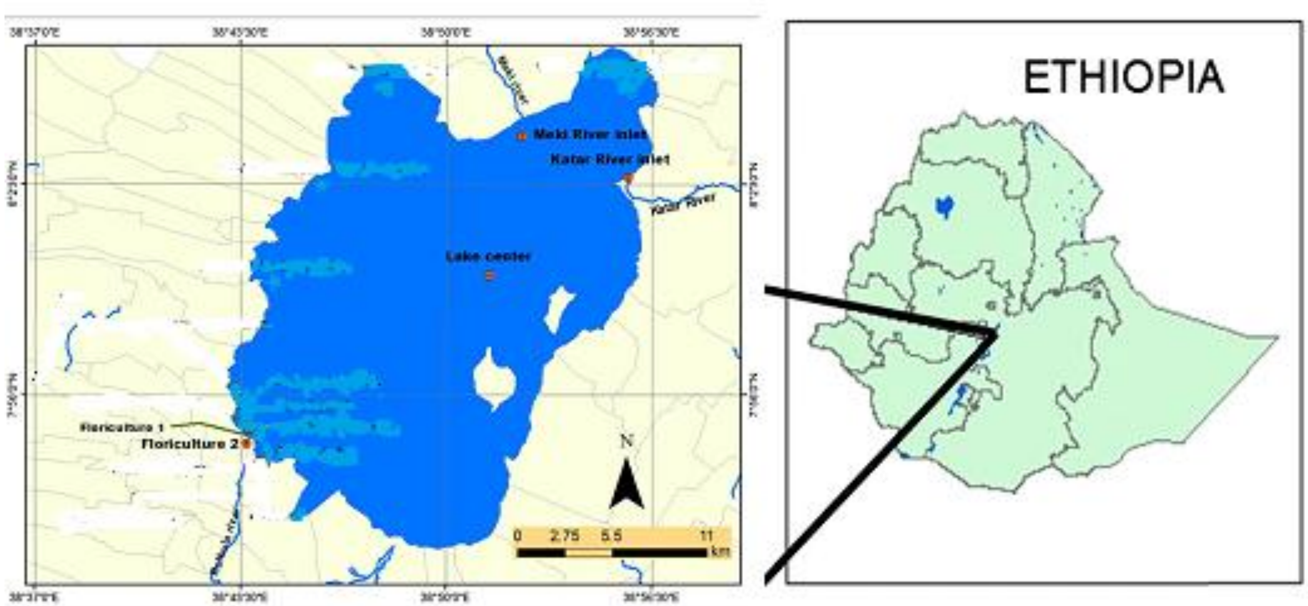

Fig.3. 3. Water sampling sites in Lake Ziway, Ethiopia. Source; (Berhan Teklu *et al.*, 2016).

The sample containers were cleaned with distilled water and rinsed three times with water at the sampling point before the sample was taken. The sample were placed in a deepfreeze container and shipped in accordance with the US-EPA protocol for shipping and sample submission procedures for analytical services (US-EPA 2014). The collected water was thoroughly mixed and checked to see if all organic matter such as leaves, rags, twigs and other floating materials had been removed. All collected 1-L samples were placed in a labeled sample container and stored in a refrigerator at 4 °C. Nutrients such as nitrate nitrogen (NO_3^-), ammonia (NH4), total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed by using spectrophotometer in accordance with the procedural manual (APHA 1999) for Nitrate (NO_3^-) and ammonia (NH4), and APHA (1995) for total phosphorus (TP) and soluble reactive phosphorus (SRP) at Addis Ababa University of limnology laboratory .

3.3. Aquarium experiment

A series of duplicate nine glass aquaria with size of 100cm x 30cm x 42cm for each of the two fish species was labeled and placed in two sides. One of the aquaria was asigned as control and the other eight were treatment aquaria with different concentrations of the effluent. One electric air pumping compressor and one heater were set into each glass aquarium for the required aeration and heat. Tap water was added in a proportion of 0%, 25%, 50%, 75% and 100% for dilution two days before addition of the effluent to remove the chlorine. Then, 100%, 75%, 50%, 25% and 0% of effluent was dispensed into each aquarium, respectively with two replicates for each test concentration. The 0% effluent was set as control for both fishes. Then, the heat and aeration system was adjusted and allowed for 30 minutes. The physicochemical parameters such as pH, Temperature (T), Electrical conductivity (EC), Dissolved Oxygen (DO) and % saturation were measured with a portable multimeter probe.

Plate.3.3. Experimental set up of effluent test

10 fingerlings each of *O. niloticus* and *C. carpio* were collected from the acclimatization tank and transferred into separate aquarium by using a hand net. This was also repeated for juveniles. Continuous aeration and heat was maintained in each aquarium. Both fingerling and juvenile fishes were fed twice daily 6% of their body weight on fish diet formulated from 45% and 30% crude protein, respectively. Then, the time when the first fish was transferred into the test

solutions was recorded. This experiment lasted for 15 (fifteen) days. In order to illustrate the acute effect or disorder caused by the effluent, the behavior and mortality of fishes was monitored and observed in the first 24hr, 48hr, 72hr and 96hr first in the control solutions and then in the treatments. At the end of the experiment, fishes were not released back into the environment to prevent contamination from the test samples except from the control. They were euthanized with an overdose of formaldehyde solution (95mg/L) and disposed off quickly in a hygienic manner. All remaining equipment such as aerator, heater and aquarium were completely cleaned.

3.4. Histological examination of fish organs

At the end of exposure period, two (2) fishes were taken from each replicate aquarium. The gill arches of the fish were excised quickly from both sides and the abdominal cavity was operated and the liver and kidney were excised and fixed in 10% formalin as a histological fixative for 24 hr (Tao *et al.*, 1999).

Plate.3.4. Tissue preparation for histology test

The specimens were prepared at Ethiopia Public health Institute (Pastor Hospital) and processed as usual in the recognized method of dehydration, cleared in xylene and finally embedded in paraffin wax before being sectioned at 5 µm using a rotary microtome (Leica TP 1020 USA) According to Humason (1967). The specimens were stained with hematoxylin and eosin. Finally, the prepared sections were examined and photographically enlarged using light microscopy.

3.5. Data analysis

A statical analysis one way ANOVA was done to assess the variation in the values of the physicochemical parameters by sites; and two ways ANOVA across concentration, experiment and time.

4. RESULTS AND DISCUSSION

4.1. Physicochemical Parameters

The physico-chemical parameters of the composite Sher Ethiopia floriculture effluent and Lake Ziway water was analyzed and compared using standardized (FAO, WHO, and Ethiopia-EPA) guidelines for the maintenance of fisheries and aquatic life use; to check if the result is within the acceptable range and is presented in Table 4.1.

Table4. 1. Physicochemical features of sampled water (Mean ± SE), with comparison to FAO, WHO and (Ethiopia-EPA 2003) standards.

