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REFERENCE BOOK

EFFECTS OF CADMIUM CHLORIDE ON *CATLA CATLA* WITH SPECIAL EMPHASIS ON HISTOLOGICAL AND BIOCHEMICAL PARAMETERS IN GILLS, MUSCLES AND LIVER

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"There is no end to the grace of god, blessings of the parents and knowledge of the guru".

I would like to thank the supreme power the almighty god who has always guided me to work on the right path of life. Without his grace this could not become a reality. No one who achieves success does so without acknowledging the help of others. I had finished, but not alone. Without these supports, especially the 'selected few 'I'm about to mention, I may not have been where I am today, at least not sanely.

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Chapter 1
INTRODUCTION

Figure 1 Photograph of *Catla catla*(Taken during experiment)

Ponds are a crucial component of the aquatic ecology. Ponds, despite their small stature, provide important environmental, social, and economic purposes. These include providing drinking water; recharging groundwater; functioning as flood control sponges; promoting biodiversity; and supplying livelihoods. But climate change has a significant impact. In India, ponds have been serving as a traditional source of water supply since the beginning of time. One of the most essential surface water resources for life is freshwater ponds. These ponds retain rainwater throughout the rainy season, and the local residents use the water they store for household purposes. Since animals primarily use it for drinking, it is important to assess the quality of the water. To determine the quality of the water, physicochemical factors should be studied. Ecological equilibrium is essential for widespread biodiversity and human life.

One of the rarest forms of nature is fresh water. The major issue of concern in the twenty-first century is the accessibility of fresh water. Water demand is growing alongside the growth in both our global population and economic position. Due to climate change, energy scarcity, land use decisions, industrialization, and mineral processing, water availability and quality have been declining over the last few decades. Water pollution results from the physical, chemical, and biological features of water resources being altered by natural or manmade processes to the point that it is harmful to people, plants, and animals.
High concentrations of contaminants in lake water, mostly organic matter, increase biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids, and excreta coli form. They render water unfit for consumption, irrigation, or any other use.

Different toxicants are present in the environment in varying amounts. Even while the dosage of some toxicants does not render the organism fatal, it may nonetheless result in severe cell-level damage in many organs. Currently, the issue of water pollution has grown significantly as a result of the advent of cutting-edge technologies that use heavy metals as raw materials for various purposes. Heavy metals are one of the most prevalent pollutants today due to their toxicity, accumulation, and bio-magnification.
They are pervasive throughout the ecosystem and are highly destructive. The level of harm that a substance (a toxin or poison) can cause to people or animals is known as its toxicity. Toxins can enter a food web by building up inside of individual species through the bioaccumulation process, but toxin concentrations can rise within a food web by transferring from one trophic level to the next through the bio magnification process.

Bioaccumulation is often a helpful integrative indicator of the chemical exposures of species in degraded settings. All trace metals are dangerous and have some bioavailability. Multiple physiological processes that may be altered in aquatic creatures exposed to abnormally high local bioavailable hazardous metal concentrations include the rate of metal intake from a source of the metal, the rate of efflux, and the rate of detoxification of accumulated metal into a relatively metabolically inert form. As heavy metals are broken down over such a lengthy period, they essentially become permanent contributions to the aquatic ecosystem, making them conservative contaminants. Measurements of bioaccumulation are required because heavy metals bio accumulate and harm aquatic ecosystems. Measurements of bioaccumulation are studies on ways of observing the absorption and retention of contaminants like metals or pesticides in organs or tissues of creatures like fish.

Competitive, anti-competitive, or non-competitive inhibition, as well as several other effects, may be seen in the bioaccumulation of metal combinations. Combinations of these could show the enhancement of taking up metal. The build-up of trace metals in aquatic creatures may have a long-term negative impact on the biosphere's biogeochemical cycle. When chemicals accumulate and become harmful, bioaccumulation creates an issue for the ecosystem.

The organism absorbs metal ions from its surroundings and stores them in several organs and tissues. Heavy metal poisoning of the aquatic environment has been given increased attention because it might have catastrophic effects on the ecological balance of the surrounding ecosystem and the diversity of aquatic creatures. Fish are one of the animal species whose inhabitants are unable to escape the negative effects of pollutants.

There are 35 metals associated with occupational and community exposure from the periodic table, of which 23 are classified as heavy metals. Even so, a variety of creatures need trace amounts of these metals, including cobalt, magnesium, iron, copper, molybdenum, and zinc. A surplus of these may be detrimental to living organisms.

Several heavy metals are regarded as macro and microelements that are vital. The most prevalent heavy metal contaminants are mercury, lead, cadmium, copper, and nickel. By analysing fish tissues and water sediments, it is essential to determine heavy metal toxicity for the ecological risk assessment of contaminants. The most well-known metal contaminant in aquatic environments is cadmium.

Due to cadmium's high solubility in water, cadmium toxicity in aquatic ecosystems is rising as a result of numerous industrial and agricultural activities. Cadmium is easily collected in plant components, contaminating the entire food chain and ultimately reaching humans. When this metal enters the food chain, it is thought to be potentially hazardous and poses a major threat to human health. Due to its non-corrosive properties, cadmium is one of the dangerous heavy metals that are usually utilized in nickel-cadmium batteries, the mining and metal production industries, dentistry, and other applications. Cadmium is also associated with the metallurgy, paints, and dyes, electroplating, and metal finishing industries. Because soil includes contaminants like fluorine and cadmium, long-term misuse of phosphorus fertilizers, lime, and agrochemicals in agricultural areas increases the number of harmful trace elements in the soil. Soil may readily reach a water source after being raised with cd. Cd is employed in the manufacturing of plastics, pigments, and electroplating, which has led to a recent spike in the contamination of soil, water, and air.

Cadmium has become more prevalent in the environment as a result of the discovery of new uses for it. Television image tube phosphorus, nickel-cadmium batteries, motor lubricants, rubber curing agents, fungicides, phosphate fertilizers, stearate stabilisers for polymers (polyvinyl chloride), and nuclear reactor shields are all made using cadmium. Cadmium is generally used to electroplate other metals or alloys to prevent corrosion and to create solders or alloys with low melting points. Because this element interacts often with sulfhydryl groups of amino acids, proteins, and enzymes, it may also substitute necessary metals like copper and zinc in several metalloproteins, changing the protein shape and impacting its function. Therefore, cadmium's harmful effects are linked to modifications in organisms' normal physiological and metabolic processes. It is well known that fish quickly adjust their detoxifying enzymes in response to cadmium and other pollutants. This enzymatic response is influenced by a variety of parameters, including temperature, age, nutritional condition, and oxygen availability.

By displacing the original metals from their normal binding sites and attaching them to protein locations that are not intended for them, these heavy metals causes cell dysfunction due to their toxicity. Heavy metal binding to DNA and nuclear proteins is the main cause of the oxidative degradation of biological macromolecules. Heavy metal salts, acids, organic debris, pesticides, and even cyanides are just a few of the toxicants found in industrial waste that degrade the physicochemical properties of water.
Additionally, a significant portion of the dissolved metals that enter aquatic ecosystems is adsorbed into colloid particles. Their dispersion and movement in the environment are improved by specific physicochemical features. Metals, in particular lead and cadmium precipitate by forming complexes at high alkalinity and pH, which has a significant impact on metal toxicity. Metals in water may change fish blood and tissues' physiological and biochemical properties, which might lead to population meltdown. Fish acquire compounds known as xenobiotic, particularly those that are poorly soluble and are carried in suspension or solution.
Industrial waste discharge or soil leaching caused by the addition of sewage sludge are two ways that cadmium reaches surface water. Pesticide use to enhance crop production is now a common practice. Its excess use is harmful not only to plants and animals but also to human beings. Large amounts of cadmium are discharged by industrial effluents into the soil's surface and groundwater systems; these excess amounts, together with naturally occurring levels, progressively build up to two hazardous thresholds that harm the aquatic ecosystem's biotic community. Since their widespread use worldwide can pose threats to the environment, especially aquatic bodies, and cause severe harm to non-target species, notably fish, the presence of pesticides in the environment has created major social and scientific development concerns around the world.

The use of sewage effluent as fertilizer has become increasingly popular in developing nations as it is thought to be an excellent source of organic matter and plant nutrients. Farmers are primarily concerned with the general advantages, such as increased agricultural production, inexpensive water sources, efficient effluent disposal methods, sources of nutrients, organic matter, etc., but they are less cognizant of the negative effects, such as heavy metal contamination of soils and crops, and quality issues related to health. Long-term irrigation with this sewage effluent contaminates the soil and crops to the point that it is hazardous to plants and degrades the soil.

Numerous factors critical to overall element uptake rely heavily on heavy metal bioaccumulation from aquatic environments. The overall amount, each metal's bioavailability in the environment, and the method for absorption, storage, and excretion are among the factorial facilitators. Accordingly, the less accessible a metal is, the less it will be accumulated.

Cadmium exhibits bio magnification and has a longer half-life due to its inability to degrade. Cadmium has been revealed to disrupt the enzymes responsible for numerous protein and carbohydrate metabolisms. The most harmful element in metals is cadmium because it produces both acute and long-term harm since it is physiologically active. When heavy metals accumulate in tissue without being absorbed by the organism, they become poisonous. As a major source of environmental pollution, heavy metals are known to have cytotoxic, mutagenic, and carcinogenic effects on animals.

Cadmium is potentially deadly to aquatic organisms, but the degree of toxicity varies depending on the test organism, its life stage, the length of exposure, and ambient factors including temperature, PH, dissolved oxygen, hardness, and organic material content. The quantity of physiologically active forms of cadmium may be affected by variables like hardness and pH, while other variables like temperature and dissolved oxygen may have an impact on an organism's tolerance level.

The tendency of metals to bind to various molecular groups found within organisms' cells as well as the degree of exposure to the metal as influenced by its metabolic traits and position in the food chain could be used to explain differences in the concentration of metals in various parts of an organism. Cadmium is an oxyphillic and sulfophillic element. It forms stable complexes with a range of chemical molecules and has an affinity for several different types of body bonds. Cadmium harms fish by impairing gill calcium absorption, stunting development, and changing liver function.

Chloride occurs naturally in all types of water. A high concentration of chlorides is considered to be an indicator of pollution due to organic wastes of animal or industrial origin. Chlorides are troublesome in irrigation water and are also harmful to aquatic life. A higher concentration of chloride is hazardous to human consumption and creates health problems. It can reduce energy levels and harm the performance of critical organs such as the brain, lungs, kidneys, liver, and blood cells.

Both in the solid and liquid phases of the aquatic ecosystem, species distribution is intimately correlated with the ecological consequences of pollutants and their bioavailability and toxicity. Plankton, aquatic plants, molluscs, and fish all absorb pollutants. Numerous macroscopic and microscopic species as well as plants inhabit the aquatic ecosystems' bottom sediments. Many of these organisms consume the organic materials in these sediments. Water quality indicators are recognized to quickly and effectively show trends in water quality even at low concentrations, as well as yearly cycles and regional and temporal changes in water quality. Fish are one of the healthiest and cheapest sources of protein, minerals, non-saturated fatty acids, and Omega 3 that help in the reduction of blood cholesterol and prevent heart malfunction (arteriosclerosis). Fish that live in polluted water are more likely to accumulate heavy metals in their tissues. If exposed to toxicants in any form, they exhibit restlessness, fast body movement, convulsions, trouble breathing, increased mucous production, colour change, and unsteadiness. The beneficial effects of fish may be negated by the presence of harmful heavy metals. Through a variety of pathways, fish bio accumulates heavy metals, including cadmium. Because fish come into very close contact with the medium that contains the chemicals in solution or suspension, as well as because fish must extract oxygen from the medium by passing massive volumes of water over the gills, fish accumulate chemicals, especially those with poor water solubility. Potential locations for chemical absorption from water include the skin, gills, and digestive system. Once absorbed, the chemicals are moved by the blood to the liver for transit or to a storage location like bone.Fish absorb these heavy metals through the consumption of food that contains suspended particulates, a continuous ion exchange mechanism that transports dissolved metal through lipophilic membranes like the gills, and dissolved metal adsorption on tissue and membrane surfaces. The modes of intake from water include diffusion-facilitated transport or absorption in gills and surface mucus. The amount of heavy metals that accumulate in fish varies according to the kind of heavy metal, the type of fish, and other environmental factors, including salinity, pH, hardness, and temperature. Ecological needs, size and age of individuals, their life cycle, feeding habits, and season of capture will also affect the accumulation. Hardness is one of the most important factors that affect fish physiology and metal accumulation. Heavy metals produce many alterations in the tissues and blood of affected fishes and render them unable to eat. Heavy metals in fishes are absorbed in the gills, skin, and digestive tract of fish and then transported by the blood to either a storage point such as bone or to the liver for transportation.

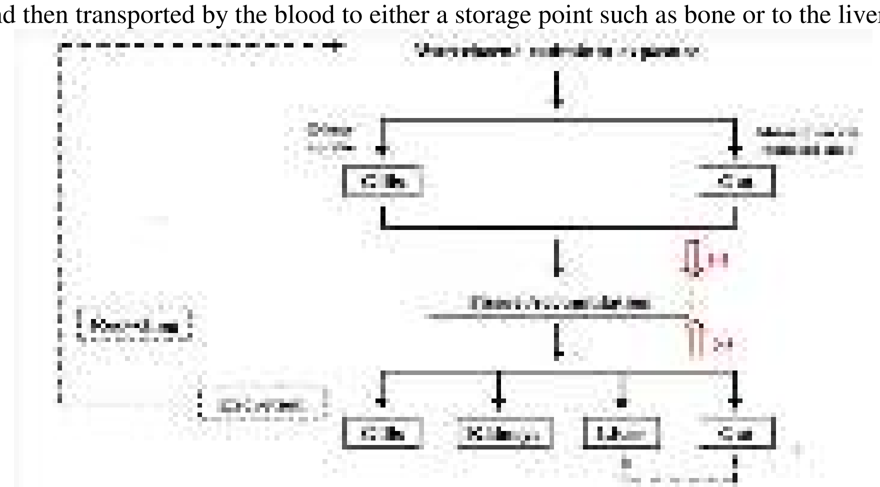


Figure 2: The Cycle of Cadmium in Aquatic ecosystem

The fish evolved a defence against the harmful effects of both necessary and inessential heavy metals, as well as other xenobiotic that cause the body to experience degenerative changes like oxidative stress. An organism's behaviour is described as an action in a specific method or exact way that an organism responds to environmental stimuli, especially those reactions that can be seen. Several biotic and abiotic elements are crucial in influencing how animals respond and behave. Since gills are in close touch with the water, they will be the major entry point for toxins into the fish's body if the water is polluted. Water intake processes include gill and surface mucus-mediated diffusion, facilitated transport, and absorption. Metals' bioaccumulation is a reflection of how far an organism consumes, how it is dispersed throughout its tissues, and how much metal is retained in each kind of tissue. Metals linked with organic components are ingested and absorbed through endocytosis through the gut, whereas metal ions are often absorbed through passive diffusion or carrier-assisted transport across the gills. It has been proposed that calcium channels allow Cd ions to enter the chloride cells in the gills. In experimental research, it has also been claimed that the gills serve as Cd storage sites.

If exposed for a long time, it may result in biochemical and histological alterations, including a reduction in the growth of fish. Oxidative stress in fish can result in hepatic and renal damage. A divalent cd is the most bioavailable type of cd. Exposure to this type may result in metallothionein production. Fish are especially vulnerable to increased cd levels during their early phases of life and reproduction. Skeletal malformations caused by CD can make it difficult for fish to obtain food and protect themselves from predators, which can have fatal consequences. These abnormalities may ultimately have an impact on fish diversity and environmental stability.

One of the main freshwater carp, *Catla catla*, is indigenous to India, Bangladesh, Myanmar, Nepal, and Pakistan. It was also introduced as an exotic species in many other nations. According to reports, *Catla catla* may grow to a maximum size of 182 cm and weigh over 50 kg. It is a particularly rich source of proteins (these figures vary). It is primarily omnivorous and feeds on the surface and middle of the water. Juveniles consume aquatic and land insects, debris, and phytoplankton. Its projecting jaw and big, upturned mouth are distinctive features. Due to its excellent nutritional content, it is a costly food fish that is in high demand on the market.

Fish respiration rates are typically altered by metal toxicants, which mostly results in a decrease in oxygen intake rates. Since the gills are the primary respiratory organ and all metabolic pathways rely on the efficiency of the gills for their energy supply, mercury and cadmium toxicity damage to this crucial organ impairs its respiratory function by reducing respiratory surface area, which ultimately results in respiratory distress.

There are several ways for aquatic creatures to absorb Cd, but Cd (II), which is the element's free ionic form, is the most easily absorbed. Metals linked with organic components are swallowed and absorbed through endocytosis through the gut, whereas metal ions are often absorbed through passive diffusion or carrier-assisted transport across the gills. It has been proposed that calcium channels allow cadmium ions to enter the chloride cells in the gills. The metal is made available for interaction with cytoplasmic elements; including enzymes (producing harmful effects) and metallothionein once it has entered the cells (probably being detoxified).

The increased gulping activity and opercula movement by the exposed fish may be a reflection of an attempt by the fish to extract more oxygen to meet the increased energy demand to withstand the cadmium toxicity. It may also be correlated to the formation of a hypoxic condition due to the interference in gaseous exchange caused by the accumulation of mucous on the gill epithelium. Higher cadmium chloride concentrations were found to have more significant effects, including oedema, mucous discharge, and noticeable leucocyte infiltration in the epithelium. The context of these includes the commencement and acceleration of epithelial lifting and leucocyte infiltration, lamellar blood sinus constriction, hyperplasia, and mucous production.

Since the separation of the epithelia from the lamellae increases the distance that waterborne contaminants must travel to reach the circulation, oedema that causes the lifting of the lamellar epithelium may act as a protective mechanism. This method also raises the pollutant's diffusion barrier. Fish have frequently been utilised as bio-indicators of metal contamination. Fish muscle tissue is the most commonly utilised sample for examination since it is a prime target for metal accumulation and the majority of the fish is edible. Because muscles do not actively acquire HMs and appear to have a very quick decontamination rate, they are the primary location for POPs (persistent organic pollutants) (such as pesticides, PAHs, PCBs, etc.) accumulation, while Cd accumulation is observed lowest in fish muscles.

Fish exposed to heavy metals had livers that displayed oedema, nuclear pyros is, and degeneration in many types of liver cells, as well as congestion of the central vein. Any foreign molecule that enters the body through the portal circulation will affect the liver, which will subsequently be damaged. The liver is a crucial organ for detoxification because it breaks down harmful chemicals and drug metabolites. Hepatocytes' endoplasmic reticulum carries out this breakdown. The hepatic tissue underwent several modifications, including hyperplasia and fatty infiltration in specific locations. Due to the organs' propensity for accumulating heavy metals, different fish organs are used to absorb heavymetals. Numerous heavymetals are concentrated during this process in various body organs to varying degrees.

The current study also focuses on ascertaining the toxicological effects of acute Cadmium (Cd) exposure on the main carp, *Catla catla* serum, and its biochemical parameters. According to this study, *Catla catla* serum biochemical parameters are significantly impacted by the presence of hazardous metals in the aquatic environment. Even though fish resist the initial attack of the poisons because of their protective adaptations, the damage induced by progressive exposure, even in tiny amounts, is manifested at later stages when the organism's resistance weakens owing to ageing. The condition of the test organism and how it reacts to the amount of metal that enters its body, as well as the degree of retention and the rate of elimination, all have an impact on the toxic effect of heavy metals.

Acute and sub-acute exposure to Cadmium may result in a variety of biochemical alterations, including modifications to the metabolism of carbohydrates; alterations in haematology; and alterations in the capacity of blood proteins to bind Cadmium. An increase or decrease in blood parameters is considered to be an indication of disease, stress from the environment, or tissue destruction. The possibility exists that changes in oxygen levels and water temperature might have an impact on fish haematological parameters.

The impact of microhabitat, environmental factors, ambient temperature, nutritional status, and potential seasonal variations on the fish's haematological parameters will require more investigation. The scarcity of trustworthy references in their native environment has been one of the challenges in determining the condition of fish population proliferation.

Chapter 2

REVIEW OF LITERATURE

Everyone on earth is in danger from the pollution of the air, water, and soil in unforeseen ways. Since a few decades ago, there has been concern about the numerous pollutants that are damaging freshwater systems. The biological balance of the surrounding environment and the biological diversity of the aquatic ecosystem may both be significantly impacted by heavy metal toxicity.

(Susanta N., Madhumita B.,2013) .

Heavy metal pollution of natural aquatic resources caused by anthropogenic sources, such as industrial, residential, and other activities, has been a major problem for the past few decades (Basis, U. E., Lokhande M.V., 2017).

Natural water contains a variety of impurities that are introduced into aquatic systems through a wide range of processes, such as the weathering of rocks and soils, the leaching of soils, the dissolution of aerosol particles from the atmosphere, and several human activities, such as metal mining, processing, and use. (Adeyeye,1994).

It depends on whether a pollutant will be bio-transformed or excreted since various pollutants in water resources have varying chemical characteristics. A multitude of processes, including soil erosion, runoff, the deposition of dust and aerosol, sediments, and the discharge of wastewater, can cause heavy metals to accumulate in aquatic organisms. (Goswani et al, 2016).

The concentration of heavy metals in fish tissue may vary due to differences in the metal content and chemical properties of the water from which the fish were harvested. (Basis, U. E., Lokhande M.., 2013). The quantity of metal in the water and the length of exposure determine how much heavy metal builds up in fish tissue. It is critical to analyse the levels of heavy metals in commercial fish to determine possible danger factors for human health.. The primary sources of metal contamination have been identified as being industry, mining, modern agriculture, household waste, and motor traffic. (Nanda P., 2014).

Cadmium (Cd) is a chemical element with an atomic number of 48. In addition to cadmium sulphate, the ionic form of cadmium (Cd2+) is frequently coupled with the ions of oxygen, chlorine, or sulphur ($CdSO_4$). This group IIB metal is soft, bluish-white, and has an atomic weight of 112.41 and a specific gravity of 8.65. Cd is one of the most hazardous HMs and a tissue-contaminating environmental agent (Okocha, R.C et al, 2011).

Twenty-three of the periodic table's 35 metals are heavy metals. Cd is one of the dangerous heavy metals that are naturally present in the environment in considerable amounts, but its level is increasing as a result of pollution from anthropogenic activities. Due to its non-corrosive nature, cadmium is commonly utilised in the creation of nickel-cadmium batteries; the metal and mining industries; dentistry; paints and dyes; cement; and phosphate fertilizers, among other things. There may be a connection between the high cadmium level in some phosphate fertilizers, like superphosphate fertilizers, and the fact that a variety of phosphate rocks naturally contain cadmium (Kamaraju S. et al,2018).

It is an element that naturally exists in the crust of the earth's crust and was ranked seventh on the "Top 20 list" by ASTDR (ASTDR, 1999). Due to its presence in common agricultural products including pesticides, fungicides, sludge, and commercial fertilizers, the percentage of cadmium in the upper soil has been rising. Additional sources of Cd contamination include motor oil, electroplating, dental alloys, and exhaust. Therefore, anthropogenic activities have enhanced the environment's Cd amplification. 10% of the entire amount of Cd in the environment comes from natural sources, while the other 90% comes from human activities. The majority of natural emissions come from volcanic activity (62%), which is also responsible for 25% of airborne soil particles and 2% of forest fire emissions.(Okada et al, 1997).

Heavy metals can be detrimental due to their ability to accumulate in the environment and their resistance to bio control. Heavy metals can accumulate in soft tissue and turn poisonous if the body does not metabolise them. K. Sobha et al. (2007).

Heavy metals are important aquatic environmental pollutants all around the world. The biological degradation of heavy metals does not result in their destruction. The global pollution of aquatic systems by heavy metals has caught the attention of researchers (Datta ,H. M. et al 2006). Cadmium is a heavy metal that is not required. It is one of the worst environmental contaminants. In the 1970s, cadmium, a widespread neurotoxic pollutant, was one of the heavy metals most often used. (Moore et al., 1989).

It has been reported that certain heavy metals are frequently consumed by food, ingested by particles, or both. Additionally, they are continuously absorbed onto tissue and membrane surfaces as well as transferred as ions across lipophilic membranes like the gills. (Yasmeen, S. et al, 2021).
Heavy metals have an immediate effect on the tissues and may interact with cell membranes. A higher concentration of dangerous metals in the water environment can negatively impact aquatic species at the cellular or molecular level, which eventually results in a change in the animals' biochemical makeup and behaviour (Holden, 1973; Kumar et al., 2019).

Since different organisms respond differently to the same amount of a poisonous agent, toxicity is species-specific . The possible toxicological effects of substances on organisms are identified and estimated through toxicity bioassays. These tests offer a data set that can be used to evaluate the risk connected to an environment where the organisms exist. Several techniques, including acute toxicity tests, sub-acute toxicity tests, and chronic toxicity tests, have been developed to assess the risk and potential toxicity of chemicals to organisms. The estimate of the dose or concentration required to eradicate 50% of a sizable population of the test species is known as the LC50.(Khan, A. et al., 2016).

Fish are an excellent indicator of heavy metal toxicity in aquatic systems. Because it is consumed widely throughout the majority of the world, fish that has been tainted can be harmful to human health. According to a study by (Zhang et al. 2007), the fish gill is a highly specialised organ with a number of essential activities, as toxicants often enter the fish body through the gill surface. Various investigations have demonstrated a significant loss of gill protein, a poor oxidative metabolism of the gill, a build-up of cadmium in fish gills, and injury from exposure to cadmium compounds. Fish with cadmium exposure had histological alterations, according to (Usha, R.,1999) Gills are a sensitive bio indicator of metal pollution in aquatic settings, and pathological studies show the precise damage that heavy metals cause to show the level of contamination.

An organism's behaviour is defined as an action, conduct, or response to environmental stimuli, particularly those actions or responses that are observable (Waisberg, 2003).

The toxicity description varies and depends on the type of test organism used with each exposure time as well as environmental factors including temperature, PH, dissolved oxygen, hardness, and organic chemical concentration. The amount of physiologically active forms of cadmium may be influenced by variables like hardness and pH, whereas the organism's tolerance level is mostly influenced by variables like temperature and dissolved oxygen. Gills serve as sensitive bio indicators of metal pollution in aquatic habitats, and pathological testing in turn reveals the level of the pollution as heavy metals selectively damage a specific organ. After being exposed to cadmium, fish gills showed damage, such as the filaments' blackening. The darkening of the gill filaments was probably brought on by the negative effects of this metal when exposed to different levels of heavy metals. The swelling of secondary gill lamellae was observed. Cyst formation was also observed. After exposure, mucus coats the skin and gill filaments to protect them, decreasing their capacity to exchange gases between blood and water.(Vijayan,P., 2017)
The amount of water contaminants that local animals accumulate depends on their body weight. The results of this study also show that, unlike zinc, not all heavy metal accumulation in fish is inversely connected with body weight. According to (Ademorati, C.M., 1996), the size of the organism is one of the key factors influencing bioaccumulation. Body size has little bearing on the bioaccumulation of metals including chromium, cadmium, copper, and zinc, according to studies on (Silene and Sandra , 2009).

Industrial waste that contains heavy metals seriously harms fish. Embryos were less understanding than adults. Adult females had more sensitivity than adult males (Sengar,N.S, et al., 2016).

When any foreign molecule hits the liver via the portal circulation, the organ is harmed. The liver is an essential detoxifying organ that eliminates poisonous substances and drug metabolites. This breakdown occurs in the endoplasmic reticulum of hepatocytes. Two changes in the hepatic tissue were hyperplasia and fatty infiltration at specific sites (Kumari, N. et al., 1989). In the present investigation, exposure to cadmium caused damage to a few hepatocytes with completely vacuolated nuclei, necrosis in blood cells, pyknosis, extra vacuoles, and hepatocyte necrosis.

According to findings, green algae species that were exposed to Cdcl2 developed cadmium granules inside their mitochondria. Additionally, mitochondrial degradation and vacuolization were caused by cadmium. The European Community has added cadmium, a heavy element that is not necessary, to its "Black List" (Mason, 1996). It has also been labelled as a b-class (soft) metal (Da-Silva and Williums, 1991).

Cadmium induces a number of biochemical changes at acute and subacute levels. These include changes in enzymatic activity modification of carbohydrate metabolism, haematological changes, and changes in the ability of blood protein to bind cadmium. (Mohanty, B.P., 2013)

Due to its non-corrosive and cumulative nature, it has particularly significant applications in electroplating and galvanising. It also functions as a pigment for paints and plastics, as well as a cathode material for nickel-cadmium batteries. Due to anthropogenic activities such as smelting, the use of phosphate fertilisers, pigment, cigarette smoke, and cars, among others, cadmium has infiltrated the food chains of humans and animals. (Okada et al., 1997; Kumar et al., 2019). With a cadmium concentration of 0.01 to 0.1 PPM, many species also produce less ATP, chlorophyll, and oxygen. It has been suggested that cadmium reduces oxygen consumption.

As the site for gaseous exchange, ionic control, acid-base balance, and nitrogenous waste excretion in fish, the gill epithelium has a significant potential to be exploited as a model system for understanding the many epithelial diseases brought on by toxic exposure. Despite fish having kidneys, the branchial epithelium is a flexible tissue that serves a variety of excretory and regulatory roles (Cicik, B. et al, 2005). Through the lungs and the digestive tract, the body can absorb cadmium from the environment. Cd is like many other heavy metals, competes with nutrient elements for binding sites in transport and storage proteins, metalloenzymes, and receptors. These critical trace elements include Fe^{2+}, Zn^{2+}, Cu^{2+}, and Ca^{2+}. It serves as a stressor, causing physiological changes resembling those seen during hunger. According to the paper, cadmium slows glycolysis by specifically inhibiting particular proteins like phosphofructokinase and lactate dehydrogenase. Additionally, it influences the organism's lipid and glycogen levels. The ability of Cd to produce reactive oxygen species—which may serve as signalling molecules in the induction of gene expression and apoptosis—depletes endogenous radical scavengers and damages a variety of transport proteins, including the Na+/K+ ATPase—making it toxic.(Waisberg et al. 2003).

Fish suffering from acute toxicity to cadmium are typically anoxic as a result of gill injury, which prevents fish from getting oxygen from the water. Similarly, it has been claimed that cadmium inhibits the action of acetylcholine esterase, which results in respiratory system depression and death by paralysis of the respiratory muscles.

However, Cearley and Coleman (1979) proposed that damage to the fish's iron-regulating mechanism should be prioritised over respiratory problems or nervous system deterioration. is more likely to be what killed the person.

The transfer of cadmium through perfused gills was studied by (Part and Svanberg,1981) who discovered that a tenfold rise in cadmium concentration led to a hundredfold increase in cadmium transfer. By adding a powerful complexing agent to the solution, the amount of cadmium that was transferred via the gills was reduced, suggesting that the concentration of free cadmium ions affects transfer.

The effects of cadmium on the biochemical, histological, haematological, and behavioural responses of the liver have been the subject of numerous investigations. (Kumda et al., 1972)

The current issue plays a crucial function in the control of gas exchange, acid balance, nitrogenous waste, and excretion, highlighting its critical significance at the environment's interface (Krishnani, K. K., et al , 2003).

This demonstrates a key point of entry for heavy metals, where large concentrations can be harmful. Heavy metal detoxification results in a significant build up in fish liver. Consequently, the liver's function in both storing and detoxifying highly accumulated heavy metals (Mckim et al., 2001).

The tendency of matter to bind to various molecular groups found within the cell of the organism as well as the degree of exposure to the metal as impacted by metabolic traits, position, and food chain could be responsible for variations in metal concentration in different sections of an organism. This may help to explain why all fish species' gills contain more metal than their muscles do. (Nanda, P. et al 2014).

Due to their affinity, different organs of fresh people can absorb heavy metals. Many of these heavy metals are accumulated at various levels in the body's organs during this process. (Rao and Padmaja, 2000).

Among the factorial facilitators are the total amount, the bioavailability of each metal in the environment, and the process for absorption, storage, and excretion. As a result, the less readily available metal is, the less it will be accumulated. Most aquatic life is exposed to individual and combined contaminants both physically and orally. Fish bio-uptake of heavy metals frequently occurs in the nearby water passively or with assistance (Regan,L., 1993).

According to (Perera, P.A.C.T. et al. 2015), fish are more susceptible to elevated Cd levels during the reproductive process and in the early stages of life. By disrupting the hypothalamic-pituitary system or other endocrine systems and limiting hormone synthesis, HMs may have their detrimental effects on fish reproduction and gamete formation (Ebrahimi, M. et al., 2011). Exposure to various HMs reduces fish population fertility, either directly by damaging sperm and ova or indirectly by accumulating in the reproductive organs (Rurangwa, E ,1998).

Because gills come into direct contact with water, the amount of metal there reflects the amount of metal in the water where the fish dwell. High metal concentrations in the liver indicate that metals are being stored there for detoxification (Vosyliene, 1999). Numerous researchers have noted that cadmium causes gill necrosis in fish, which may also contribute to decreased oxygen consumption and increased ventilation frequency . The respiratory distress is made worse by the fish exposed to cadmium's decreased haemoglobin, hematocrit, and erythrocyte levels (Yasmeen, S. et al ,2021).

Diverse fish species experience considerable histopathological, biochemical, and physiological changes as a result of the disruption of their biological activities. Fish with heavy metal toxicity may have changes in their physiologic processes, including growth, reproduction, and mortality (Wardah, H., 2018).

