Chapter 5

FISH HEALTH

5.1 Introduction

5.1.1 Disease

Fisheries and aquaculture constitute an important source of food production substituting greatly to agricultural production ensuring food security. Considering the significance as food, income and employment generation, fisheries and aquaculture has been given importance all over the world especially in developing countries. International organizations like FAO are keen to promote this sector as it provides the cheap animal protein food to human being, and improve the livelihood status of millions of rural people. Aquaculture, the fast developing food production sector is getting importance all over the world, and research is focused towards the inputs in aquaculture such as feeds, seeds and fertilizers mainly to boost production potential. Research is also being concentrated on factors that limit aquaculture production so as to identify and solve such issues. One of the important factors that limit production potential is the poor health status of the fish. The health of the fish is greatly affected by parasites, diseases and deteriorated environmental conditions. Parasites and diseases are gaining exceptional significance in the context of enhancing food production and to supply healthy and hygienic food. It has been reported that the fish species that live in natural waters and culture conditions are affected mainly due to onset of infections. The aquatic environment which was so pristine in some decades back has become deteriorated and that, the system is now not conducive for fisheries development.

Disease is an abnormal condition of fish where body functions are greatly altered as a consequence of stress, inherent weakness and infection. Disease is the outcome of the interaction between three main factors, and they are host, parasite and the environment. Host is defined as an organism that can be either resistant or susceptible to a given disease. Resistant or susceptibility of the host depends on the species of host, age, size, defense mechanism, health of fish and its nutritional state. The host may be from wild or culture systems. Pathogens may be physical, chemical or biological. Physical pathogens include temperature variation, ultraviolet radiation, light and photoperiod, sound, crowding, handling and transportation. All these factors in one way or other produce stress to fish if the values are above or subnormal. Fish behavior and activities are
directly related to water temperature. Thermal stratification if not controlled would lead to stressful situation. Day light and especially direct sunlight affect fish behavior and production performance causing stress to fish. Unnatural and loud sounds affect fish behavior and the stress caused due to this will reduce production performance.

Chemical pathogens include pesticides, heavy metals, toxins, drugs, plant and animal poison. These chemical agents enter the aquatic systems produce diseases in aquatic organisms in a variety of ways. Further, prolonged use of chemicals will create immunity among several microbial pathogens and thus the control of infection by microbial organisms becomes difficult in the long run.

Biological agents such as bacteria, fungi, viruses and metazoan parasites cause diseases of different kinds. They are responsible for infectious diseases. Microbial pathogens are present in water, sediment or fish as part of normal flora. Their presence and number are largely influenced by different environmental factors such as temperature, dissolved oxygen and pH. The normal microbial flora under unfavorable environmental characters becomes pathogenic. These pathogens enter the host through direct transmission or through vertical means. Environment plays a significant role in disease development.

The physico-chemical characteristics of the environment determine the health of the fish. Any alterations in temperature, pH, salinity or dissolved oxygen beyond optimum range for the host may lead to stress. Stress predisposes fish to most diseases and affects fish health, thereby decreasing the production performance. Stress occurs when an environmental factor extends to or beyond the normal optimum range of the fish and disturbs its physiology. Stressors reduce the ability of fish to function and behave normally. Stressors are acute or chronic and their impacts on fish are additive and accumulative at least for a short period.

The physiological responses are numerous. Incidence and severity of diseases are direct results of suppression of the fish’s immune system caused by stress induced secretions from the endocrine system. In laboratory techniques perhaps the most widely used physiological indicator of stress is the quantity of the hormone cortisol in fish blood. Generally higher the cortisol the greater the stress level. Other physiological indicators of stress include changes in blood glycogen, glucose, lactic acid and osmolarity.

Stressors cause a serious morphological, biochemical and physiological changes to occur in fish. The impact of stress on fish depends on the duration and magnitude of the stress condition. Death is the ultimate result, but sublethal stress conditions cause reduced fish growth, low yields, poor feed conversion and poor health, including pathological
diseases. The stressed fish thereafter becomes susceptible to infection by diverse groups of parasites and pathogens. The disease thus produced may lead to mass mortality producing significant loss to fishing industry.

There is strong relationship exists between environmental degradation and onset of fish diseases. The exponential population rise and the consequent increase in human activities have produced immense pressure on land. Agricultural activities, deforestation and grazing of land by livestock have all to a large extent lead to soil erosion. The eroded soil ultimately reaches the aquatic environment, and the siltation thus formed affects the fish health. The untreated or partially treated Industrial and municipal wastes that enter the aquatic environment alters the water and soil quality characteristics causing stress to aquatic environment. The aquatic organisms live in such stressful environment become susceptible to infection by diverse groups of parasites and pathogens.

A number of studies have been carried out on disease manifestation in fish as a function of adverse environmental characteristics. The chemical factors involved in diseases of aquatic organisms are acidity, alkalinity, ammonia, bicarbonates, chlorine, carbondioxide, dissolve oxygen, dissolved suspended and settled solids, harness, temperature, heavy metals and pesticides. These chemical factors either singly or in combination with other chemicals cause stress to aquatic organisms. The stress response in organisms is specifically significant as it alters the metabolism of the fish. Specifically, metabolic changes in stressed fishes are similar in many ways to those recognized in higher vertebrates (Hoar, 1957). Besides metabolic alterations, the fish species subjected to environmental stress show symptoms of various kinds of diseases; especially to microbial diseases. This is because, the natural microflora present in water and sediments become virulent under unfavorable environmental conditions and they become pathogenic and affect the fish species. Microbial diseases due to environmental stress are very common, and are well known in freshwater, brackiswater and sea water fishes. The fish under stress always show hematological alterations and biochemical changes. The fish under initial stages of infection show increased levels of blood and biochemical values and this is mainly to cope with the initial attack as a compensatory reaction. The values thereafter will decrease considerably leading to emaciation, weak and ultimately the fish will die. Though elaborative studies have been carried out elsewhere in the world on diseases of fish, the aspect on fish diseases especially health status of fish owing to environmental stress is rather scanty in Ethiopia. This chapter elaborates specifically on environmental degradation and bacterial infection in Tilapia,
Oreochromis niloticus collected from the Koka Reservoir. The aspects dealt in this chapter include: Collection and identification of bacterial pathogen, bacterial diseases, hematological and biochemical changes and histopathology of infections.

5.1.2 Pathogenic bacteria in water sediment and fish

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. These bacteria only become pathogens when fishes are physiologically unbalanced, nutritionally deficient, other stressors.

The quality of fish is affected by bacteria which are either spoilers or pathogens, capable of bringing great human health hazards in the form of temporary illness or even death depending on the type and the population density in the fish body. In the wild as well as in the confined water bodies, fishes are affected by bacterial pathogens leading to development of diseases sometimes causing mass mortality of fishes. The most common pathogens that are detrimental to fish populations are species of Aeromonas, Pseudomonas, Vibrio, Shigella, Salmonella, Staphylococcus, Streptococcus, Escherichia coli, Clostridium etc. Disease causing agents in fish population exert important effects on host through enzootic or epizootic through the interaction of pathogen, host and environment.

Pathogenic bacteria are found in all aquatic environments, fishes and other organisms are constantly exposed to them (Stickney, 2009). Under healthy conditions, the wild and cultured species are not affected by the pathogens found in the environment. However, unfavorable circumstances intensify the stress and the fish become susceptible to infections. The environmental stress may be due to extreme variability in the water quality, resulting from introduction of pollutants of diverse origin and their persistence could affect the metabolic processes of aquatic organisms.

Many of the pathogens that cause diseases in fish and shellfish are facultative forms that are ubiquitous in aquatic systems. In nature, a high percentage of apparently normal and healthy animals harbor potential pathogens without evidence of clinical signs of disease (Wedemeyer, 1970).

Some of the bacterial pathogens of fishes are fastidious and require special growth media for laboratory culture, others grow at different temperatures, depends on the aquatic environmental temperature. Aeromonas salmonicida is the most common bacterial pathogen of fishes worldwide. The Aeromonas bacteria species is widely found in rivers, freshwater lakes, drinking water, sea water and sewage. The bacteria initiate the
formation of a sticky bacterial layer on the surface water storage sources and piping systems. The summer season enhances the growth and activity of *Aeromonas* bacteria. Thus, the hot season is a witness to an enhanced number of bacterial infections as compared to the colder season. This bacteria strain or species can survive in aerobic and anaerobic conditions.