Parameters	Sampling sites					Guidelines		
	FF1	FF2	Meki	Katar	Center	Eth. (EPA)	FAO	WHO
DO (mg/l)	6.3±.5	5.3±.5	7.3±.5	5.99±.5	7.4±.5	≥ 4	6–8	7.3-10.9
Temp. (^{O}C)	23.8±.78	23.9±.78	22.0±.78	24.1±.78	22.4±.78	15-30	15-30	15-30
pH	8.3±.24	8.75±.24	9.0±.24	8.7±.24	9.1±.24	6-9	6.5 -8.5	6.5-9
Cond. (µS/cm)	145.8±14	291.4±14	98.8±14	105.2±14	113.8±14	<1000	<1000	<1000
TP (mg/l)	.125±.02	.095±.02	.053±.02	.045±.02	.015±.02	< 10	Nd	<10
SRP (mg/l)	.026±.02	.029±.02	.0085±.02	.012±.019	.006±.019	0.005-0.1	Nd	Nd
NO^{-3}(mg/l)	.054±.038	.022±.038	.098±.038	.038±.038	.081±.038	<10	Nd	Nd
NH4 (mg/l)	.096±.074	.096±.074	.186±.074	.188±.074	.192±.074	<1.5	Nd	Nd

4.1.1. Dissolved oxygen (DO)

The result of this study demonstrates that the mean values of DO showed significant difference ($p<0.05$) between sampling sites of FF2 and center as well as FF2 and Meki. But, DO level did not show significant difference among the other sampling sites. Dissolved oxygen (DO) levels 5.3±.5 at FF2 were less than 7.3±.5 at Meki site and 7.4±.5 at the center of the lake (Table 4.1). The work of Tibebe Ayele (2017) was also similar report; “DO level around flower farm area was close to the minimum permissible values’’. Dissolved oxygen is very crucial for the survival and functioning of aquatic biota because it is required for respiration of all aerobic organisms. It also provides a useful measure of the health of aquatic ecosystem (Tesfalem Fikresilasie, 2011).

The reduction of dissolved oxygen level at FF2 could be due to nutrient enrichment from the farm effluent which promotes excessive growth of algae and aquatic vegetation; when the vegetation decomposes the level of dissolved oxygen declines. Besides, bacteria in water can consume oxygen as organic matter decays. Thus excess organic material can cause an oxygen-deficient situation to occur (Malefia Tadele, 2009). In addition, the values were also compared with Ethiopian EPA, FAO and WHO standards and the level of dissolved oxygen in all sites were within the permissible range for effluent discharge and ranges of the standard values for maintenance of fisheries and aquatic life.

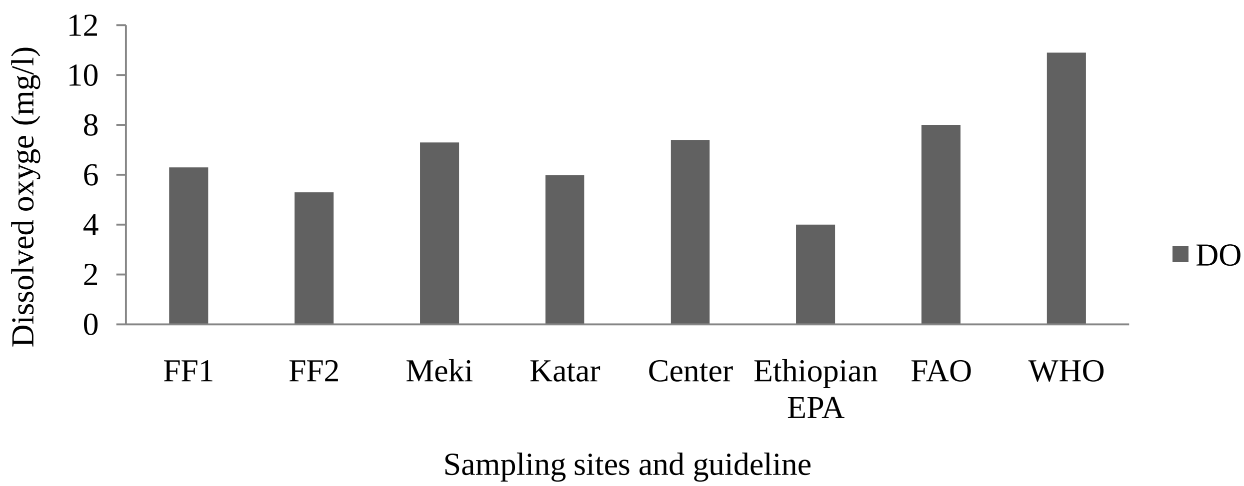

Fig.4.1. Mean values of DO as compared to EPA, FAO and WHO standards

4.1.2. Temperature (T^oc)

In this study, the mean values of temperature did not show significant difference between sampling sites ($p>0.05$). The values were also compared with Ethiopian EPA, FAO and WHO standards and the level of temperature in all sites were within the ranges of the standard values for maintenance of fisheries and aquatic life. In this case, there is no indication of thermal pollution and this may be due to the sampling time which is in the morning around four and five o'clock. Water temperature is a controlling factor for aquatic life (Carr and Neary, 2006). It controls the rate of metabolic activities, reproductive activities and life cycles. If water temperatures increase, decrease or fluctuate too widely, metabolic activities may speed up, slow down, malfunction, or stop altogether (Murdoch and Cheo, 1991). The same result was also reported by Tamiru Sisay and Seyoum Leta (2017) who studied on the ecological impacts of floriculture industries effluent using physico-chemical parameters along Wedecha River in Debrezeit.

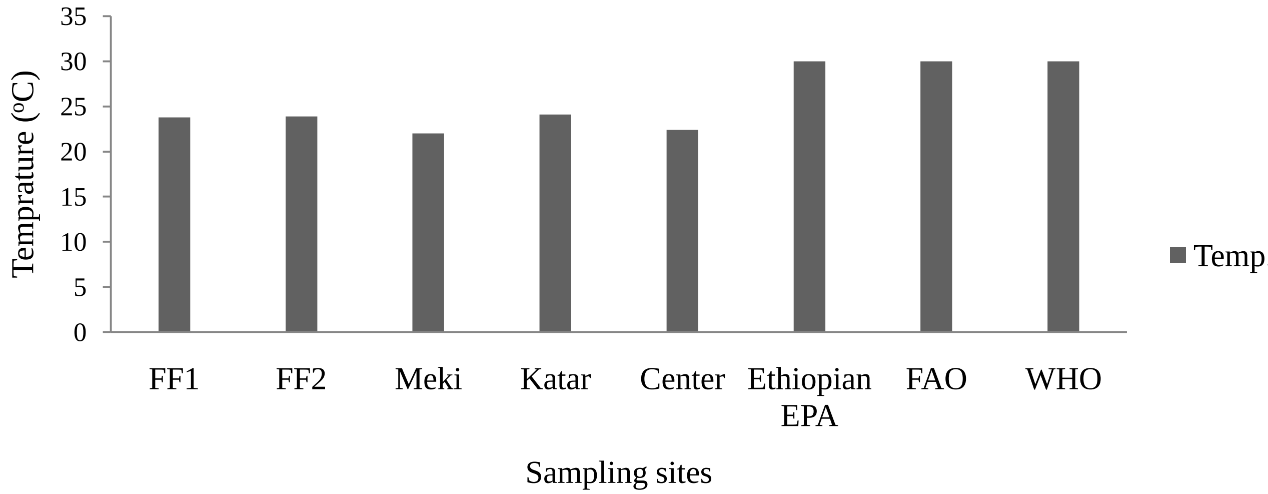

Fig.4.2. Mean values of temperature as compared to EPA, FAO and WHO standards

4.1.3. pH

Mean values of pH did not show significant difference between the sampling sites ($p>0.05$). The values were compared with Ethiopian EPA, FAO and WHO standards and the level of pH in all sites were within the ranges of the standard values. But, the mean values of pH at the center slightly exceeded Ethiopian EPA and FAO water quality guidelines for the maintenance of fisheries and aquatic life. In this study, Lake Ziway is slightly alkaline with an average of around 8.7 as shown in Table 4.1. As stated by different authors, this is due to the geology of the rocks in the area. The work of Malefia Tadele (2009) also supported this; the pH of Lake Ziway is around 8.5 because of the geology and rocks of the surrounding catchments. This shows that the lake water is becoming less suitable for fishes. On other hand, the freshness of Lake Naivasha in Kenya is decreasing by decreasing the amount of water in the lake, the cut flowers and other agricultural practices are increasing the salinity of the lake which affects aquatic and terrestrial organisms, as well as its suitability as irrigated and potable water, as reported by Loukes (2008).