Heavy metals pose a major threat to many aquatic animals and can alter their genetic, physiological, biochemical, and behavioural make-up (Scott & Sloman, 2004). Compared to other aquatic creatures, fish are more susceptible to heavy metal contamination. The hypothalamic-pituitary-adrenal axis (HPI axis), which is widely dispersed in fish head kidneys, secretes the steroid. Although sublethal pollution may only make fish unwell, dead fish are a blatant sign of excessively contaminated water. Extremely low pollution levels may not immediately impact fish because they would not show any signs of sickness, but they may lower fish reproduction, leading to a long-term decline and eventual extinction of this priceless natural resource (Krishnani et al., 2003; Burger and Gochfeld, 2005). Such low-level pollution may affect reproduction either directly on the reproductive organs or indirectly by building up on the free gametes (sperm or ovum) that are released into the water.Skin accumulates more slowly than other tissues like the kidney, liver, and gills because it is the sole tissue with the largest surface area and direct contact with the exposure medium. Given that people eat their skin along with their muscles, skin is a very important tissue from an accumulation aspect (Yousafzai and Shakoori, 2006).

It is necessary to examine the effects of different metals in combinations and to understand how the different metals could combine to produce antagonistic, additive, or synergistic effects (Marr et al., 1998).

The principal gill lamellae are leaf-like structures that are laterally compressed and alternately connected to each side of the interbranchial septum. Secondary gill lamellae that are perpendicular to the long axis of each primary gill lamellae are present on both of its sides. The primary gill lamellae are firmly attached to the gill ray and consist of a central core of cartilaginous rods and linings of epithelial cells. (Triebskorn, R. et al. ,2008) noted that mild to severe hyperplasia was found in almost all fish from the lower Amur River basin studied and that hyperplasia, the most common type of gill pathology, was found in most of the fish studied. Numerous papers have discussed hyperplasia, which is thought to be a non-specific defensive response to heavy metals and mixed pollutants. The secondary defence was suggested by the appearance of inflammatory cells in the gill tissue (moderate to pronounced). Due to the size of the surface area that is in touch with the environment outside and the thin membranes that separate the internal and external media, fish gills are major target organs for heavy metals (Ferrari, L. et al., 2009). As a result, the heavy metals present in the water are rapidly exposed to the gills, causing morphological and functional changes. Modifications to the gill structure impair the proper operation of crucial physiological processes such as gas and ion exchanges, osmoregulation, the excretion of nitrogenous waste, and acid-base balance.

Additionally, according to an experimental study, the gills act as a cadmium storage site (Allen 1995). (Ramesh and Nagaranjan, 2007). (Wong and Wong ,2000) looked at the morphological and biochemical changes in fish gills following experimental cadmium exposure. They added that fish with hypocalcemia were most commonly affected by cadmium toxicity, which was most common in chloride cells.

Using fish blood as a technique for monitoring the environment and illness changes. It is therefore essential to determine the functional and systemic health of fish that have been exposed to pollutants (Hassan et al. 2018). Metals can alter the haematological markers in fish. Blood parameter elevations or decreases are thought to be symptoms of disease, environmental stress, or tissue damage (Li et al. 2010; Hassan et al. 2018).

Fish haematological indicators can be changed by metals. Blood parameter variations are thought to be a sign of illness, stress from the environment, or tissue damage (Li et al. 2010; Hassan et al. 2018).

Previous research has demonstrated that the liver of fish exposed to heavy metals exhibited edoema, nuclear pyknosis, and degeneration in hepatic cells of several species, as well as congestion of the central vein (Kaoud and El-Dahshan ,2010).

Fish have a close link with their environment, and variations in fish haematology are significantly influenced by water quality .A fish under stress and expending its energy reserves will have a high blood glucose concentration, which is one of the most sensitive indicators of an organism's stressed state (Vosyliene, 1999). Because of their critical role in storing excess energy, proteins are the most significant organic component of fish tissues.

Fish protein content used to steadily decrease when exposed to sub-lethal doses of the heavy metal cadmium. Pollutants may not enter the liver directly, but they do pass through physiological fluids and affect it indirectly. The liver also functions as a sensitive poison detector. Fish exposed to different toxicants exhibited decreased tissue protein levels (Baskaran and Palanisamy ,1990). It has been determined that the fish's liver, kidney, gills, and blood have the highest protein concentrations. The liver's greater protein content may be due to the higher concentration of enzymes in the tissue. The liver is the principal site for protein synthesis and metabolism, so hepatic tissue has a higher protein concentration. The principal site for protein synthesis and metabolism is the liver, and as there are more enzymes in the liver tissue, it may have higher protein content. Intensive proteolysis in those tissue or tissues from cadmium-toxinated fish that are inhibiting protein synthesis may be the source of the decrease in protein content in all tissues. in the 1990's (Baskaran,and Palanisamy, 1990).

The reduced protein content indicated proteolysis in tissue, which forms amino acids and is used in the TCA cycle for energy production during stress conditions. Fish accumulation of chemicals, especially those with poor water solubility, occurs because of the very intimate contact with the medium that carries the chemicals in solution or suspension and also because fish have to extract oxygen from the medium by passing enormous volumes of water over the gills. The gills, skin, and digestive tract are potential sites of absorption of water-borne chemicals (Vinodhini and Narayanan, 2008). The chemicals, once absorbed, are transported by the blood to either a storage point such as bone or to the liver for transportation.

According to (Stebbing and Fandino ,1983), the complexity of heavy metals is largely to blame for the negative biological impact they have on aquatic life. Fish die when the quantity of toxicants in the body of water is excessively high. Thus, the conclusion of toxicological investigations was formerly considered to be the death of an organism (Jones and Reynolds, 1997).
Overall, the effects of trace metal poisoning on aquatic ecosystems include a decline in biological species variety and richness as well as a shift in species composition (Javed, M. 2005).

Chapter 3

Materials and Methods

A. Material
1.Test fish –

In this research, a *Catla catla* was chosen as the test subject. Since the beginning of human history, fish, which make up the biggest category of vertebrate animals, have been consumed as food, being surface feeders with great consumer preference and regarded as the greatest dietary supplement with adequate amounts of crucial elements like protein and fat. Apart from its nutritional content, fish food is distributed for its flavour and ease of digestion. It contains vitamins A, D, E, and K as well as minerals like calcium and phosphorus.

In India, 9.6 million hectares of inland water or rivers provide about 7 million tonnes of freshwater fish each year. Ponds, lakes, and canals make up this field, which has 35% major carp. India has already created a strategic plan for increasing acreage and productivity to double the amount of freshwater aquaculture produced. *Catla catla* production in India is likely to continuously increase because it is a major element of the carp polyculture system. Production of farmed *Catla catla* is also anticipated to increase. Several tropical Southeast Asian and Middle Eastern nations have taken notice of the Indian large carp's great development potential.

Basic Features

Short and deep body, slightly compressed laterally, with a depth greater than half the length of the head; large head, with a depth greater than half the length of the head; The body is covered in conspicuously large cycloid scales; the head lacks scales; the snout is bluntly rounded; the eyes are large and visible from the underside of the head; there are no barbels; The upper lip is non-existent; the lower lip is very thick; the mouth is broad and curved up with a noticeable protruding lower jaw; the lower jaw lacks a conspicuous process and a moveable symphysis; 5.3.2/2.3.5; A dorsal fin with 14 to 16 branches and simple, non-osseous rays that are inserted just in front of the pelvic fins; a short anal fin; long pectoral fins that reach the pelvic fins; a forked caudal fin; and a lateral line with 40 to 43 scales. The fins are dark and the body is greyish on the back and flanks.

Study Area-
Site-1

The Bilawali Tank is located on Khandwa Road, southwest of Indore, Madhya Pradesh. Tank's catchment area is 290 acres (1.17 square kilometers) In the past, the tank supplied water to the textile industry. Today, the tank serves a specific area's needs for its numerous applications, including drinking, fish cultivation, etc.

Site – 2

Sirpur Lake is located in Indore-Dhar road. The lake and its surrounding protected area have a combined area of 800 acres (around 3.6 square kilometres) early in the 20th century; the Holkar's of Indore State built Sirpur Lake. Following India's independence and the dissolution of the royal houses, religious sites began to spring up everywhere around the lake, and as time went on, the people who lived nearby began to infringe upon it. The ecology of the lake was nearly destroyed by illegal activities including fishing, poaching, cattle grazing, waste dumping, etc.

B. Methods

Physicochemical analysis of water –

Different physicochemical characteristics must be used to test the water. The primary factor in choosing the parameters for a water test is the intended use of the water and the degree to which its quality and purity are required. Different kinds of floating, dissolving, suspended, microbiological, and bacteriological pollutants are present in water. Physical and chemical tests must be carried out to evaluate the substance's BOD, COD, dissolved oxygen content, alkalinity, hardness, and other characteristics. Physical tests should be carried out to evaluate the substance's temperature, colour, odour, pH, turbidity, and TDS. Water should be tested for trace metals, heavy metals, and organic contaminants, such as pesticide residue. Water quality is periodically checked using the following physical and chemical characteristics:

1. **pH:** Water's pH is the most important factor in determining how corrosive it is. Different dissolved gases and solids cause pH to fluctuate. The more acidic the water is, the lower the pH value. Total alkalinity and electrical conductivity both showed a positive correlation with pH. Low oxygen levels corresponded with high temperatures throughout the summer due to diminished photosynthetic activity and the absorption of carbon dioxide and bicarbonates, which ultimately cause pH to rise. The pH of water can alter due to several circumstances. The higher pH values indicate that the change in physicochemical conditions has a greater impact on carbon dioxide and carbonate-bicarbonate balance.
   Procedure: Take about 100 ml of sample, add eight 0.5 ml of saturated KCl solution, and use a pH metre to measure the pH at 25° C.
2. **Temperature:** In a well-established system, the water temperature regulates the speed of all chemical processes and has an impact on fish immunity, development, and reproduction. Fish can die from sudden temperature changes.
   Procedure: Temperatures were recorded by using a thermometer on the spot. Use thermometer with accuracy of 0.1° C
3. **Total dissolved solids:**
   Procedure: Total dissolve solids are those that remain in water after it has been filtered through pores with a diameter of one micrometre. The process is carried out by heating the sample in the oven at 103° C until the evaporated dish has dried.
4. **BOD:** Organic material contamination in water. Water pollution is measured by BOD, which is expressed in mg/L. The quantity of dissolved oxygen necessary for the metabolic breakdown of organic molecules and the oxidation of some inorganic elements is known as BOD (e.g., iron, sulphites).
   Procedure: One is initially measured by fixing 1 ml of $MnSO_4$ in first bottle, and then add alkaline-iodide-azide to the other incubate in the lab for five days at 20° C, then execute bubble by inverting many times.when ppt is settled half the bottle volume add 1ml of $H_2SO_4$and then titrate with N/40 Na_2SO_3.Add starch as indicator,the blue colour disappear. This is an indicator of how much oxygen was used by microbes to break down the organic material in the sample while it was incubating.
5. **Acidity:** The amount of acid in a solution is measured as acidity. The ability of water to quantitatively neutralise a strong base to a particular pH level is referred to as acidity. Mineral acids, carbon dioxide, and hydrolysed salts like ferric and aluminium sulphates are the main causes of water acidity. Acids can have an impact on a variety of biological, chemical, and corrosion processes. When dissolved in water, carbon dioxide from the atmosphere or aquatic creatures' respiration generates acidity by creating carbonic acid (H_2CO_3).
   Procedure: Titration with standard sodium hydroxide (0.02 N) and phenolphthalein as an indicator is used to measure the acidity level.
6. **Alkalinity:** The sum of all titratable bases determines the alkalinity of water, which is its ability to neutralise acids. To calculate the amount of lime and soda required for water softening (e.g., for corrosion control in conditioning the boiler feed water), the alkalinity of the water must be measured. The main contributors to water's alkalinity are the ions hydroxide (OH), bicarbonate (HCO_3), carbonate (CO_3), or a combination of two of these ions.

Procedure: Titration with a standard acid solution (H_2SO_4 of 0.02 N) employing selective indicators is used to measure alkalinity (methyl orange or phenolphthalein).

7. **Hardness** is due to the presence of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in a water supply. It is expressed. Hardness minerals exist to some degree in every water supply

Standardization:for correction factor Pipette 25ml of standard calcium solution in a conical flask and adjust the volume to 50ml with distilled water. Add 1ml buffer solution. Add 1 to 2 drops of indicator, titrate slowly with continuous stirring until the reddish tinge disappears, adding last few drops at 3 to 5 interval. At the end point the colour is sky blue.

Procedure: Pipette 50 ml of water sample in 150 beaker .Add 1 ml hydroxylamine hydrochloride solution. Add 1 to 2 ml buffer solution so as to achieve pH of 10.0 to 10.1 and add 2or 3 ml of eriochrome black t solution, titrate with standard EDTA solution. Red and purple colour disappears and solution is clear sky blue in colour. Blank titration carried out in a similar way.

Calculation: Total hardness $CaCO_3$ mg/l = $\frac{1000 (V1 - V2) \times CF}{V3}$

8. **Chloride** source could be a water additive used to control microbes and disinfect**.** It is determined by titrating a known volume of material with a standardised silver nitrate solution while employing an indicator such as potassium chromate in water or eosin/fluorescein in alcohol. The former creates a red-coloured combination with silver as soon as the chlorides are precipitated out of the solution, whereas the latter is an adsorption indicator.

Procedure- For 10 ml of sample and 1 ml of 2 M nitric acid and 0.2 ml of 0.1 M silver nitrate, the appearance of the solution does not change for at least 15 minutes.

9. **Cadmium**: Test solution: In a glass evaporating dish, vaporise 150 ml to 15 ml of the solution in 12 ml increments by placing the dish in a water bath. Pipette 10 ml of the lead standard solution at 1 ppm pb.

Procedure- Mix 2.0 ml of the test solution into the cylinder containing the standard solution. Add 2 ml of the acetate buffer pH 3.5 to each cylinder, mix it, and then add 1.2 ml of the thioacetamide reagent. After allowing the mixture to stand for two minutes, look downward over a white surface; the colour produced by the test solution is not more intense than the colour produced by the standard solution.

Heavy metal detection and chloride content in fish tissues

Procedure- On the same day, fish were carried to the lab and dissected into separate organs (muscles, gills, and liver) using clean stainless steel tools according to FAO guidelines. To obtain a sample for analysis, the organs from five individuals of fish species from both sites were collected. To achieve a consistent weight, a 48-hour drying period at 110 °C with individual Petri dishes for each tissue was used. Weighed and placed in a different test tube was 0.5g of each dried tissue. Each sample was then dissolved with nitric acid (HNO_3) and per chloric acid ($HClO_4$) in a 2:1 ratio and permitted to dry at room temperature for the next day. The contents of the samples were heated for around 2 hours until all the tissues had broken down in a water bath that was heated to 100 °C. After allowing the digest to cool, 5 mL of distilled water was added. The mixture was transferred to a 25 ml volumetric flask and diluted to the desired strength with 1 percent HNO_3 (FAO, 1984).The digests were maintained in plastic bottles, and an atomic absorption spectrophotometer was used to measure the accumulated heavy metal content in mg/g dry weight.

Chloride :

Procedure- In fish tissue is determined by titrating a known volume of material with a standardised silver nitrate solution while employing an indicator such as potassium chromate in water or eosin/fluorescein in alcohol. The former creates a red-coloured combination with silver as soon as the chlorides are precipitated out of the solution, whereas the latter indication is an adsorption indicator.

Biochemical analysis from fish blood serum

Procedure- A plastic disposable syringe equipped with a heparin-pre-moistened tip was used for the cardiac puncture, which was used to obtain blood from the heart area. Blood was drawn from all the fish, and the individual heparinized plastic vials were then put on ice. For the purpose of estimating plasma glucose and protein, pooled blood samples were centrifuged for 15 min at 10,000 rpm. Plasma was then removed and put into clean vials. The o-toluidine technique (Cooper and McDaniel, 1970) was used to measure plasma glucose, while the Lowry et al. method was used to quantify plasma total protein (1952).

Histological slide preparation and study of fish tissues-

The gills, liver, and muscles of each group of fish were removed during dissection. They were fixed by FAA (Formalin, Acetic acid, and alcohol). Tetrahydrofuran was used for clearing after alcohol dehydration to prepare the fixed tissues. Transverse and longitudinal serial slices of 5-8 mm were obtained after they had been immersed in paraffin wax. The tissue slices were stained with hematoxylin and eosin. The portions underwent two ten-minute long xylene changes to deparaffinize them. The hydrated sections were then distinguished in acid alcohol by simply dipping, staining with haematoxylin for five minutes, and rinsed in running water for five minutes. The portions were simply dipped in eosin after dehydrating them. The extra stain is removed by immersing for 30 seconds in 90% alcohol and then for 5 minutes in absolute alcohol. The dehydrated sections were then blotted again, cleaned in two changes of xylene over ten and fifteen minutes, and blotted again before being mounted in DPX. After being viewed under a microscope, the tissues were micro photographed.

Chapter 4

RESULTS

Physicochemical analysis of water (2016-2017)

pH-During Pre-monsoon season the mean pH values were 8.44, 8.95 ,In Post-monsoon season values were 7.92, 7.53 at Site A(Bilawali tank), Site B (Sirpur lake) respectively.

Temperature- During Pre-monsoon season the mean Temperature values were 27.30°C, 26.92 °C, in Post-monsoon season values were 19.85°C, 21.2°C at Site A(Bilawali tank), Site B (Sirpur lake) respectively.

Total dissolved solid- During Pre-monsoon season the mean Total dissolved solid values were 308 ppm , 140 ppm , in Post-monsoon season values were 287.35 ppm ,137.85 ppm at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

BOD- During Pre-monsoon season the mean BOD values were 2.6 mg/l ,2.8 mg/l , in Post- monsoon season values were 2.4 mg/l, 2.6 mg//l at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

Acidity- During Pre-monsoon season the mean Acidity values were 10.86 mg/l, 10.26 mg/l, in Post-monsoon season values were 9.70 mg/l, 8.39 mg/l at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

Alkalinity- During Pre-monsoon season the mean total Alkalinity values were 165, 152.55 (mg/l) ,In Post-monsoon season values were 81.35 ,72.7(mg/l) at Site A (Bilawali tank), Site B (Sirpur lake) respectively .

Hardness- during Pre-monsoon season the mean total Hardness values were 205, 195 (mg/l) , In Post-monsoon season values were 145,186 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

Chloride- during Pre-monsoon season the mean Chlorides values were 70.68, 68.68 (mg/l), In Post-monsoon season values were 59.03, 60.77 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

Cadmium- During Pre-monsoon season the mean Cadmium values were 24.10 , 33.18 (mg/l), In Post-monsoon season values were 23.89 , 29.74 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

(The seasonal variations in Physicochemical parameters of the freshwater ponds are mentioned in Table 1. and Graph(1- 9) for (2016-2017)

Physicochemical analysis of water (2017-2018)

pH- During Pre-monsoon season the mean pH values were 8.35,7.58 ,In Post-monsoon season values were 7.52 7.03 at Site A(Bilawali tank), Site B (Sirpur lake) respectively.

Temperature- During Pre-monsoon season the mean Temperature values were 27.19°C, 27.03°C, in Post-monsoon season values were 21.77°C, 20.15°C at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

Total dissolved solid- During Pre-monsoon season the mean Total dissolved solid values were 329.24 ,208.76, in Post-monsoon season values were 288.47, 198.79 at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

BOD- During Pre-monsoon season the mean BOD values were 2.34, 2.08, in Post-monsoon season values were 2.08 , 1.98,at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

Acidity-During Pre-monsoon season the mean Acidity values were 9.76, 10.50, in Post-monsoon season values were 8.63, 10.05, at Site A (Bilawali tank), Site B (Sirpur lake) respectively.

Alkalinity-During Pre-monsoon season the mean total Alkalinity values were 150.34 , 108.73 (mg/l), In Post-monsoon season values were 143.52 , 96.78 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake) respectively .

Hardness-During Pre-monsoon season the mean total Hardness values were 175,139.60 (mg/l), In Post-monsoon season values were 158,114.07 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

Chloride- During Pre-monsoon season the mean Chlorides values were 68.23, 56.88 (mg/l), In Post-monsoon season values were 62.45, 56.34 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

Cadmium-During Pre-monsoon season the mean Cadmium values were 25.66 , 20.52 (mg/l), In Post-monsoon season values were 20.39 , 19.34 (mg/l) at Site A (Bilawali tank), Site B (Sirpur lake).

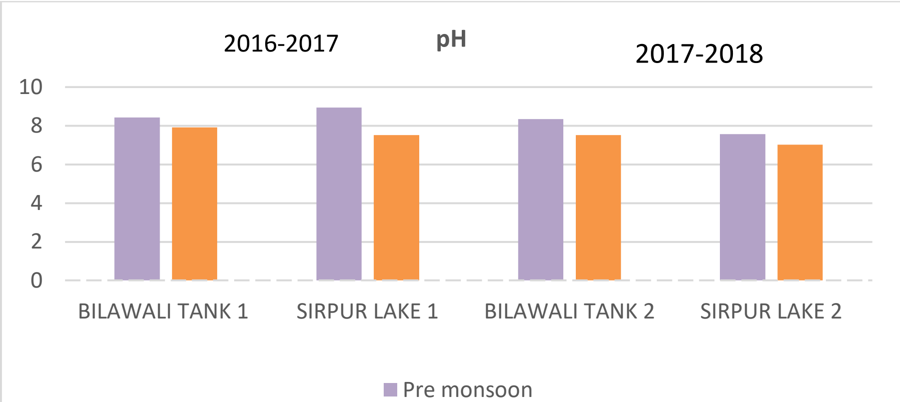
(The Seasonal variations in Physicochemical parameters of the freshwater ponds are mentioned in Table 2. and Graph (1- 9) for (2017-2018)

Table 1 Physicochemical parameters of Bilawali tank and Sirpur Lake (2016-2017)

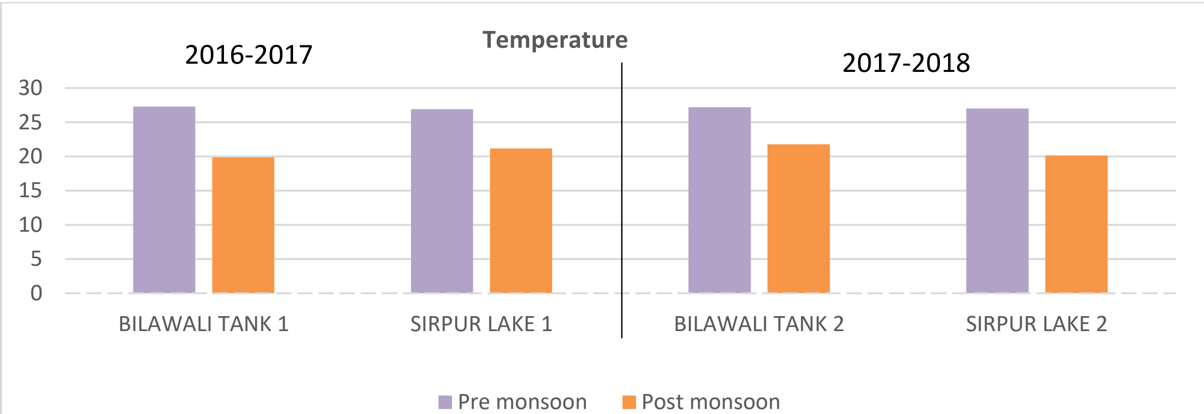
Parameters	Bilawali Tank 1		Sirpur Lake 1	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
pH	8.44	7.92	8.95	7.53
Temperature	27.30	19.85	26.92	21.2
Total dissolved solid	308ppm	287.35ppm	140ppm	137.85 ppm
BOD	2.6 mg/l	2.4 mg/litre	2.8mg/litre	2.6mg/litre
Acidity	10.86 mg/litre	9.70 mg/litre	10.26 mg/litre	8.39 mg/litre
Alkalinity	165 mg/litre	83.35 mg/litre	152.55 mg/litre	72.17 mg/litre
Hardness	205mg/l	145mg/l	195 mg/l	186 mg/l
Chloride	70.68 mg/l	59.03 mg/l	68.68 mg/l	60.77 mg/l
Cadmium	24.10 ppm	23.89 ppm	33.18 ppm	29.74 ppm

Table 2 Physicochemical parameters of Bilawali tank and Sirpur lake (2017-2018)

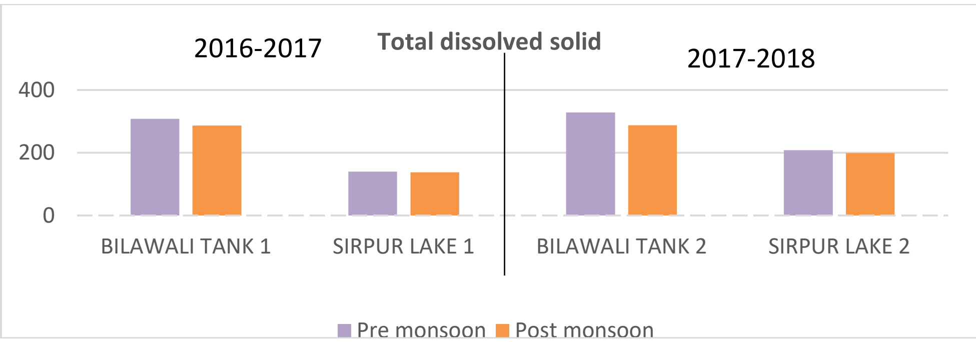
Parameters	Bilawali Tank 2		Sirpur Lake 2	
	Pre-monsoon	Post-monsoon	Pre-monsoon	Post-monsoon
pH	8.35	7.52	7.58	7.03
Temperature	27.19	21.77	27.03	20.15
Total dissolved solid	329.24 ppm	288.47 ppm	208.76 ppm	198.79 ppm
BOD	2.34 mg/l	2.08 mg/l	2.08 mg/l	1.98 mg/l
Acidity	9.76 mg/l	8.63 mg/l	10.50 mg/l	10.05 mg/l
Alkalinity	150.34	143.52	108.73	96.78
Hardness	175 mg/l	158 mg/l	139.60 mg/l	114.07 mg/l
Chloride	68.23 mg/l	62.45 mg/l	56.88 mg/l	56.34 mg/l
Cadmium	25.66 ppm	20.39 ppm	20.52 ppm	19.34 ppm



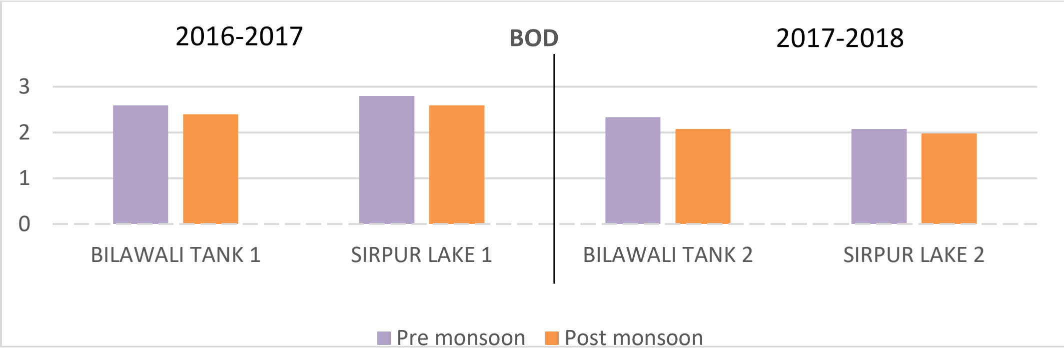
Graph 1: Seasonal comparison of pH of water sample of Bilawali tank and Sirpur lake in two consequent years shows higher level of pH during Pre-monsoon in both years.



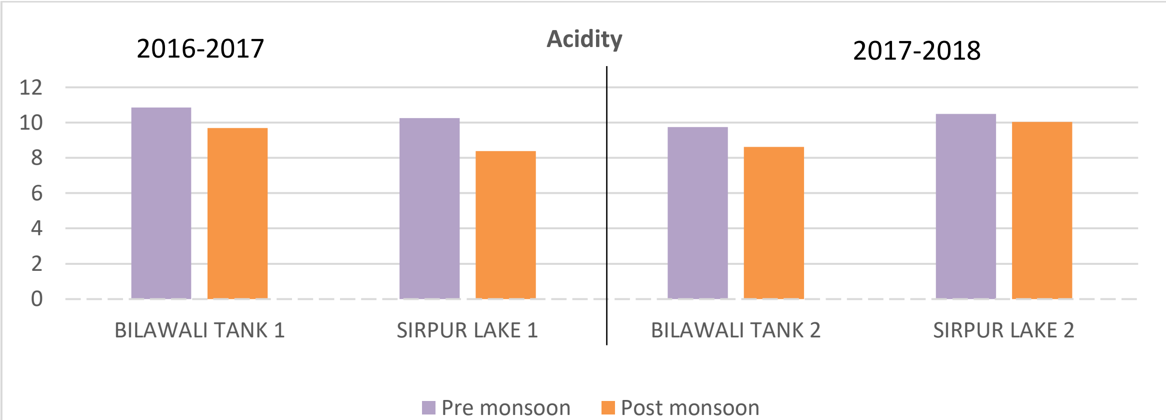
Graph 2: Seasonal comparison of Temperature of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level of temperature during Pre-monsoon in both years.



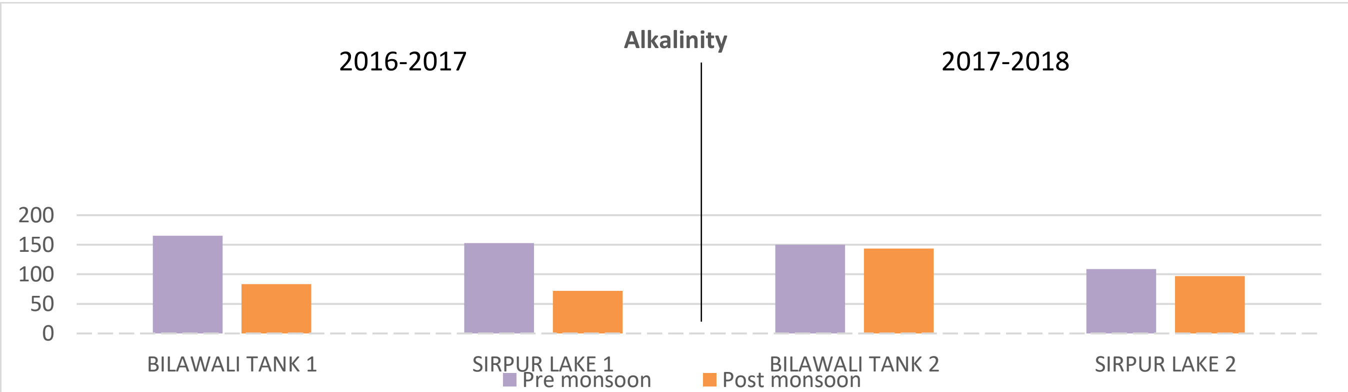
Graph 3: Seasonal comparison of Total dissolved solid of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre monsoon in both years.



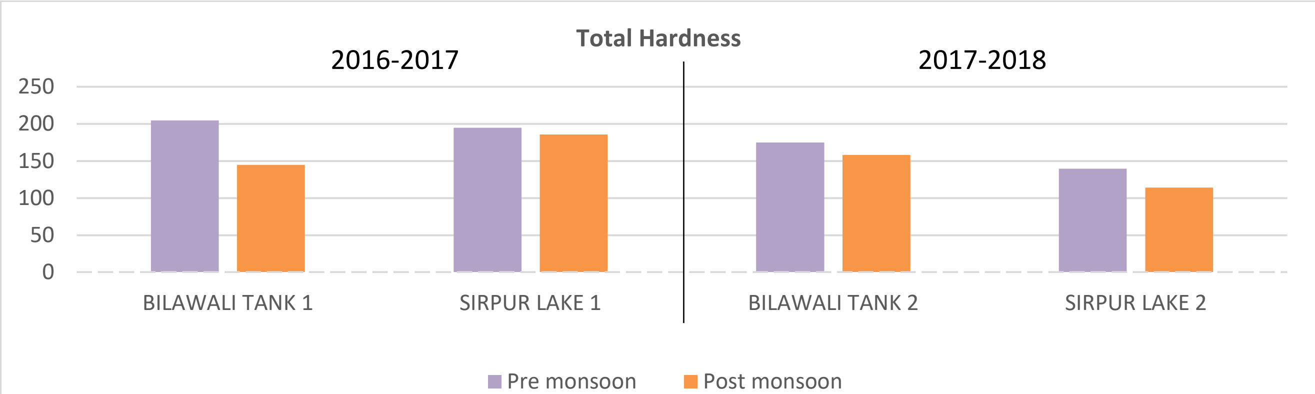
Graph 4: Seasonal comparison of BOD of water sample of Bilawali tank and Sirpur lake in two consequent years shows higher level during Pre-monsoon in both years.



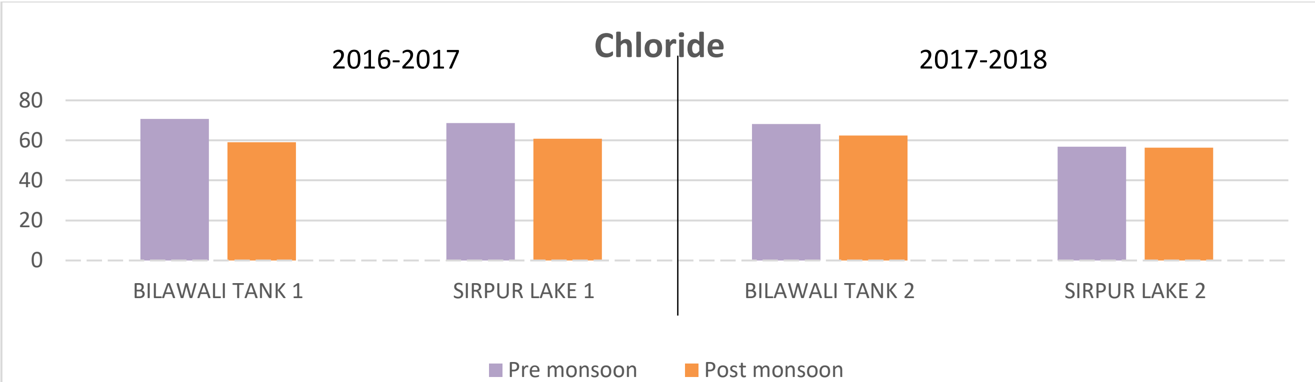
Graph 5: Seasonal comparison of acidity of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre-monsoon in both years.