Bacterial infections are likely to found in or around open sores, tissue damages as well as on the moist outer surface of fish. Pathogenic bacteria can develop diseases and infections in the body tissues through gills, gut and skin. The genus *Aeromonas* includes gram negative, facultative anaerobic rods. The non motile *A. salmonicida* and motile *A. hydrophila* are potent pathogens of fish in freshwater and marine habitats (Gonzalez-Serrano et al., 2002). *Aeromonas* species cause diseases such as motile Aeromonas septicemia, Hemorrhagic septicemia, Ulcer or Red sore disease. However, *A. hydrophila* is the most common pathogen causing fin rot, tail rot and hemorrhagic septicemia in freshwater fishes. They are also found in the alimentary tract of fishes and considered as normal gut flora. However, it is an opportunistic pathogen causing diseases in weakened fish (Austin & Austin, 2007). *Aeromonas salmonicida* has been considered as a fish pathogen to develop Furunculosis in salmonids, eels and trouts (Tom Wiklund, 1995). It is most prevalent in the cold water bodies affecting the fishes. Hernandez et al. (2009) and Mian et al. (2009) reported that *Aeromonas* and *Streptococcus* are the major flora responsible for diseases in tilapia in culture conditions. Reports also revealed that the temperature changes, handling, inadequate food and oxygen are the factors which favors the infection of *Aeromonas* in fishes (Leung et al., 1994; Roberts, 2001). The diseased fishes show morphological, anatomical and physiological alterations in their tissues (Ugwem et al., 2011). *Aeromonas* is a type of bacterium, commonly found in aquatic environments and in fish tissues capable of surviving in a wide range of water temperatures. There are several varieties of this bacteria *Aeromonas hydrophila* *Aeromonas caviae*, *Aeromonas subria*, *Aeromonas schubertii*. The most common bacterium in this group is *Aeromonas hydrophila* which is considered to be a normal part of the intestinal flora in healthy fish. This bacteria is already present in the fish's environment, therefore *Aeromonas* infections are most often secondary to other stressors such as: poor water quality, parasitism and nutritional deficiencies. It is known from literature that *Aeromonas* are widely spread in water reservoirs (Biamon & Hazen, 1983) and close to the discharge of wastes in water bodies. Constant microbial pollution by the discharge of untreated or only partly treated wastewaters including industrial,
agricultural and domestic wastes are the major pathways of enteric bacterial pathogens into the natural surface water resources.

Fresh fish contains high load of bacteria on the surface slime of the skin, gills and digestive tract. These bacteria include Gram negatives of the genera *Pseudomonas, Shewanella, Psychrobacter, Vibrio, Flavobacterium, and Cytophaga* and some Gram-positives such as coryneforms and specific spoilage organisms (SSO) including *Pseudomonas, Shewanella putrefaciens, Photobacterium phosphoreum, Aeromonas hydrophila, and Alteromonas putrefaciens*, *Vibrionaceae, Aeromonas, Moraxella, Acinetobacter, Enterobacteriaceae; Micrococi* (Shewan, 1962; Liston et al., 1976). The long term survival of these organisms in natural waters constitutes a significant public health concern because of the dangers they pose to humans through consumption and recreation (Chandran et al., 2011). Sediment associated bacteria have the potential to contaminate the overlying water column through microbial re-suspension during human and boat activity or natural turbulence (An et al., 2002; Craig et al., 2004). The results also indicated that small particle size and high organic carbon content enhanced the survival of indicator and pathogenic bacteria in the sediments. In lakes, the most important factors explaining sediment bacterial biomass were sediment water content and hydraulic flushing rate (Schallenberg, 1993). Previous studies have demonstrated that pH, temperature and certain inorganic and organic contaminants are the most important drivers of the bacterial communities in sediment (Tranvik, 1988, Jansson et al., 2007). Gudasz et al. (2012) reported that bacterial biomass was positively correlated with bacterial production and organic matter mineralization. Therefore, it is reasonable to conclude that increasing allochthonous organic carbon in lake sediment could enhance sediment bacterial metabolism.

All species and strains of *Pseudomonas* are gram-negative rods, and have historically been classified as strict aerobes. *Pseudomonas aeruginosa* is increasingly recognized as an emerging opportunistic pathogen of clinical relevance. Several different epidemiological studies indicated that antibiotic resistance is increasing in clinical isolates (Van Eldere, 2003). Among the *Pseudomonas* species in fish (*P. chlororaphis, P. anguilliseptica, P. fluorescens, P. putida, P. plecoglossicida*), *Pseudomonas anguilliseptica* is considered the most significant pathogen in cultured fish (Toranzo & Barja, 1993; Austin & Austin, 1999). *P. anguilliseptica* was originally described in 1972 as the aetiological agent of bSekiten-bioQ or bred spot diseaseQ, which caused massive mortality of Japanese eel in Japan (Wakabayashi & Egusa, 1972). Since then, this
bacterium has been recorded in European eel reared in Taiwan, Scotland and Denmark (Kuo & Kou, 1978; Stewart et al., 1983). The pathogen was subsequently isolated from other fish species such as black sea bream and Ayu in Japan (Nakai et al., 1985), salmonids in Finland (Wiklund & Bylund, 1990). Recently, P. anguilliseptica was also recovered as an emerging pathogen of turbot and black spot sea bream (Pagellus bogaraveo) cultured in Spain (Lo’pez-Romalde et al., 2003 a,c).

Considering the environmental requirements of the common fish pathogens Pseudomonas and Aeromonas the distribution of these organisms in the soil, sediment as well as the various tissues of the food fish Cyprinus carpio were considered for investigation. The common carp inhabiting the reservoir showed disease symptoms and hence it has been considered for the examination of the prevalence of the two common pathogens in the Koka Reservoir.

5.1.3 Histopathology

Histology and cytology offer approaches and techniques for studying changes structure of tissues, and their composite cells, thus giving indications of degree of stress and of the adaptive capability of organisms. Besides diseases and stress, pollutants can also produce pathological changes in various tissues altering their shape, structure and function. The pathological changes induced by pollutants in fishes have fascinated fishery biologists and fish pathologists. The primary objective of such studies is to identify the nature and extent of tissue damages induced by different pollutants more out of academic interest and to some extent in getting an insight in to the nature of action of the pollutant on organisms.

Histopathology provides a sensitive indicator of stress induced by xenobiotics, chemicals and pathogens. The degree of contamination in aquatic environment is frequently assessed by comparing contaminant concentration in associated biota (Yang & Chen, 1996). Both biotic and abiotic factors can be adversely affected by the presence of toxicants even in trace concentrations. Prolonged exposure to water pollutants even at sub-lethal concentrations have been reported to induce morphological, histological and biochemical alterations in the tissues of fish. Accumulation of pollutants has been associated with alteration in immune function, decreased fertility and other reproductive abnormalities in vertebrates like birds, fish, and mammals and shellfishes.

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Metals are unique environmental and industrial pollutants in the sense that they are neither created nor
destroyed by human beings but are only transported and transformed into various products (Shukla & Pandey 1985). Metal contamination of aquatic ecosystems has long been recognized as a serious pollution problem. Most heavy metals released into the aquatic environment as a result of direct input, atmospheric deposition and erosion due to rainwater, therefore aquatic animals may be exposed to elevated levels of heavy metals. When fish are exposed to elevated levels of metals in a polluted aquatic ecosystem, they tend to take up these metals from their direct environment (Farombi et al., 2007).

Heavy metals such as cadmium, lead, copper and more specifically mercury are potentially harmful to most organisms which are able to accumulate along the aquatic food chain with severe risk for animal and human health. The accumulation of these metals in the tissues of organisms is at different concentrations in various tissues of fishes (Canli & Kalay, 2000). According to Nimmo et al. (1978), and Nevo et al. (1986) heavy metals like mercury and cadmium are known to accumulate in aquatic organisms and cause rapid genetic changes.

Hence, the present histopathological studies are conducted to know the extent of tissue damages caused by different biological and chemical agents in the Koka reservoir and Awash River mouth.
5.2 REVIEW OF LITERATURE

5.2.1 Diseases

Bacteria are microscopic single celled organisms that can be found in every environment and thousands of species are classified on the basis of their pathogenicity. The bacteria can infest through any open sores or any area where the organism lost its protective covering due to injury or damage.

The studies of Burkholder and Glasgow (1997), revealed that the ulcerative lesions at or near the vent is the characterization of an infected fish in the polluted environment. Outbreaks of epizootic ulcerative syndromes were noticed in fishes inhabiting freshwater lakes during dry season in many tropical countries (Rodger & Bruke, 1981; Philips & Keddie, 1990; Sareena, 1998).

Mears and Sherwood (1974) studied the fin erosion in Dover sole in relation to toxic bottom sediments. Niewolak and Opieka (2000) observed high density of Pseudomonas sp. and Aeromonas sp. in bottoms sediments in river water. Davis (1922) first observed the columnaris diseases in warm water fishes from Mississippi River and identified the causative agent as Cytophaga. Anderson and Conroy (1969) reported that all freshwater fishes are susceptible to columnaris disease under stressful condition.

According to Austin and Austin (1999) bacteria can spread disease throughout the fish body if they enter through gill, gut or skin, leads to a systemic infection in fishes. Furguson (1989) stated that bacterial infection cause localized surface diseases such as fin rot and ulcers; however, if these are not resolved they can lead to a systemic infection. Fishes infected with Aeromonas and Pseudomonas have different symptoms like; lack of appetite, swimming abnormalities, pale gills, blotted appearance, skin ulceration and in extreme cases death of fish (Austin and Austin 2007).