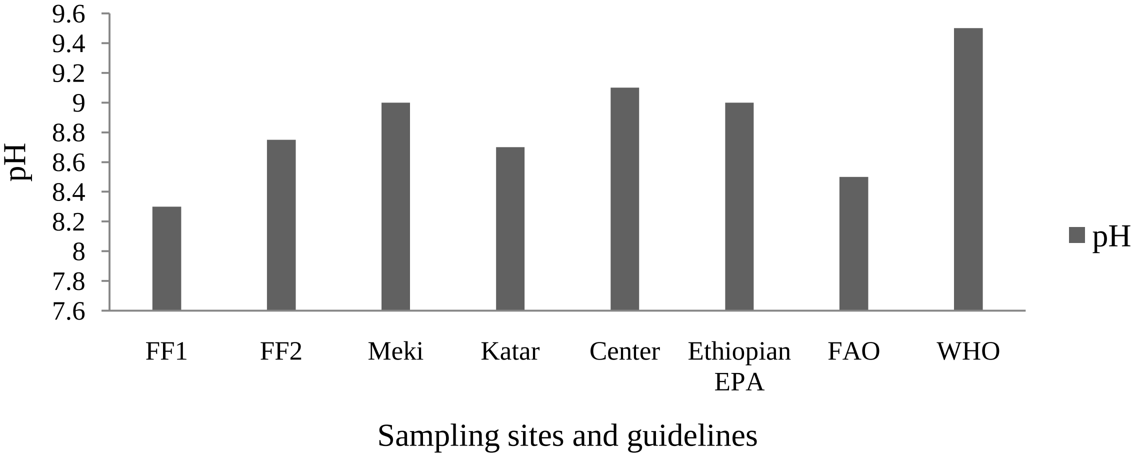

Fig.4.3. Mean values of pH as compared to EPA, FAO and WHO standards

4.1.4. Conductivity

The mean of electrical conductivity in this study did not show significant difference between all sampling sites ($p>0.05$). During the comparison with Ethiopian EPA, FAO and WHO guidelines, the level of conductivity (EC) in all sites were within the ranges of the standard values for agriculture and aquatic life. The federal democratic republic of Ethiopia EPA surface water EC limit is 1000 µS/cm (20 °C) (FEPA, 2003).However, EC was relatively greater at FF1 and FF2 than in the other sites (Table 4.1); this could be due to the concentration of ions in different chemicals from the flower farms effluents discharged in to the lake which led to a negative effect on the lakes water quality.

The work of Malefia Tadele (2009) also agreed with this; the farms around Lake Ziway discharged untreated effluents in-to the lake which led to a negative effect on the lakes water quality. Similar result was also obtained by Birhan Teklu *et al.* (2016) the higher EC values were found for sampling points near the floriculture area of Lake Ziway. An increase in conductivity indicates that there is a slight increase in dissolved ions, which is an indicator for the pollution status of Lake Ziway and may have an impact on aquatic life and water quality. In an aquatic environment, EC is an important and simple indicator to characterize the pollution status of b surface water, a sudden increase in conductivity can indicate the presence of more dissolved ions, which have an impact on aquatic life and water quality (Birhan Teklu *et al.*, 2016).

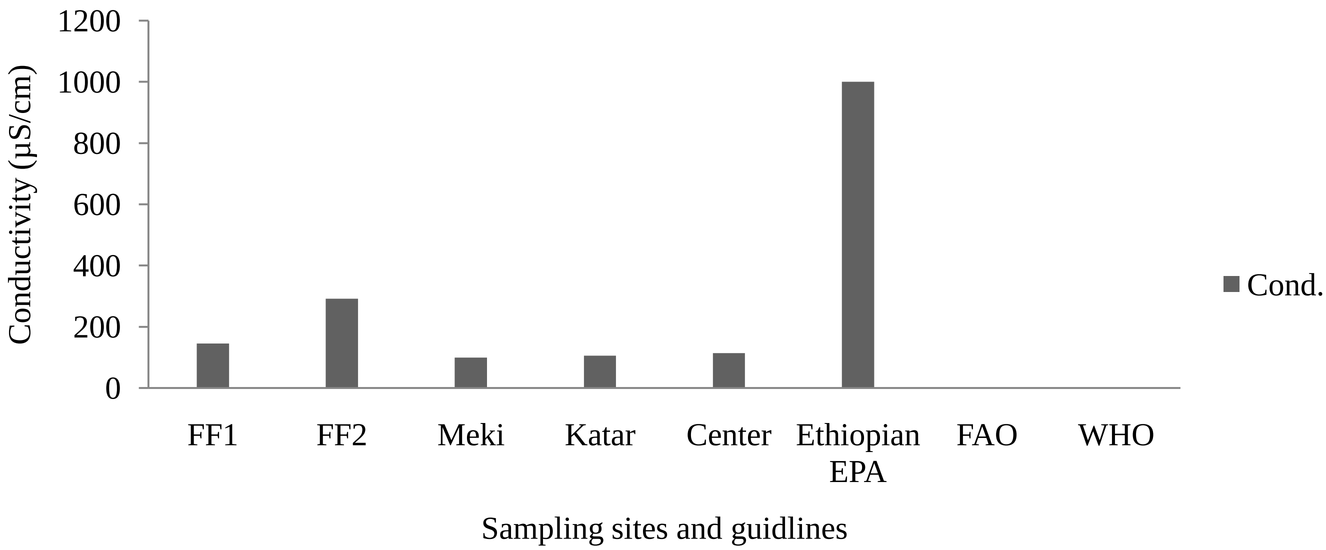

Fig.4.4. Mean values of Conductivity as compared to EPA, FAO and WHO standards

4.1.5. Total phosphorus (TP)

The result of this study indicated that there was no significant difference ($p>0.05$) in the value of total phosphorus (TP) among the sampling sites. The levels of total phosphorus in all sites were also within the ranges of the standard values for the maintenance of fisheries and aquatic life as indicated in Table 4.1. The work of Birhan Teklu *et al.* (2016) also supported this; the amounts of phosphorus found in Lake Ziway were either zero or too low to be detected (<0.05 mg/L) so, it can be considered to be below the MPL. It can therefore, be considered as posing no risk to human health and aquatic life. But, the mean values of TP at FF1 and FF2 were relatively greater than the other sites as shown in Table 4.1. This indicates that there is use of fertilizers by the flower farms located at the shore areas and which is discharged into the lake. Excessive amounts of phosphorous in a system can lead to an abundant supply of vegetation and excessive growth of algae, consequently low dissolved oxygen.