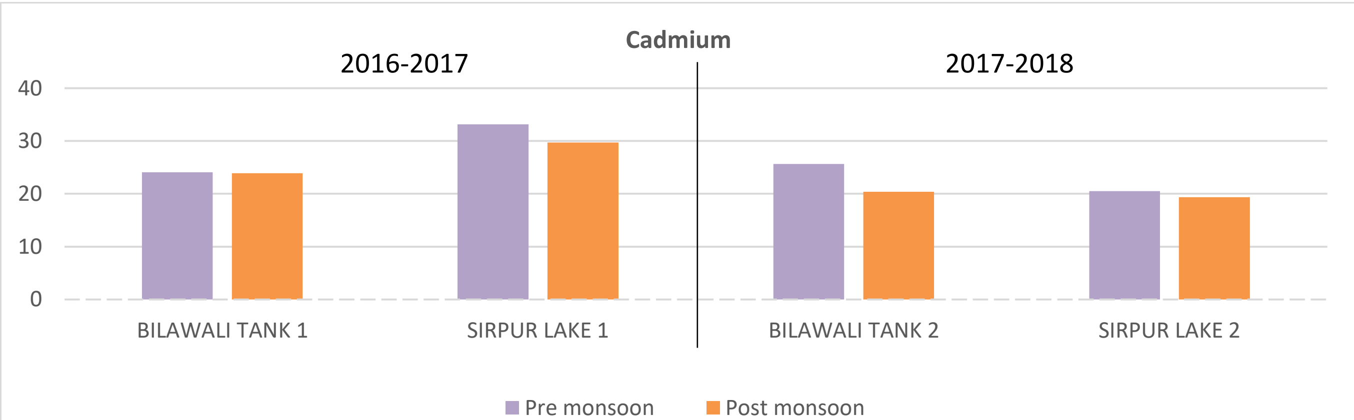
Graph 6: Seasonal comparison of Alkalinity of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre-monsoon in both years.



Graph 7: Seasonal comparison of Total Hardness of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre-monsoon in both years.



Graph 8: Seasonal comparison of Chloride of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre-monsoon in both years.



Graph 9: Seasonal comparison of Cadmium of water sample of Bilawali tank and Sirpur Lake in two consequent years shows higher level during Pre-monsoon in both years.

Heavy metal and Chloride detection in fish tissues (2016-2017)

Gills

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 6.43±2.87, 3.03±1.35, In post monsoon season values were 2.20±0.98, 1.98±0.85 at Site A (Bilawali tank), Site B (Sirpur lake).

(Chloride)- During Pre-monsoon season the mean Chlorides values were 33.14± 14.82 , 31.5±18.5, In Post-monsoon season values were 23.85±95.16 , 21.15 ±14.8 at Site A (Bilawali tank), Site B (Sirpur lake).

Muscles

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 3.12±1.39, 2.76±1.23, In Post-monsoon season values were 0.44±0.19, 0.10±0.04 at Site A (Bilawali tank), Site B (Sirpur lake).

(Chloride)- During Pre-monsoon season the mean Chlorides values were 12.94±5.78, 12.77±19.2 , In Post monsoon season values were 12.46±5.82 ,6.64±5.58 at Site A (Bilawali tank), Site B (Sirpur lake).

Liver

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 2.70±0.13, 2.80±1.25, In Post-monsoon season values were 1.21±0.5, 0.30±0.13 at Site A (Bilawali tank), Site B (Sirpur lake).

(Chloride)- During Pre-monsoon season the mean Chlorides values were 14.2±6.37, 11.5±8.07, In Post-monsoon season values were 7.03±18.4 ,9.91±1.80 at Site A (Bilawali tank), Site B (Sirpur lake).

Heavy metal and chloride detection in fish tissues (2017-2018)-

Gills

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 1.85±0.59, 1.28±0.14, In Post-monsoon season values were 0.35±0.14 , 0.52±0.17 at Site A(Bilawali tank), Site B (Sirpur lake).

(Chloride)- During Pre-monsoon season the mean Chlorides values were 24.82±4.96, 18.96±3.53, In Post-monsoon season values were 32.06±11.87 , 25.14±13.01 at Site A (Bilawali tank), Site B (Sirpur lake).

Muscles

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 0.64±0.5, 1.33±1.09, In Post-monsoon season values were 0.30±0.15 , 0.52±0.17 at Site A (Bilawali tank), Site B (Sirpur lake).

(Chloride)- During Pre-monsoon season the mean Chlorides values were 11.86±9.52 ,9.65±3.53, In Post-monsoon season values were 10.52±0.90 , 9.33±2.90 at Site A (Bilawali tank), Site B (Sirpur lake).

Liver

(Cadmium)- During Pre-monsoon season the mean Cadmium values were 7.67±0.75, 5.49±0.22, In Post-monsoon season values were 6.22±0.72 , 0.66±0.14 at Site A (Bilawali tank), Site B (Sirpur lake).

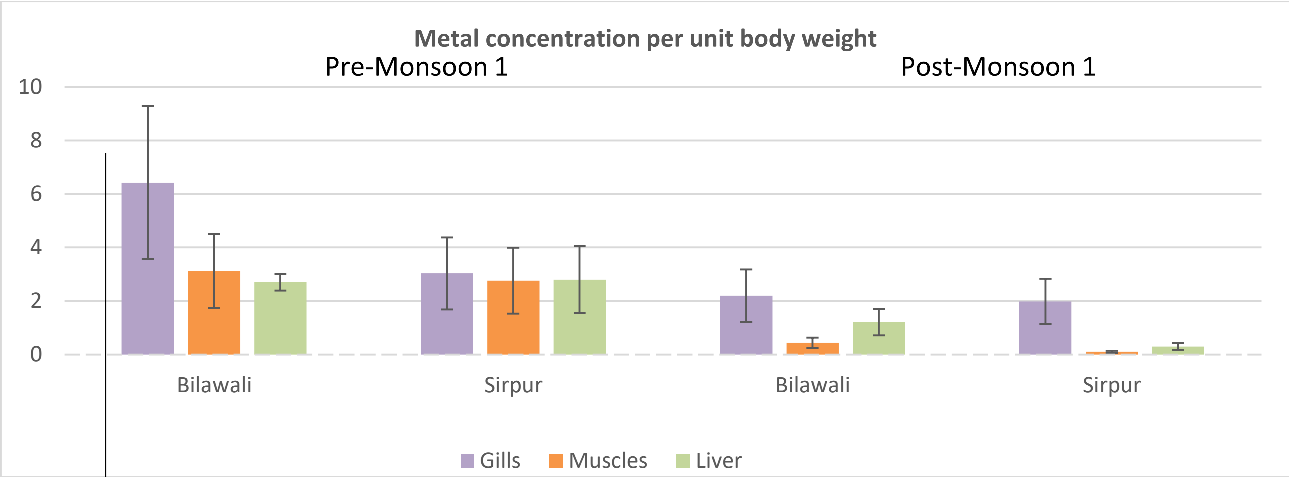
(Chloride)- During Pre-monsoon season the mean Chlorides values were 9.76±1.73 , 12.62±1.36, In Post-monsoon season values were 9.52±2.60, 0.93±0.76 at Site A (Bilawali tank), Site B (Sirpur lake).

Table 3: Seasonal variations in Cadmium and Chloride concentration in fish tissue (Gills , Muscles, Liver)(2016-2017)

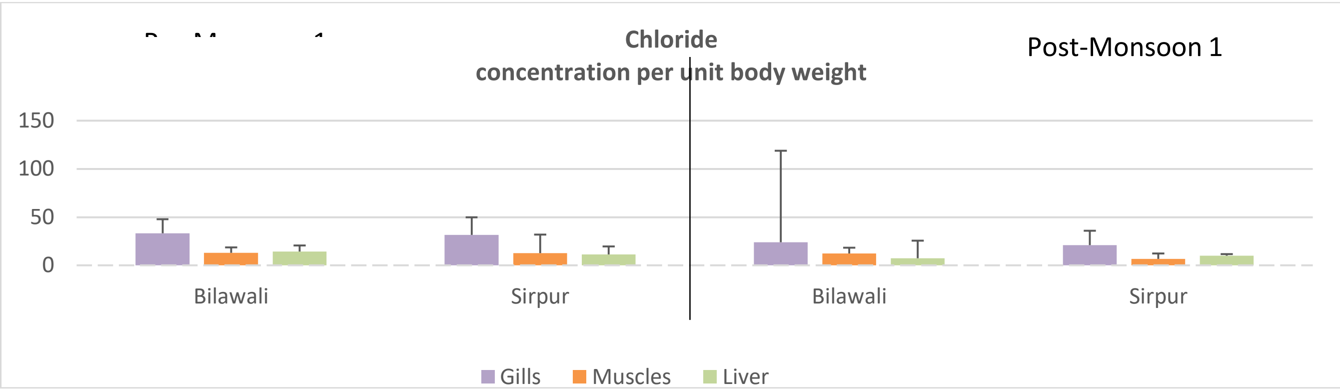
Parameters	Tissue type	Pre-Monsoon 1		Post-Monsoon 1	
		Bilawali	Sirpur	Bilawali	Sirpur
Dry weight of tissues	Gills	139.08±62.19	53.76±24.04	95.20±42.5	155.22±64.69
	Muscles	95.42±42.57	145.22±64.64	230.91±95.42	196.24±87.76
	Liver	30.08±13.08	230.91±103.2	123.22±55.10	55.82±24.96
Metal concentration per unit body weight	Gills	6.43± 2.87	3.03± 1.35	2.20±0.98	1.98± 0.85
	Muscles	3.12± 1.39	2.76± 1.23	0.44± 0.19	0.10± 0.04
	Liver	2.70± 0.31	2.80± 1.25	1.21±0.5	0.30± 0.13
Chloride concentration per unit body weight(µg/g)	Gills	33.14± 14.82	31.5± 18.5	23.85±95.16	21.15± 14.8
	Muscles	12.94±5.78	12.77± 19.2	12.46±5.82	6.64± 5.58
	Liver	14.2± 6.37	11.5± 8.07	7.30±18.4	9.91±1.80

Table 4: Seasonal variations in Cadmium and Chloride concentration in fish tissue (Gills , Muscles, Liver) (2017-2018)

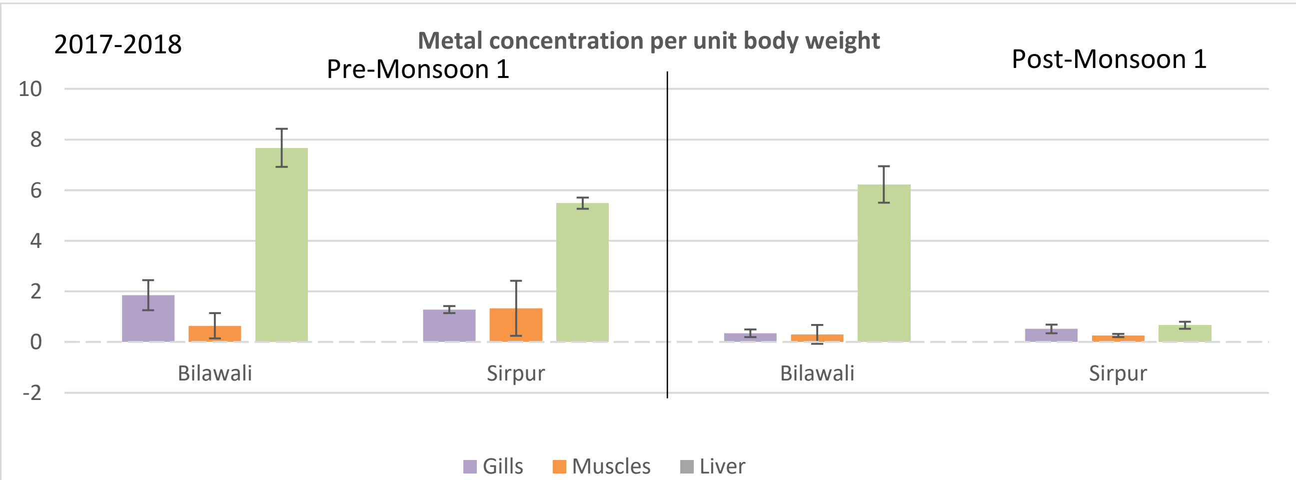
Parameters	Tissue type	Pre-monsoon 1		Post-monsoon 2	
		Bilawali	Sirpur	Bilawali	Sirpur
Dry weight of Tissues	Gills	103.22±35.10	95.42±32.57	75.82±24.96	99.22±64.64
	Muscles	86.20±22.5	120.08±62.69	133.22±59.10	85.82±14.36
	Liver	81.30±1.64	139.07±2.55	67.12±1.81	82.50±1.64
Metal Concentration per unit body weight	Gills	1.85±0.59	1.28±0.14	0.35±0.15	0.52±0.17
	Muscles	0.64±0.5	1.33±1.09	0.30±0.37	0.26±0.06
	Liver	7.67±0.75	5.49±0.22	6.22±0.72	0.66±0.14
Chloride concentration per unit body weight (µg/g)	Gills	24.82±4.96	18.96±3.53	32.06±11.87	25.14±13.01
	Muscles	11.86±9.52	9.65±5.08	10.52±0.90	9.33±2.90
	Liver	9.76±1.73	12.62±1.36	9.52±2.60	10.93±0.76



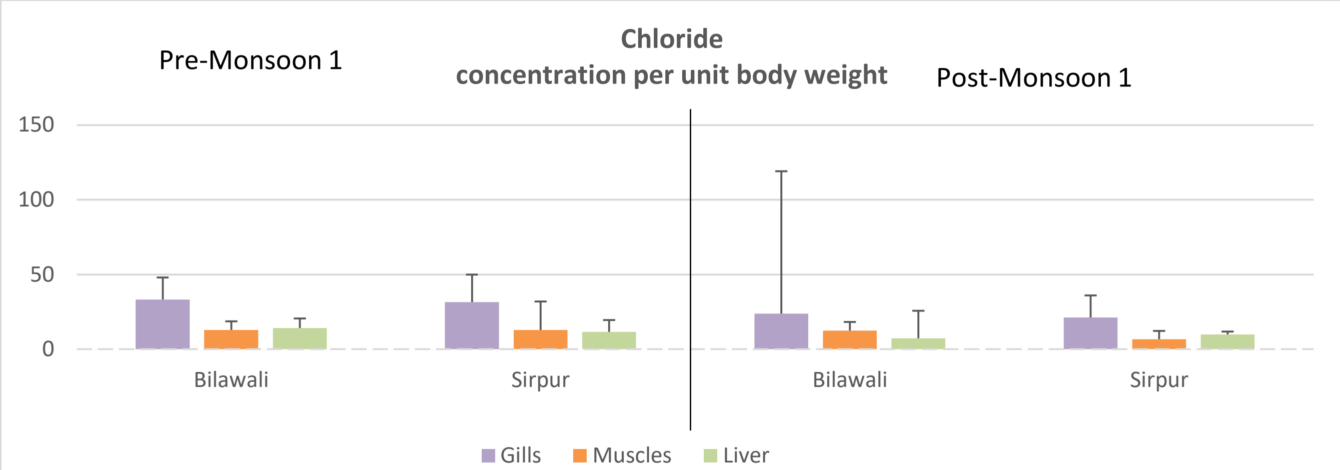
Graph 10: Seasonal variations of Cadmium concentration in fish tissues (Gills, Muscles, Liver) of Bilawali tank and Sirpur lake shows higher concentration of Cadmium in Gills during Year(2016-2017).



Graph 11: Seasonal variations of Chloride concentration in fish tissues (Gills, Muscles, Liver) in Bilawali tank and Sirpur lake shows higher concentration of Chloride in Gills during (2016-2017).



Graph 12: Seasonal variations of Cadmium concentration in fish tissues (Gills, Muscles, Liver) in Bilawali tank and Sirpur lake shows higher concentration in liver during Year (2017-2018).



Graph 13: Seasonal variations of Chloride concentration in fish tissues (Gills, Muscles, Liver) in Bilawali tank and Sirpur lake shows higher concentration of Chloride in Gills during (2017-2018).

Biochemical parameters in fish 2017-2018)

Blood glucose- During Pre-monsoon season the values of Blood glucose in fish blood sample were 17.34±0.17 , 25.69±0.60, In Post-monsoon season values were 13.72±0.86 , 15.11±0.78 at Site A (Bilawali tank), Site B (Sirpur lake).

Total protein- During Pre-monsoon season the values of Total protein in fish blood sample were 1.50±0.33 , 1.39±4.39, In Post-monsoon season values were 3.55±0.08 , 4.61±0.14 at Site A (Bilawali tank), Site B (Sirpur lake).

Biochemical parameters in fish (2016-2017)

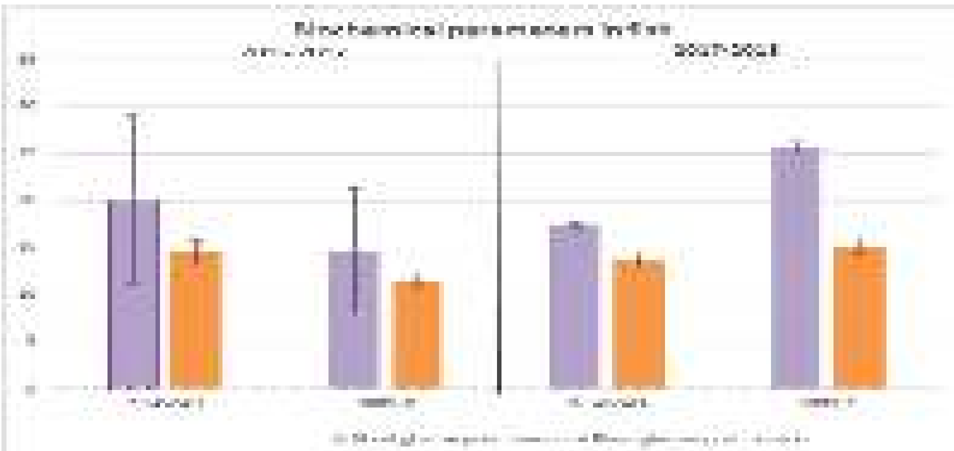
Blood glucose- During Pre-monsoon season the values of Blood glucose in fish blood sample were 20.07±8.97 , 14.71±6.57, In Post-monsoon season values were 14.53±1.21, 11.36±0.55 at Site A (Bilawali tank), Site B (Sirpur lake).

Total protein- During Pre-monsoon season the values of Total protein in fish Blood sample were 1.29±0.58 , 1.48±6.66, In Post-monsoon season values were 2.33±0.14 , 3.45±0.10 at Site A(Bilawali tank), Site B (Sirpur lake).

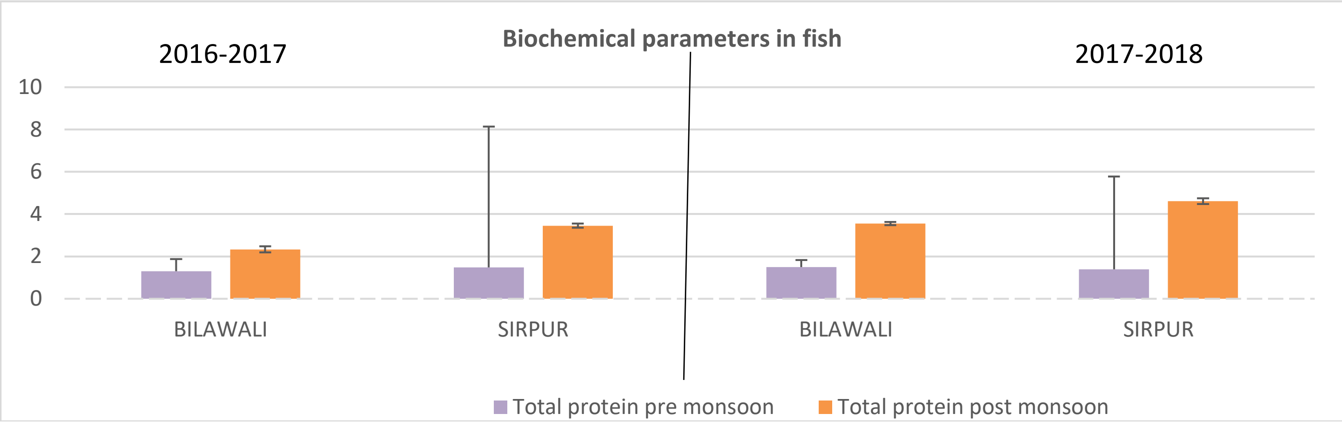
BIOCHEMICAL PARAMETERS-

Table 5: Biochemical parameters (Blood glucose and Total protein) of fish of Bilawali tank and Sirpur lake in year (2016-2017) and (2017-2018)

Parameters	Bilawali	Sirpur
Blood glucose pre monsoon1	20.07±8.97mg/dl	14.71±6.57
Total protein pre monsoon 1	1.29±0.58mg/ml	1.48±6.66mg/ml
Blood glucose post monsoon 1	14.53± 1.21	11.36±0.55
Total protein post monsoon 1	2.33±0.14mg/ml	3.45±0.10mg/ml
Blood glucose Pre-monsoon 2	17.34±0.17	25.69±0.60
Total protein Pre-monsoon 2	1.50±0.33mg/ml	1.39±4.39mg/ml
Blood glucose post monsoon 2	13.72±0.86	15.11±0.78
Total protein post monsoon 2	3.55±0.08mg/ml	4.61±0.14mg/ml



Graph 14: Seasonal variations of Blood Glucose level of fish in Bilawali tank and Sirpur lake, In year(2016-2017) (2017-2018) in Pre-monsoon season fish from both sites showing higher level than in pre monsoon season in both the year.



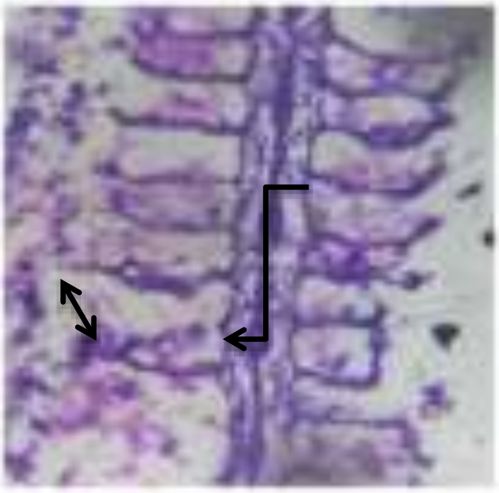
GRAPH 15: Seasonal variations of Total protein in Blood serum of fishinBilawali tank and Sirpur lake, In year(2016-2017)(2017-2018) in Pre-monsoon season fish from both sites showing higher level than in Post monsoon season in both the year.

HISTOLOGICAL PARAMETERS-

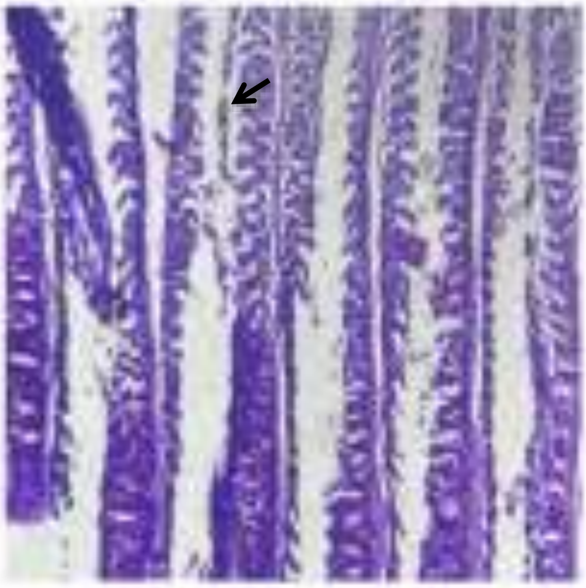
Histological analysis in fish tissues (2016-2017) (Bilawali tank) (Pre-monsoon)

Gills –

Microphotograph-1: T.S of gill of *Catla catla*-1 of Bilawali tank in year (2016-2017) Pre-monsoon showing inflamed blood vessels.



Microphotograph-2: T.S of gill of *Catla catla*-2 of Bilawali tank in year (2016-2017) Pre-monsoon showing dis-organisation of cartilage core and dis-organisation of lamella and lamellar adhesion.

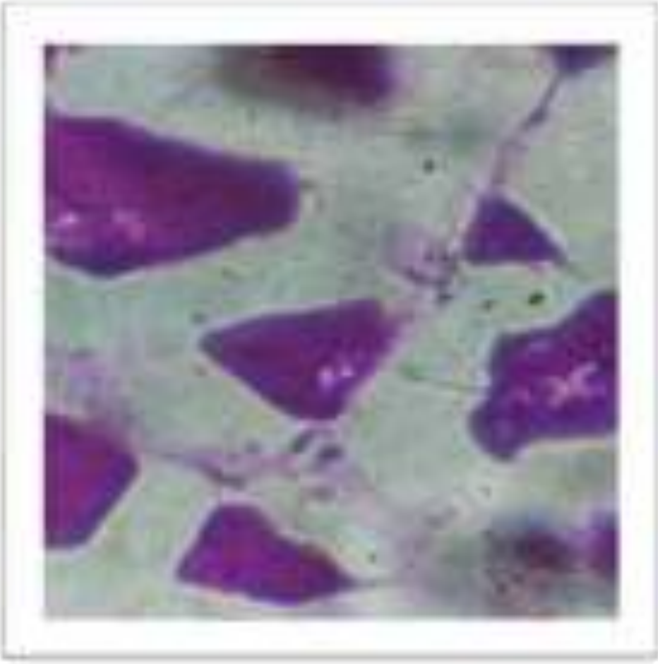


Microphotograph-3: T.S of gill of *Catla catla*-3 of Bilawali tank in year (2016-2017) pre-monsoon showing hyperplasia of epithelial cells,shortening of secondary lamella.

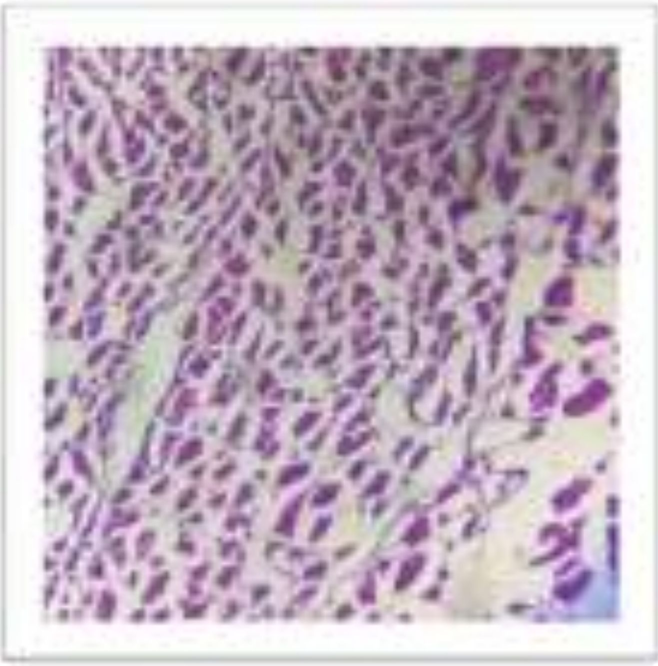
Microphotograph-4: T.S of gill of*Catla catla*-4 of Bilawali tank in year (2016-2017) pre-monsoon showing Sub-epithelial edema, in many areas of secondary epithelium collapse and form ladder like structure.

Microphotograph-5: T.S of gill of*Catla catla*-5 of Bilawali tank in year (2016-2017) pre-monsoon showing Haemorrhage in the center of primary lamella.

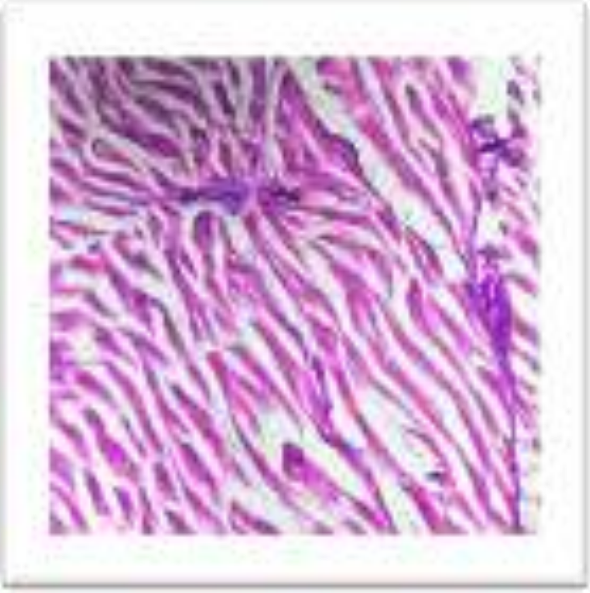
Muscles-



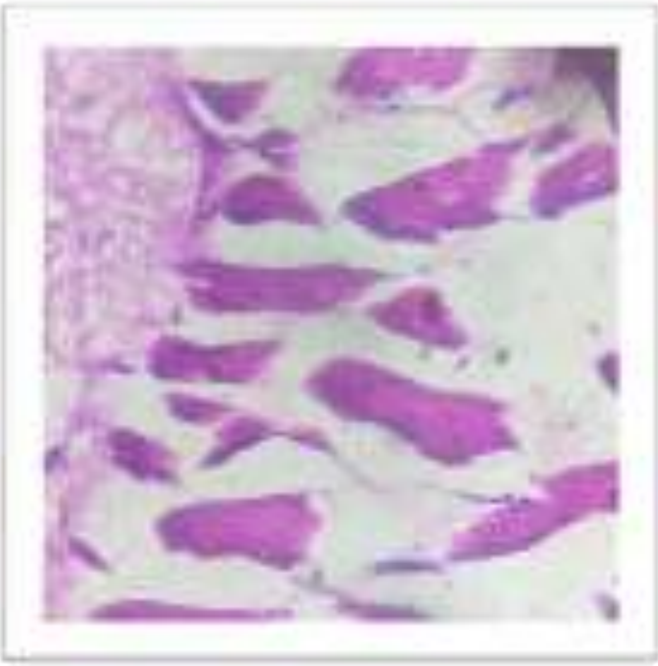
Microphotograph-6: T.S of Muscle of *Catla catla*-1 of Bilawali tank in year (2016-2017) pre-monsoon showing loosely packed muscle fibres.



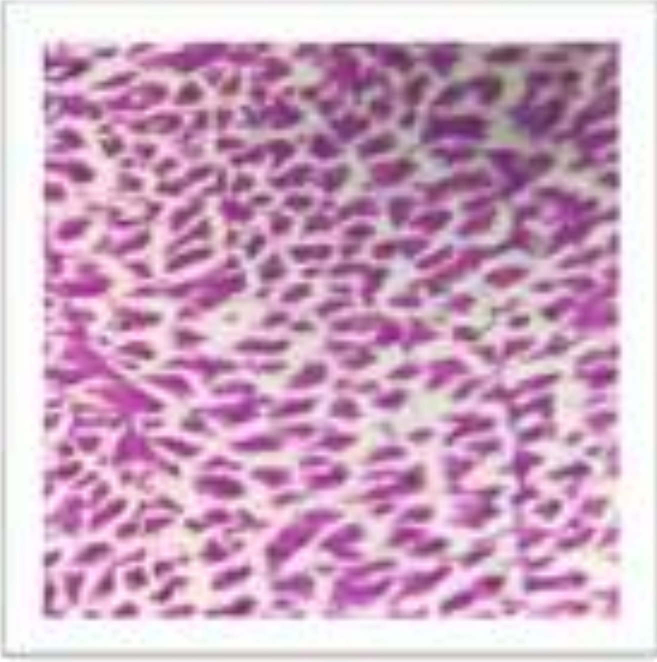
Microphotograph-7 T.S of Muscle of *Catla catla*-2 of Bilawali tank in year (2016-2017) pre-monsoon showing degeneration of muscle bundles.



Microphotograph-8: T.S of Muscle of *Catla catla*-3 of Bilawali tank in year (2016-2017) pre-monsoon showing atrophy of muscles.

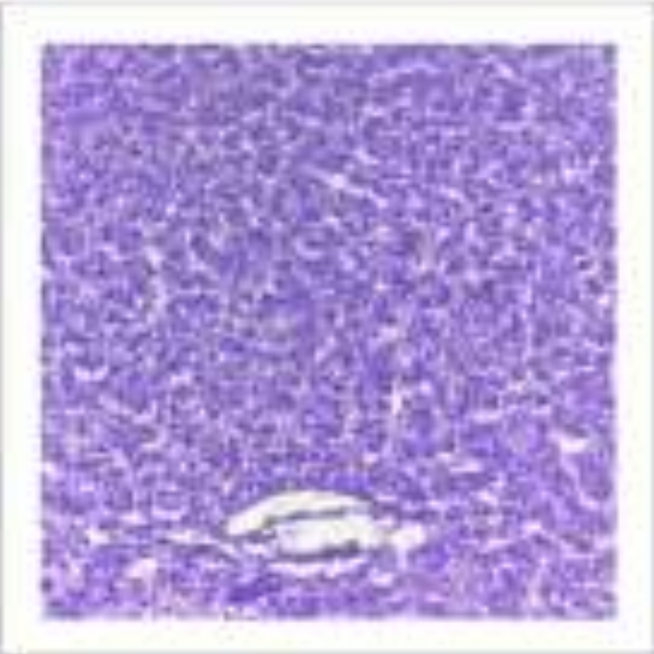


Microphotograph-9: T.S of Muscle of *Catla catla*-4 of Bilawali tank in year (2016-2017) Pre-monsoon showing large inter-myofibrillar space.