Pseudomonas septicemia is one of the serious diseases in fish culture systems caused by the genus Pseudomonas leading to severe economic loss. Miyazaki et al. (1984) reported that Hemorrhagic septicemia causes mortality in cultured Nile tilapia in Japan. They also noticed pale gills, ulcers, abscesses, exophthalmia and abdominal swelling in the infected fishes.

Schneider and Nicholson (1980) reported that the tail and fin rot disease in freshwater and marine fishes are associated with poor quality of the water. Bright Singh et al. (1981) observed that bacteria belonging to the genera Pseudomonas, Vibrio and Aeromonas as causative agents of fin rot disease in Etropus soratensis. In addition to
presence of bacteria, fin rot has been linked with traumatic damage, pollution and in appropriate nutrition (Turnbull, 1992).

Karunasagar and Sugumar (1993) reported that the causative agents for epizootic ulcerative syndromes in fishes are Aeromonas, Pseudomonas, Vibrio and Rabdovirus in a polluted environment. Bullock (1968) stated that though bacteria are secondary invaders in the case of fin and tail rot, they cause significant effect on pathogenesis.

5.2.2 Pathogens

The estimation of structure of microbial communities in freshwater bodies became obvious, since they play a key role in biogeochemical cycles through destruction and mineralization of organic substance (Cole et al., 1988; Cotner & Biddanda, 2002). Lakes and reservoirs play an important role in the global budget of carbon and are the first indicators as well as global ecosystem changes (Magnuson, et al., 1990; Williamson, et al., 2008). Therefore, the estimation of diversity and structure of microbial communities of inland freshwater reservoirs are of great fundamental and practical interest. Microbial activity declines rapidly when the dissolved electron acceptors, dissolved oxygen (O2) and nitrates reduced within just a few millimeters below the sediment water interface (Nealson, 1997; Thamdrup, 2000; Falkowski et al., 2008).

The appearance and development of a fish disease is the result of the interaction among pathogen, host and environment. Therefore, studies involving the characteristics of potential pathogenic microorganisms for fish, and their biology and environmental factors will allow the application of adequate measures to prevent and control the diseases limiting the production of fishes (Alicia et al., 2005). Kothary and Babu (2001) reported that the bacterial pathogens present in the animals or reservoir may be high or as low depends on the nutrients available.

Parasites and bacterial pathogens of Nile tilapia (Oreochromis niloticus), catfish (Clarias gariepinus), Barbus sp., Tilapia zillii in Lake Ziway, Ethiopia, were examined for bacterial pathogens. Among the bacteria, Edwardsiella tarda, Shigella sp., Escherichia coli, Citrobacter, Klebsiella oxytoca, and Yersinia enterocolitica were identified from various tissues of healthy fishes by Yimer (2000).

Gerba and Mcleod (1976), Burton et al. (1987), Davies et al. (1995) and Craig et al. (2004) revealed the presence of much higher number of pathogenic bacteria in sediments than in overlying waters. This might be due to the combination of sedimentation and sorption which provides protection from bacteriophage and microbial toxicants. This includes protection from protozoan bacteriovory (Davies & Bavor, 2000), attack by
bacteriophage (Roper & Marshall, 1979), rich supply of nutrients (Craig et al., 2004) and substratum for adherence (Davies et al., 1995). They are also very well protected against environmental stress factors such as heavy metals, pH shifts, salt stress, or grazing. These characteristics make biofilms the preferred lifestyle of microorganisms in most habitats. In these microcolonies, the bacterial cells are protected by a layer of extracellular polymeric substances against heavy metals like aluminium, iron, or manganese which selectively adsorbs these ions (Nielsen et al., 2000).

Pellet et al. (1983) stated that *Pseudomonas aeruginosa* is an autochthonic organism of biological biogenesis of waters and algal blooms may stimulate their development. Moreover, bacteria occur on epiphyte in surface water as well as sediments indicates the degree pollution of water and bottom sediment by organic substances (Albinger, 1992). *Pseudomonas* septicemia is one of the most serious septicemic diseases for fish farming industry caused by bacteria belonging to the genus *Pseudomonas* leading to sever economic losses. In addition, a species of *Pseudomonas* was found responsible for the mortality of tilapia fry in Taiwan (Tang, 1998). *Pseudomonas fluorescens* has been reported to cause disease in a wide range of fish species, including silver carp (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*), goldfish (*Carassius auratus*), tench (*Tine a tinea*), grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*), unnamed species of carp and rainbow trout by Austin and Austin (2007). He further described the exophthalmia and ulcers on the dorsal surface of rainbow trout in grow out ponds in Turkey. Generally, *Pseudomonas fluorescens* is associated with fin or tail rot in which the infected area is eroded away. Tang, (1998) reported that the bacterial hemorrhagic ascites is descriptive, with affected regions displaying lesions in the gills, heart, intestine, kidney, liver and spleen necrosis, extensive skin lesions and erosion, which extended over the entire flank from the operculum to the tail. From the literature, it is understood that though microbial pathogens are opportunistic pathogens and enter the host as secondary invaders, their effects are severe, once they colonize on the host tissue. They also invade different parts of the host tissue and produce significant effects and as a result, the function of different organs is impaired.

### 5.2.3 Histopathology

Water quality play an important role in the health of aquatic organisms and any alteration will definitely influence on their biology. The contamination of aquatic ecosystem is highly vulnerable to wide range of pollutants and has become a matter of concern over
the last few decades. The principle anthropogenic processes in the aquatic environment lead to eutrophication, increase in turbidity, disturbance of ionic equilibrium, acidification and ultimately alters their physico-chemical and biological characteristics. These changes are influence on physiological and metabolic activities of the aquatic organisms including fish. The possible effects may range from impairment of growth, reproduction and metabolic functions of organisms or changes in the physical and chemical properties of the ambient medium that indirectly affect the resident biota in water. Jiraungkoorskul et al. (2002) reported that the fishes are most sensitive to aquatic pollutants during their early stages. Further they also reported the herbicide applications leads to decrease in dissolved oxygen in the water, and increase in temperature, which intern affects some cold water fish species. There are several reports on the impact of environmental toxicants on fish, which revealed the histopathological changes in the vital organs such as gills, liver and kidney (Singhaseni & Tesprateep 1987; Gill et al., 1988; Richmond & Dutta, 1989). Several studies have also reported on the response of fish to sediments contaminated by pesticides, heavy metals, and persistent organic pollutants (Amo Kaschl et al., 2002; Audry et al., 2004; Athikesavan et al., 2006; Ayas et al., 2007). Considerable interest has been shown in recent years in histopathological study while conducting sub-lethal tests in fish. Tissue changes in test organisms exposed to a sub-lethal concentration of toxicants are a functional response of organisms which provides information on the nature of the toxicants. Histological changes associated with pesticides in fish have been studied by many authors (King, 1962; Eller, 1971; Bruno & Ellis, 1988; Narayan & Singh, 1991; Mercy et al., 1996; Gupta & Srivastava, 2006). Adams et al. (1989) and Johnson et al. (1999) reported that histology of tissues provides a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs. Velkova-Jordanoska and Kostoski, (2005) widely reported that the histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem. These histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to provoke cellular change in the affected organisms.

Brown et al. (2002) reported that effluents from sewage treatment plants are oestrogenic to fish which disrupts endocrine function and causes intersex, changes in gonadal structure and alterations in germ cell development. Heavy metal contamination of aquatic ecosystems has long been recognized as a serious fish health problem even at
micro level. Farombi et al. (2007) stated that heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Stebbing and Fandino (1983) reported that, the biological effect of heavy metals in the aquatic environment is adverse mainly due to their complex nature. Rocha, (2002) reported that the heavy metal such as copper is an essential micronutrient used in agriculture as fertilizers and pesticides, leaches into the aquatic environment resulting alteration in gill epithelium of teleost fish.

Roberts & Sommerville (1982) reported that haemorrhagic septicaemia due to *Aeromonas hydrophila* in tilapia, the liver is usually pale and there may be focal haemorrhages over the visceral and peritoneal surfaces. Reports also indicated swelling and haemorrhaging of tissues and accumulation of ascitic fluid due to aggregation of bacteria at the necrotic areas. They also found that cellular inflammatory infiltrate consists of macrophages with ingested bacteria.