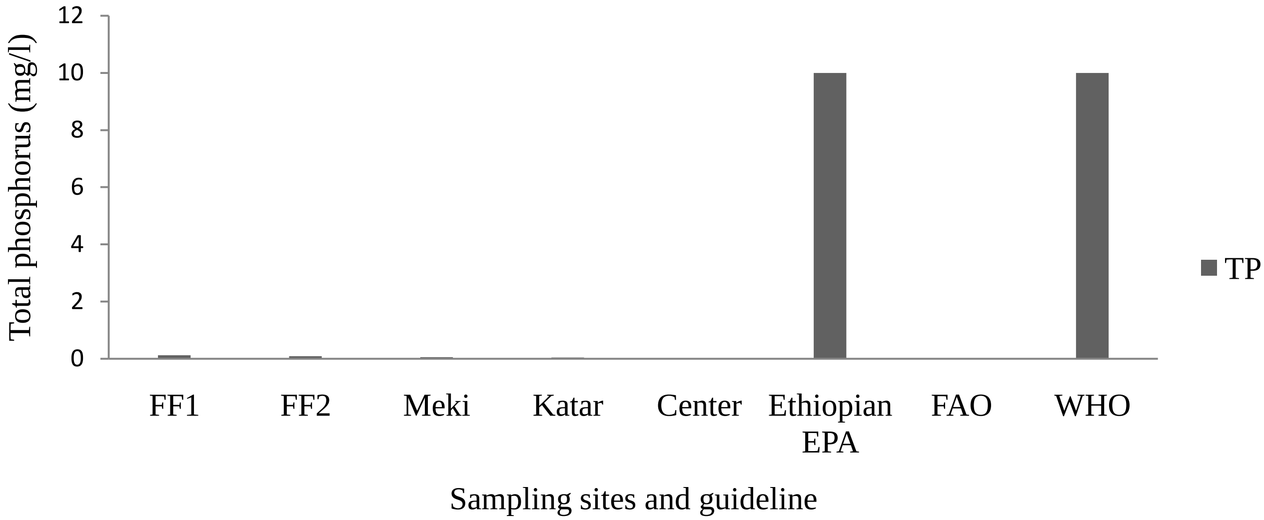

Fig.4.5. Mean values of (TP) as compared to EPA, FAO and WHO standards

4.1.6. Soluble reactive phosphorus (SRP)

The mean values of SRP did not show significant difference between all sampling sites ($p>0.05$). The values were also compared with Ethiopian EPA, FAO and WHO standards and the level of soluble reactive phosphorus in all sites was within the ranges of the standard values. Although it is within the range of the standards, the mean values of SRP in FF1 and FF2 were also relatively greater at FF1 and FF2 than the other sites; this could be due to use of excessive fertilizers by the flower farms located at the shore area. Increased SRP levels near FF1 and FF2 caused excessive growth of macrophytes (Fig. 4.5), which might be the reason for reduced levels of oxygen. The work of Abayneh Tilahun (2013) also reported similar result; excessive amounts of phosphorous in a system can lead to an abundant supply of vegetation and excessive growth of algae, and consequently low dissolved oxygen.

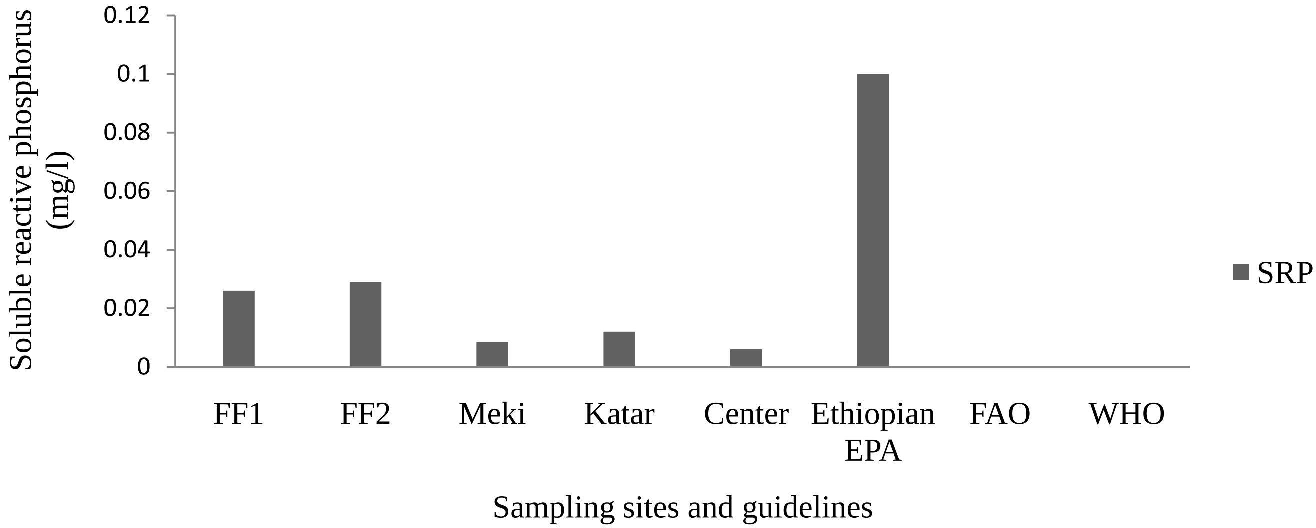

Fig.4.6. Mean values of SRP as compared to EPA, FAO and WHO standards

Plate 4.1: Image showing floating macrophyte of the effluent discharge

4.1.1. Nitrate (NO_3^-)

In this study, the value of nitrate (NO_3^-) did not show significant difference between sampling sites (P>0.05). The values were compared with Ethiopian EPA, FAO and WHO standards and the level of nitrate in all sites were beneath the ranges of the standard values. The maximum permissible limit of Nitrate for effluent discharge is 10 (Ethiopian EPA 2003). The ambient standard of nitrate to protect aquatic ecosystems is 10 mg/L (FEPA, 2003). In this regard, the concentration of nitrate-nitrogen in all sites was within the acceptable range. However, in the report of Abayneh Tilahun (2013), the concentration of nitrate at Wedecha River was 16.6 mg/l; high concentration of nitrate in the water body is due to the fertilizer that the flower farms used and discharged.

Even if it is within the range of the standards, the mean values of Nitrate around the center of the lake and Meki were slightly higher than the other sites as shown in Table 4.1. The most probable reasons for slight changes in the vertical distribution of nitrate might be the result of intensive turbulent mixing in the water column by wind current and usually have minimum consumption by phytoplankton around the lake center (Prokopkin *et al.*, 2010; Dessie Tibebe, 2017).

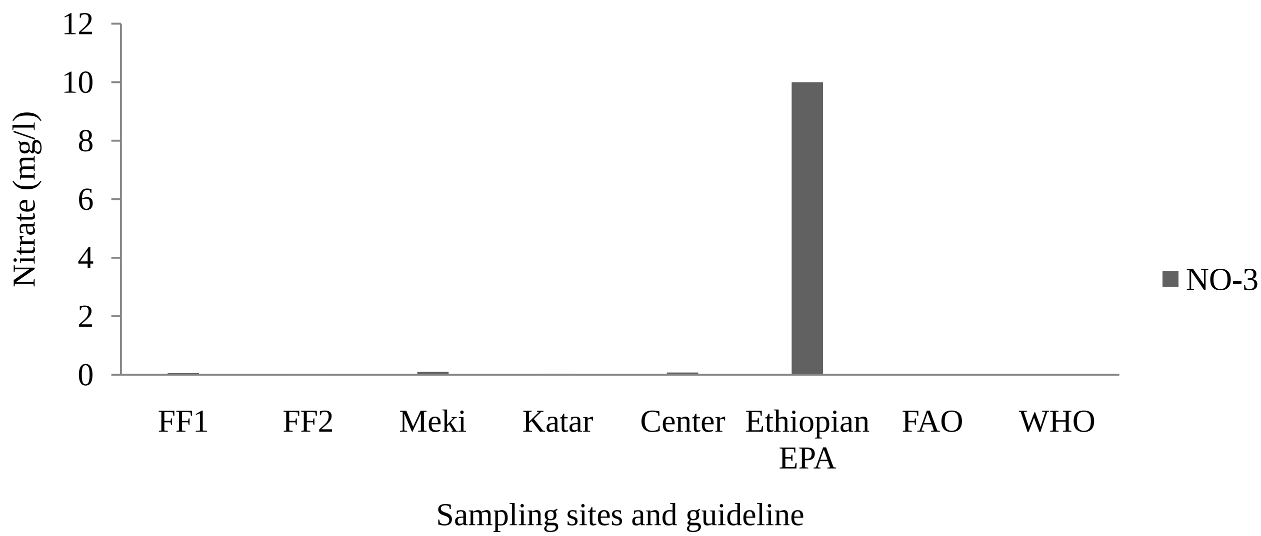

Fig.4.7. Mean values of nitrate as compared to EPA, FAO and WHO standards

4.1.2. Ammonium (NH_4)

In the present study, the mean of ammonium concentration in the sample ranges from 0.096-0.192mg/l; and did not show significant difference between all sampling sites ($P>0.05$). The values were compared with Ethiopian EPA, FAO and WHO standards; but the standard by EPA (1989) states that the optimum NH4 for surface water is < 1.5mg/l. The level of ammonia in all sites was within the ranges of the standard values for surface water and fisheries and aquatic life.