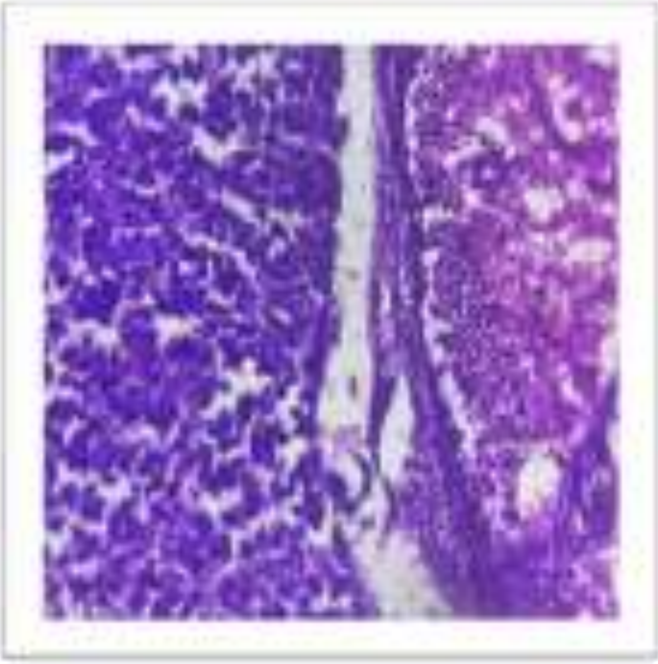


Microphotograph-10: T.S of Muscle of *Catla catla*-5 of Bilawali tank in year (2016-2017) Pre-monsoon showing Normal tissue.

Liver –

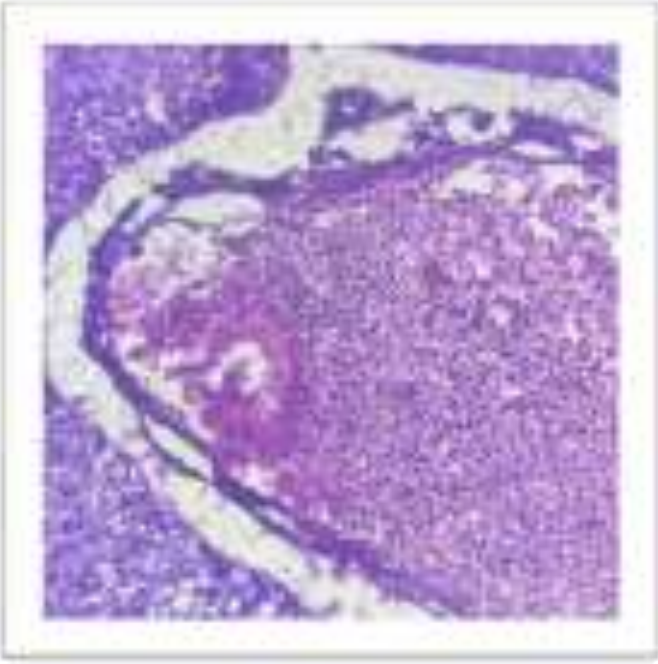


Microphotograph-11: T.S of Liver of *Catla catla*-1 of Bilawali tank in year (2016-2017) Pre-monsoon showing Normal tissue.

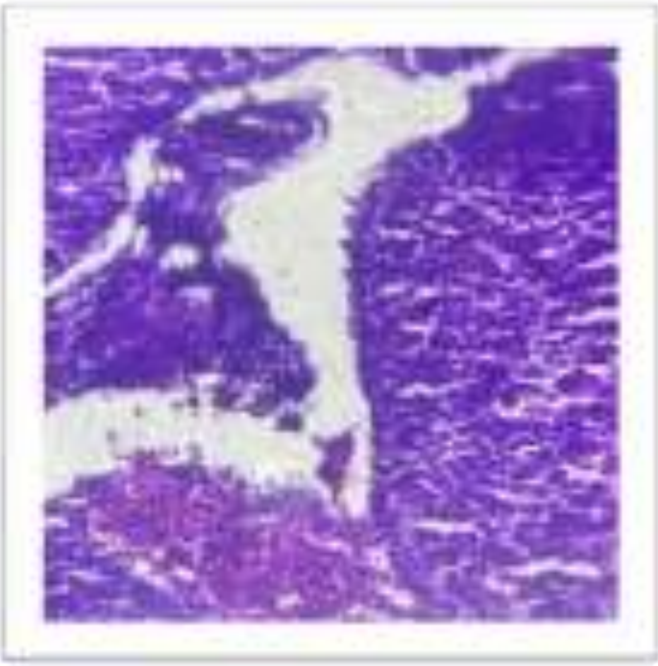


Microphotograph-12: T.S of Liver of *Catla catla*-2 of Bilawali tank in year (2016-2017) Pre-monsoon showing Cytoplasmic vacuole and Blood congestion.

Microphotograph-13: T.S of Liver of *Catla catla*-3 of Bilawali tank in year (2016-2017) Pre-monsoon showing Normal tissue.



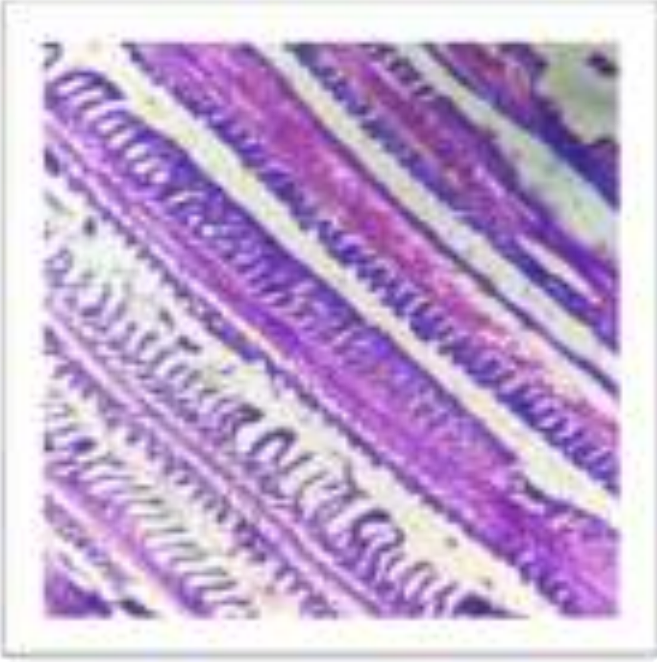
Microphotograph-14: T.S of Liver of *Catla catla*-4 of Bilawali tank in year (2016-2017) Pre-monsoon showing Macrophages Aggregation and Hepatocyte Hypertrophy.



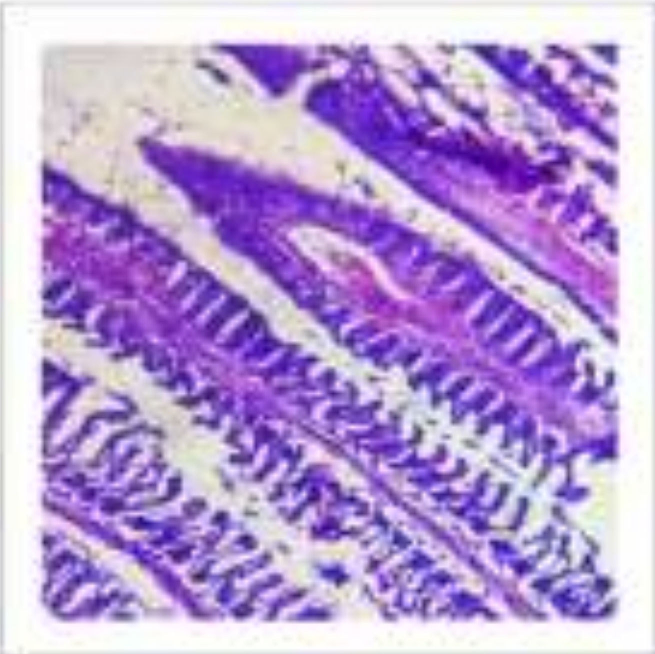
Microphotograph-15: T.S of Liver of *Catla catla*-5 of Bilawali tank in year (2016-2017) Pre-monsoon showingNuclear Hypotrophy.

Histological analysis in fish tissues(2016-2017) (Sirpur tank)(Pre-monsoon)

Gills -

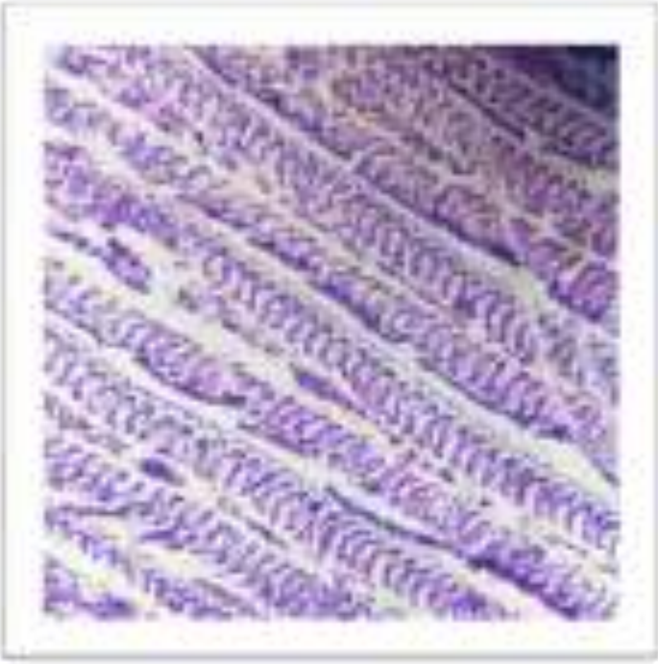


Microphotograph-16: T.S of Gill of *Catla catla*-1 of Sirpur lake in year (2016-2017) Pre-monsoon showing Proliferation of cartilage.



Microphotograph-17: T.S of Gill of *Catla catla*-2 of Sirpur lakein year (2016-2017) Pre-monsoon showing lamellar fusion and lamellar shrinkage.

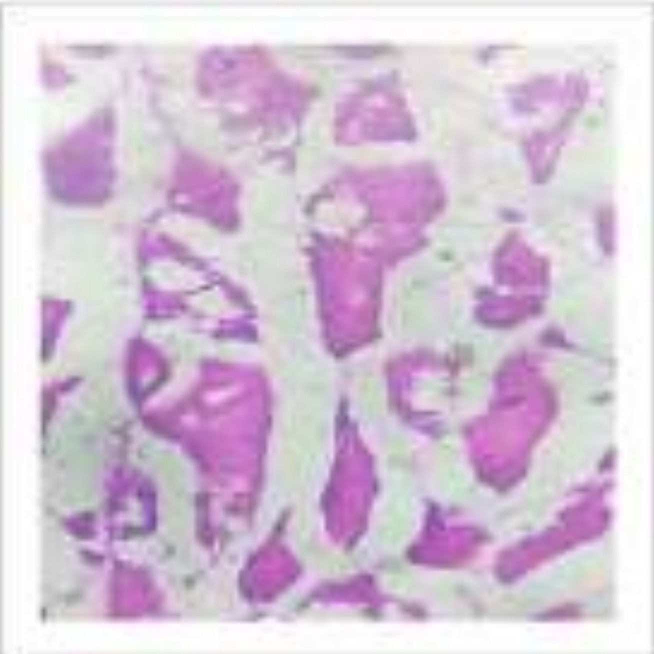
Microphotograph-18: T.S of Gill of *Catla catla*-3 of Sirpur lake in year (2016-2017) Pre-monsoon showing shortening and fusion of lamella.



Microphotograph-19: T.S of Gill of *Catla catla*-4 of Sirpur lake in year (2016-2017) Pre-monsoon showing cellular Necrosis and Epithelial rupture.

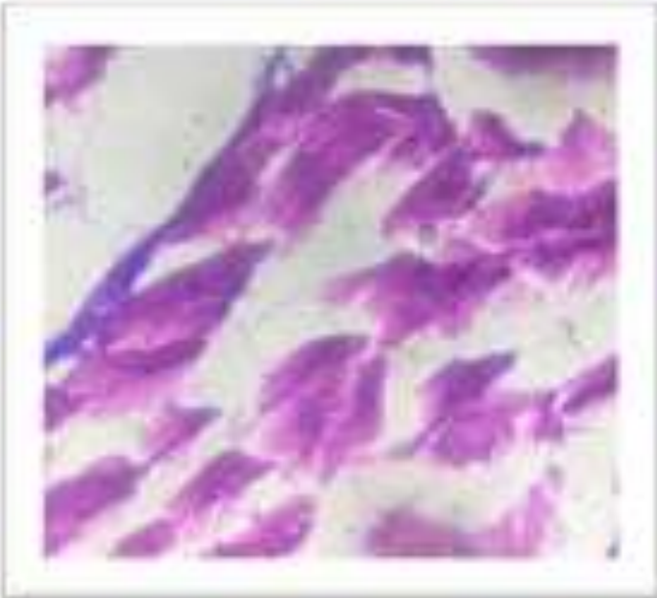
Microphotograph-20: T.S of Gill of *Catla catla*-5 of Sirpur lakein year (2016-2017) Pre-monsoon showing interstitial edema.

Muscles-

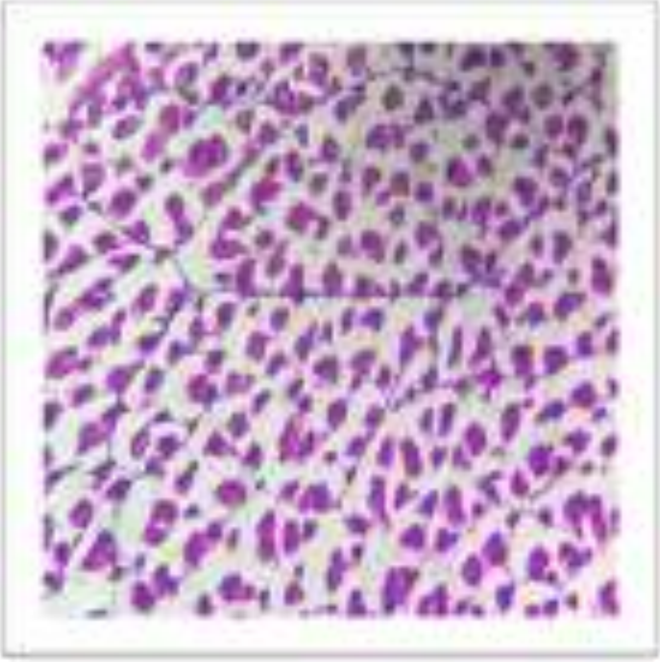


Microphotograph-21: T.S of Muscles of *Catla catla*-1 of Sirpur lake in year (2016-2017) Pre-monsoon showing disintegrated myofibrils.

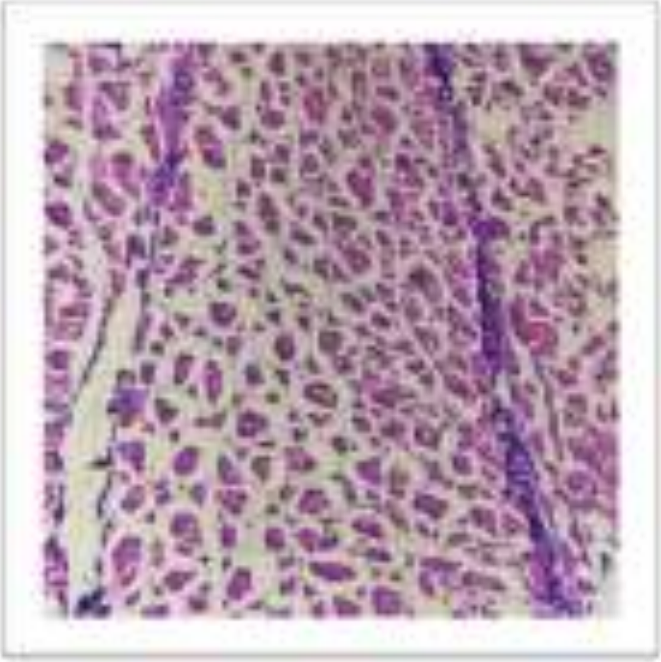
Microphotograph-22: T.S of Muscles of *Catla catla*-2 of Sirpur lake in year (2016-2017) pre-monsoon showing Increase in IMFS.



Microphotograph-23: T.S of Muscles of *Catla catla*-3 of Sirpur lake in year (2016-2017) Pre-monsoon showing Intra myofibril gap.

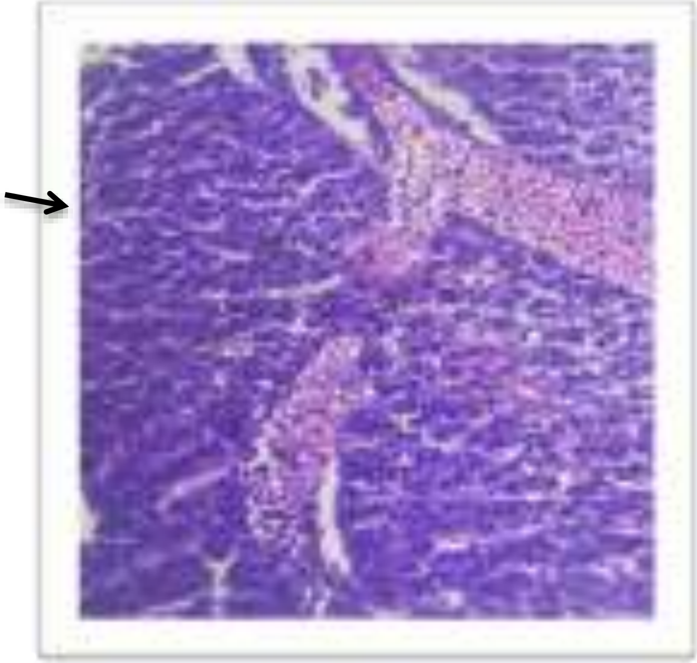


Microphotograph-24: T.S of Muscles of *Catla catla*-4 of Sirpur lake in year (2016-2017) Pre-monsoon showing normal tissue.

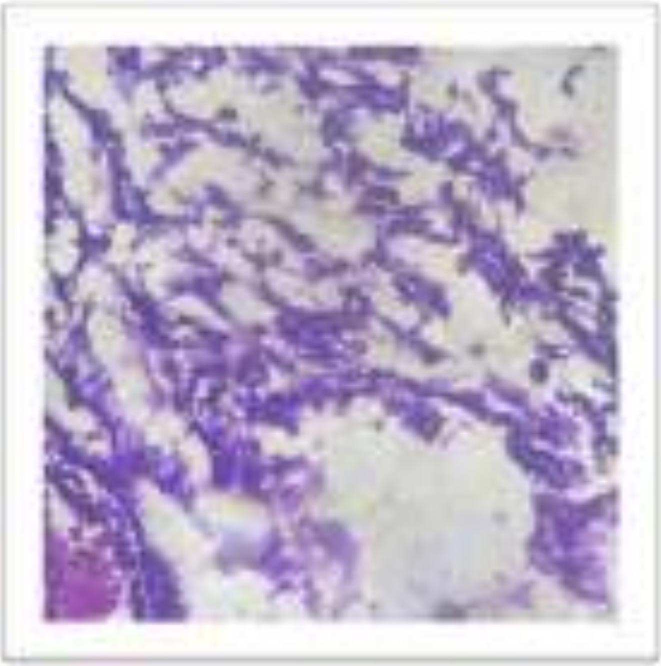


Microphotograph-25: T.S of Muscles of *Catla catla*-5 of Sirpur lake in year (2016-2017) pre-monsoon showing intramuscular edema.

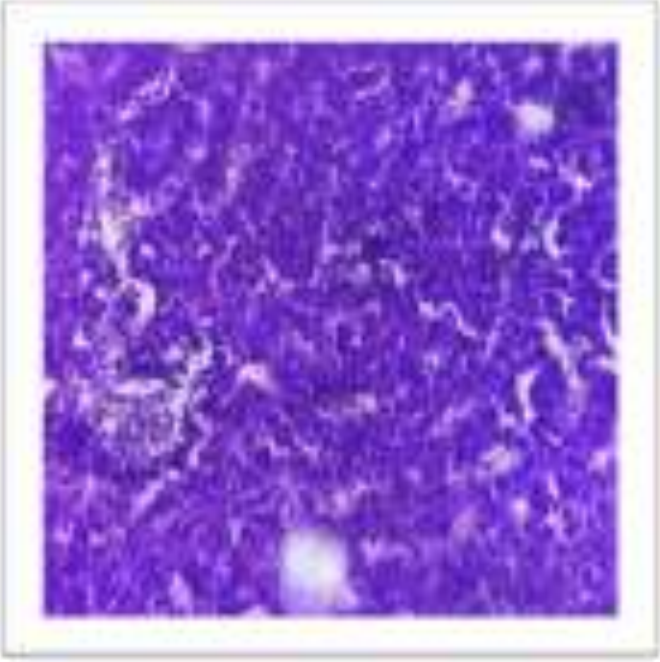
Liver –



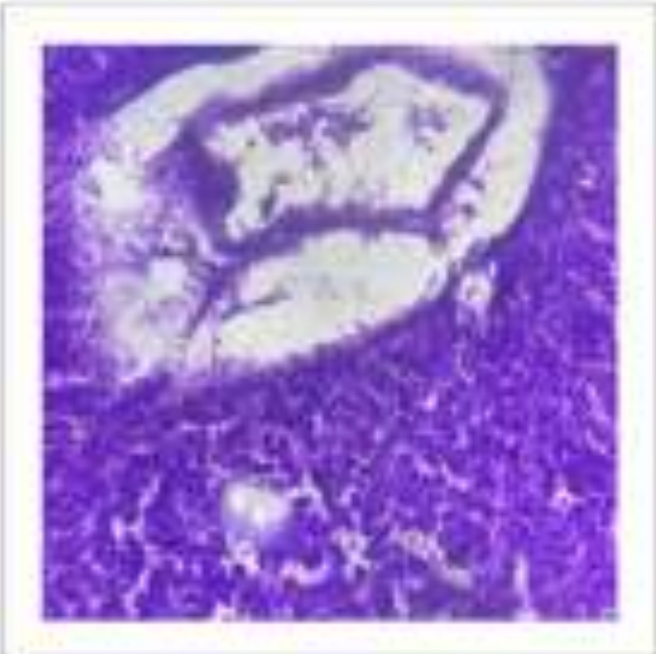
Microphotograph-26: T.S of Liver of *Catla catla*-1 of Sirpur lake in year (2016-2017) Pre-monsoon showing Blood Congestion in Sinusoid.



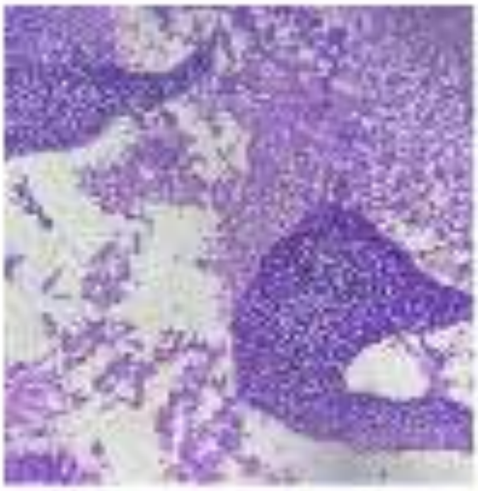
Microphotograph-27: T.S of Liver of *Catla catla*-2 of Sirpur lake in year (2016-2017) Pre-monsoon showing irregular hepatocytes.



Microphotograph-28: T.S of Liver of *Catla catla*-3 of Sirpur lake in year (2016-2017) pre-monsoon showing normal structure.



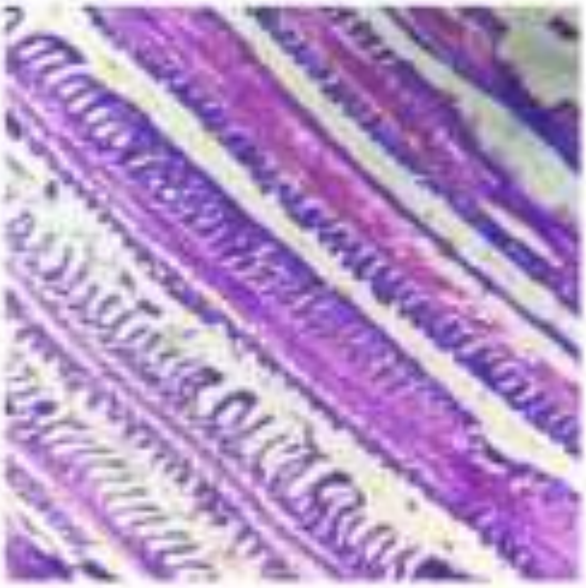
Microphotograph-29: T.S of Liver of *Catla catla*-4 of Sirpur lakein year (2016-2017) Pre-monsoon showing Cytoplasmic Vacuolization.



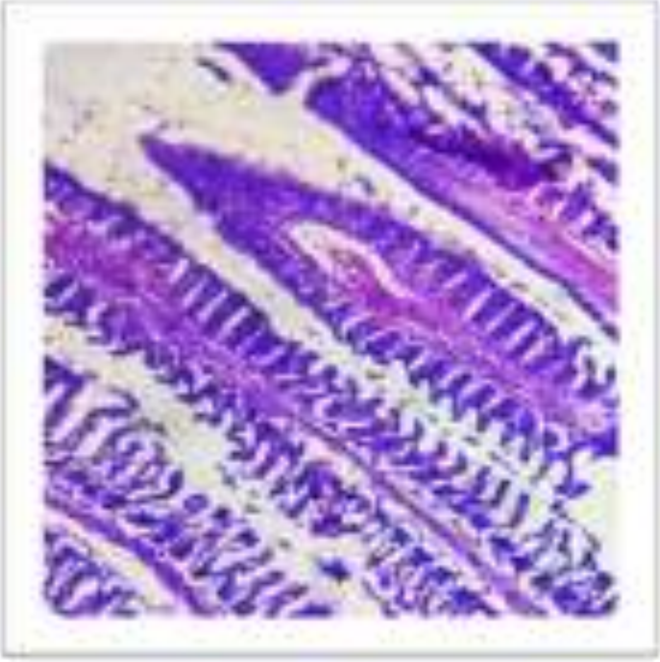
Microphotograph-30: T.S of Liver of *Catla catla*-5 of Sirpur lake in year (2016-2017) Pre-monsoon showing Necrosis.

Histological analysis in fish tissues(2016-2017)(Bilawali tank)(Post-monsoon)

Gills -

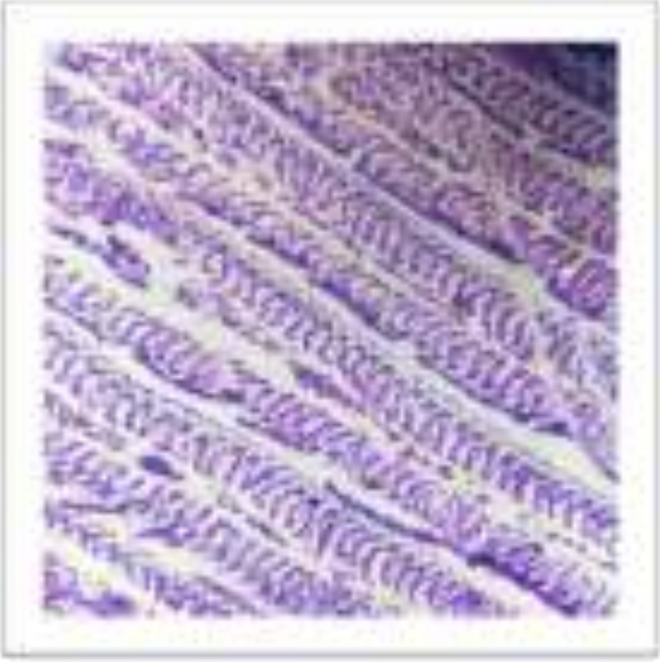


Microphotograph-31: T.S of Gill of *Catla catla*-1 of Bilawali tank in year (2016-2017) Post-monsoon showing fusion of secondary Gill lamella and Multifocal Hyperplasia.



Microphotograph-32: T.S of Gill of *Catla catla*-2 of Bilawali tank in year (2016-2017) post-monsoon showing mucous cell population.

Microphotograph-33: T.S of Gill of *Catla catla*-3 of Bilawali tank in year (2016-2017) Post-monsoon showing shortening of secondary gill lamella and fusion of gill lamella.

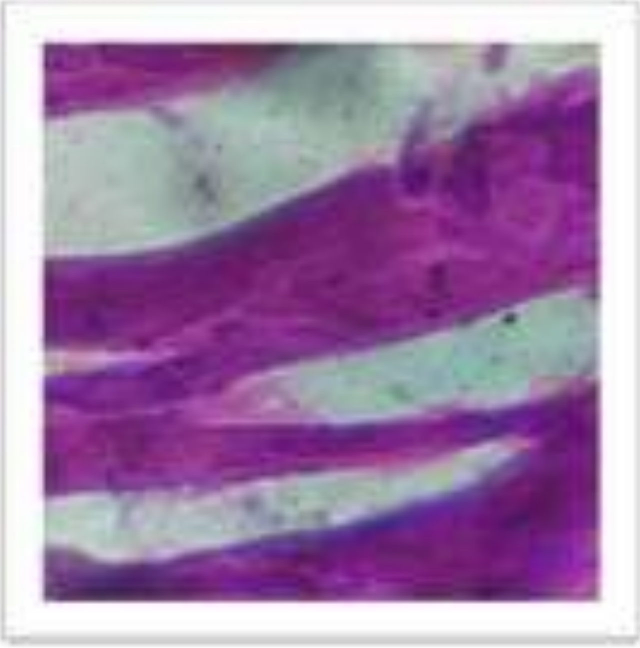


Microphotograph-34: T.S of Gill of *Catla catla*-4 of Bilawali tank in year (2016-2017) Post-monsoon showing vasodilation in Blood vessel.

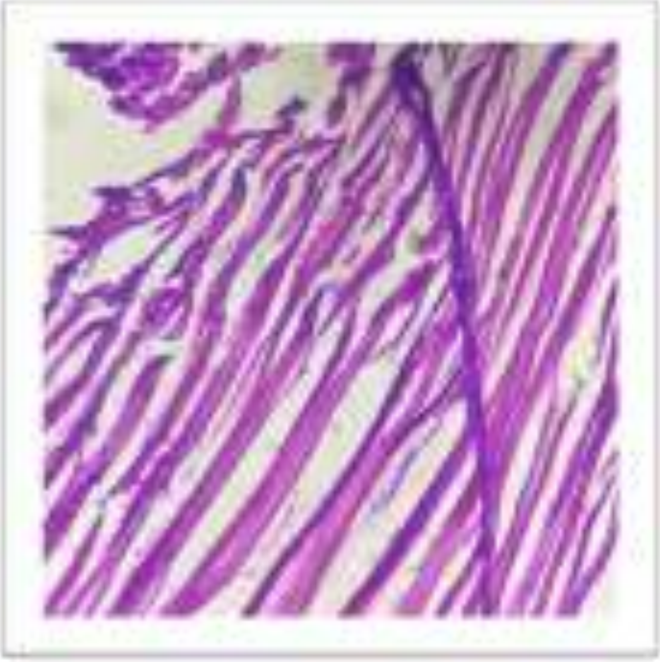
Microphotograph-35: T.S of Gill of *Catla catla*-5 of Bilawali tank in year (2016-2017) Post-monsoon showing Partial fusion of lamella, Stasis in Central Venous Sinus.

Muscles-

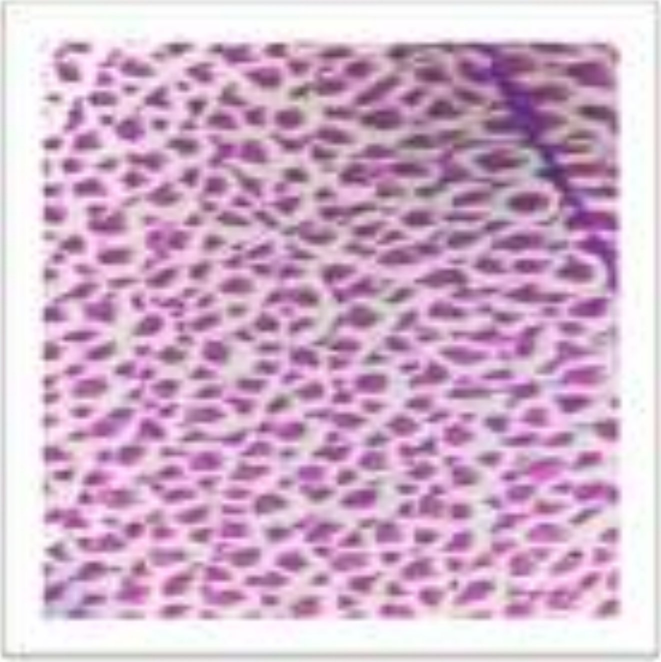
Microphotograph-36: T.S of Muscles of *Catla catla*-1 of Bilawali tank in year (2016-2017) Post-monsoon showing normal structure.



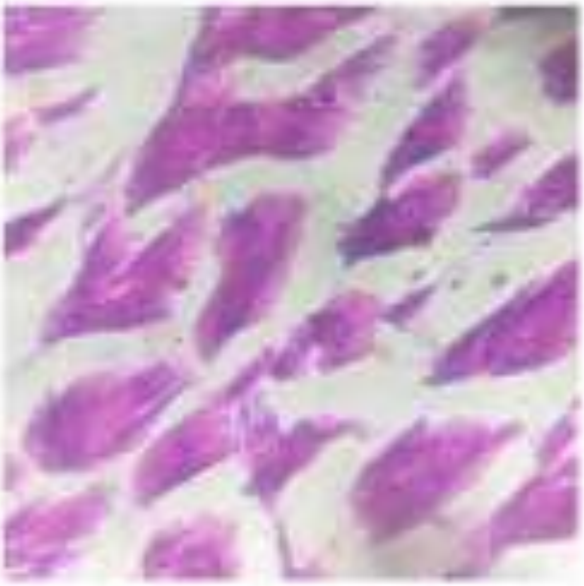
Microphotograph-37: T.S of Muscles of *Catla catla*-2 of Bilawali tank in year (2016-2017) Post-monsoon showing Normal tissue.