Paperna (1977) and Paperna & Sabnai (1980) studied the cellular changes at the benign bacterial infections and reported that there are formation of a thin capsular structure around the hypertrophic cells on the respiratory capillaries. They have also noticed proliferative condition results in massive epithelial hyperplasia which may embed the hypertrophic infected cell as well as part or all of the gill lamellae of the filament. In mullets and carp, the mere overload of the gills with hypertrophic cells cause severe erosion of the gill architecture, which interferes with the respiratory function.
5.3 MATERIALS AND METHODS

5.3.1 Disease
Fish species *Cyprinus carpio*, *Oreochromis niloticus* and *Clarias gariepinus* inhabiting the Koka Reservoir were examined for the evidences of diseases and disorders on the body surface, fins, eyes and gills. The anterior, middle and posterior regions of the body surface were thoroughly examined for the disease symptoms like, hemorrhages, scale and fin erosion.

5.3.2 Pathogen
The enumeration and characterization of the bacteria were carried out for one year from June 2010 to May 2011. Those fishes showing disease symptoms were considered for enumeration and characterization of pathogens. The skin, gill, liver, kidney and intestine of *Cyprinus carpio* were dissected aseptically after collection and preserved in sterile glass tubes kept in ice boxes.

The tissues were weighed and macerated with sterile water in tissue grinder. The tissue extracts were serially diluted with deionized water. The prepared tissue inocula were used for culturing in selective media for isolation of *Pseudomonas* and *Aeromonas*. The collected water and sediment samples from the reservoir and river were subjected to serial dilution for plating by following the standard procedures mentioned in APHA (1985). The serially diluted water, sediment and the tissue samples were inoculated into various selective media such as Brain heart infusion Agar, Pseudomonas agar, MacConkey agar and Blood agar following pour plate method. The plates were incubated at 37°C and examined after 24 hours. The positive and suspected colonies were counted and isolated following streaking technique. The pure isolates were used for various morphological and biochemical characterization by following the methods as described in the Bergh’s Manual of Determinative Bacteriology (Garrity, 2011) and Botzenhart and Langhamer (1986). The bacterial population in the tissues, water and sediments are represented as cfu/ml for water and cfu/g for sediment and tissues.

5.3.3 Histopathology
For the histological and histopathological details, the gills, liver, stomach, intestine, ovary and kidney were aseptically removed soon after collection of *Oreochromis niloticus* from the Koka reservoir and Awash River mouth. The tissues were trimmed appropriately for required size, rapidly rinsed with distilled water and excess moisture in the tissues was blotted off using moisture absorbent tissues paper. The tissue slices were fixed in alcoholic Bouin’s fixative and 10% neutral formalin solution.
After fixation for the required duration, Bouin’s fixed tissues were washed in 70% alcohol containing excess lithium carbonate until the yellow colour of the tissue was removed. The washed tissues were stored in 70% Alcohol for further processing. Further, the tissues were dehydrated using grades of alcohol (70%, 90% and Absolute alcohol), the duration in each grade was of 1 hour maximum. The dehydrated tissues were cleared in methyl benzoate until the tissues became transparent and transferred to xylene for maximum period of 30 min. The tissues were transferred to 1:1 xylene and paraffin wax (kept in slightly melted state) for a maximum of 30 min and then transferred into pure molten wax for embedding. The tissues were further made into wax blocks using ‘L’ block. The tissue block was trimmed and the sections were taken at 5-6 μ thickness using rotary microtome. The sections were stained with Harri’s Haematoxylin and alcoholic eosin for general histological evaluation. The stained sections were observed under compound microscope and photomicrographs were taken for further details. The histological changes of the organs were assessed according to the standard method described by Bernett et al. (2004).
5.4 RESULTS

5.4.1 Diseases
A total of 150 fishes, *Cyprinus carpio*, *Oreochromis niloticus* and *Clarias gariepinus* were collected during the study period for external examination of diseases like fin and tail rot, hemorrhagic septicemia. On careful examination, it was found that common carp and cat fish showed symptoms of diseases (Plate 3&4).

Tail and fin erosion were more common in common carp. The caudal fins are the most affected among the body parts. The caudal fin rays were fragmented and free from each other. In acute stages the infection was in the form of complete or partial loss of fin rays especially caudal fins (Plate 4 E &F). The fin surface showed excess mucus secretion which is more cloudy and sticky in nature. In some fishes white patches appear on the caudal fin as well as on the body surface. The ventral, anal and pectoral fins showed extensive hemorrhage at the base of the fins (Plate 3). The diseases appeared as erosion of margins of the fins with black patches progressing towards the base. Few fishes with fin rots showed exophthalmic condition and protrusion of jaws (Plate 3 B).

Hemorrhages on the anterior abdominal and lateral part of the body of the common carp, tilapia and cat fish were frequently noticed. Inflammatory abdomen with protruded vent was also observed (Plate 4C) in common carp.

The distribution of hemorrhagic lesion was found throughout the body surface with excessive mucus secretion, scale erosion and skin lesions with blood streaks. In acute cases, the lesion extended from tail region to central parts on either side of the body or from the dorsal to ventral region in the case of cat fishes.
5.4.2 Pathogen
The pathogenic bacteria *Aeromonas* and *Pseudomonas* population in the water and sediments collected from the reservoir site are presented in Tables 25 & 26, and Fig. 27 & 28.
The distribution of *Aeromonas* bacterium in the surface water and sediment recorded from June to May is shown in Table 25 and Fig. The bacterial colony increased gradually from November onwards and reached a peak in May. However, the maximum count (2.9x10⁴ cfu/ml) was noticed in September and the lowest (1.3x10⁴ cfu/ml) in October. The sediment bacteria population was lowest (1.2x10⁴ cfu/g) in October and highest (3.3x10⁴ cfu/g) during March and further the population remained almost stable. The *Aeromonas* bacterial colony counts in the tissues of *Cyprinus carpio* were comparatively lower in the skin, gill, liver and kidney than the intestine (Table 27 and Fig. 29). In the kidney, liver and gills, the population density declined in July. In the skin and intestine, the lowest counts were recorded in October. The number of *Aeromonas* in the skin was maximum 2.02 x10⁴ cfu/g tissue in March and minimum in October 1.12x10⁴ cfu/g tissue. The bacterial load in the gills was maximum (1.3x10⁴ cfu/g) and minimum (1.0x 10⁴ cfu/g). In Kidney, the population varied from 1.2x10⁴ cfu/g in April to 1.0x10⁴ Cf/g in July. The liver tissues showed bacterial count varying between 1.0x10⁴ cfu/g and 1.13x10⁴ cfu/g. In the intestine, the bacterial population was high throughout the period of study, varied between 1.8x10⁴ cfu/g in October and 3.02x10⁴ cfu/g in April. However, the bacterial population was found stable from March to May. The *Pseudomonas* bacterium showed high numbers (2.86x10⁴ cfu/ml) in the water during January and the lowest (1.86x10⁴ cfu/ml) was noticed in June. From October to April, the bacterial density remained at high level. In sediments the bacterial numbers were always higher than the surface water. The maximum number was found to be 3.3x10⁴ cfu/g during February. The bacterial density was high from December to March. The *Pseudomonas* bacteria developed (Table 26 and Fig. 30 enumerates the presence of bacterial population in skin varied between 1.11 and 1.81x10⁴ cfu/g in the study period. The population was comparatively high except in March).
In gills the density of *Pseudomonas* was pronounced during October and November and also from May to July. In other months low counts were recorded. The minimum
bacterial count was recorded in March (1.09x10^4 cfu/g) and the maximum was in October (2.5x10^4 Cfu/g) (Table 28).

In the Kidney of *Cyprinus carpio* the bacterial count ranged between 1.05 to 1.68x10^4 cfu/g. The range of variation between months are not significant. The highest and lowest densities were observed during February and April respectively.

In the Liver extracts, *Pseudomonas* bacterium did not show much variation between months. The bacterial density varied from 1.03 and 1.8x10^4 cfu/g. The maximum and minimum values were recorded in April and March respectively.

The *Pseudomonas* population in the intestine, was greater than that of other tissues, varied from 1.13x10^4 cfu/g (March) and 2.82x10^4 cfu/g (June). In general, the intestinal bacterial population was high during April to July.