Ammonia is a common pollutant contributing to eutrophication of aquatic environment. Ammonia emanates from the decomposition of nitrogenous organic matter such as urea and proteins (CCME, 1999; Tesfalem Fikresilassie 2011). Ammonia affects the respiratory system of macro-invertebrates through increased ventilation of gills. It is the main poisonous component and is toxic to aquatic organisms either by inhibiting cellular metabolism or by decreasing oxygen permeability of cell membrane. In addition to its toxicity ammonia requires large amount of oxygen to undergo oxidation. Thus, it exerts additional oxygen demand in the presence of nitrifying bacteria.

But, in terms of the different taxonomic groups of aquatic animals that have been exposed to NH4- toxicity, certain freshwater invertebrates and fish seem to be the most sensitive, exhibiting acute toxicities (96-hour LC50) lower than 0.6 mgL-1 and chronic toxicities (72-day LC50) of 0.05 mgl-1 (Dessie Tibebe 2017). On the basis of acute and chronic toxicity data, the level of ammonia ranging 0.05 - 0.35 mgl-1 for short-term exposures, 0.01- 0.02 mgl-1 for long-term exposures, have been estimated and recommended to protect sensitive aquatic animals (USEPA, 2000; Camargo and Alonso, 2006; Dessie Tibebe 2017). The level of ammonia in this study is between the ranges of acute and chronic toxicity data; in this case, the concentration of ammonia in the lake is less suitable for sensitive animals like fishes.

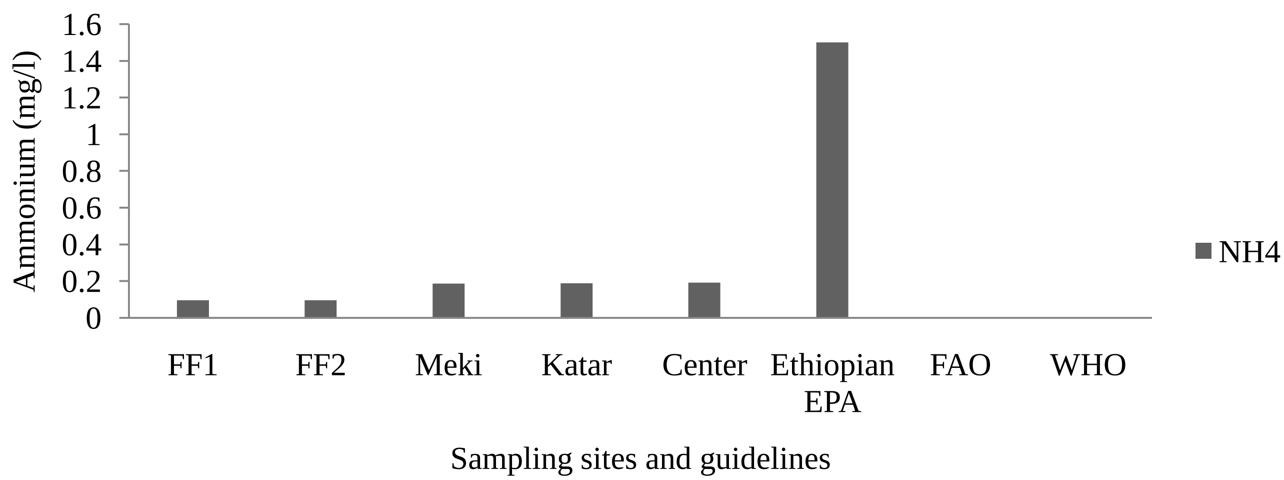

Fig.4.7. Mean values of ammonium as compared to EPA, FAO and WHO standards

4.2. The acute toxicity, Survival and Behavioral responses of the Control and Exposed Fishes in the effluent

Results of the physico – chemical analysis of the test effluent (floriculture effluent) in different concentration (C for Common carp &T for Nile tilapia) shown in Table4.2. The mean values of water temperature ranged from 25 to 27^{0}C during the experiments which was within the range of the standard values of Ethiopian EPA guidelines.

Table4.2. Physicochemical features of the test effluent (Mean± SE) with comparison to Ethiopian EPA standards

		Parameters			
		DO (mg/l)	Temp (^{O}C)	pH	Cond. (µS/cm)
Eth. EPA for effluent		>4	15-30	6-9	<1000
Experiment	EX. 1	6.795±.1	26.821±.16	8.635±.34	203.788±2.9
	Ex. 2	6.961±.1	25.797±.16	8.662±.34	126.614±2.9
Concentration (%)	C100	7.307±.21	27.272±.35	8.788±.76	332.338±6.56
	C75	7.239±.21	26.488±.35	8.773±.76	222.438±6.56
	C50	7.134±.21	26.609±.35	8.753±.76	160.081±6.56
	C25	6.833±.21	26.475±.35	8.701±.76	159.869±6.56
	C0	6.935±.21	26.556±.35	8.816±.76	53.719±6.56
	T100	6.682±.21	26.174±.35	8.684±.76	232.009±6.56
	T75	6.592±.21	25.388±.35	8.491±.76	182.863±6.56
	T50	6.609±.21	25.597±.35	8.487±.76	156.266±6.56
	T25	6.736±.21	26.013±.35	8.580±.76	97.938±6.56
	T0	6.714±.21	26.519±.35	8.411±.76	54.487±6.56

Dissolved oxygen varied from 6.5 to7.3 mgl^{-1} and this was also within the acceptable range by Ethiopian EPA. The mean values of conductivity ranged from 54.5 to 333µscm^{-1} during study period and the level of conductivity was also within the ranges of the permissible limit as well as the standard values for maintenance of fisheries and aquatic life. The result of the analysis, however, indicated that the effluent was safe and not lethal to aquatic organisms when compared with Ethiopian Environmental Protection Agency EEPA (2003) specification and safe limit for effluent discharge into any categories of water bodies.

4.2.1. The behavioral responses of the control fishes

In the present study, the behavioral response of the control fish were observed in 24hrs, 46hrs, 72hrs, 96hrs and up to the end of 15 days of the experiment; no acute effect was observed on the behavior of control fishes and they were active for feeding and alert to slightest of the disturbance with their well-synchronized movements and normal swimming behavior. Natural color was also observed throughout the exposure period. The behavior did not significantly vary between the control groups of common carp and Nile tilapia with different sizes; therefore, these results were taken as standard for the entire experiment.

Table4.3.The survival rate of *C. carpio* and *O. niloticus* in 24 hrs, 48 hrs, 72 hrs, 96 hrs and in 15 days exposure period

Effluent Conc. %	% mortality in 24 hrs	% mortality in 48 hrs	% mortality in 72hrs	%mortality in 96hrs	%mortality in15 days
0	0	0	0	0	0
25	0	0	0	0	0
50	0	0	0	0	0
75	0	0	0	0	0
100	0	0	0	0	0

4.2.2. The behavioral responses of the exposed fishes

In this study, both the fry and the fingerlings of common carp and Nile tilapia exposed to different concentrations of floriculture effluent showed similar reactions. The behavioral responses of fingerlings were observed more clearly than those of the fry due to their size. They were active for feeding and alert to slightest of disturbance with well-synchronized movements. Normal swimming behavior and natural color were also seen and it should also be noted that no mortality occurred throughout the exposure period. This may be due to the water quality of the tested effluent in different concentration in which it was within the acceptable range as described (Table 4.2) and it may not have an observable effect on the behavioral response as well as it may not have lethal effect for the tested fish species as indicated in Table 4.3. The behavioral abnormalities have been attributed to nervous impairment as a result of blockage of nervous transmission between the nervous systems and various effecter sites, enzyme dysfunctions that may induce paralysis of respiratory muscles or depression of respiratory centre and disturbances in energy or metabolic pathways which results in depletion of energy (Ogundiran *et al*., 2010). Due to this, behavioral changes are the most sensitive indicators of potential toxic effects of waste water in fishes (Banaee *et al.,* 2011; Nwani *et al.,* 2013).