Microphotograph-38: T.S of Muscles of *Catla catla*-3 of Bilawali tank in year (2016-2017) Post-monsoon showing intra Muscular edema.

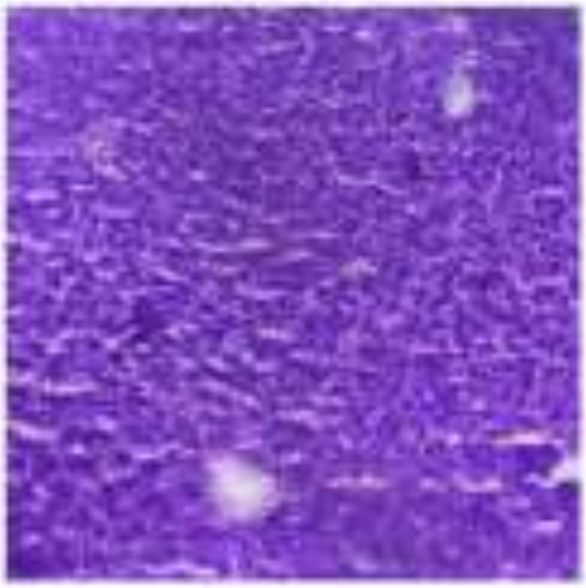


Microphotograph-39: T.S of Muscles of *Catla catla*-4 of Bilawali tank in year (2016-2017) Post-monsoon showing Normal structure.

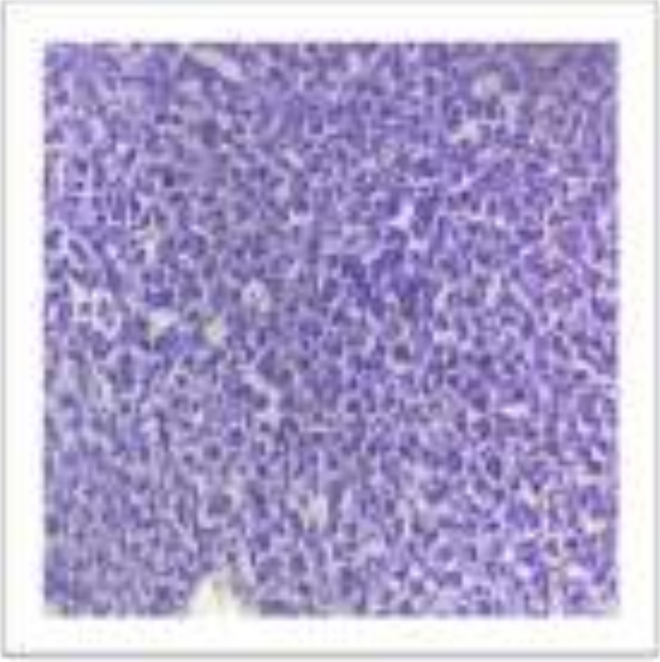


Microphotograph-40: T.S of Muscles of *Catla catla*-5 of Bilawali tank in year (2016-2017) Post-monsoon showing Muscle degradation.

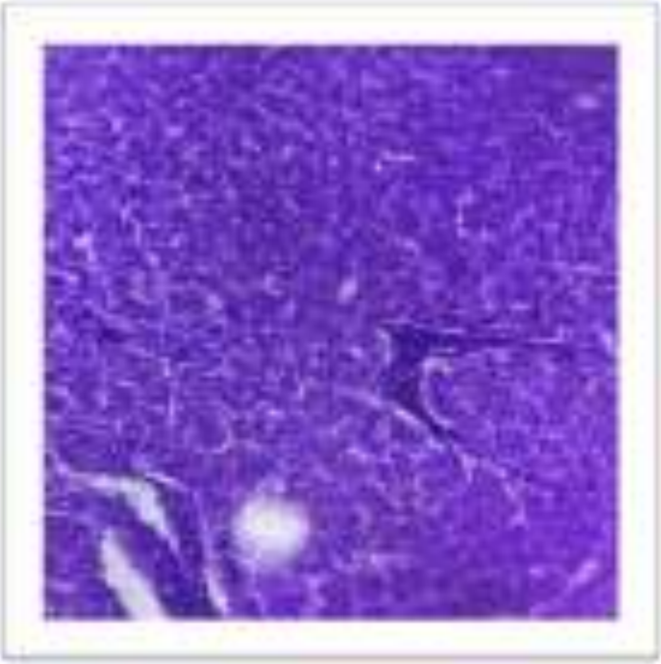
Liver –



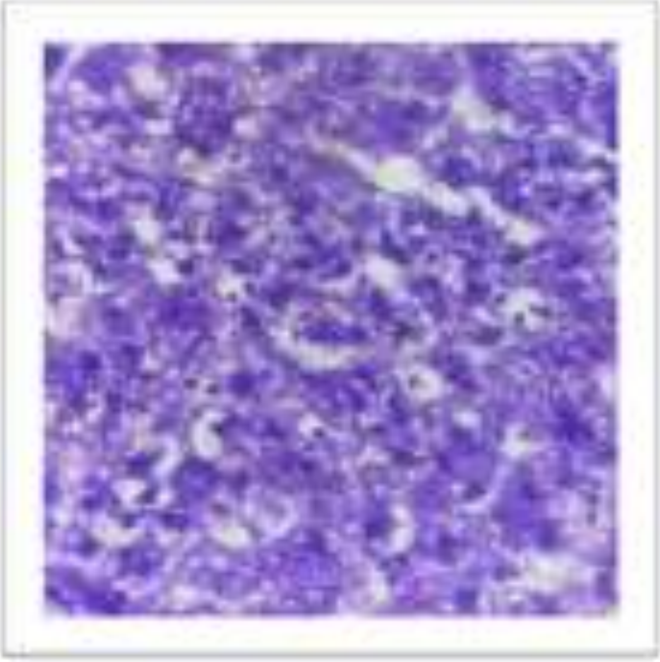
Microphotograph-41: T.S of Liver of *Catla catla*-1 of Bilawali tank in year (2016-2017) Post-monsoon showing Normal tissue.



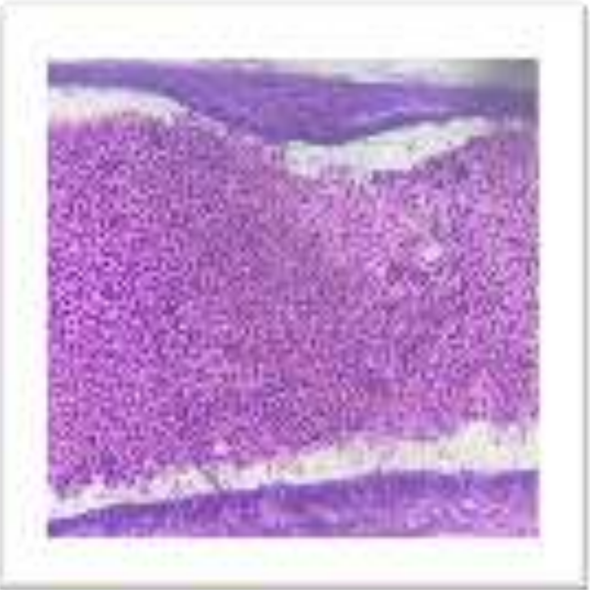
Microphotograph-42: T.S of Liver of *Catla catla*-2 of Bilawali tank in year (2016-2017) Post-monsoon showing hepatocyte hypertrophy.



Microphotograph -43: T.S of Liver of *Catla catla*-3 of Bilawali tank in year (2016-2017) Post-monsoon showing Normal structure.



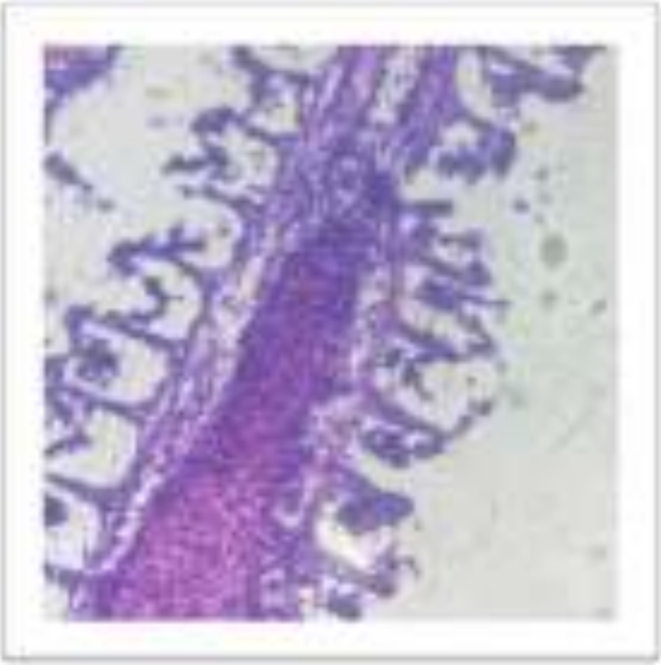
Microphotograph-44: T.S of Liver of *Catla catla*-4 of Bilawali tank in year (2016-2017) Pre-monsoon showing Cytoplasmic Vacuolization.



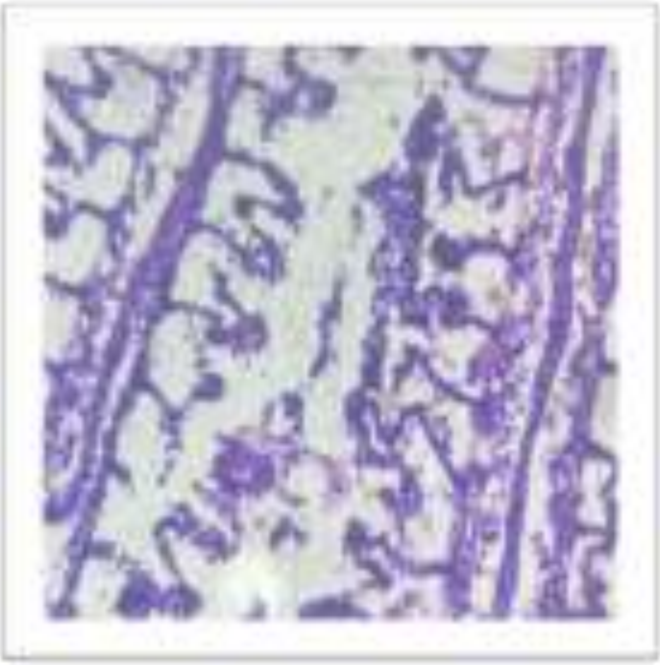
Microphotograph-45: T.S of Liver of *Catla catla*-5 of Bilawali tank in year (2016-2017) Post-monsoon showing Multifocal Necrotic areas.

Histological analysis in fish tissues (2016-2017) (Sirpur lake) (Post-monsoon)

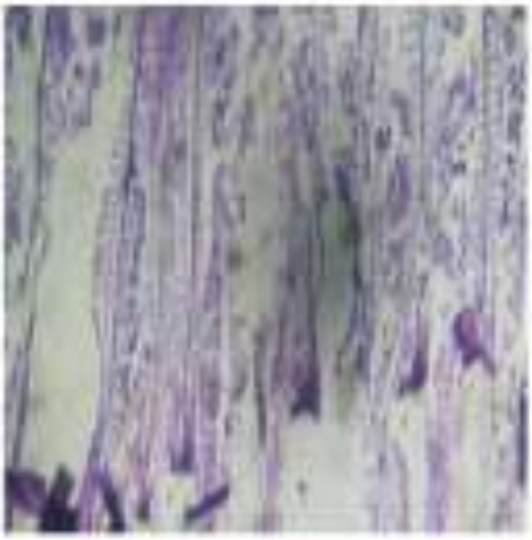
Gills -



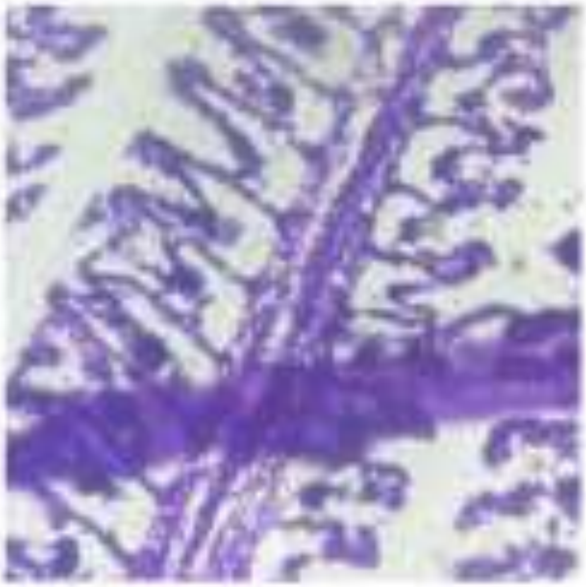
Microphotograph-46: T.S of Gill of *Catla catla*-1 of Sirpur lake in year (2016-2017) Post -monsoon showing Proliferation of Mucous cells and Haemorrhage.



Microphotograph-47: T.S of Gill of *Catla catla*-2 of Sirpur lakein year (2016-2017) Post-monsoon showing Separation of Gill filament from Basal membrane.



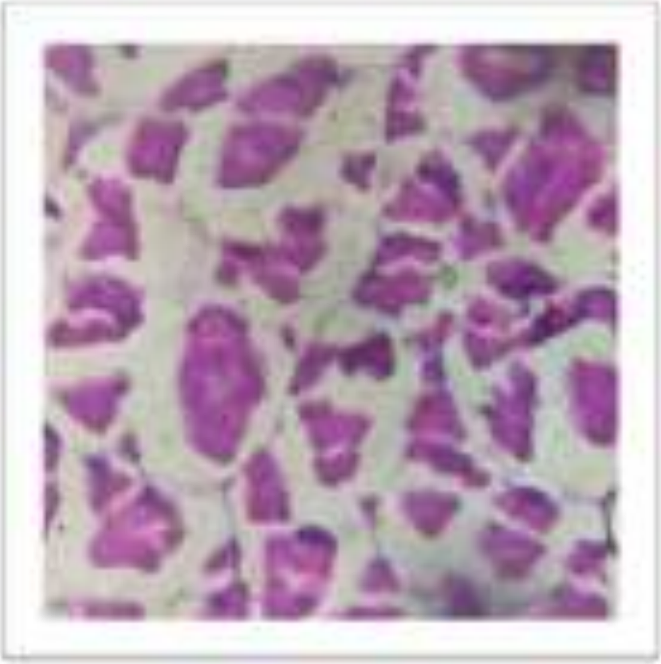
Microphotograph-48: T.S of Gill of *Catla catla*-3 of Sirpur lake in year (2016-2017) Post- monsoon showing Atrophy of Gill filament.



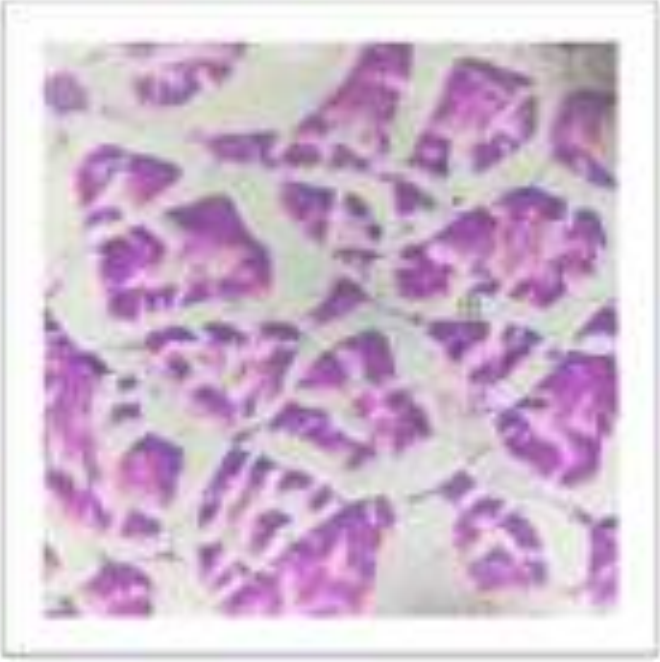
Microphotograph-49: T.S of Gill of *Catla catla*-4 of Sirpur lake in year (2016-2017) Post-monsoon showing Fusion of lamella.

Microphotograph -50: T.S of Gill of *Catla catla*-5 of Sirpur tank in year (2016-2017) Post-monsoon showing Hyperplasia.

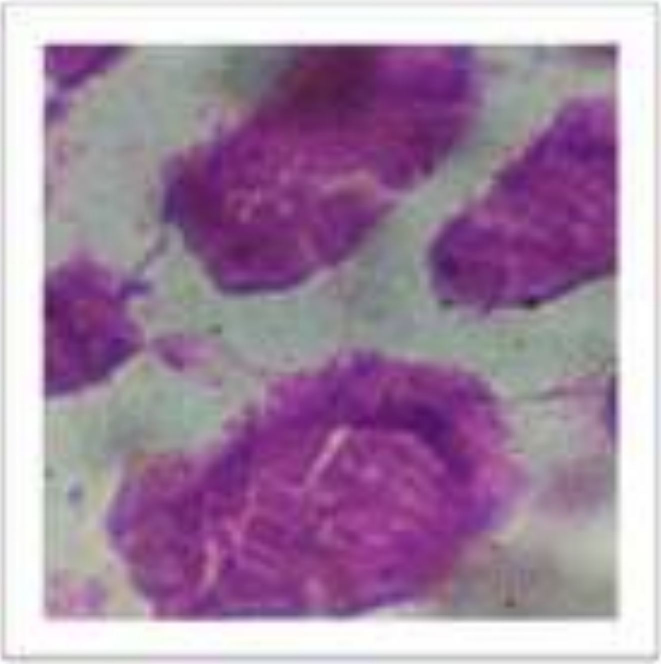
Muscles-



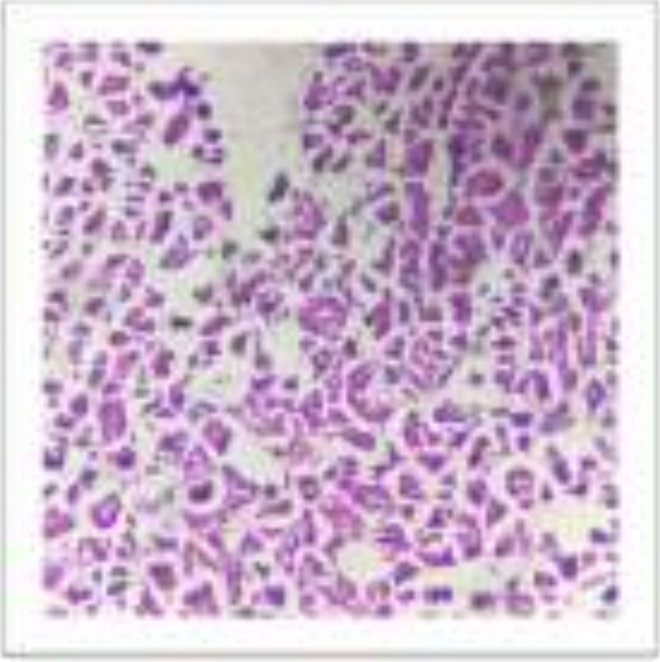
Microphotograph-51: T.S of Muscles of *Catla catla*-1 of Sirpur lake in year (2016-2017) Post-monsoon showing Scattered Myofibrils.



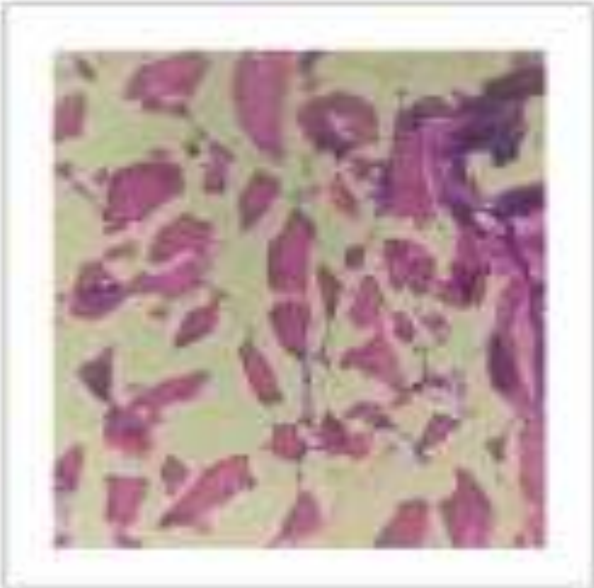
Microphotograph-52: T.S of Muscles of *Catla catla*-2 of Sirpur lake in year (2016-2017) post-monsoon showing increased Intra Myofibrillar space.



Microphotograph-53: T.S of Muscles of *Catla catla*-3 of Sirpur lake in year (2016-2017) Post-monsoon showing Splitting of Muscle fibre.

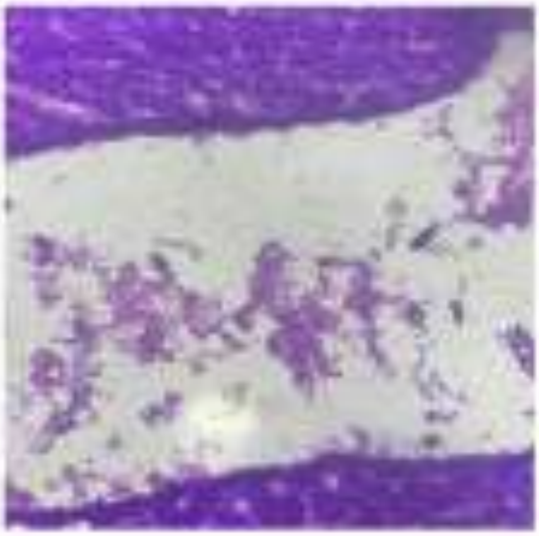


Microphotograph-54: T.S of Muscles of *Catla catla*-4 of Sirpur lake in year (2016-2017) Post-monsoon showing Structural degradation.

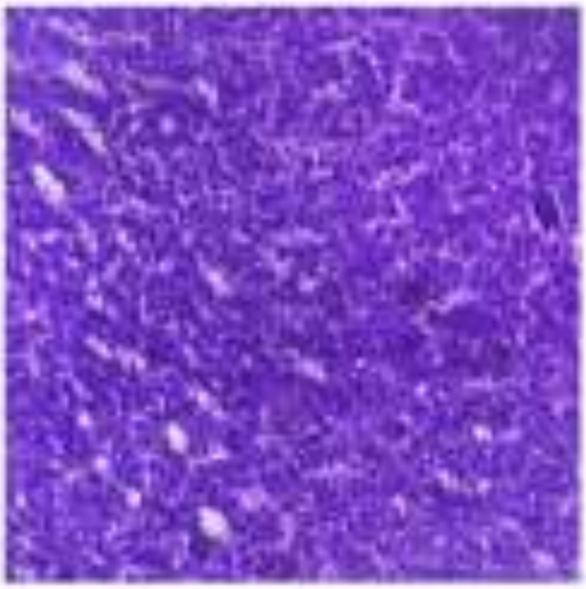


Microphotograph-55: T.S of Muscles of *Catla catla*-5 of Sirpur lake in year (2016-2017) Post-monsoon showing infiltration of Inflammatory cells.

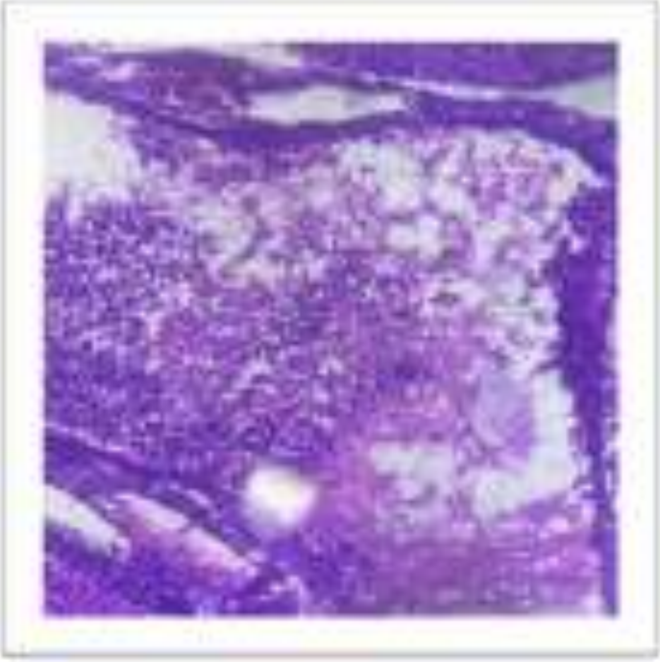
Liver –



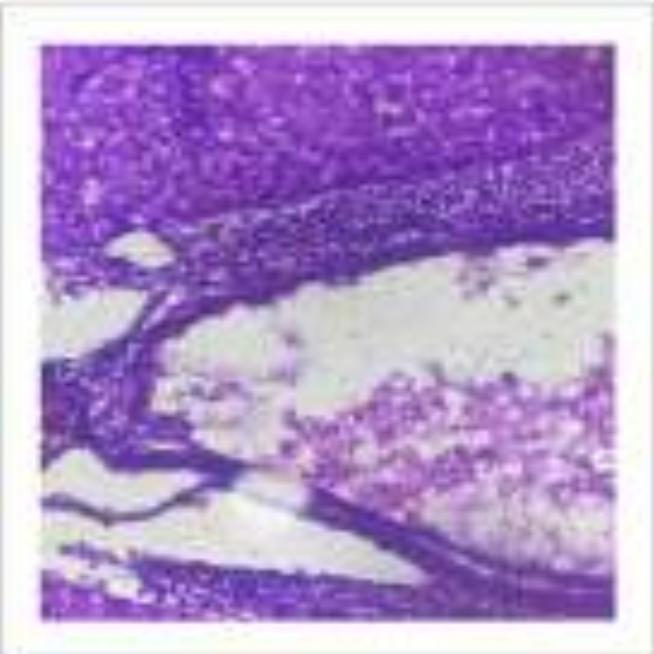
Microphotograph-56 T.S of Liver of *Catla catla*-1 of Sirpur lake in year (2016-2017) post-monsoon showing large vacuolization.



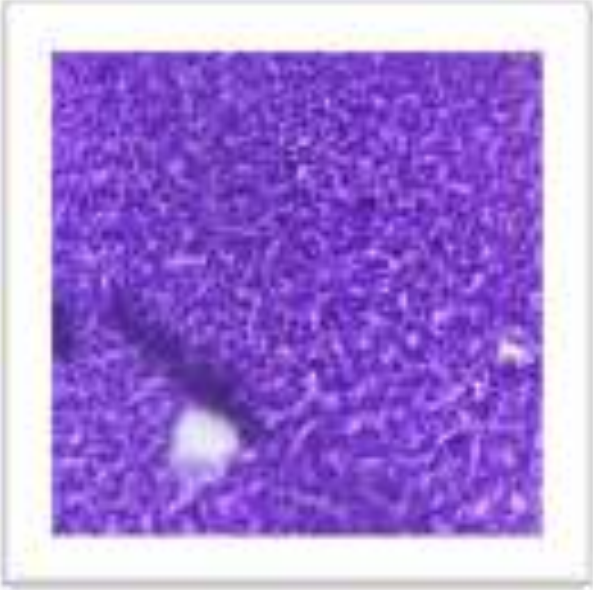
Microphotograph-57: T.S of Liver of *Catla catla*-2 of Sirpur lake in year (2016-2017) Post-monsoon showing Nuclear degeneration and Nuclear pyknosis.



Microphotograph-58: T.S of Liver of *Catla catla*-3 of Sirpur lake in year (2016-2017) post-monsoon showing Blood congestion and Degeneration of tissue.



Microphotograph-59: T.S of Liver of *Catla catla*-4 of Bilawali tank in year (2016-2017) Post-monsoon showing Necrosis.

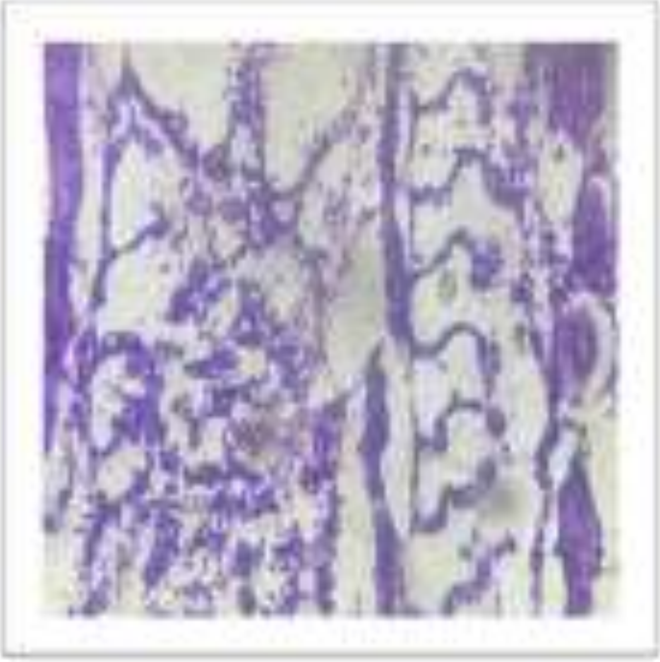


Microphotograph-60: T.S of Liver of *Catla catla*-5 of Sirpur lake in year (2016-2017) post-monsoon showing Normal tissue.

Histological analysis in fish tissues (2017-2018) (Bilawali tank) (Post-monsoon)

Gills-

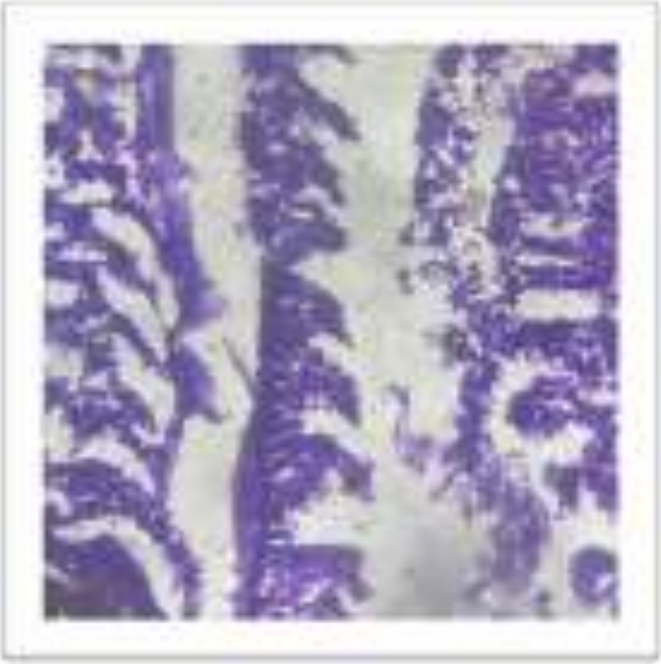
Microphotograph-61: T.S of Gill of *Catla catla*-1 of Bilawali tank in year (2017-2018) Pre-monsoon showing severe Degeneration in gill lamella.



Microphotograph-62: T.S of Gill of *Catla catla*-2 of Bilawali tank in year (2017-2018) pre-monsoon showing severe degeneration in gill lamella.

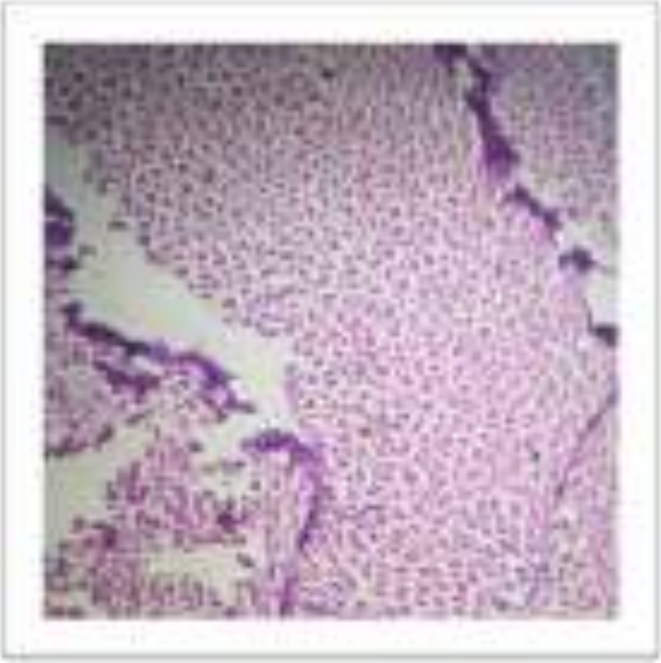
Microphotograph-63: T.S of Gill of *Catla catla*-3 of Bilawali tank in year (2017-2018) Pre-monsoon showing rupture of Primary and Secondary lamellae.

Microphotograph-64: T.S of Gill of *Catla catla*-4 of Bilawali tank in year (2017-2018) Pre-monsoon showing hyperplasia of epithelium leading to fusion of lamella.