**Table 25: Distribution of Aeromonas sp. in Water and Sediment in the Reservoir**

<table>
<thead>
<tr>
<th>Month</th>
<th>Water CFU/mlx10^4</th>
<th>Sediment CFU/gx10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>2.51</td>
<td>2.8</td>
</tr>
<tr>
<td>July</td>
<td>2.78</td>
<td>2.98</td>
</tr>
<tr>
<td>August</td>
<td>2.84</td>
<td>3.04</td>
</tr>
<tr>
<td>September</td>
<td>2.9</td>
<td>3.1</td>
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<tr>
<td>October</td>
<td>1.3</td>
<td>1.2</td>
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<tr>
<td>November</td>
<td>1.56</td>
<td>1.6</td>
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<tr>
<td>December</td>
<td>1.72</td>
<td>1.82</td>
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<tr>
<td>May</td>
<td>2.62</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Fig. 27. Variation in distribution of Aeromonas sp. in Water and Sediment in the Reservoir
Table 26: Distribution of *Pseudomonas sp.* in the Water and Sediment in the Reservoir

<table>
<thead>
<tr>
<th>Month</th>
<th>water CFU/mlx10^4</th>
<th>Sediment CFU/mlx10^4</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>1.86</td>
<td>2.56</td>
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<tr>
<td>July</td>
<td>2.06</td>
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<td>September</td>
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<tr>
<td>Oct ober</td>
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<td>2.5</td>
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<tr>
<td>November</td>
<td>2.1</td>
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<tr>
<td>April</td>
<td>2.32</td>
<td>2.86</td>
</tr>
<tr>
<td>May</td>
<td>1.98</td>
<td>2.63</td>
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</table>

Fig. 28. Variation in *Pseudomonas sp.* in the Water and Sediment in the Awash River
Table 27. Population density of Aeromonas sp. in the tissues of Cyprinus carpio (cfu/g X 10^4)

<table>
<thead>
<tr>
<th>Months</th>
<th>Skin</th>
<th>Gills</th>
<th>Kidney</th>
<th>Liver</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
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<td>1.12</td>
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<tr>
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<td>1.36</td>
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<td>1.0</td>
<td>1.0</td>
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<td>1.42</td>
<td>1.8</td>
<td>1.12</td>
<td>1.08</td>
<td>2.63</td>
</tr>
<tr>
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<td>1.5</td>
<td>2.6</td>
<td>1.8</td>
<td>1.12</td>
<td>3.02</td>
</tr>
<tr>
<td>October</td>
<td>1.12</td>
<td>1.03</td>
<td>1.02</td>
<td>1.1</td>
<td>1.8</td>
</tr>
<tr>
<td>November</td>
<td>1.62</td>
<td>1.12</td>
<td>1.08</td>
<td>1.0</td>
<td>1.92</td>
</tr>
<tr>
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<td>1.3</td>
<td>1.12</td>
<td>1.1</td>
<td>2.02</td>
</tr>
<tr>
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<td>1.13</td>
<td>1.14</td>
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<td>2.68</td>
</tr>
</tbody>
</table>

Fig.29. The variation in population of Aeromonas sp. in the tissues of Cyprinus carpio
Table 28: Population density of *Pseudomonas* in the tissues of *Cyprinus carpio* (cfu x10^4/g)

<table>
<thead>
<tr>
<th>Months</th>
<th>Skin</th>
<th>Gills</th>
<th>Kidney</th>
<th>liver</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.5</td>
<td>2.67</td>
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<td>1.27</td>
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<td>1.78</td>
<td>2.5</td>
<td>1.23</td>
<td>1.38</td>
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<tr>
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<td>1.68</td>
<td>1.54</td>
<td>1.47</td>
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<td>1.13</td>
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<td>1.62</td>
<td>1.05</td>
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</tr>
</tbody>
</table>

Fig. 30. Population density of *Pseudomonas* in the tissues of *Cyprinus carpio*
5.4.3 Histopathology

Gills
The structure of the gills of the fish collected from Koka reservoir and Awash river mouth showed four gill arches on each side of the branchial cavity. Each arch is composed of numerous gill filaments with two rows of secondary lamellae that run perpendicular to each filament. Secondary gill lamellae composed of a single layer of epithelial cells supported by pillar cells, which were contractile and separated by capillary channels. Chloride cells were identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae. Mucous cells were present in the epithelium of the filament at the base of lamellae, but devoid of light cytoplasm and smaller than chloride cells (Plate 5 A). The photomicrograph of the vertical section of the gills also showed the arrangement of the lamella with the support of cartilaginous rod and blood vessels with traces of sinusoidal blood spaces. The outer covering of squamous epithelium forms a continuous lining. The primary lamella is rounded at the apices, while the projecting secondary lamellae are clearly interspaced (Plate 5B). In some fishes the gills showed extensive hypertrophy and hyperplasia of chloride cells and mucous cells at the base of the gill filament and secondary lamellae. Cyto-architectural distortion of the lamella with overlapping of primary and secondary lamellae was common in some fishes. Thus there was occlusion of inter lamellar spaces. The shrinkage of cartilaginous supporting mass resulted in decrease in size of the gills (Plate 5C). The architecture of gills also showed disrupted epithelium owing to the lysis of the cells. The increase in intracellular vacuolation (Plate 5D). The pillar cells appeared much reduced in size. The gill arch, especially at the bases of the gill filaments, showed numerous mononuclear leukocytic infiltration, edema and congestion. This may be due to cell lysis throughout the epithelium and the cartilaginous core became absolutely obscured. Severe edema, hyperplasia, fusion and focal desquamation of the epithelial lining of the secondary lamellae was common in some fishes (Plate 5D).

Liver
The liver is a large reddish-brown organ close to the transverse septum, separating the peritoneal cavity from the pericardial cavity and is usually present on the left side of the abdominal cavity. It is a digestive gland, secretes bile juice by its hepatic cells into the intracellular bile canaliculi and carried into the extracellular bile canaliculi to form the bile duct, which subsequently joins with the hepatic duct and opens into the duodenum.
The microsections of the liver of *O. niloticus* collected from the Koka reservoir and river showed a typical parenchymatous appearance (Plate 6A). The histological examination of liver revealed normal architecture of the hepatic lobules. The hepatic lobules contain hepatocytes supported by lattice fibers. Hepatocytes are polygonal cells, containing a spherical nucleus with a nucleolus and large quantities of lipid and glycogen granules. The central vein contains a number of blood cells. The blood sinusoids in between the hepatic cord were prominent with blood cells. Hepatocytes with glycogen vacuoles and eccentric nuclei are found in the transverse section of the liver. The interlobular bile ducts are distinct. Branches of interlobular portal vein and interlobular hepatic arteries are clearly visible in the portal triad (Plate 6C).

However, some fishes collected from the reservoir showed histological changes in the liver which includes loss in shape and arrangement of hepatocytes as well as vacuolation between the cells and along the sinusoids. Sinusoidal capillaries are found congested and blood vessels packed with erythrocytes. The fishes collected from the reservoir showed isolated degenerated elements around the parenchyma cells with large number of fibro-connective tissue along the marginal area of liver lobules. Erythrocyte infiltration/ hemorrhage was also noticed in the micro section (Plate 6B). The hepatic cell differentiation was not prominent throughout the lobule due to cell rupture/necrosis and infiltration of nuclear material. Acute and extensive vacuolation were also observed. Dilatation of the central vein accompanied by blood congestion was also detected (Plate 6D).

**Stomach**

The stomach of *Oreochromis niloticus*, appears as a tube with slight anatomical differences from the esophagus. Generally the stomach has two distinct histological regions: the anterior cardiac region with gastric glands and the posterior pyloric region without gastric glands. The fish stomach has been defined as a widening of the intestine i.e., an intestinal bulb because of the absence of gastric glands and a similar epithelium, in both regions. The photomicrograph of *O. niloticus* revealed that the gastric wall shows mucosa, submucosa, muscularis and serosa layers. The serosa along the whole stomach is a thin connective tissue composed of a single layer of flat mesothelial cells. The lamina muscularis consists of two or three layers of smooth muscle cells. The muscle layer in the proximal cardiac region of the stomach is single, comprised by circularly arranged muscle cells (Plate 7A &B). At the distal
cardiac region the thick muscular layer and has two distinct layers: the thick inner circular layer and the thin outer longitudinal muscular layer. In the pyloric region the inner circular layer increases in thickness and the outer longitudinal layer remains unchanged. The sub-mucosa layer showed well developed lamina propria surrounded by epithelial glands. The gastric glands are lined with a single type of secretary cell and extend throughout the sub-epithelial (lamina propria) connective tissue. The tunica mucosa is thrown up into a number of longitudinal folds projecting into a lumen. The mucosal surface has a mosaic appearance due to the hexagonal borders of the surface epithelial cells (Plate 7 A). In each tubular gland at the gastric region, there are cells secreting mucus, distributed around the gland aperture (Plate 7 C). This structure is found only at the cardiac stomach. Glands present on the pyloric region are deeper and have higher mucous production than those of the gastric region. Gastric pits (foveolae) are present as invaginations of the mucosal surface (Plate 7 C & D). Simple, straight, tubular unbranched gastric glands occupy most of the depth of the mucosa, and are lined with a single type of cell which has eosinophilic granules. In few fishes several histopathological alterations were seen in the stomach which included degeneration in serosa and muscularis layer with aggregations of inflammatory cells between them and focal areas of necrosis. Also, atrophy and edema of muscle bundles as well as splitting of muscle fibers were seen. The pathological changes like atrophy, degeneration and necrotic changes in the mucosa and submucosa with necrotized cells aggregated in the lumen were also found (Plate 7 C & D). Widening of Gastric pits, uneven distribution of gastric glands due to cellular necrosis, and disrupted mucosal folding with fragmentation were also common in the fish stomach (Plate 7 D).