4.3. Histological changes of tissues

The present study is concerned on three organs, namely gill, liver and kidney; which are major sites of respiration, accumulation and biotransformation and excretion of xenobiotic substances in fish. All these three organs showed significant differences in the cytological response to the effluent exposure under different concentrations. This was because, histological alterations are good biomarkers for both field and laboratory assessment, particularly in the areas that are naturally subjected to a multiplicity of environmental variations or depletion due to chemical contamination (Ogundiran, 2010). In this context, histological study through light microscopy is a rapid investigation method to detect the toxic effects of different xenobiotics in various tissues and organs (Samanta *et al.,* 2016).

4.3.1. Histological changes in the Gill of control and exposed fishes

In the control fishes of this study, gills had normal morphological structure. Each gill arch supported perpendicularly many distinct and regular filaments arranged in two rows; without any lesions. But, the gill of exposed fishes to different effluent concentration showed several alterations which includes; epithelial cell lifting, losing or shortening of filaments, distortion of chloride cells; high deformation (distortion) of the primary and secondary gill lamellae (filaments) and fusion of adjacent lamellae, which were more prevalent and more pronounced in both carp and tilapia exposed to the effluent (Fig.4.1). The severity of the lesions observed in this study increased with the increase of effluent concentration namely; the lifting of the epithelial cells with proliferation of filamentary epithelium and fusion and severe distortion of lamellae at 100% of concentration as follows.

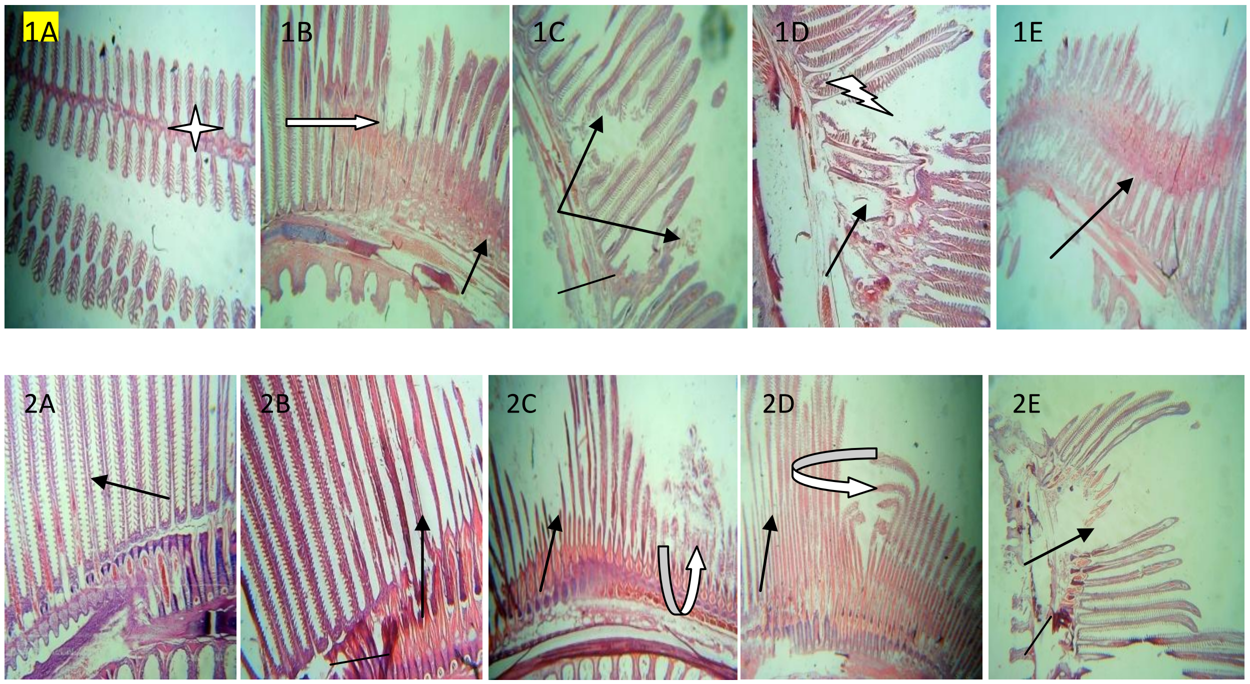

Fig.4.1. Histological changes in the gill of control and exposed fishes

1A: Control of common carp (0%): 4 point star showing normal (regular) arrangement of primary and secondary gill lamellae; 1B (25% treated effluent): right arrow shows (losing or shortening of some filaments) and arrow (fusion of chloride cells); 1C (50% effluent) arrow (complete or partial losing or shortening of some filaments), line (fusion of chloride cells); 1D (75%effluent) lightning bolt arrow shows complete or partial losing or shortening of filaments, line (distortion of chloride cells); 1E (100% effluent) severe fusion of primary and secondary gill lamellae or filaments. 2A: Control of Nile tilapia (0%): arrow shows regular arrangement of primary and secondary gill lamellae; 2B (25%) arrow (proliferation of filaments); 2C (50% effluent) curved up arrow (proliferation and shortening of filament cells), arrow (filament cell proliferation); 2D (75% effluent) curved right arrow (Epithelial cells lifting), arrow (proliferation of filaments); 2E (100%effluent) arrow (Shortening, loosing & sever distortion of filament cells) and line (distortion of chloride cells).

The investigation by Fontaínhas-Fernandes *et al.* (2008) reported several histological lesions namely epithelial cell lifting, epithelial hypertrophy and hyperplasia, slight deformations of lamellae and fusion of adjacent lamellae were more prevalent and more pronounced in tilapia exposed to treated sewage water, than in the control group, which was the same result to the present study.

Similar alterations in the gills of *Cyprinus carpio* and *Oreochromis mossambicus* have also been reported in the fishes exposed to industrial effluents in urban stream (Engelhardt *et al.,* 1981;

Camargo and Martinez, 2007). Alterations like epithelial cells lifting, hyperplasia and hypertrophy of the epithelial cells, partial fusion of some primary and secondary lamellae are examples of defense mechanisms. Since, these results the increase of the disagreement between the external environment and the blood serve as a barrier to the entrance of contaminants (Mallatt, 1985; Hinton and Laurén, 1990; Poleksic and Mitrovic-Tutundzic, 1994; Fernandes and Mazon, 2003; Camargo and Martinez, 2007).

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

4.3.2. Histological changes in the liver of control and exposed fishes

In this study, the control group (0% treated effluent) of both Nile tilapia and common carp, the liver tissue showed normal structure of hepatocytes with a homogenous cytoplasm and a large central or sub-central spherical nucleus and normal lattice network of parenchymatous cells or central vein prominently without a central nucleus and partial sinusoidal distortion (SD) without cell lyses. This result was similar to the findings of Ogundiran *et al.*, (2010) and Hadi and Alwan, (2012). Histologically liver is made up of hepatocytes that are not oriented into distinct lobules but arranged in branched laminae of two cells thick; separated by sinusoids cells. Hepatocytes are polygonal cells with a central spherical nucleus and a densely stained nucleolus (Figueiredo-Fernandes *et al.,* 2007; Hadi and Alwan, 2012).