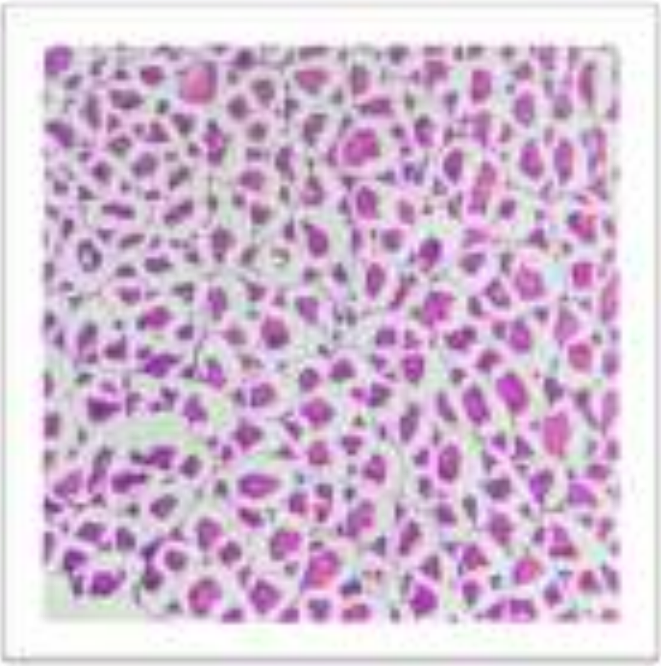


Microphotograph-65: T.S of Gill of *Catla catla*-5 of Bilawali tank in year (2017-2018) pre-monsoon showing epithelial lifting.

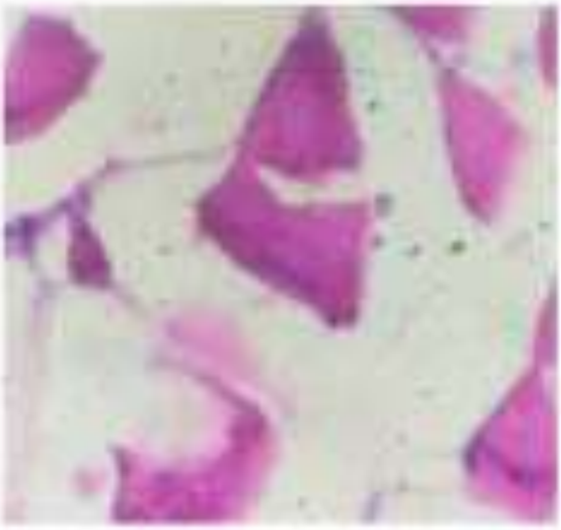
Muscles-



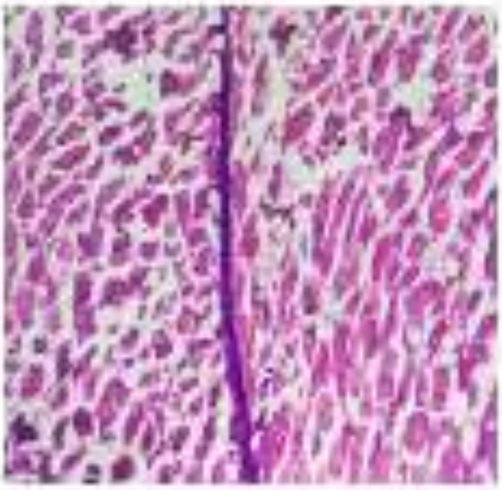
Microphotograph-66: T.S of Muscles of*Catla catla*-1 of Bilawali tank in year (2017-2018) pre-monsoon showing normal structure.



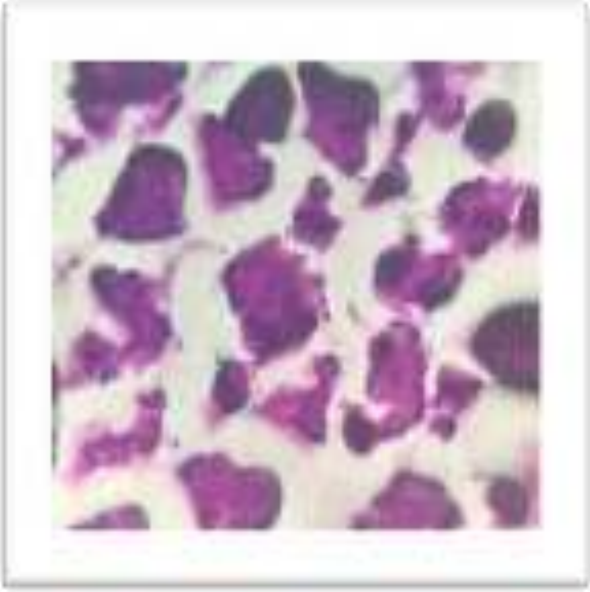
Microphotograph-67: T.S of Muscle of *Catla catla*-2 of Bilawali tank in year (2017-2018) pre-monsoon showing normal tissue.



Microphotograph-68: T.S of Muscles of *Catla catla*-3 of Bilawali tank in year (2017-2018) pre-monsoon showing degeneration.

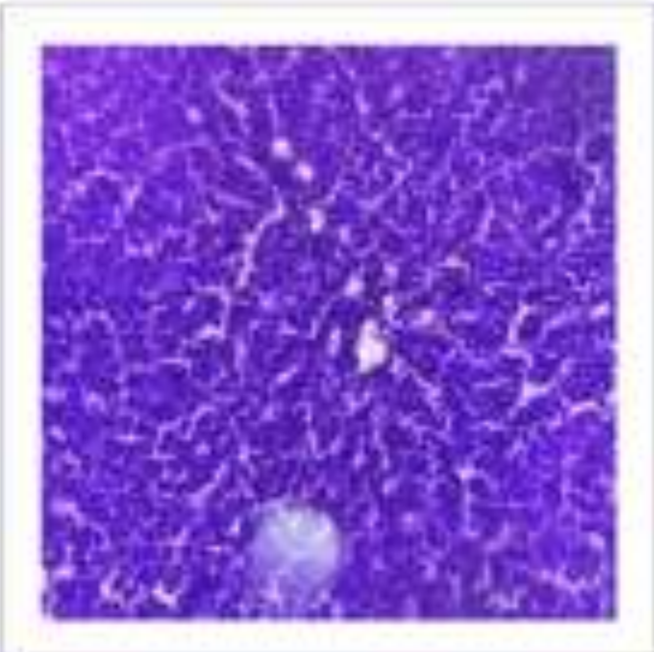


Microphotograph-69: T.S of Muscles of *Catla catla*-4 of Bilawali tank in year (2017-2018) Pre-monsoon showing Damaged tissue.

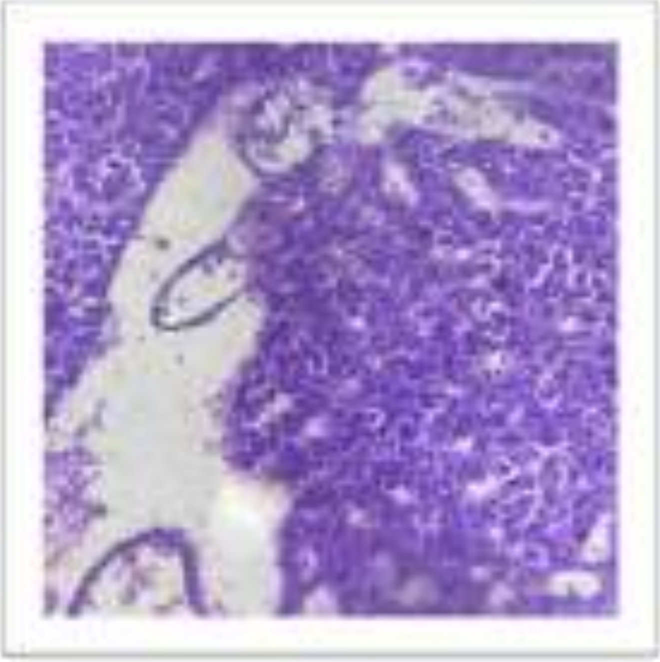


Microphotograph-70: T.S of Muscles of *Catla catla*-5 of Bilawali tank in year (2017-2018) Pre-monsoon showing necrosis.

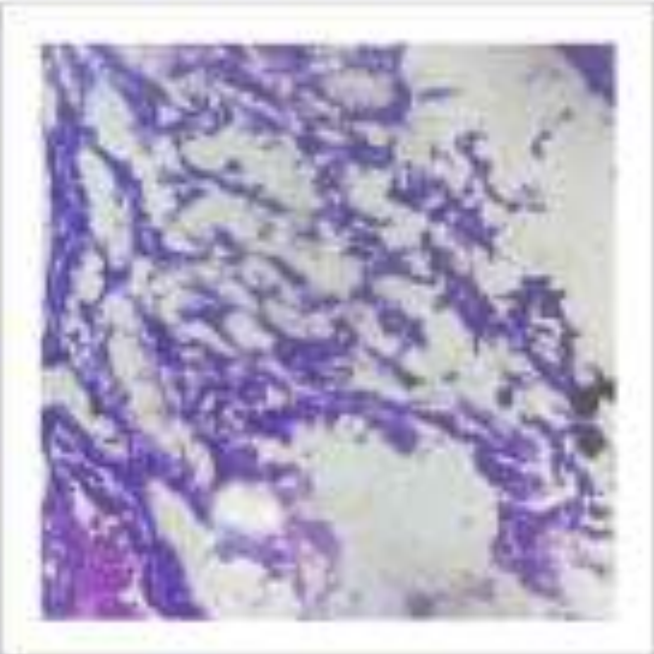
Liver-



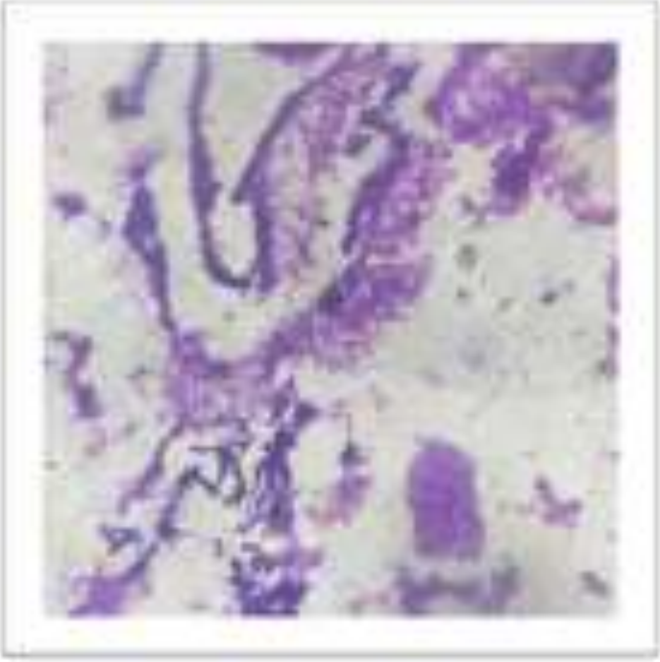
Microphotograph-71: T.S of Liver of *Catla catla*-1 of Bilawali tank in year (2017-2018) Pre-monsoon showing normal tissue.



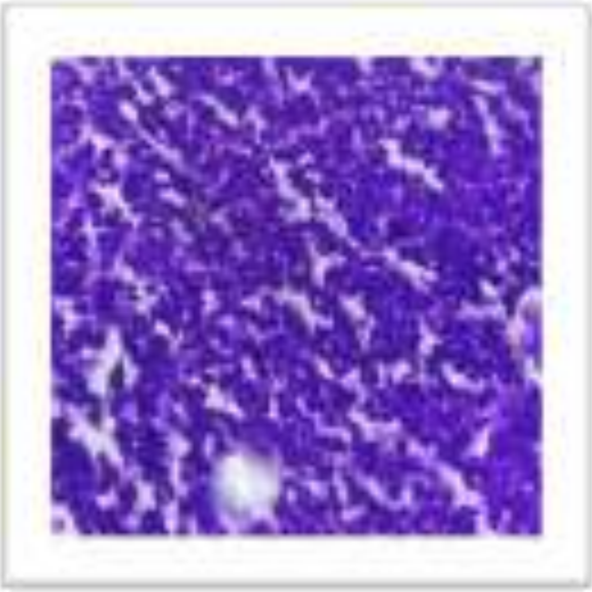
Microphotograph-72: T.S of Liver of *Catla catla*-2 of Bilawali tank in year (2017-2018) pre-monsoon showing large vacuolization.



Microphotograph-73: T.S of Liver of *Catla catla*-3 of Bilawali tank in year (2017-2018) Pre-monsoon showing degeneration of tissue.



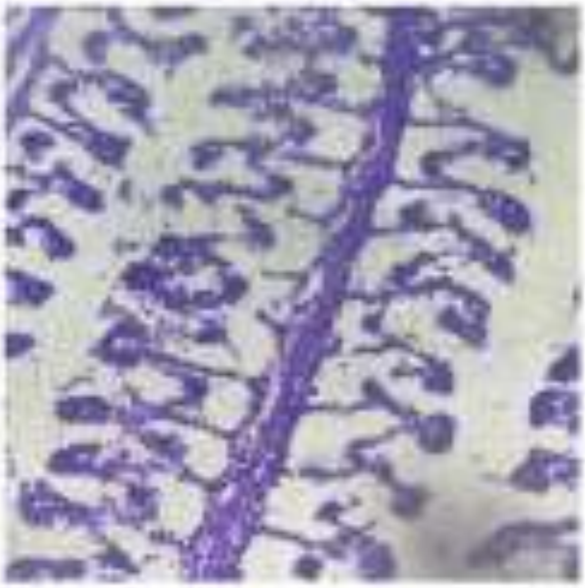
Microphotograph-74: T.S of Liver of *Catla catla*-4 of Bilawali tank in year (2017-2018) pre-monsoon showing degeneration of cell.



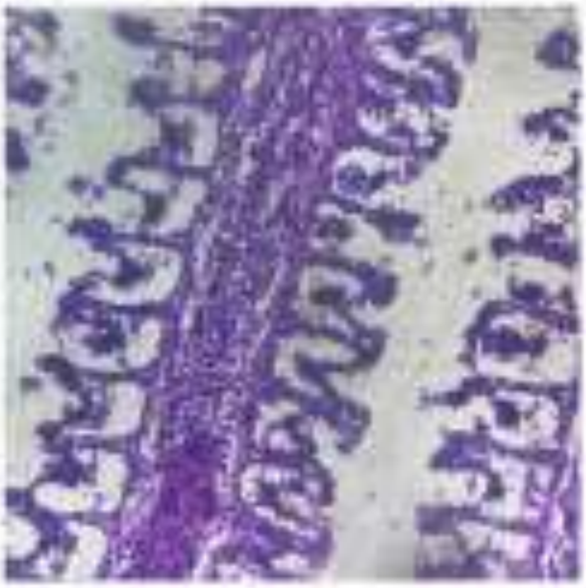
Microphotograph-75: T.S of Liver of *Catla catla-5* of Bilawali tank in year (2017-2018) Pre-monsoon showing Nuclear degeneration.

Histological analysis in fish tissues (2017-2018) (Sirpur lake) (Post-monsoon)

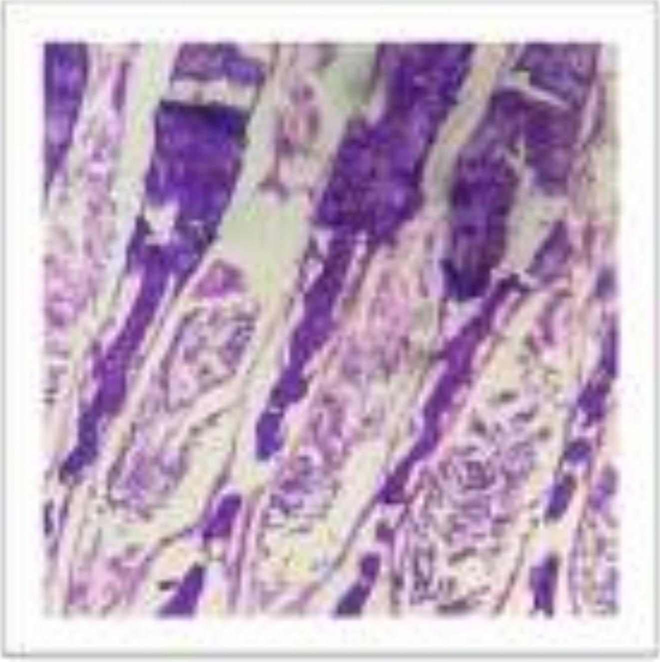
Gills-



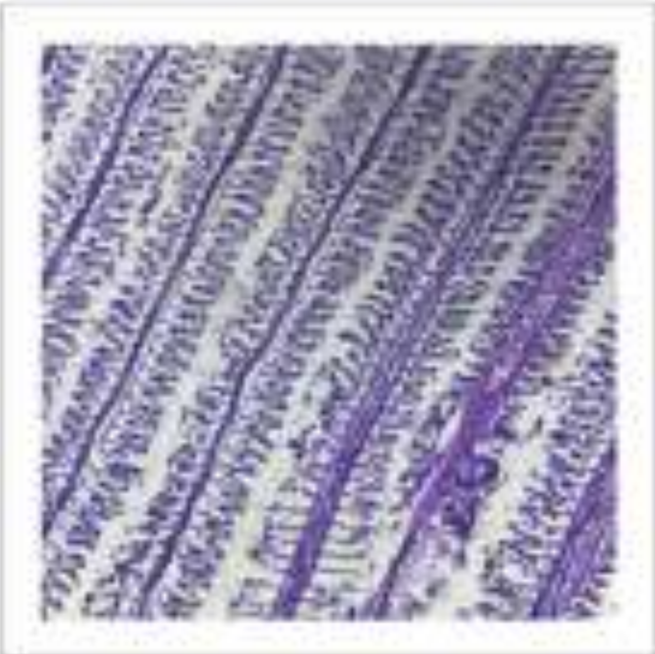
Microphotograph-76: T.S of Gill of *Catla catla*-1 of Sirpur lake in year (2017-2018) Pre-monsoon showing Degeneration of lamella.



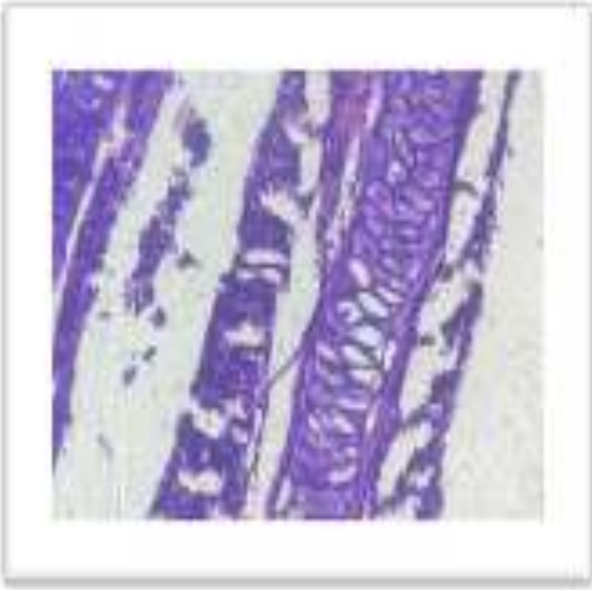
Microphotograph-77: T.S of Gill of *Catla catla*-2 of Sirpur lake in year (2017-2018) Pre-monsoon showing Blood congestion.



Microphotograph-78: T.S of Gill of *Catla catla*-3 of Sirpur lake in year (2017-2018) Pre-monsoon showing Necrosis.

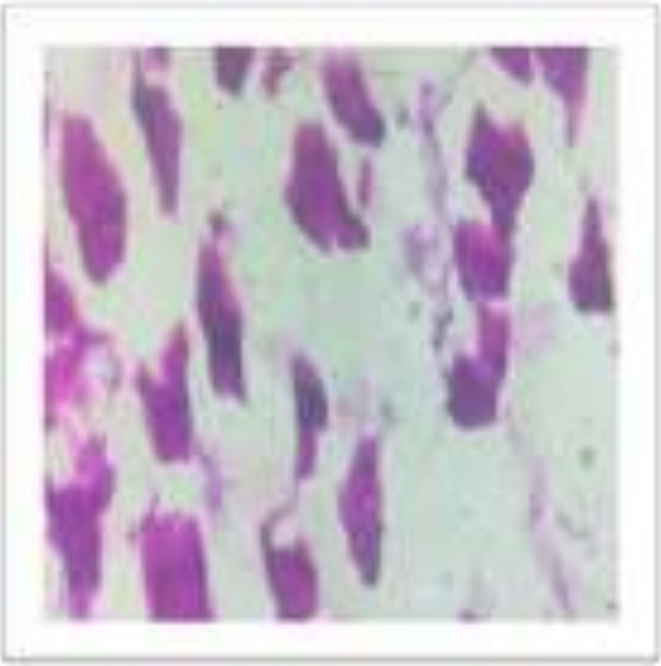


Microphotograph-79: T.S of Gill of *Catla catla*-4 of Sirpur lake in year (2017-2018) Pre-monsoon showing Fusion of lamella and Shortening of secondary lamella.

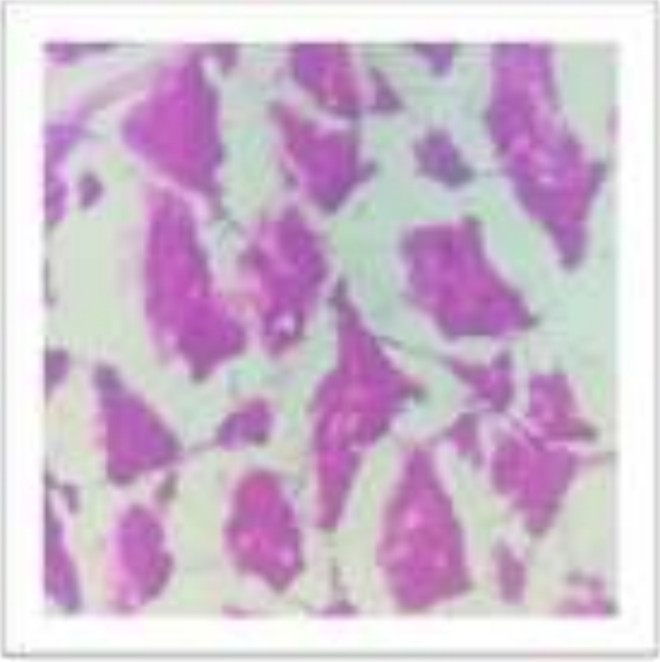


Microphotograph-80: T.S of Liver of *Catla catla*-5 of Sirpur lake in year (2017-2018) pre-monsoon showing haemorrhage in center of lamella.

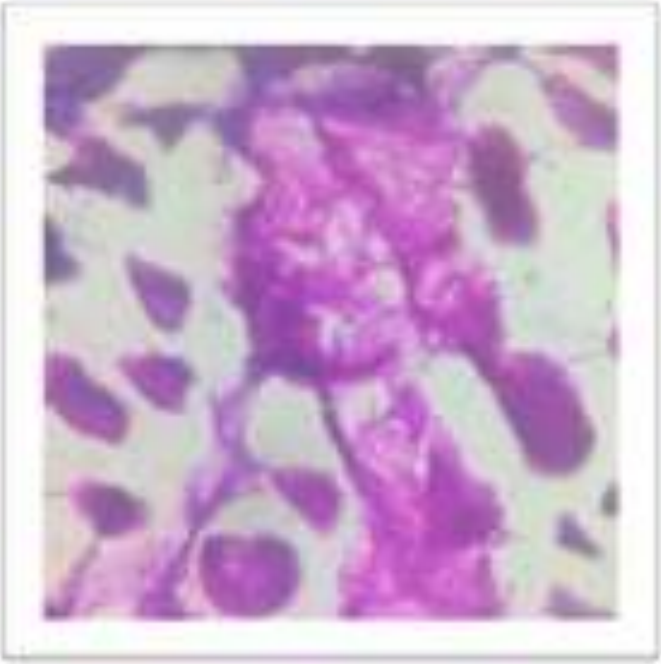
Muscles-



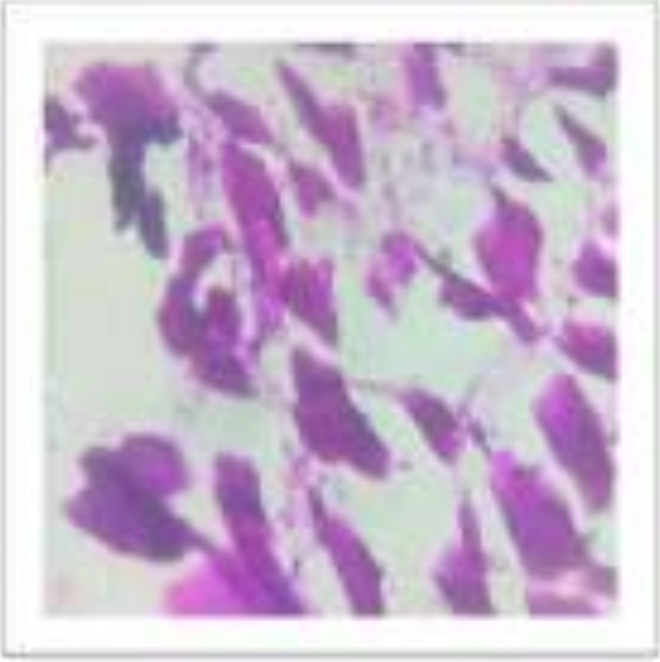
Microphotograph-81: T.S of Muscles of *Catla catla*-1 of Sirpur lake in year (2017-2018) pre-monsoon showing loosely packed fibres.



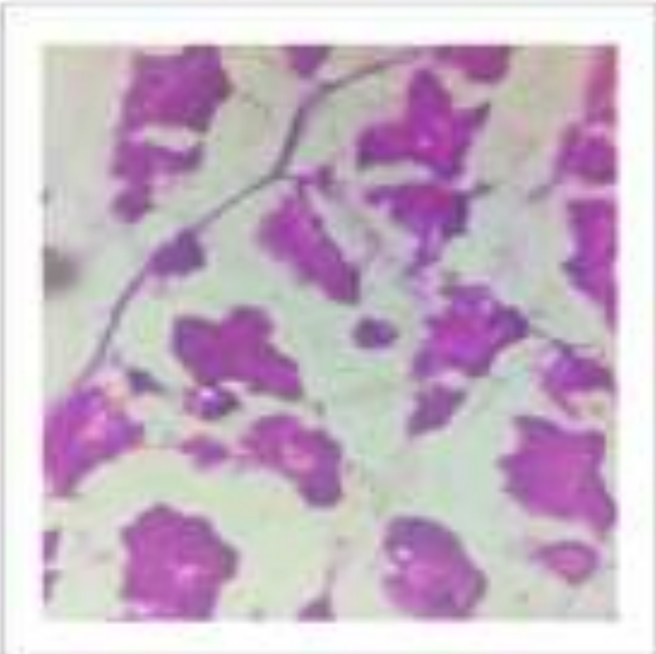
Microphotograph-82: T.S of Muscles of *Catla catla*-2 of Sirpur lake in year (2017-2018) pre-monsoon showing structural deformation.



Microphotograph-83: T.S of Muscles of *Catla catla*-3 of Sirpur lake in year (2017-2018) pre-monsoon showing splitting of muscle fibres.

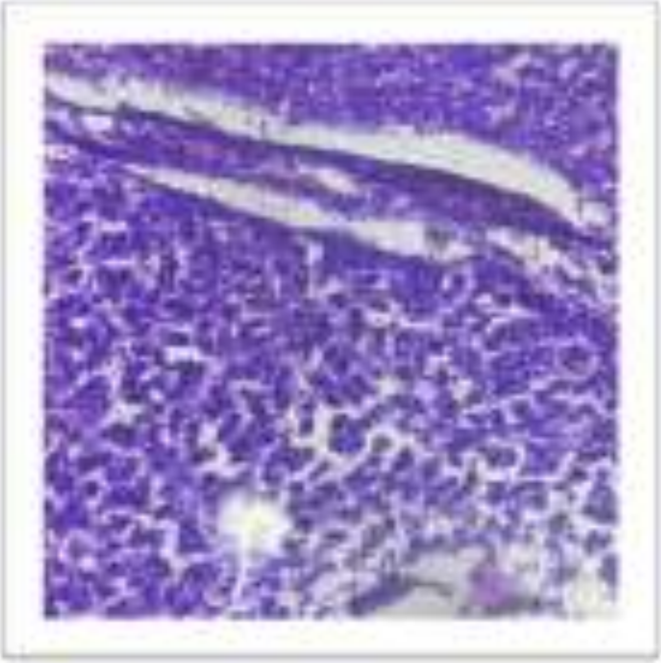


Microphotograph-84: T.S of Muscles of *Catla catla*-4 of Sirpur lake in year (2017-2018) pre-monsoon showing lesion that is less compact.

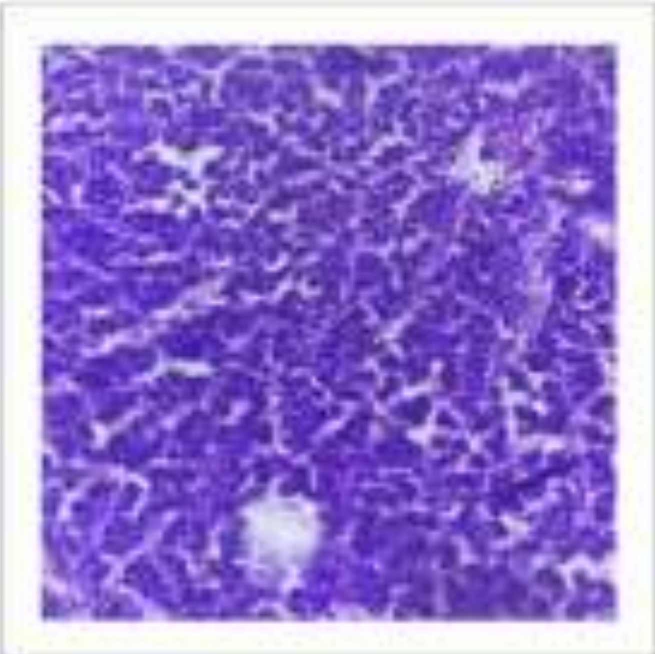


Microphotograph-85: T.S of Muscles of *Catla catla*-5 of Sirpur lake in year (2017-2018) pre-monsoon showing loosening of muscle bundles.

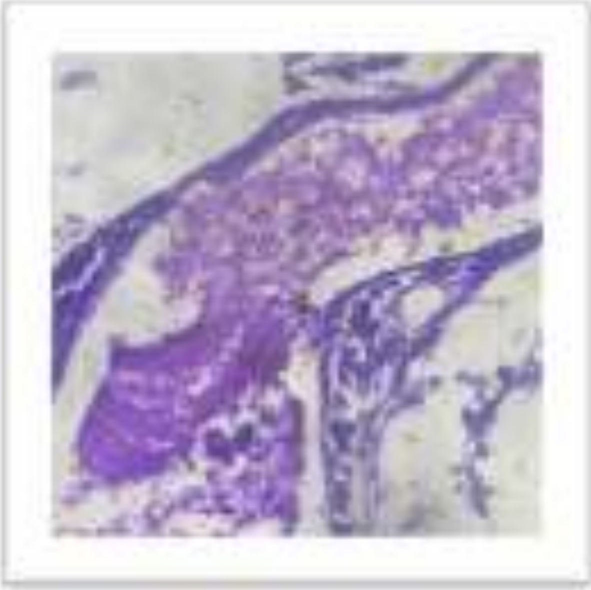
Liver-



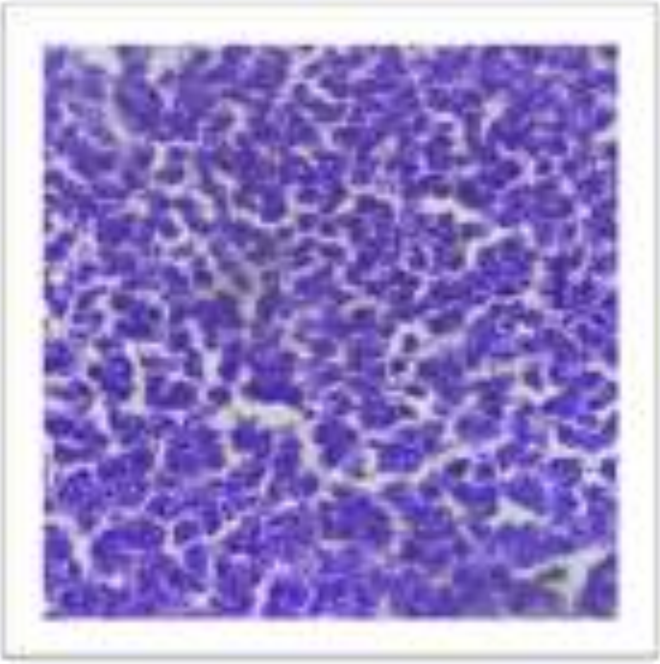
Microphotograph-86: T.S of Liver of *Catla catla*-1 of Sirpur lake in year (2017-2018) pre-monsoon showing blood congestion and cytoplasmic vacuolization.



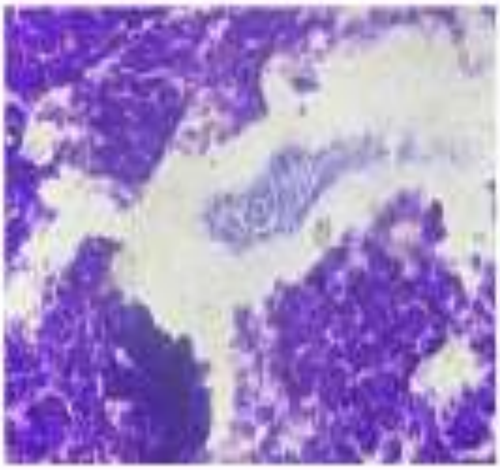
Microphotograph-87: T.S of Liver of *Catla catla*-2 of Sirpur lake in year (2017-2018) pre-monsoon showing cytoplasmic vacuolization.



Microphotograph-88: T.S of Liver of *Catla catla*-3 of Sirpur lake in year (2017-2018) pre-monsoon showing degeneration of tissue.