**Intestine**

The segment of the digestive canal following the stomach is called the intestine. In contrast to other vertebrates there is no marked distinction between small and large intestine in fishes. Intestinal length is variable and is generally correlated with feeding habits; carnivorous species often have shorter intestine than herbivorous fish. The basic architecture of intestine is similar to that of stomach of tilapia, having mucosa, submucosa, muscularis and serosa layers (Plate 8). The mucosal epithelium consists of single columnar layer with absorptive cells, bearing a brush border (microvilli). The goblet cells were found numerous and scattered along the epithelium. The lamina propria and submucosa contained large number of wandering eosinophilic granular cells and
variable quantities of lymphoid tissue. The submucosa composed of a loose connective
tissue containing blood vessels, macrophage possessing large granules in the cytoplasm.
They are grouped mainly close to the epithelial base as well as apical region. The section
of intestine of *O. niloticus* displayed the presence of elongated villi, deep finger-
like processes of the intestinal mucosa, extending in the organ lumen. The villous
core filled with connective tissue of the lamina propria contained blood and lymph
capillaries. The outer columnar epithelium of lamina propria composed of
absorptive cells (enterocytes) and mucus-secreting or goblet cells. In the
micrograph the columnar absorptive enterocytes were numerous. The nuclei of the
enterocytes are clearly visible and lie just below the centre of the cell. The goblet
cells show a strong positive stain (Plate 8 A). In some fishes, the intestine showed
severe degenerative and necrotic changes in mucosa and submucosa. Atrophy and
aggregations of inflammatory cells with edema are also observed in the
photomicrographs. The finger shaped villi appeared in the form of ball due to
degeneration of underlying tissue (plate 8 C &D). The mucosal layer showed flattened
and reduced knob like protuberance. Vacuolization and degeneration of mucosal cells are
noticed. Fragmentation of the columnar cells and reticular cells are also noticed. The
submucosal layer disintegrated and blood vessels are found scattered throughout the
layer (Plate 8 D).

**Kidney**
The kidneys are paired, elongated structures placed above the alimentary canal and are
close to the vertebral column. Kidneys play the most important part in the excretion of
nitrogenous wastes and in maintaining homeostasis. Fish kidney consists of a large
number of tubules or nephrons which are the basic functional unit of the kidney. Kidneys
are generally divided into two portions, the head kidney and the trunk kidney. The head
kidney is generally made up of lymphoid, hematopoietic, interrenal and chromaffin
tissues and devoid of renal corpuscles, tubules, and glomeruli. The head kidney is
therefore, not excretory in function in *O. niloticus*. Thus it is derived from the pronephric
hematopoietic tissue. The trunk kidney is broad in the middle and gradually narrows in
the hinder part. Histologically, the trunk kidney is made up of a large number of
nephrons, each consisting of a renal corpuscle or the Malpighian body and the tubule.
The intertubular space is full of lymphoidal tissue which is unevenly distributed. A
typical nephron consists of vascularised glomerulus, renal corpuscle, proximal
convoluted tubule with brush borders, distal convoluted tubule and collecting ducts. The
glomerulus is round with tuft of capillaries which enter the renal tubule at the urinary pole of the glomerulus. The photo micrographs of kidney shows enlarged glomerulus, enclosed by layers of epithelium (Bowman's capsule). Cells of the outer or parietal layer of Bowman's capsule form a simple squamous epithelium. Cells of the inner layer, podocytes in the visceral layer, are extremely complex in shape. Proximal tubules are characterised by their less eosinophilic (pink), columnar cells and by large amounts of fuzzy material, filled the entire lumen with tubules. The proximal tubules are formed of columnar epithelium, eosinophilic cells, have a wide brush border. In some species, the glomerulus has a central avascular core surrounded by capillary loops which effectively reduces its functional area. The photomicrographs of kidney was composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent. The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border (Plate 9).

The fishes collected from the reservoir site also showed hydroptic swelling and hypertrophy of tubules with dilated nuclei. Glomerular alteration was also observed (Plate 9 C). The size of the glomerulus is reduced than the usual structure and displayed dilation of blood capillaries with granulomatous inflammation. Thickening of Bowman’s capsular endothelium and fibroblast proliferation around tubules are common. Some fishes showed hyaline droplet accumulation. Some tubules showed dilation and necrosis. In some fishes the renal tubules exhibited high degree of congestion leading to reduction in tubular space. Nuclei of epithelial cells found detached and scattered in the renal tissue. Cloudy appearance of renal tissue is also observed. Tubules at the peripheral region are found damaged and blood cells seen distributed more than the interstitial tissue between the renal tubules. The blood vessels showed fibrous nature. Vacuoles are common and cytoplasm highly flaccid and dense at the terminal region of the epithelial cells (Plate 9 C).

**Ovary**

The ovary of *Oreochromis niloticus* is sac-like organ formed of numerous ovigerous folds lined by germinal epithelium. The micro sections of ovary of O. niloticus revealed different stages of oocytes with interstitial tissues encapsulated by a connective tissue, consisting of germinal epithelium and tunica albuginea with blood vessels. Oogonial cyst
of early meiotic oocytes are noticed in the ovarian tissues. Postovulatory structure, corpora lutea, intra-oocytic and extra-oocytic deposition of yolk granules and yolk materials are also found in the ovary. Primary oocytes, covered generally by two layers of follicle cells, an outer thecal layer and an inner granulosa layer are seen. Histological characteristics of ovarian tissues of the fish Oreochromis niloticus showed developmental stages of oocytes as described by West (1990). Ovarian stages of oocyte development include the small sized chromatin nucleolar oocyte and perinucleolar oocyte, the medium size cortical alveolar oocyte and vitellogenic stage and the large size ripened oocytes (Plate10).

**Chromatin nucleolar stage**
The oocytes are found to be small, spherical containing a central nucleus. The nucleus contained one to four nucleoli together with chromatin network. Cytoplasm appeared as a thin layer and strongly basophilic (Plate 10 A).

**Perinucleolar stage**
The number of nucleoli increased and arranged along the inner side of nuclear membrane. Nucleus is large and surrounded by cytoplasm which appeared less basophilic. Follicular cells are monolayer of simple squamous lining surrounding the oocyte (Plate 10 B).

**Cortical alveoli formation stage**
This stage is characterized by the appearance of clear vesicles (cortical alveoli) in the cytoplasm. The vesicles found accumulated from the periphery of the oocytes. The nuclear membrane showed convolutions with nucleoli in the periphery. The zona radiata or primary envelope was visible. Follicular layers consist of simple cuboidal or columnar layer surrounded with stratified squamous thecal layer (Plate 10 C).

**Vitellogenic (yolk) stage**
In this stage, the size of the oocyte increased with small yolk granules appeared as a ring of deep eosinophilic in the cytoplasm. The nucleus found convoluted. The zona radiata was clearly visible as a noncellular deep eosinophilic band. Follicular layers well developed with cuboidal or columnar layer surrounded by stratified squamous thecal layer (Plate 10 D)

**Ripe (mature) stage**
This stage was characterized by the enlargement of both cortical alveoli and yolk granules. The size of the oocyte increased markedly. The zona radiata clearly visible. Follicular cells are cuboidal or low cuboidal surrounded by thin thecal layer (Plate 10 E).
5.5 DISCUSSION

5.5.1 Disease

The environment plays an important role in fish health. Any adverse condition may decrease the immunological responses and increases susceptibility to different diseases in fishes. Virgona (1992) and Callinan et al. (1995) reported the seasonal occurrence of diseases in fishes due to climatic variations in aquatic environment. In the present study, the fishes Cyprinus carpio, Oreochromis niloticus and Clarias gariepinus showed various disease symptoms and infestation in the form of hemorrhages, lesions, septicemia, tail and fin rot. The study clearly demonstrated that the occurrence of infestation in fishes is correlated with environmental factors influencing the growth of bacterial pathogens which causes disease in fishes. Wedmeyer and Wood (1974) noticed the effect of environmental stress on outbreaks of microbial diseases in fishes inhabiting oxygen deficient waters due to organic overload. Further this condition leads to eutrophication which enhances the population of bacterial fish pathogens such as Aeromonas and Vibrio which can initiate bacterial epizootics in fishes (Sniezko 1974). Rehulkka (2002) observed that Aeromonas cause severe skin lesion in trout and finally results the mass mortality.

Earlier reports indicated that tail and fin rot disease were caused by microbial pathogens like Aeromonas, Pseudomonas, Vibrio and Escherichia in game fishes (Davis 1953) and Mystus gulio (Beena 1993; Jisha 2008).

In the present study the fishes were found with Hemorrhagic septicemia which might be caused by the common pathogens like Aeromonas and Pseudomonas which are more prevalent in sediment and water of Koka reservoir. The result of the present study is in conformity with Shapherclaus (1957). However, the prevalence of Hemorrhagic septicemia is comparatively lower than other disease condition. Similarly, the disease was more pronounced in carp than tilapia and catfish. Similar observation was reported in different fishes by Jisha (2008). Abdominal swelling in carp in the present observation might be due to fluid accumulation in the body cavity. Similar dropsical condition was observed by Flemming (1958), Amalacher (1958c) and Bank (1960).