Different concentrations of the effluent in the present study have induced discrete pathological changes in the liver tissue of both common carp and Nile tilapia. These changes include blood congestion in the central vein, focal area hemorrhage, sinusoidal distortion, vacuoles in the central nucleus of hepatocytes, degeneration of hepatocytes with a hypertrophied nucleus, vacuole formation of focal area, hypertrophy of hepatocytes with nuclear hypertrophy, nuclear degeneration and decrease in the size of nucleus as shown in Figure 4.2. The same alterations such as hypertrophy of hepatocytes, nuclear hypertrophy, blood congestion in the central veins was also observed by Hadi and Alwan (2012), in the liver of fresh water fish (*Tilapia zillii*) exposed to aluminum in different concentration. They have also reported that the severity of histological changes increased with 100 µg /liter.

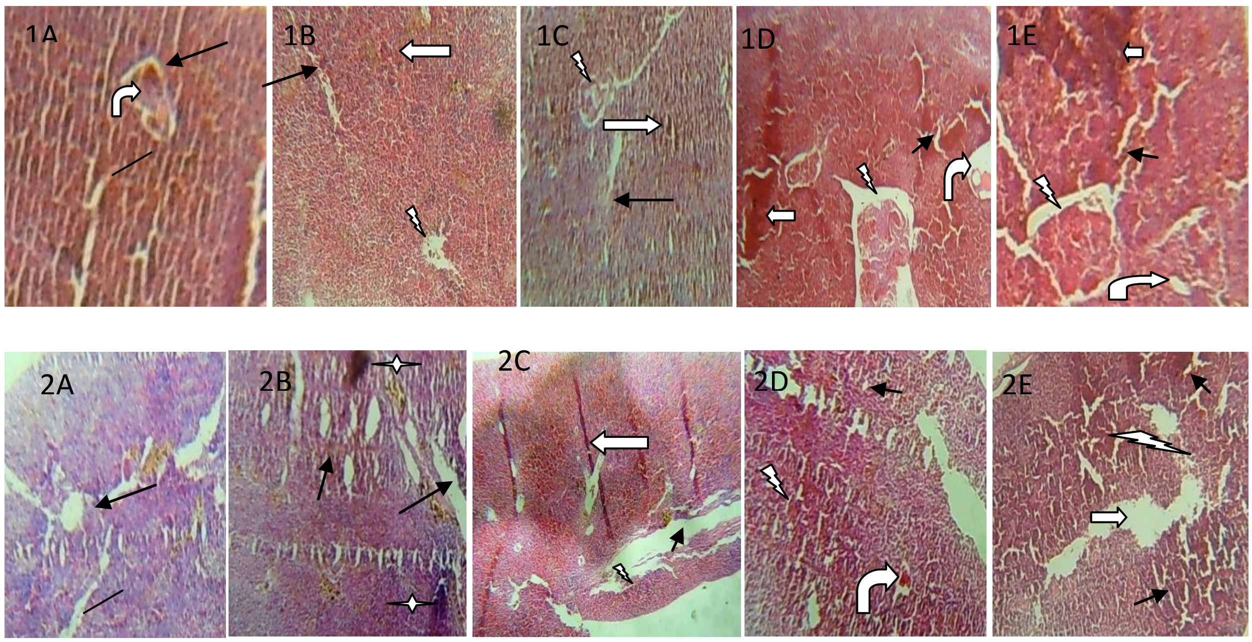

Fig4.2. Histological changes in the liver of control and exposed fishes

1A: control experiment of common carp (0%), arrow shows (hepatocytes with a large densely stained central nucleus and Line (Partial sinusoidal distortion (SD) without cell lyses) and bent arrow (central nucleus). 1B (25%), Lightning bolt arrow (blood congestion in the central vein), right arrow (slight focal area hemorrhage) and arrow (sinusoidal distortion). 1C (50%), right arrow (focal area hemorrhage), arrow (sinusoidal distortion) and Lightning bolt arrow (vacuoles in the central nucleus). 1D(75%) Lightning bolt arrow (degenerated hepatocytes with a hypertrophied nucleus), right arrow (focal area hemorrhage) and arrow (sinusoidal distortion) and bent arrow (vacule formation of focal area). 1E (100%) Lightning bolt arrow (hypertrophy of hepatocyets with nuclear hypertrophy), right arrow (Focal area hemorrhage), arrow shows (sinusoidal distortion) and bent arrow (vacule formation of focal area).

2A: (control experiment of Tilapia (0%), arrow shows (Central vein prominently without central nucleus) and Line (Partial sinusoidal distortion without cell lyses); 2B, (25%) point star shows (degeneration of hepatocyets with nuclear hypertrophy or degeneration); arrow shows (cellular hypertrophy and an elaborate sinusoidal distortion). 2C, (50%) arrow (sever vacuolization and disruption of hepatocytes), Lightning bolt arrow (nuclear degeneration) and right arrow (focal area hemorrhage). 2D, (75%) Lightning bolt arrow shows (vacuolization and disruption of hepatocytes without central nucleus), arrow shows (focal area hemorrhage) and bent arrow (blood congestion in central vein). 2E, arrow (focal area hemorrhage and vacuole formation), right arrow (degeneration or irregular shape central vein) and Lightning bolt arrow (blood congestion in the CV).

Similarly, anomalies such as severe focal necrosis (hemorrhage), blood congestion of the central vein, cellular hypertrophy and an elaborate sinusoidal distortion with congestion of sinusoidal lumen, were observed in the liver of juvenile catfish exposed to detergent effluent; all these anomalies were categorized under indications of cirrhosis; whereas histological alterations such as irregular shaped central vein and cellular vacuolation may be attributed to the accumulation of lipids and glycogen due to liver dysfunction as a result of exposure to the toxicants (Ogundiran, 2010). The severity of the lesions observed in this study increased with the increase of effluent concentration and also the liver of tilapia is more affected than liver of Common carp.

The organ most associated with the detoxification and biotransformation process is the liver, and this is due to its function, position and blood supply (Vander-Oost and Vermeulen 2003). It is also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998; Marina and Camargo, 2007). So, liver plays a major role in the accumulation and detoxification of several components of wastes (effluents) (Frieberg *et al.*, 1971). Therefore, all histological changes observed in the liver of both common carp and Nile tilapia in the present study indicated that those fishes were responding to the direct additive effects of contaminants. This idea has also been supported by Ogundiran (2010) who studied the liver of juvenile catfish exposed to detergent effluent.

4.3.3. Histological changes in the kidney of control and exposed fishes

The kidney of both Nile tilapia and common carp examined from the control group showed normal structure of renal tubules consisted of epithelial cells that are differentiated into proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) as indicated on fig.4.3. Histologically, kidney is made up of a large number of nephrons, each consisting of a renal corpuscle or the malpighian body and the renal tubules. Renal capsules are numerous in number, spherical or oval in shape and contain vascularised glomerulus. The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Peebua *et al*., 2006; Hadi and Alwan 2012). Renal tubules consisted of columnar epithelial cells and are differentiated into proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and collecting ducts (Samanta *et al.*, 2016).

The results of this study demonstrated that the concentrations of effluent produced a variety of histological changes in the kidneys of exposed fishes. These changes are degenerative proximal convoluted tubule and distal convoluted tubule, vacuole formation of hematopoietic tissue; severe vaculation of tubular epithelium, maximum damages like necrosis of proximal convoluted tubule and distal convoluted tubules, glomerular shrinkage and shrinkage of renal tubules, degeneration of renal tubules. The severity of the lesions observed in this study also increased with the increase in effluent concentration and also the kidney of Nile tilapia was more affected than the kidney of common carp. The fish kidney is one of the first organs to be affected by contaminants in the water (Thophon *et al.*, 2003; Camargo and Martinez, 2007).