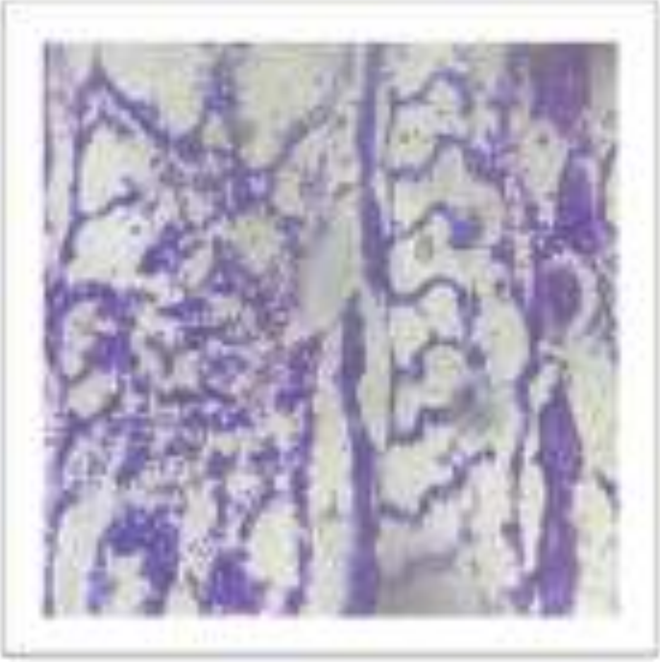
Microphotograph-89: T.S of Liver of *Catla catla*-4 of Sirpur lake in year (2017-2018) pre-monsoon showing beginning of cytoplasmic vacuolization.



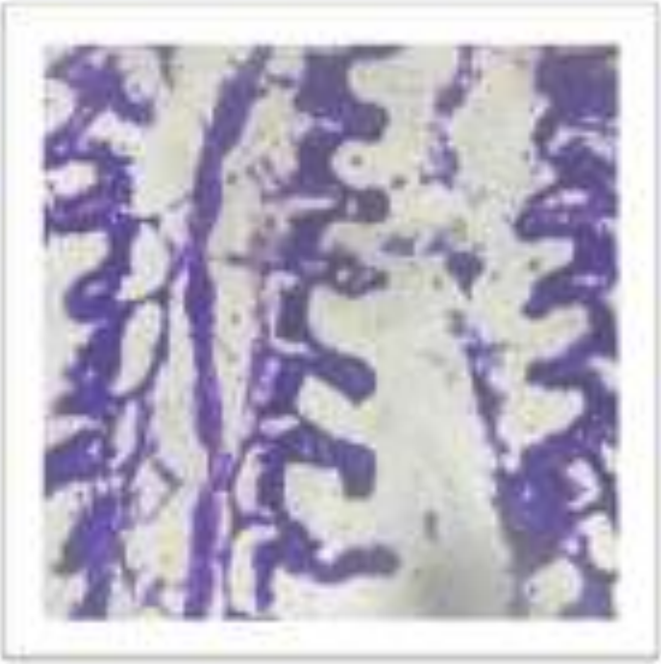
Microphotograp-90: T.S of Liver of *Catla catla*-5 of Sirpur lake in year (2017-2018) pre-monsoon showing large vacuole with hepatocyte hypertrophy.

Histological analysis in fish tissues (2017-2018) (Bilawali tank) (Post-monsoon)
Gills

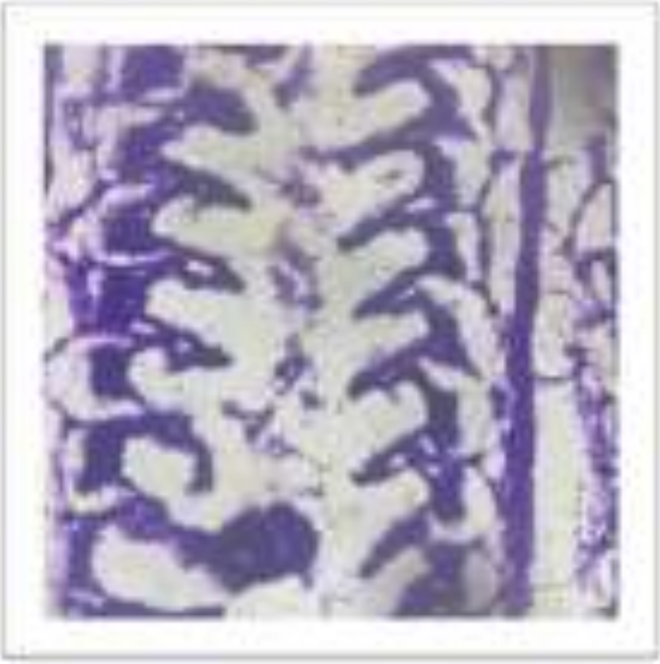
Microphotograph-91: T.S of Gill of *Catla catla*-1 of Bilawali tank in year (2017-2018) post-monsoon showing shortening and fusion of lamella.



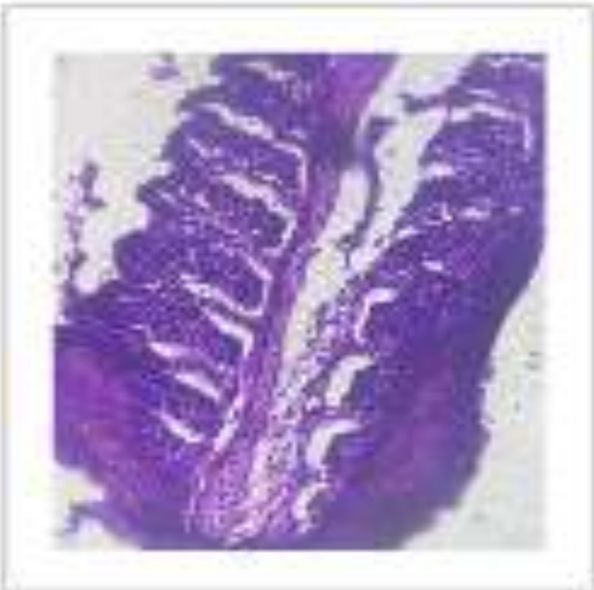
Microphotograph-92: T.S of Gill of *Catla catla*-2 of Bilawali tank in year (2017-2018) post-monsoon showing ruptured of lamella.



Microphotograph-93: T.S of Gill of *Catla catla*-3 of Bilawali tank in year (2017-2018) post-monsoon showing hyperplasia.



Microphotograph-94: T.S of Gill of *Catla catla*-4 of Bilawali tank in year (2017-2018) post-monsoon showing curling of secondary lamella.



Microphotograph-95: T.S of Gill of *Catla catla*-5 of Bilawali tank in year (2017-2018) post-monsoon showing inflamed and hypertrophy.

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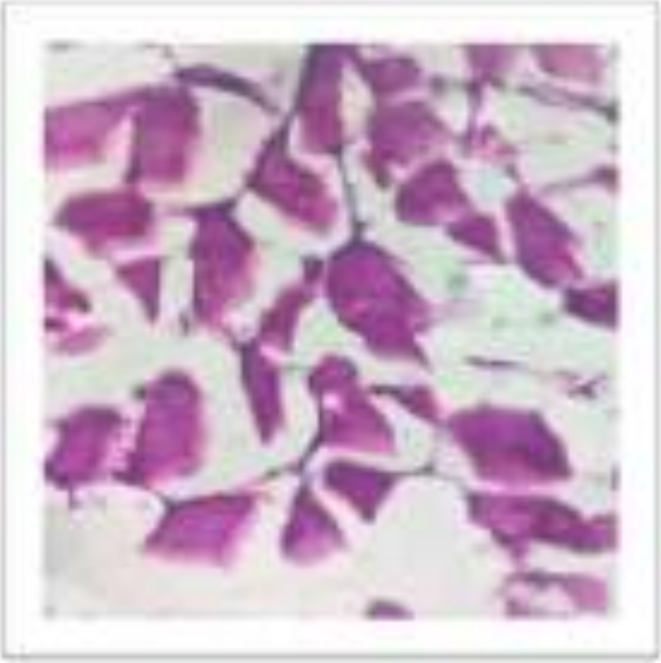
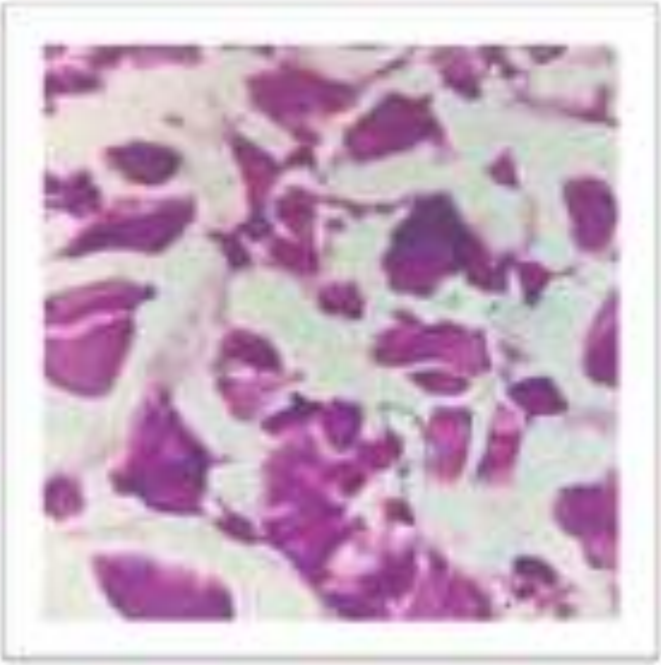
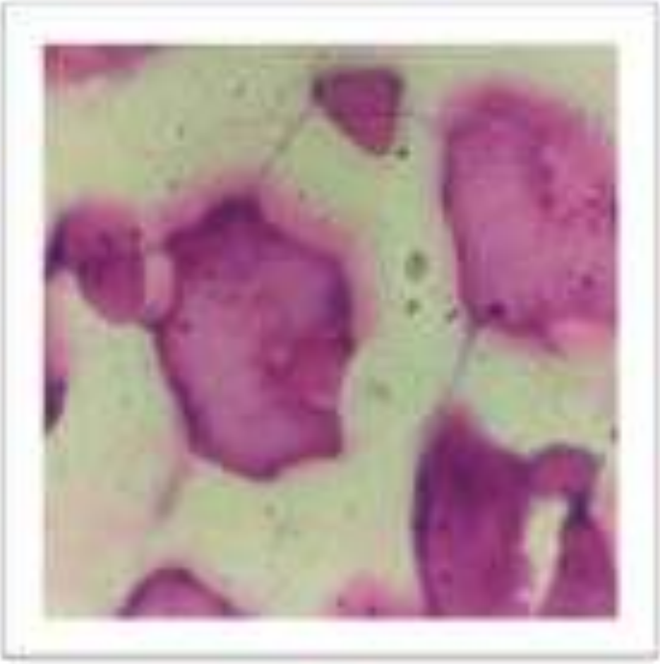
Muscles-



Microphotograph-96: T.S of Muscles of *Catla catla*-1 of Bilawali tank in year (2017-2018) post-monsoon showing normal tissue.

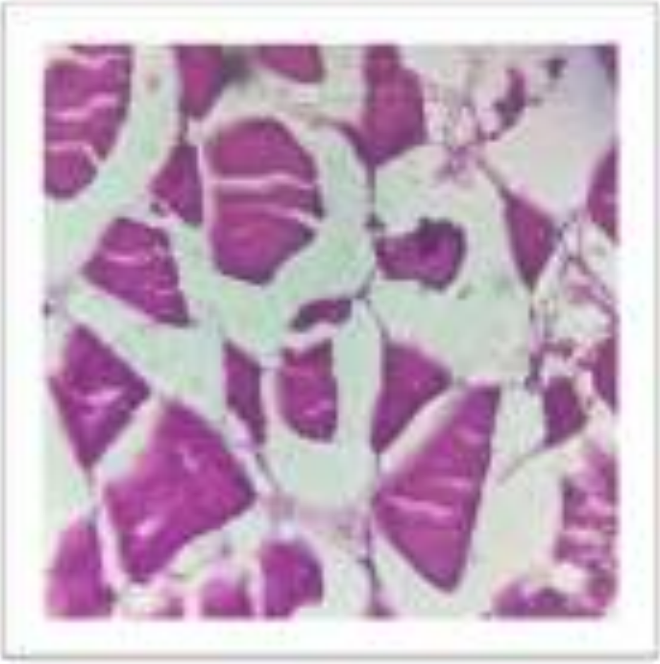


Microphotograph-97: T.S of Muscles of *Catla catla*-2 of Bilawali tank in year (2017-2018) pre-monsoon showing splitting of muscle fibres.



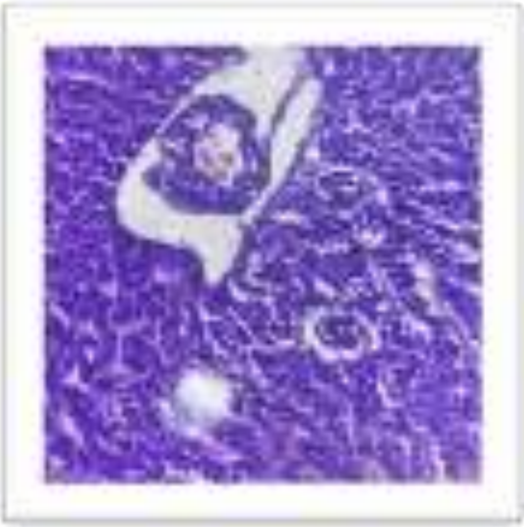
Microphotograph-98: T.S of Muscles of *Catla catla*-3 of Bilawali tank in year (2017-2018) post-monsoon showing structural changes decreasing size of muscle bundle.

Microphotograph-99: T.S of Muscles of *Catla catla*-4 of Bilawali tank in year (2017-2018) post-monsoon showing degeneration of muscle bundles.

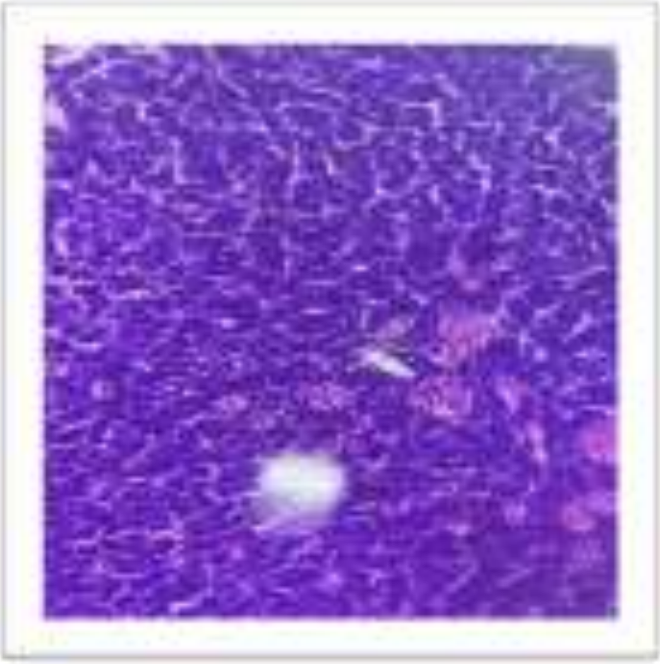


Microphotograph-100: of T.S of Muscles of *Catla catla*-5 of Bilawali tank in year (2017-2018) post-monsoon showing splitting of my muscle bundles.

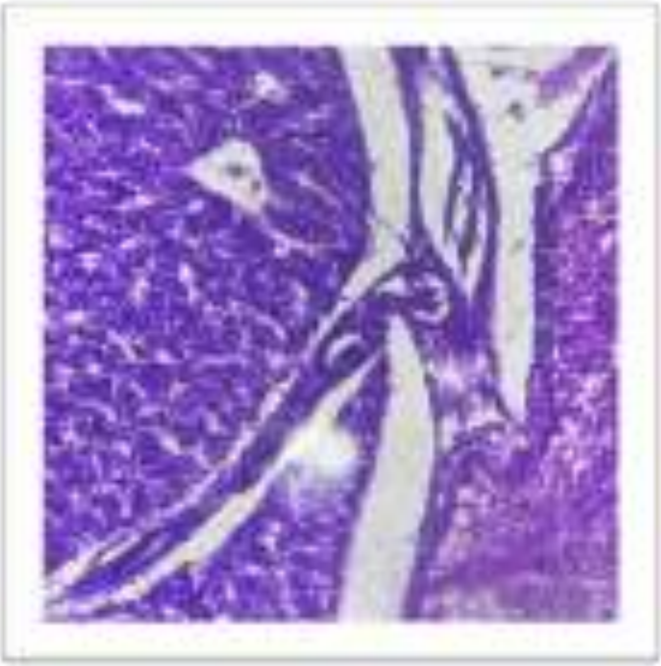
Liver-



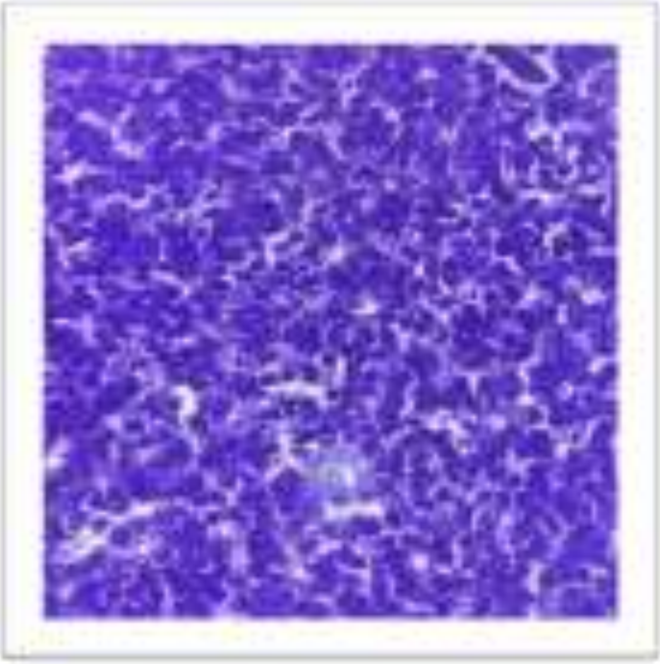
Microphotograph-101: T.S of Liver of *Catla catla*-1 of Bilawali tank in year (2017-2018) post-monsoon showing degeneration of arteriole.



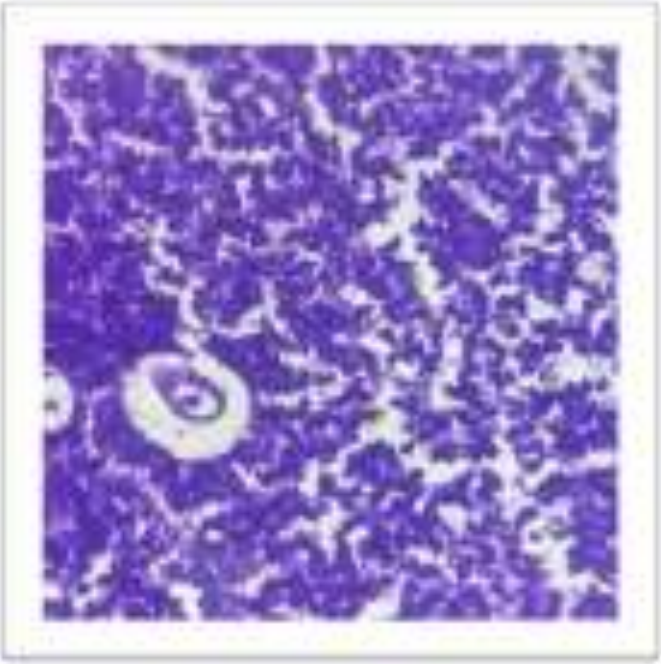
Microphotograph-102: T.S of Liver of *Catla catla*-2 of Bilawali tank in year (2017-2018) post-monsoon showing blood congestion.



Microphotograph-103: T.S of Liver of *Catla catla*-3 of Bilawali tank in year (2017-2018) post-monsoon showing large cytoplasmic vacuoles.

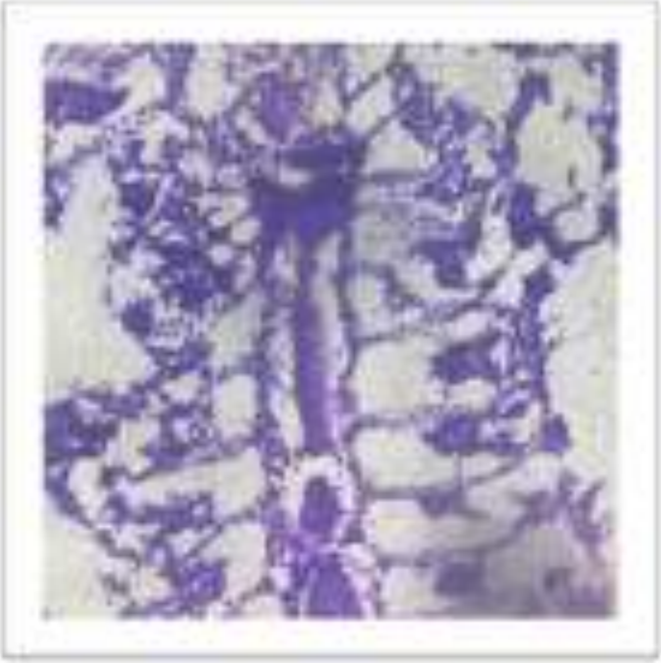


Microphotograph-104: T.S of Liver of *Catla catla*-4 of Bilawali tank in year (2017-2018) post-monsoon showing normal tissue.

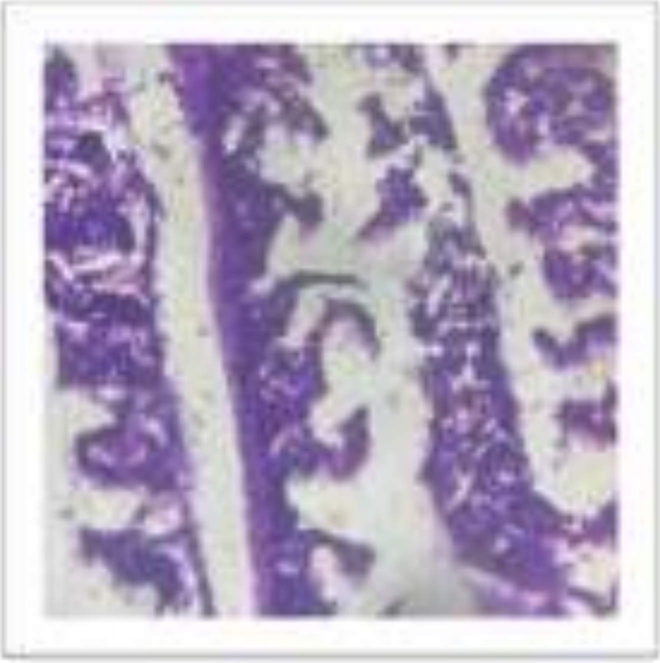


Microphotograph-105: T.S of Liver of *Catla catla*-5 of Bilawali tank in year (2017-2018) post-monsoon showing cytoplasmic vacuolation.

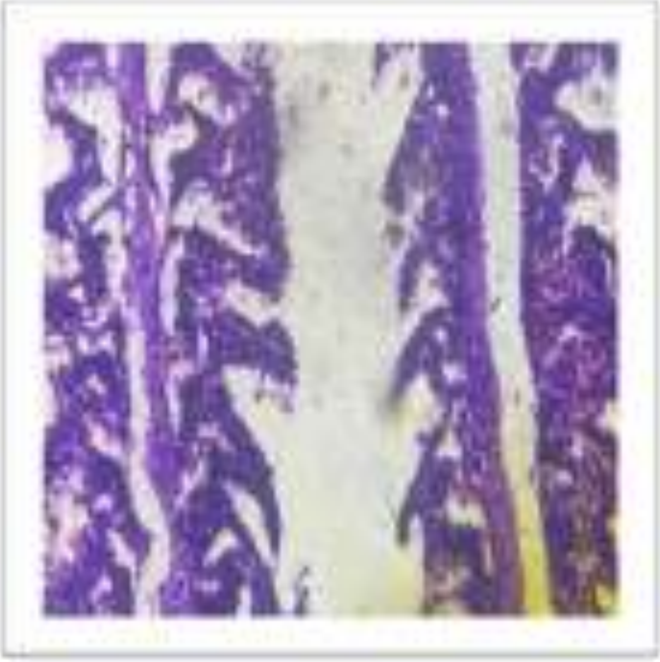
Histological analysis in fish tissues (2017-2018)(Sirpur lake)(Post-monsoon)
Gills-



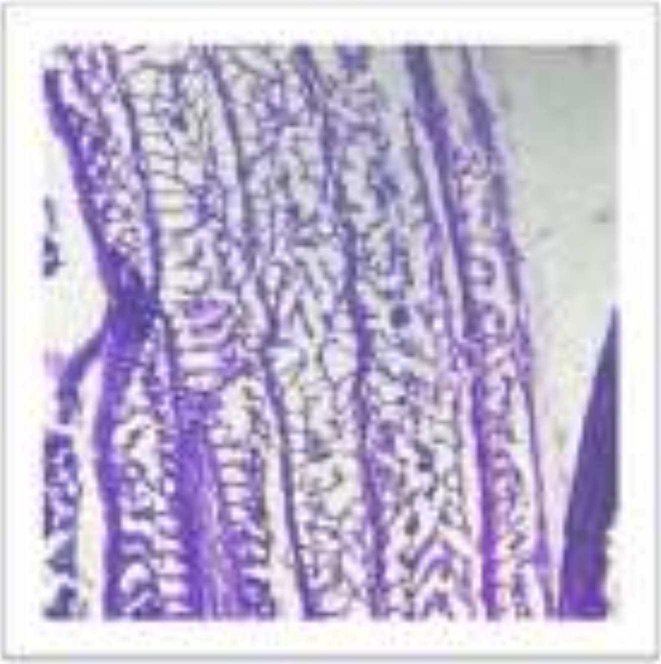
Microphotograph-106: T.S of Gill of *Catla catla*-1 of Sirpur lake in year (2017-2018) post-monsoon showing dis-organisation of cartilaginous core.



Microphotograph-107: T.S of Gill of *Catla catla*-2 of Sirpur lake in year (2017-2018) post-monsoon showing degeneration of lamella.



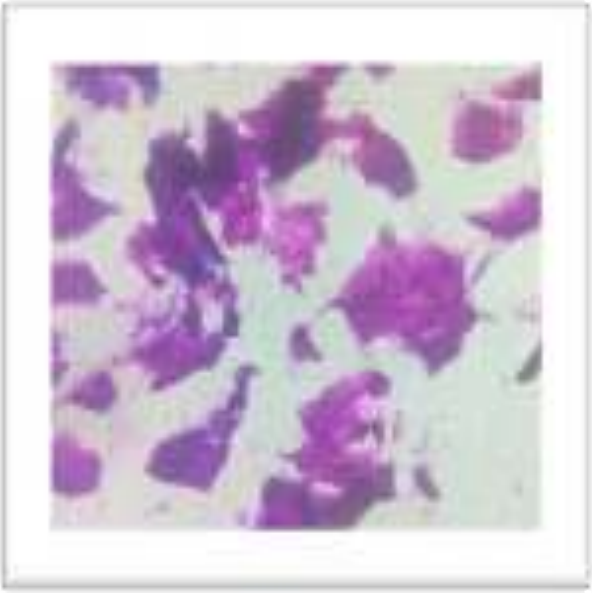
Microphotograph-108: T.S of Gill of*Catla catla*-3 of Sirpur lake in year (2017-2018) post-monsoon showing shortening of secondary lamella and cellular hypertrophy.



Microphotograph-109: T.S of Gill of *Catla catla*-4 of Sirpur lake in year (2017-2018) post-monsoon showing rupture of primary and secondary lamella.

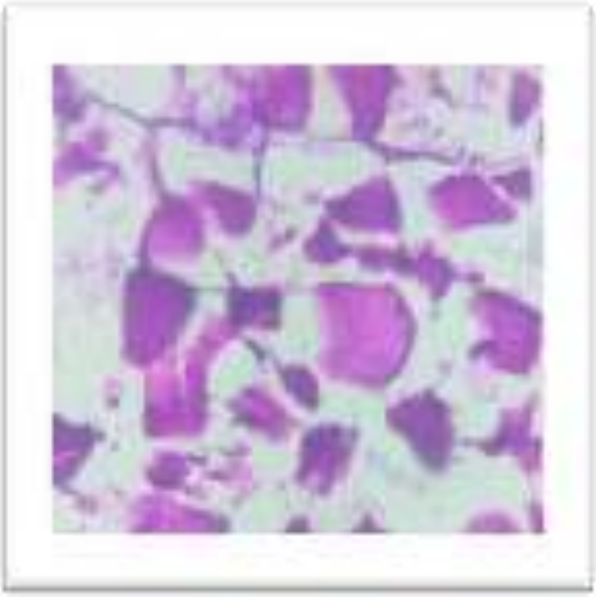
Microphotograph-110: T.S of Gill of *Catla catla*-5 of Sirpur lake in year (2017-2018) post-monsoon showing Chloride cells.

Muscles-

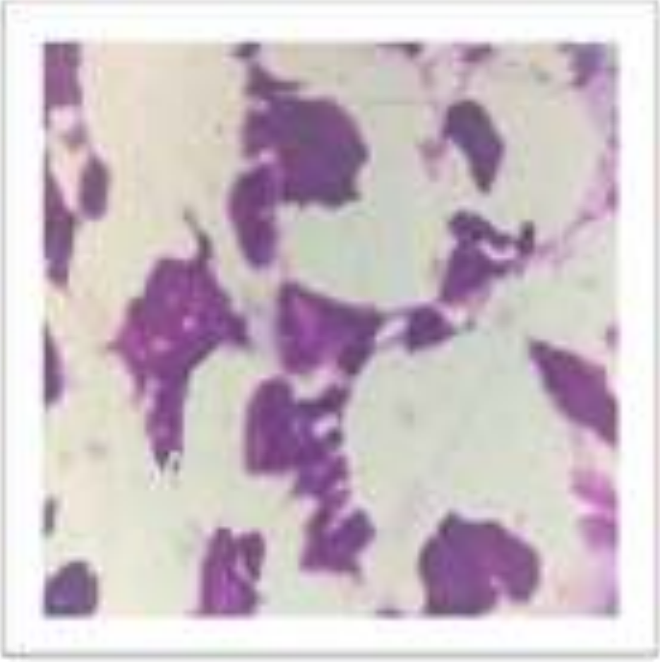


Microphotograph-111: T.S of Muscles of *Catla catla*-1 of Sirpur lake in year (2017-2018) post-monsoon showing focal area of necrosis.

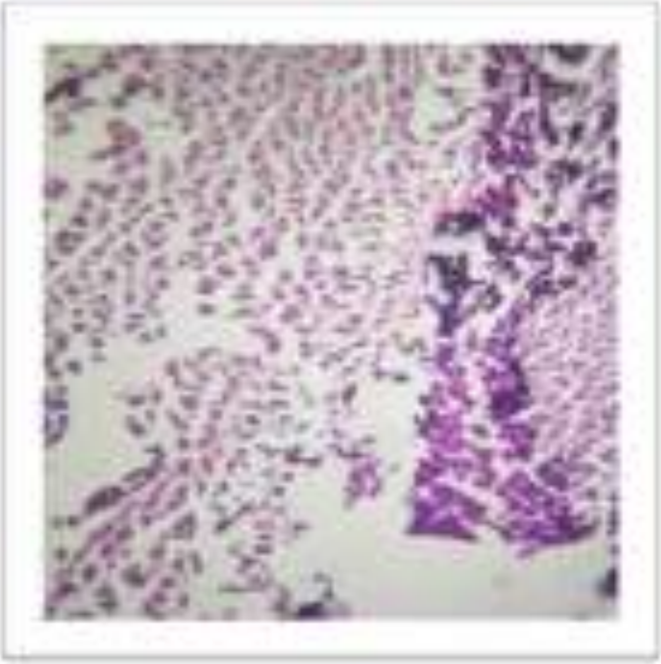
Microphotograph-112: T.S of Muscles of *Catla catla*-2 of Sirpur lake in year (2017-2018) post-monsoon showing splitting of muscle fibre .



Microphotograph-113: T.S of Muscles of *Catla catla*-3 of Sirpur lake in year (2017-2018) post-monsoon showing muscular atrophy.

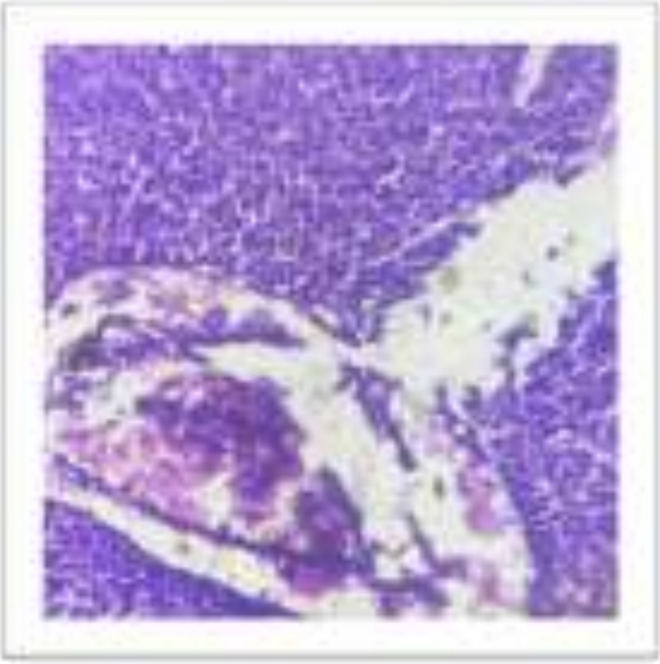


Microphotograph-114: T.S of Muscles of *Catla catla*-4 of Sirpur lake in year (2017-2018) post-monsoon showing degeneration of muscle fibre.