The exophthalic condition observed in tilapia is mainly due to the excessive hemorrhage in acute infection of pathogenic bacteria especially Aeromonas. Similar result has been reported by Leung et al. (1994) and Roberts (2001), who also reported that abrupt temperature change, crowding of fishes, inadequate food and oxygen are known to be the factors which contribute to the infection.
5.5.2 Pathogens

In the present study, the total **pathogenic bacterial** density was found higher in sediments than water samples. The results are in agreement with the findings of Fosch *et al.* (2002) and Dang *et al.* (2010) who reported that the sediments usually harbor the most diverse bacterial population. Schallenberg (1990) found that sediments of epilimnetic water have higher bacterial biomass than hypolimnetic region. Yaohui Bai *et al.* (2012) observed that among the environmental parameters, the total organic carbon accounted for the greatest proportion of variability in bacterial community. Further, they reported that increasing allochthonous organic carbon could enhance bacterial diversity and biomass in the sediment. Anon (1997) also reported the higher bacterial population density in the sediments than water due to the rich organic content of the former and the lesser residence time of the microorganisms in the water column.

Martin, (1981) and Sathiyamurthy *et al.* (1992) reported that the bacterial population occur during monsoon may be due to the rain water flow which brings huge quantities of nutrients. In contrast, during summer the population level would be low in water column than sediment (Natarajan *et al.*, 1980; Velammal, 1993).

The present result also indicated that the highest population of Aeromonas was found during rainy reason (July) and minimum after rainy season (October). However, the March month is said to be dry season in Ethiopia, some time rain occurs during this period. Hence, the Aeromonas population was found highest in sediments during March, and minimum in October. This could be due to high temperature and salinity which enhances the availability of nutrients in the water (Carlucci, 1974; Wollast, 1991).

In the present study the Pseudomonas population was found high during January and February in both water and sediments. However, minimum number in water during rainy season (June) and in sediment at the end of rainy season (October). The present trend in the population of Pseudomonas is mainly due availability of nutrients and other physiological characteristics of water in different seasons. The results also indicated that Pseudomonas population is higher in sediments than in water. The results are in agreement with the findings of Pirnay *et al.* (2005) and Wolf-Rainer *et al.* (2011) who stated that the river water serve as a source of potentially pathogenic *pseudomonas* strains in sediments. Microbes in rivers are diverse and dynamic in composition due to environmental stresses and therefore, the composition of microbial community in rivers have been suggested as an indicator for microbial pollution in sediments (Atlas, 1984).
The population composition of *Aeromonas* in different tissues showed distinct seasonal variation. The *Aeromonas* and *Pseudomonas* population followed the same trend of distribution in different tissues i.e., both microbial populations were found higher in intestine followed by skin, gills, kidney and liver.

The present results are in conformity with that of Henebry *et al.* (1988) and Shiranee *et al.* (1998) who reported that higher bacterial load in the gut of fish than in the surrounding waters. This study thus confirms the suggestion that fish selectively feed on detrital particles with high number of bacterial biomass per unit weight (Odum, 1968; Moriarty, 1976). Henebry *et al.* (1988) observed increased bacterial population in silver carp and suggested that bacterial populations may increase in the midgut before being ultimately digested. Further, Herbst, (2000) reported that the pathogenic bacteria are introduced into the aquatic ecosystems with the wastes through influx water from the land and hence the risk of transfer of diseases to the fish and humans is high.

Next to intestine, the higher bacterial load was found in skin because of the frequent contact of body surface to the water and sediments. In addition, the presence of scales could harbor the detritus particle which serve as substratum for the growth of bacteria. It is also noticed that the scale erosion and hemorrhages in fish could have facilitated the bacteria to adhere in the skin and fins. Gills are the organs in which the microbial population was found to be higher than kidney. It is mainly because of the role played by gills in filtering minute particles which are entrapped among the gill filaments in a mucus film. The mucus film could facilitates flocculation and retain very small particles including bacteria. This could be the reason for the high density of pathogenic bacteria in the gills. The present results are supported by Drenner *et al.* (1987) and Northcott and Beveridge (1988). It is apparent that fish are continuously exposed to the microorganisms present in water and in sediment including the contaminants in sewage (Trust, 1975; El-Shafai *et al.*, 2004). The kidney and liver being the internal organs, less susceptible to the pathogenic bacteria. However, there are reports of presence of bacteria in the kidney and liver of healthy fish (Evelyn & McDermott, 1961; Apun, *et al.*, 1999). Toranzo *et al.* (1993) reported that the liver and kidney of healthy turbot contains mostly *Pseudomonas* population. Similarly, Decostere *et al.* (1996) recovered the *Shewanella* spp. in kidney and liver of fishes. The reasons for the presence of some of these bacteria in these tissues are unclear. Moreover, it is speculative whether or not the fish are at the earliest stage of an infection cycle.
5.5.3 Histopathology

Histological study of the gills showed the typical structural organization of the lamella. However, the architectural distortion was noticed in some of the collected fishes (Plate 5). The change in physiological properties of gill is evident in the form of shrinkage of lamella. The abnormal shortening of the secondary lamella was probably the result of edematous change as seen in plate 5 D. It may be a form of adaptive measure to overcome the effect of the toxic material in aquatic system (Smith & Piper, 1975). Histopathological changes of gill such as hyperplasia, hypertrophy, epithelial lifting, aneurism and increase in mucus secretion have been reported in fishes exposed to a variety of chemical compounds such as pesticides, phenols and heavy metals (Mallatt, 1985; Nowak, 1992). Alvarado et al. (2006) reported that, the dramatic increase of chloride cells in the gills causes epithelial thickening and due to migration of chloride cells up to the edge. results hypertrophy and fusion of secondary lamellae. Benson et al. (1987) and Ayoola (2008) stated that lethal concentrations of herbicide cause damage to gill filament leading to impairment in gaseous exchange efficiency. Eller (1975) and Richmond and Dutta (1989) stated that the destruction of the epithelial layer of the secondary lamellae is due to undesirable detergents and pesticides entering into the water bodies.

The lamellar epithelial lining reacts to some heavy metals like lead creating tissue osmoregulatory imbalance (Fernandes et al., 2008). According to Roberts (1978), heavy metals causes dilation of the lamellar capillaries and pooling of blood which thromboses, eventually fibroses and fuses with adjacent lamella. Swelling and hyperplasia of the gill epithelium could serve as a defense function, as these alterations increase the distance across waterborne irritants must diffuse to reach the bloodstream. Lamellar fusion could be a protective adaptation once it reduces the amount of vulnerable gill surface area (Mallatt, 1985). In the present study, congestion of nucleated blood cells in the lamellae implies shortage of oxygen in the water and blow up of blood vessels. Similar alterations in the gills have been reported in fishes exposed to different toxicants in the water (Gardner & Yevich, 1970; Karlsson-Norrgren et al., 1985; Pratup & Wendelaar Bonga, 1993; Thophon et al., 2003) and this can be sometime referred as a first sign of pathology. It is inferred that such damages noticed in the present study could be mainly due to direct contact of gills to the pollutants in the water. The incidence of histological alterations in liver is an evidence of the poor environmental quality in which fishes live. The liver is associated with the
detoxification and biotransformation processes and hence it is one of the organs most susceptible to the contaminants in the water (Camargo & Martinez, 2007; Soufy et al., 2007). Liver of fish is sensitive to environmental contaminants because they tend to accumulate and causes alteration in the cellular organization (Heath, 1995). In the present study the type of histopathological lesions observed in liver indicates that the fish from Koka reservoir respond to toxicants in water and sediments. Most of the histopathological alterations observed such as hepatocellular necrosis, vacuolation and cystic degeneration in liver could be interpreted as a nonspecific response to stress in fish exposed to a wide spectrum of pollutants (Plate 6 B & D). The present results are in conformity with the observations of Khan et al. (1994), Brand et al. (2001), and Sepulveda et al. (2004). These cytological changes might be of adaptive or compensatory nature as observed in carps (Rutschke & Brozio, 1974; Rojik et al., 1983; Benedeczky et al., 1984, 1986; Storch et al., 1984). Hinsen et al. (1971) and Metelev et al. (1971) observed wide varieties of insecticides tend to accumulate in high concentrations in liver and suffers harmful effects. Normally, in the liver of fish, chemically induced cytotoxic lesions are almost exclusively restricted to parenchymal cells (Hinton et al., 1988). In the study of Risbourg and Bastide (1995), the exposure of fish to Atrazine herbicide increased the size of lipid droplets, and vacuolization. The most frequently encountered types of degenerative changes like hydropic degeneration, cloudy swelling, vacuolization and focal necrosis shows the evidence of fat degeneration. These changes may be attributed to direct toxic effects of pollutants on hepatocytes. Necrosis of some portions of the liver tissue were observed in the present study (Plate 6 B) would have resulted from the excessive metabolism required by the fish to get rid of the toxicant from its body during the process of detoxification or inability to regenerate new liver cells. Similar results were reported by Shastry and Sharma (1979) in C. punctatus exposed to a sublethal concentration of Endrin. King (1962) found many small vacuoles in hepatic cells in brown trout fry and adult guppies exposed to DDT. The vacuolation of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulatory system (Gingerich, 1982). In the present study also vacuolization and degeneration of hepatocytes, focal area of necrosis, thrombosis formation in central veins, dilation and congestion in blood sinusoids and fibrosis were observed. Eder and Gedigk (1986) stated that oxygen deficiency as a result of gill
degeneration is the most common cause of the cellular degeneration in the liver. According to Mohamed (2001) the vascular dilation, intravascular haemolysis and thrombosis formation in the blood vessels may be also responsible for the cellular degeneration of liver. The present results are in agreement with the results of Mohamed (2001), Ptashynski et al. (2002) and Fanta, et al. (2003) who have studied the effects of different pollutants on fish liver. Also, El-Demerdash and Elagamy (1999) and Olojo et al. (2005) observed degeneration of the hepatocytes and focal necrosis in the liver of Clarias gariepinus exposed to lead, and tilapia to cadmium and mercury respectively.