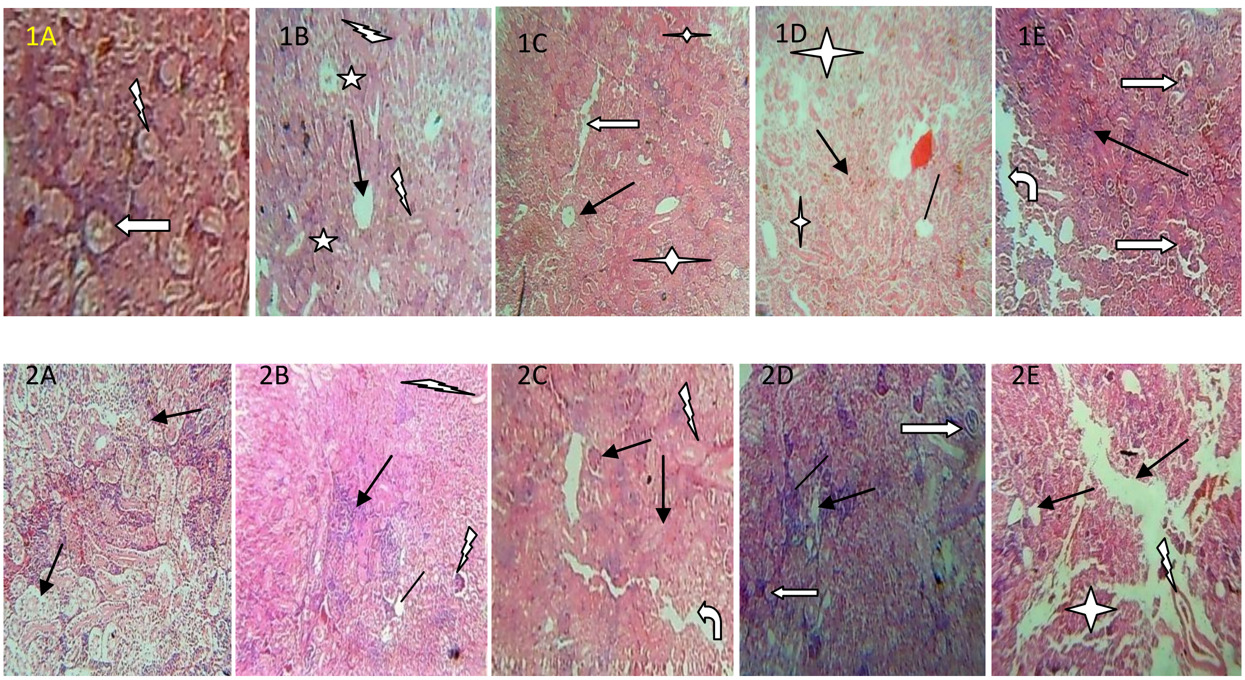

Fig.4.3. Histological changes in the Kidney of control and exposed fishes

1A: Kidney of *C. carpio* under control condition showing normal proximal convoluted tubule (lightning bolt arrow), distal convoluted tubule (arrow), 1B (25%) degenerative PCT and DCT (lightning bolt arrow), shrinkage of glomerulus (star), vacuolation in the hematopoietic tissues (arrow); 1C (50%) vacuole formation of hematopoietic tissue (arrow), sever vaculation of tubular epithelium (right arrow), degeneration of PCT and DCT (4- point star). 1D (75% effluent), maximum damages like Necrosis of PCT and DCT(4- point star), Glomerular shrinkage and shrinkage of renal tubules (arrow), Vacuolizationin the hematopoietic tissues (line). 1E (100%), Renal tubular degeneration (right arrow), degeneration of Interstitial tissue & Renal tubules (arrow); sever vacuolizationin the hematopoietic tissues (bent arrow).

2A: Kidney of *O. niloticus* under control condition showing normal PCT and DCT (arrow); 2B (25%), damage of PCT (arrow), vaculation in hematopoietic tissue (line), Renal tubule degeneration (lightning bolt arrow); 2C (50%) degenerative PCT and DCT (arrow), sever vacuolization in the hematopoietic tissues (bent arrow), Glomerular shrinkage (lightning bolt arrow). 2D (75%) sever degeneration of Renal tubules (line), shrinkage of Glomerular (right arrow) and vaculation in hematopoietic tissue (arrow). 2E (100%) sever vacuolization in the hematopoietic tissues (arrow), Glomerular shrinkage (lightning bolt arrow), maximum damages like Necrosis of PCT and DCT (4- point star).

Similar deformities were also reported by Samanta *et al.* (2016) that showed degenerative changes in PCT and DCT, shrinkage of glomerulus, severe vacuolation in the haematopoietic tissues after long term exposure to almix herbicide. Necrosis and vascuolation were observed by

Dhanapakiam and Premalatha (1994) in *Cyprinus carpio* exposed to malathion. Sastry and Sharma (1979) also observed a number of striking changes in the histological structure of the kidney of *Channa punctatus* exposed to sub lethal concentration of 0.01ppm of endrin for a span of 30 days and found that the shrinkage of glomerulus was the visible sign of toxicity. Konar, (1979) and Prashanth (2011) observed shrinkage and degeneration of glomerulus and vacuolation of tubules in *Cyprinus carpio* chronically treated with heptachlor.

5. CONCLUSION

In this investigation, the results of physicochemical parameters and nutrient of the effluent from Sher Ethiopia floriculture and water of Lake Ziway as well as the test effluent were analyzed. All parameters in the effluent and the lake was within the range of maximum permissible limits (MPL) set by Environmental Protection Authority (EPA) and FAO, WHO, and (Ethiopia-EPA 2003) guideline for the maintenance of fisheries and aquatic life. But, some parameters like pH, conductivity, total phosphorus and soluble reactive phosphorus in the effluent indicate nutrient enrichment and organic loading than the lake water and show the pollution status. The effect of the effluent was also tested on the behavioral responses and survival rate of fingerling and juvenile of *O. niloticus* and *C. carpio*.

The result indicated that there were no visible effect on the behavioral response of fish and no death was observed. This shows that Sher Ethiopia floriculture effluent discharge located at the shore of Lake Ziway may not be lethal for those fishes and it was less toxic to kill the tested fish species. This study however demonstrated that the tested effluent caused several histological lesions in Nile tilapia and common carp. The results have also proved that the degree of distortion of the tissues was proportional to the concentration of the effluent. In addition, the degree of lesion in kidney and liver of Nile tilapia was higher than Common carp; indirectly, this shows that the floriculture effluent may be one of the problems for the decrease in catch size of *O. niloticus* than *C. carpio* in Lake Ziway. This is the first study that has tried to assess histological alterations in the gill, liver and kidney caused by wastewater (floriculture effluents) in Ethiopia.

6. RECOMMENDATION

In this study, Sher Ethiopia floriculture industry is affecting the water quality and the ecology (fishes) of Lake Ziway. Based on results of this study and experience of other countries producing floriculture products, the following best management practices are important:

- Waste water treatment and vegetation buffer (effluent treatment plants) have to be in place before the wastewater is discharged to the water body to improve the water quality of the discharged effluent loaded with fertilizer and pesticide residues.
- The wastewater has to be recycled rather than being disposed to the environment and to reduce excessive pollution of water and to protect the lake water from being polluted.
- Environmental impact assessment and environmental audit about the inputs and discharges in-to and out of the farm should be undertaken to reduce the problem.
- Pesticides are harmful to aquatic organisms and dangerous to fishes as well. So, Integrated Pest Management should be used to minimize the effect.
- In addition, as this study is the first investigation in Ethiopia that has tried to assess histological alterations in the gill, liver and kidney of fishes caused by floriculture effluent (wastewater), further study is needed to get more evidences and to reduce the problem.
- Researchers should have to get a permission to undergo their study in the flower farm compound from all over the place they need and take sample from the septic tanks at night and day in different time interval without any influence by the farm owners.
- In addition to this, sample from inside plants may also be important to get more evidences.

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