Histological structure of stomach shows the mucosa, which consists of the lamina epithelia and lamina propria, muscular layer and serosa (Poleksic et al., 2006). The mucosal epithelium of the stomach of O.niloticus is similar to that of other teleosts (Elbal & Agulleiro, 1986; Gargiulo et al., 1997) and it is entirely composed of a columnar epithelium. The existence of neutral mucus substances in gastric epithelial cells could indicate that these cells play a certain role in absorptive processes, as in other teleosts (Ezeasor & Stokoe, 1980; Grau et al., 1992). However, in the present study the appearance of granules dispersed in the cytoplasm of the surface epithelium were probably secretory granules and appeared weakly positive reaction with haematoxyline and eosin.

The gastric glands found throughout the stomach mucosa were found edematous, accompanied with hyperemia of various degrees of erosion. In some fishes, severe damage in submucosa and mucosal layer and congestion of whole mucosal folding (Plate 7C) were observed. Shrinkage and atrophy of glandular structures resulted in the formation of large spaces at the periphery. However, the muscularis showed compact architecture. The photomicrographs also revealed hyperchromatic epithelial cells, disintegration of glandular epithelium and desquamation of gastric mucosa. Similar observations were made by Bhatnager and Shrivastava (1975) in Heteropeustus fossilis exposed to copper and Sastry and Gupta (1978b) in Channa punctatus exposed to lead nitrate.

The intestinal sections of O. niloticus shows degenerative changes in the form of loss of structural integrity of mucosal folds, hyperemia, degeneration of villi, necrosis, desquamation of mucosal epithelium, cellular debris, excessive mucus in lumen and inflammatory infiltration of submucosa were noticed (Plate 8 B & D). In the present study, necrotic condition might be due to problem in digestion of contaminated food materials. The proliferation of intestinal mucous cells in the sections most likely
represents an adaptive mechanism to protect the epithelium from contaminated food stuffs that enter in the digestive tract. Gardner and Yevich (1970), Newman and MacLean (1974) and Gutierrez et al. (1978) reported that toxic lesions are common in the intestine, along with hyperemia, loss of villi and vacuolation in fishes exposed to Cadmium Chloride. Gardner and Yevich (1970) also mentioned that the histopathological changes in intestine like hypertrophy leads to the increased serum glucose in estuarine teleost fish exposed to cadmium. They also concluded that this condition is possibly due to the fulfillment of extra energy requirement for absorption of nutrients under stress condition. Establier et al. (1978) also noticed such alteration in intestine of Mugil auratus exposed to inorganic and organic mercury and Walsh and Ribelin (1975) in Cyprinus carpio exposed to Atrazine.

The kidney of the fishes receives the largest proportion of post branchial blood and therefore renal lesions might be expected to be good indicators of environmental pollution. In the present study, tubular degeneration and esinophilic protenacious intertubular cast and hyaline droplets are common as mentioned by Meyers and Hendricks (1985) and Rand (1995). Further, they also reported that these changes might lead to cellular necrosis. The hyaline droplets in the cells may be produced within the cell itself or formed by re-absorption of excess amounts of proteineous substances, filtered through the glomerulus (Hinton & Laur’en, 1990). Rand (1995) suggested that the cell injuries may result in decrease in the level of intracellular ATP, which in turn would impair the action of the cation pump of the cell, allowing the influx of sodium, chloride, calcium and water, causing an increase in the cell volume and damage to the cell membrane. Occlusion of the proximal or distal segments of the renal tubule can occur by the accumulation of certain materials in the lumen and also as a consequence of the swelling of the epithelial cells (Takashima and Hibiya, 1995). Since the kidney is an organ through which a large volume of blood flows, xenobiotics present in the blood can cause some pathological changes in Bowman’s capsule, such as proliferation of epithelial cells and thickening of the basal lamina, leading to reduction in Bowman’s space. Similar histological changes were noticed in the section of kidney of O. niloticus in the present study. Furthermore, the excess of red blood cells in the medullar region indicates that rupture of capillaries in Bowman’s space as suggested by Hinton et al. (1992) or might be due to pesticide action (Oliveira Ribeiro et al., 1996; Schwaiger et al., 1997; Gernhofer et al., 2001; Pacheco & Santos, 2002; Veiga et al., 2002). Besides, these degenerative processes, could be due to the urban contaminants, agricultural and
land run offrs enters in the reservoir causes stress to the fish as quoted by Hinton and Laur’en, (1990) and (Rahman, et al., 2002) in different fishes. Oulmi et al. (1995) studied the effects of Linuron (herbicide) on the rainbow trout (Oncorhynchus mykiss) and their results showed cytoplasmic vacuoles and nuclear deformation in the epithelium of the first and second segments of the proximal tubule. The present study also revealed that the kidney cells have distortion of renal corpuscles, causing disorganization and obstruction to their physiological functions. These findings are in agreement with that of Omoniyi et al. (2002), and Rahman et al. (2002).

The present study on ovary of O. niloticus revealed the presence of various stages of oocyte development which indicates maturation and imminent spawning as reported by Hussain et al.(1996). In contrast, Limsuwon et al. (1987) observed 6 stages of oocytes in Clarias batrachus with similar histological characteristics. However, in the present study, few fishes showed disorganization of complete architecture of ovarian structure. The major changes are prominent inter follicular spaces, appearance of atretic follicles, change in nucleus and degeneration of ovarian follicles (Plate 10 A). In the present study it is also noticed the interstitial connective tissue became fibroid in nature with vacuolation. These changes are also attributed to the variation in water quality due to agricultural and industrial effluents like heavy metals entering in to the water. The present result is in conformity with the findings of several other researchers. Shivani Sharma et al. (2011) reported that the long term exposure of cadmium chloride in Heteropneustes fossilis resulted in marked degenerative changes in the ovary including prominent interfollicular spaces, appearance of atretic follicles, change in nucleus and degeneration in the ovarian follicles. Similar to this study, Kumar and Pant (1984) and Baruah and Das (2002) also reported a significant atresia in the ovary with major damage to younger oocytes in Puntius conchonius, after exposure to zinc on gonads. Mukherjee et al. (1992) concluded that the effect of certain toxicants on gonadotropin-induced ovarian non-esterified cholesterol depletion. Further it is also reported that excess of nutrients in the water along with high temperature would causes severe follicular damage. The problems associated with dense algal blooms causes a low or moderate buffering capacity (alkalinity), wide fluctuations in pH during the day which depress fish follicular growth. Algal die-offs can result in high ammonia concentrations that can affect fish appetites and growth rates too (Martin et al., 1994). Further, Landry et al. (2004) suggested that the female fishes in the hypoxic water found to have ovaries that were half as large as those in normal oxygen levels. Further, Boyd (1979), and Rand and
Petrocelli, (1985) reported the temperature, hardness, pH, alkalinity of water and sex, age and other physiological status of the test animals have profound effects on the toxicity of agro-chemicals.

Accumulation of more yolk granules outside the oocyte is the evidence of damage of caused to vitellogenin transport system due to cellular damage in medullar region. This observation is in conformity with the statement of Ho (1987) who stated that vitellogenin is being transported from the liver for synthesis of yolk in the ovarian follicles.

From the above discussion, it can be concluded that the histopathological changes in the tissues of *O. niloticus* are attributed to the poor water quality including entry of pollutants, occurrence and duration of algal blooms and accumulation of toxicants in the water sediments in addition to various anthropogenic activities in the catchment area of Koka Reservoir.