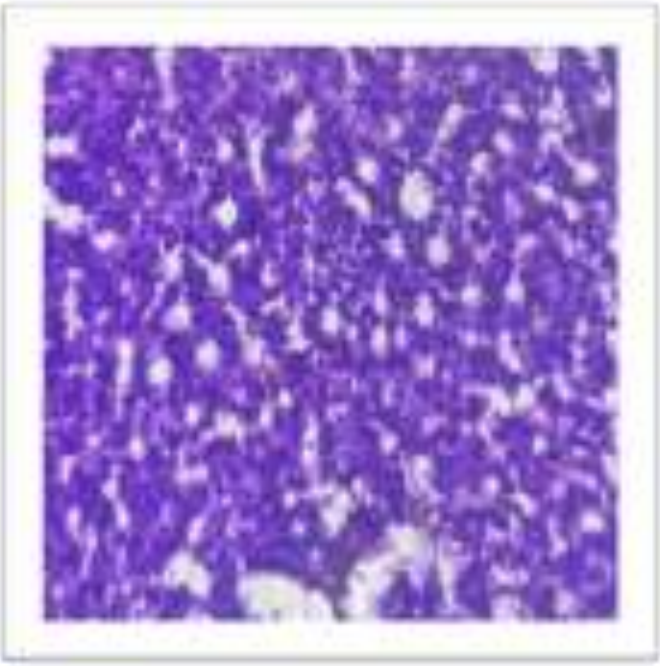


Microphotograph-115: T.S of Muscles of *Catla catla*-5 of Sirpur lake in year (2017-2018) post-monsoon showing cell degeneration.

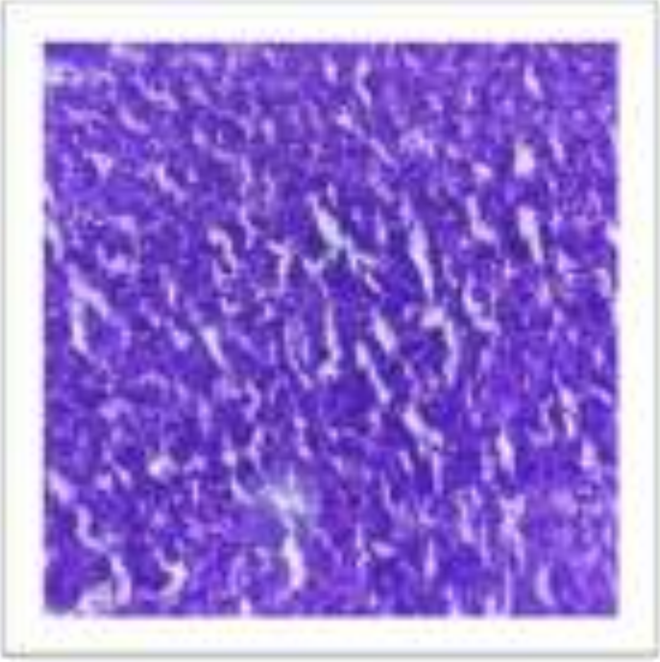
Liver-



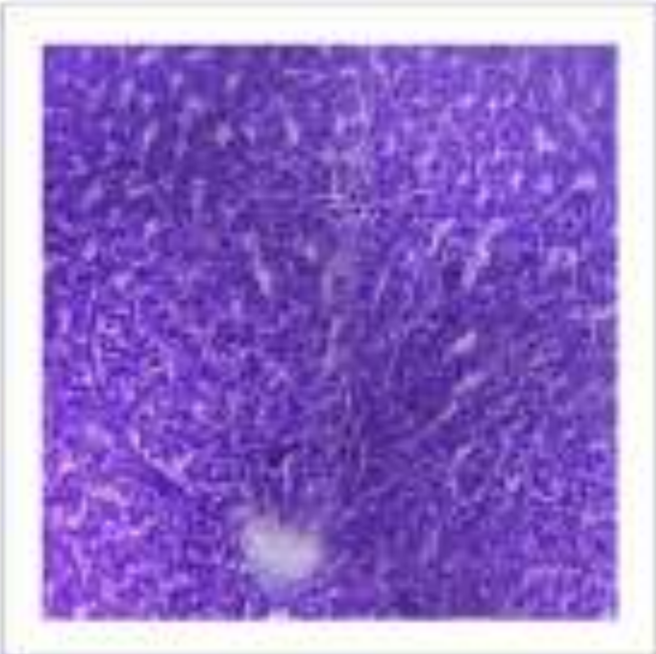
Microphotograph-116: T.S of Liver of *Catla catla*-1 of Sirpur lake in year (2017-2018) post-monsoon showing cellular infiltration.



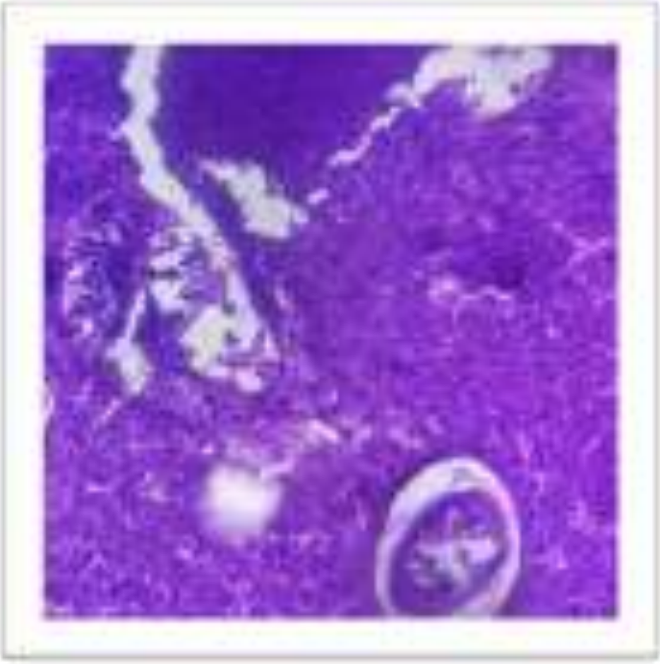
Microphotograph-117: T.S of Liver of *Catla catla*-2 of Sirpur lake in year (2017-2018) post-monsoon showing partially vacuolated hepatocytes.



Microphotograph-118: T.S of Liver of *Catla catla*-3 of Sirpur lake in year (2017-2018) post-monsoon showing hepatocyte hypertrophy.



Microphotograph-119: T.S of Liver of *Catla catla*-4 of Sirpur lake in year (2017-2018) post-monsoon showing normal hepatic tissue.



Microphotograph-120: T.S of Liver of *Catla catla*-5 of Sirpur lake in year (2017-2018) post-monsoon showing blood congestion .

Chapter 5

DISCUSSION

Drinking water cadmium contamination has become a significant issue in Bangladesh, West Bengal, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, Korea, and Pakistan. Additionally, it has been shown that groundwater, marine habitats, and freshwater ecosystems are all polluted. Recently, cadmium poisoning in the aquatic environment has worsened, mostly due to humanitarian goals (Waisberg, M, et al , 2003).

Heavy metals are conservative pollutants because they degrade over such a long period of time that they essentially become a permanent part of the aquatic ecosystem (Mason, 1996).Cadmium is a heavy metal that is not essential. It is one of the most dangerous threats for the environment. Industrial activity is one of the causes of cadmium in aquatic environments. Zinc smelting, used batteries, e-waste, paint, sludge, incineration, and fuel combustion are some of the industrial sources of Cd (Stohs, 1995). Cd may accumulate within organisms and ascend up the food chain. Cadmium's acute toxicity to aquatic organisms varies, and it is even tightly correlated with the metal's free ionic concentration. Cadmium toxicity in fish has been demonstrated to be species-specific .Yasmeen, S. and Pathan, T.S.,2021)

A variety of aquatic creatures and the ecological balance of the receiving habitat may be severely harmed by heavy metal pollution (Ashraj, 2005; Farombi & Adedlowo, 2007). Bioaccumulation of metals happens when organisms take in and retain metals from their immediate environment, which includes water, sediments, suspended particles, and prey creatures. Bioaccumulation occurs when chemical incorporation exceeds chemical metabolism or excretion. As a result, tissue analysis can detect pollutants that might otherwise go unidentified (Avenant-Oldewage,A., 2000)

The most of fish have been tested in assessments of aquatic system quality. These species may concentrate significant amounts of metals from the nearby waters since they are frequently at the top of the aquatic food chain (Rajkowska and Protasowicki, 2011) . We can affirm from this study that the freshwater edible fish *Catla catla* serves as a biological indicator organism to scale the degree of pollution in Indore's Bilawali and Sirpur ponds. Strong evidence existed for a relationship between the levels of heavy metals in various fish tissues and those in the contaminated areas' surface waters. Seasonal fluctuations in heavy metal accumulation in fish tissues and physicochemical parameters, heavy metal analysis in water both were observed. The current study's findings revealed that Site A (Bilawali tank) had higher mean physicochemical, cadmium and chloride values than Site B (Sirpur lake).

Blood glucose and total proteins are two biochemical parameters that reported declining levels. The lower total protein level was a sign of stress. The lower total protein level was a sign of stress. Different abiotic environmental conditions, such as variations in water temperature, pH, and oxygen content, and water contaminants such heavy metals, can cause stress in fish. (Vijayan, P,2017) The physiological effects of stress include alterations in the immune system and blood composition. Fish that are stressed might also come from fish farming and fish rearing.Since the values in the current study are within the range that is suitable for the living things, temperature is an important factor that regulates the biogeochemical activities in the aquatic environment, including fish (Boyd C.E,1990)

In experimental specimens, the structure of the gills, liver, and muscles changed in a number of ways. Gills are immediately exposed to pollution since they are frequently in touch with water. In the first year of sampling, several samples showed secondary lamellae fusing together as well as inflammatory cell infiltration and hypertrophy. Lamellae were shown to dilate and curl in the secondary gill lamella. In second year, It is seen that the primary gill lamellae enlarge, the epithelium rises, and the secondary gill lamellae merge. Around the axis of the main and secondary lamellae, the gill epitheliums displayed necrosis, expansion of the gill filament, and clogging of the respiratory lamellae. Lamellar epithelium hyperplasia was observed. There is muscular tissue injury along with muscle atrophy, exhibiting necrosis and loss to muscular tissues. Hyaline degeneration and muscle splitting were seen. Lesions that are less compact were observed in year 1. Displaying oedema in the muscular tissue and injury to the muscle tissues. Tissue degradation with intra-myofibril space. With internal edoema, vacuolar degeneration, atrophy, and a localised region of necrosis, the muscular bundle thickens and separates in year 2. Hepatic tissue damage accompanied with sinusoid dilatation (SD) and necrosis, hepatocyte vacuolization and consequent damage to liver tissues is seen during year 1. Degeneration of hepatocytes and an increase in mitochondria are observed in histological slides in year 2.

This study shows that the bioaccumulation of a certain heavy metal has reached an alarming level in Indore's fresh water aquatic systems (Bilawali tank and Sirpur talab). Therefore, stringent laws should be put in place to manage industrial waste coming from point sources. Detoxification techniques are therefore necessary to restore the health of economically significant fish in a stressful environment. In order to avoid the exploitation of precious water resources, monitor their water quality, and restore them for the benefit of society and the environment, monitoring programmes need to be periodically initiated. In order to prevent epidemics like those that occurred in Japan owing to the ingestion of fish and fisheries products contaminated with heavy metals, food authorities should screen fish before they are consumed by people. The study of physicochemical parameters is essential for obtaining a precise understanding of the quality of water, and it allows us to compare the findings of various physicochemical parameter values with guideline values. Both Sirpur Lake and Bilawali Tank water reservoirs are always under attack from anthropogenic contaminants coming from different sources either in the catchment areas or in other remote locations. The majority of its water is utilised to cultivate farmland and provide drinking water to local towns, villages, and industrial locations. Monitoring the reservoir's heavy metal pollution is therefore urgently necessary. The majority of researchers have focused on the physico-chemical characteristics of the reservoir rather than conducting bio-monitoring studies to find levels of heavy metal pollution in the water, sediment, and biota. For this reason, the current study was undertaken.

Because different individual react differently to the same dose of a toxic agent, toxicity is species-specific. The rate of metal intake from a source of the metal, the rate of efflux, and the rate of detoxification of deposited metal into a relatively metabolically inert form are three physiological processes that may change in aquatic creatures exposed to very high local bioavailable hazardous metals.(Vijayan, P., 2017).

Fish either absorb heavy metals through their diet or through the water they ingest. The water pathway has been shown to be more significant in aquatic biota . Since heavy metals are toxic, persistent, bioaccumulative, and biomagnifying in the food chain, they poses a serious threat to aquatic organisms. It's important to measure bioaccumulation. Studies on techniques to track the absorption and retention of contaminants like metals or pesticides in organs or tissues of creatures like fish are known as "bioaccumulation measures".The ability of harmful contaminants, such as metals, to collect in an organism's organs is one of their most crucial properties (Palniappan and Karthikeyan, 2009) .

According to estimates, roughly 2000 tonnes of cadmium are released each year as a consequence of human activity and 500 tonnes are released each year as a result of natural weathering. Heavy metal cadmium is well-known for being poisonous and having a negative impact on fish.

Heavy metals from industrial, agricultural, and household wastes contaminate water sources, making them chronic metal toxins since they enter human tissues through the food chain. It has been noted that Cd is present in the water and in several fish tissues, including *Catla catla* that have been taken from water bodies. The amount of cadmium (Cd) in the water varies significantly depending on a number of variables, including the season, hydrological parameters, biological availability, and other chemical complexes. The seasonal change in the metal concentrations showed that pre-monsoon had a high concentration of Cd.

It was shown that diluting during the rainy season significantly reduces the metal content level. However, it is acknowledged that the enrichment of these metals through bio-magnification and bioaccumulation in food components made in water has a significant impact on the water.(Basis, U.E.,2017)

According to earlier descriptions , typically reported low Cd concentrations in water during summer are caused by this metal's absorption by organisms, adsorption on to particulate matter, and the possibility that some of the metal may be torn off .

The cadmium content was highest in the pre-monsoon and lowest during the post-monsoon seasons. Local fishing activity, anthropogenic influences, and municipal sewage and sludge discharges may have increased the concentration of Cd in the sediments; the lowest Cd concentrations, which were observed during the post-monsoon seasons, were likely caused by the use by the micro-phyto-benthic community. The high concentration of Cd in the water may be reflected in the build-up of Cd in fish tissues. The fact that the gills are one of the body's most permeable parts and the primary sites of respiration in addition to transporting ions during osmosis regulation may be the primary cause of the increased build-up of Cd there.During the pre-monsoon, all heavy metal values were quite prevalent. This might be explained by the differences in heat flow conditions between winter and rainy season and summer. Consequently, this research may attest to the biological indicator organism status of the freshwater edible fish *Catla catla* as a means of determining the degree of pollution(Shobha, k.,2007).

According to this research, the body's elevated metabolic rate brought on by the hot weather and reproductive activity is the reason why most haematological measurements in the premonsoon were higher than they were at other times of the year. On the other side, a low metabolic rate and a low ambient temperature could have contributed to the lowest result, which happened during the postmonsoon.

According to the current research, Cadmium causes respiratory distress in fish, and opercular beats per minute can be used as a good biomarker to assess the health of these prized and valuable fish as well as the deteriorating situation of aquatic bodies with regard to metallic contaminants, particularly Cadmium.

The decrease in surface area available for gaseous exchange is the immediate physiological effect of the lamellar fusion, which might negatively impact the fish's respiratory physiology. Fish mucus is thought to serve a variety of purposes, including defence against UV radiation and environmental toxins (McKim and Lien, 2001; Häkkinen et al., 2003). According to many researchers, some fish mucous constituents likely the acidic and/or sulphated glycoprotein moieties—have a metal-binding property (Pärt and Lock, 1983).

Protein-rich muscle creates mechanical tissue meant for motion and is not involved in metabolism. The liver, which serves as the hub for several metabolisms, is also a protein-rich organ. The current study found that the majority of fish tissues had lower protein contents than expected. This might be because the gluconeogenesis pathway uses keto-acids to make glucose, or because free amino acids are directed toward protein synthesis or the maintenance of osmosis and ionic balance. It could also be brought on by the synthesis of heat shock proteins, harmful free radicals, or heavy metal-induced apoptosis.

It is obvious that the glycogen stores are being used to cope with the stress generated by the increase in tissue glucose levels in exposed fish. Fish under stress have higher blood glucose levels, according to(Choudhary, P. et al.2004). The particular activity of several enzymes including phosphofructokinase, lactate dehydrogenase, and citrate kinase that reduce the capacity of glycolysis is one of the causes of this, which can be attributed to a number of circumstances.

Studies on the acute toxicity of cadmium on *Catla catla*, an edible carp, indicated considerable alterations in the fish's metabolic components, including glucose and total proteins. The deceased fish's hemorrhagic circumstances clearly show the cadmium's harmful effects. It revealed the fish's general cadmium stress response. Furthermore, the increased proteolysis in those tissues or a decrease in protein synthesis brought on by those tissues coming from cadmium-toxic fish may be the source of the drop in protein concentration across all tissues.

Chapter 7

CONCLUSION

This thesis provides a quick overview of the cadmium levels in fish tissues and aquatic bodies, as well as the sources of cd emissions, as well as freshwater fish intake and effect.

Due to a growth in human population, urbanisation, and industrialization, there is an increase in sewage water, industrial effluents, and inorganic fertilisers, which contribute to environmental degradation. Environmental contaminants have a dangerous effect on the planet's living organisms. Animal and human health is negatively impacted by environmental contaminants. The most prevalent environmental toxins with the ability to bio-accumulate and stay in the body includes cadmium and its compounds, which also cause a variety of biotic alterations in the aquatic ecosystem. In order to protect the public's health, it is important to keep an eye on the levels of harmful metals in aquatic ecosystems, particularly in fish.

Pollutants in the aquatic environment have risen due to extensive industrialisation and urbanisation. Fish poisoning can be acute or chronic as a result of toxic pollution discharge into waterways. Beyond that, domestic vegetables, cotton, grains, and pulses are all grown. To safeguard the crops, a staggering number of pesticides and insecticides are employed to combat insects and other pests. The non-degraded contaminant was discharged into ponds or other bodies of water utilised for fish cultivation. Fish habitats in rural, agricultural, and industrial areas of India are directly impacted by the production of large carps, which are raised for human consumption as a key source of protein. assess the effects of toxins and heavy metal stress on Indian major carps. The current investigation was conducted, as per *Catla catla*.

Heavy metals are subtle, conservative contaminants that harm the aquatic ecosystem by bio-accumulating and biomagnifying as they go up the food chain. Freshwater fish have a wide range of affinities with CD. The origins and effects of heavy metal pollution in fish organs are highlighted in this study. There was convincing evidence of a relationship between the levels of heavy metals in fish tissue and those in the surface waters of Indore's water bodies. Metal buildup in fish organs may result in structural damage and functional problems.

Fish eating is a significant source of protein for the general population; hence the levels of Cd contamination in fish are of great importance. The majority of the Cd in fish or other seafood is highly absorbable in the form of Cadmium chloride.

The initial goal of this study is to emphasise the effects of the bioaccumulation of heavy metals in various fish organs and the variables influencing their dispersion. The second is to keep an eye on the sources, concentrations, and pollution caused by heavy metals. The function that histo-pathological investigations have in the identification of fish disorders brought on by heavy metals. Numerous studies show that fish exposed repeatedly accumulate Cd in certain tissues. However, different tissues have different chances of accumulating Heavy metals.

Additionally, it is more challenging to comprehend aquatic systems because of their dynamic character. The alleged effect of Cd on freshwater fish may be more difficult to explain due to cyclical fluctuations in the kind and intensity of pollution.

The relationship between Cd accumulation in freshwater fish and potential environmental effects is not well understood, despite the presence of other metals and their interactions with Cd as well as our awareness of the relationship between water quality conditions and Cd in fish. An initial alarm stage, the ensuring stages of resistance, and a final stage of exhaustion all were characterised by stress research in experimental animals.

More than 100 million people are at a high risk of being exposed to elevated amounts of cadmium, mostly through their drinking water as well as through the metalloid's prevalence in the air in areas where there are considerable industrial and coal-fired power plant emissions. People who eat cadmium-infected seafood that was captured in polluted waters may also bio-accumulate the metal. Thus, understanding how cadmium affects the major organ systems of fish utilised for human consumption. Therefore, it is crucial to understand the effects of cadmium on the various organ systems of fish utilised for human consumption.

The fact that adding a strong complexing agent to the solution decreased the amount of cadmium that would transfer via the gills indicates that the amount of free cadmium ions plays a role in the transfer. The organism's response to the toxicant stress is reportedly shown by the raised glucose levels. Additionally, eating such fish causes the heavy metal to accumulate in soft tissues of the body, exposing people to negative health risks.

A statistically negligible level of Cd content was found in the fish organ. The presence of Cd in fish diet may have caused this build-up. Through organ accumulation, this experiment demonstrated how fish are affected by prolonged exposure to Cd. Given that humans are fish's primary eater, it may have negative repercussions. Ensure that these and other heavy metals do not exceed the permitted Federal Environmental Protection Agency (FEPA) and World Health Organization (WHO) permissible levels. All environmental policies should be strengthened and public awareness campaigns on the value of preserving aquatic systems and their resident biota should be launched.

Originally, the quantities of heavy metals in water were analysed to evaluate the level of heavy metal contamination. This method was able to detect extremely low concentrations in water and does not provide a relationship between environmental metal substance levels and the availability of biological metal substance inside the bodies of organisms. Furthermore, there is no evidence that this method can be used to identify adverse effects on the organism or molecular effects. The adoption of a bio-monitoring methodology, which is more sensitive than previous practices and involves the study of bioaccumulation and their reactions (biomarkers), has been suggested as a solution to this issue .When evaluating changes in water quality, bio-monitoring regularly and systematically uses live creatures to determine either bioaccumulation, biological influence, or health (occurrence of disease). Bio-indicators are the foundation of the conventional bio-monitoring methodology. Bio-monitors are typically thought of as bio-accumulative indicators.

Although the majority of the fish in a pond have been sampled, the interpretation of the data is restricted by the limited numbers in any particular fish populations, prompting further confirmation. More investigation will be required to ascertain the impacts of microhabitat, environmental factors, ambient temperature, nutritional status, and potential seasonal oscillations on the fish haematological parameters since reproductive programmes affect fish physiology. It is advised that heavy metal monitoring equipment be used to monitor the cadmium levels and prohibit their discharge in any way to the aquatic environment in order to protect them.

BIBLIOGRAPHY

Abu-Hilal, A.H. and Ismael, N.S. (2008) Heavy metals in eleven common speciesof fish from the Gulf of Aqaba, Red Sea. Jordan Journal of Biological Sciences, 1, 13- 18.

Ademorati,C.M. (1996) “Environmental Chemistry and Toxicology. Foludx press Ibadan, p.215.

Adeyeye, E. I. (1994). Determination of trace heavy metals inIllisha Africanafish and in associated water and soil sediments from some fish ponds. International Journal of Environmental Studies, 45(3–4), 231–238.

Afshan,S.,Ali,S.etal(2014)EffectofDifferentHeavyMetalPollutiononFish,Research Journal of Chemical and Environmental Sciences Res J. Chem. Environ. Sci. Vol 2 [2] 35-40

Allen, P. (1995). Chronic accumulation of cadmium in the edible tissues of Oreochromis aureus (Steindachner): Modification by mercury and lead. Archives of Environmental Contamination and Toxicology, 29(1), 8–14.

Ambreen, F., Javed, M. and Batool, U. (2015) Tissue Specific Heavy Metals Uptake in Economically Important Fish, Cyprinus carpio at Acute Exposure of Metals Mixtures ,Pakistan J. Zool., vol. 47(2), pp. 399-407.

Anbu, K.(2014) Primary and secondary stress responses in Indian major carps when exposed to heavy metals, Int.J.Curr.Biotechnol.,2(8):7-12.

APHA (1998) Standard method for examination of water and wastewater American Public Health Association. 17th ed. WashingtonD.C.

APHA(2005)StandardMethodsfortheExaminationofWaterandWastewater.21st ed. Washington, DC: American Public HealthAssociation.

Ashraj, W. (2005)Accumulation of heavy metals in kidney and heart tissues of Epinephelus micodon fish from the Arabian Gulf Environmental Monitoring Assessment,1-3(103):311-316.

ATSDR(1999)Toxicol.ProfileofCadmium.AgencyforToxicSubstancesandDrug Registrar,Atlanta,GA.USDeptt.ofHealthandHumanServices.

Avenant-Oldewage, A. , Marx,H.M .(2000)Bioaccumulation of chromium, copper and iron in the organs and tissues of Clarias gariepinus in the Olifants River, Kruger National Park. Water SA. vol. 26, no. 4, pp.569–582.

Babu, A.R. (2013)Effects of Cadmium on Digestive Organs of Teleost Fish Ophiocephalus (Channa),Universal Journal of Environmental Research and Technology , eISSN 2249 0256 ,Volume 3, Issue 2:173-180.

Bais,U.E,Lokhande,M.V.(2012)EffectOfCadmiumChlorideOnTheBiochemical Content In Different Tissues Of The Freshwater Fish Ophicephalus Striatus . International Research Journal Of Biological Sciences .Issn 2278-3202 Vol.1(7),pp- 55-57.

Bais,U.E.,Lokhande,M.V.(2017)ToxicityevaluationOfcadmiumchlorideInfresh water fish Ophiocephalus Striatus . International Journal Of Fisheries And Aquatic Studies, 5(1)pp:519-521.

Baskaran, S and Palanisamy, N.(1990) Studies on the effect of lead Nitrate on the histology of liver and kidney and glycogen levels in liver and muscle of Clarias gariepinus, M. Phil. Dissertation, MadrasUniversity.

Bawuro, A. A., Voegborlo, R. B., & Adimado, A. A. (2018). Bioaccumulation of Heavy Metals in Some Tissues of Fish in Lake Geriyo, Adamawa State, Nigeria. Journal of Environmental and Public Health, 2018, 1–7. https://doi.org/10.1155/2018/1854892.

Bhagde, R. V. , Pingle , S. A.,Bhoye , M. R., Pansambal, S.S. and Deshmukh , D. R. (2020)A Comparative Study Of Physico-Chemical ParametersOf The Freshwater Ponds From Sangamner Taluka Of Ahmednagar, Maharashtra, India,International JournalofBiologicalInnovations,DOI:https://doi.org/10.46505/IJBI.2020.2209.

Boyd, C.E. (1990) Water Quality in ponds for aquaculture. Agriculture Experiment Station, Auburn Univ., Alabama, U.S.A.,482P.

Burger, J. and Gochfeld, M. (2005) Heavy metals in commercial fish in new jersey. Environtal Research, 99(3),403-412.

Cearley,J.andColeman,C.N.(1979)AtomicAbsorptionSpectroscopy:Applications in Agriculture, Biology and Medicine. Robert E.Krieger Publishing Company, New York.

Choudhary, P., Dhakad, N.K, Jain, R. (2014) Studies On The Physico-Chemical Parameters Of Bilawali Tank, Indore (M.P.) India, Iosr Journal Of Environmental Science,ToxicologyAndFoodTechnology(Iosr-Jestft).8(1.I),pp:37-40.

CiCiK, B.and ENGiN, K.(2005) The effects of Cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of Cyprinus carpio(L., 1758); Turk.J.Vet.Anim.Sci., (29):113-117.

Das, S.,Gupta, A. (2012),Effects of cadmium chloride on oxygen consumption and gill morphology of Indian flying barb, Esomus danricus ,J. Environ. Biol. 33, 1057- 1061 , issn: 0254-8704 coden: jebidp.

Da-Silva, J.J.R.F. and Willium, R.J.P. (1991) The biological chemistry of the elements. Clarendon Press,Oxford.

Devis, S.A, et al. (1998) Heavy metals and aquatic environmental 2nd .ed.36. Springer, Pub,480.

Dhaneesh, K.V., Gopi, M., Ganeshamurthy, R., Kumar, T.T., Balasubramanian, T.(2012) Bio-accumulation of metals on reef associated organisms of Lakshadweep Archipelago. 2012, Food Chemistry, vol. 131, no. 3, pp.985–991.

Dubale, M.S. and Shah, P. (1981). Biochemical alterations induced by cadmium in theliveronChannapunctatus.Environmentalresearch.26(1):110-118.

Dutta, H. M., Misquitta, D., & Khan, S. (2006,). The Effects of Endosulfan on the Testes of Bluegill Fish, Lepomis macrochirus: A Histopathological Study. Archives of Environmental Contamination and Toxicology, 51(1), 149–156.

Ebrahimi, M., Taherianfard, M. (2011). The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. Iranian Journal of Fisheries Sciences. 10(1) 13-242011.

Ebrahimi, M.; Taherianfard, M.(2011)The effects of heavy metals exposure on reproductive systems of cyprinid fish from

Kor River ,Iranian Journal of Fisheries Sciences 10(1)13-24
Fafioye, O.O., Adebisi, A.A. and Fagade, S.O. (2004). Toxicity of Parkia biglobosa and RaphiaviniferaextractsonClariasgariepinusjuveniles.AfricanJ.Biotechnol.3(11): 627-630.
Farombi O, Adedlowo, Y.R.(2007) Biomarkers of oxidative stress and heavy metal levelsas indicators of environmental pollution in African cat fish Clarias gariepinus from nilgerie Ogan river, International Journal of Environmental Research and Public health ;4(2):158-165.
Ferrari, L., Eissa, B. L., Ossana, N. A., & Salibian, A. (2009). Effects of sublethal waterborne cadmium on gills in three teleosteans species: scanning electron microscope study. International Journal of Environment and Health, 3(4), 410.
Goswami,P.,Kaushik,U.,DamorS.,Sharma,P.,Sharma, N..(2016)Effect Of Cadmium Chloride On Biochemical Profile And Enzyme Activity In Tilapia Mossibic. 2016, Int J Pharma Res Health Sci. ; 4 (6): 1462-1465 1462 Iiiiiiii© International Journal of Pharma Research And Health Sciences. All Rights Reserved Doi:10.21276/Ijprhs.2016.06.05.
Häkkinen J., Vehniäinen E. and Oikari A. (2003): Histopathological responses of newlyhatchedlarvaeofwhitefish(CoregonusavaretusS.l)toUV-Binducedtoxicity of retene. Aquat. Toxicol., 63,159-171.
Omer, H.N.,(2019)WaterQualityParameters,DOI:10.5772/intechopen.89657.
Hedayati A.(2020) Toxicological Effects Of Heavy Metal Cadmium On Two Aquatic Species:RutilusRutilusAndHypophthalmichthysMolitrix.Ip:157.34.99.174.
Holden A. V. (1973). 'Effects of pesticides on fish,' in Environmental Pollution by Pest icides, C. A. Edwards, Ed., Plenum, New York, NY, USA. 213-253p.

Hussan,A.,Choudhury,T.G.,Ahmed,I.,Gita,S.,Das,A.,Udit,U.K.,Chakrabarti,P.P., & Mandal, R. N. (2016). Effect of Mercury and Cadmium on the Oxygen Consumption and Gill Histology of Catla catla (Ham. 1822). Proceedings of the NationalAcademyofSciences,IndiaSectionB:BiologicalSciences,88(2),729–738. https://doi.org/10.1007/s40011-016-0806-z.
Nwamaka, I.K. (2013)Bioaccumulation And Toxic Effects Of Some Heavy Metals In Freshwater Fishes ,Animal Research International (2013) 10(3): 1792 –1798.
Reddy, J.S. and Reddy, D.C.(2013) Impact Of Cadmium Toxicity On BehaviouralAndHaematologicalBiomarkersOfFreshwaterFish,CatlaCatla,International Journal of Bioassay , ISSN: 2278-778X.
Javed,M.(2005).HeavyMetalContaminationofFreshwaterFishandBedSediments in the River Ravi Stretch and Related Tributaries. Pakistan J. Biolog. Sci., 8(10): 1337-1341.
Jezierska,B.,Witeska,M.,(2001)MetalToxicityToFish”,WydawinctwoAkademii Podlaskiej Siedlce.2001, Pp:318.
Jones,J.C.andReynolds,J.D.(1997).Effectsofpollutionofreproductivebehaviorof fishes. Rev. Fish Biol. Fisheries, 7:463-491.
Kaoud, H.A. and El-Dahshan, A.R. (2010) Bioaccumulation and Histopathological Alterations of the Heavy Metals in Oreochromis niloticus Fish. Nature and Science, 8, 147-156.
Kamaraju,S.AndRamasamy,K.(2018)EffectOfHeavyMetal,CadmiumChloride On Lipid Level Alterations In Freshwater Exotic Fish, Hypopthalmichthys Molitrix. IndoAmericanJournalOfPharmaceuticalSciences,Ajps2018,05(02),903-908.
Kargin, F. (1998) Metal Concentrations in Tissues of the Freshwater Fish Capoeta barroisi from the Seyhan River (Turkey). Bulletin of Environmental Contamination and Toxicology , vol. 60, no. 5, pp.822–828.
Kasherwani,D., Lodhi, H.S., Tiwari, K. J., Shukla, S. and Sharma, U.D.(2009)Cadmium Toxicity to Freshwater Catfish, Heteropneustes fossilis (Bloch),Asian J. Exp. Sci., Vol. 23, No. 1, 2009;149-156.
Khan,A.,Shah,N.,Muhammad,M.,Khan,M.,Ahmad,M.,Farooq,M.,Adnan,M., Jawad, S., Ullah, H., & Yousafzai, A. (2016) Quantitative Determination of Lethal ConcentrationLc50ofAtrazineonBiochemicalParameters;TotalProteinandSerum AlbuminofFreshwaterFishGrassCarp(Ctenopharyngodonidella).PolishJournalof EnvironmentalStudies,25(4),1555–1561.https://doi.org/10.15244/pjoes/61849.
Klaassen, C.D., Liu, J. and Choudhuri, S. (1999). Metallothionein: an intracellular proteintoprotectagainstcadmiumtoxicity.Annu.Rev.Pharmacol.Toxicol.,39:267- 274.
Krishnani, K. K., Azad, I. S., Kailasam, M., Thirunavukkarasu, A. R., Gupta, B. P., Joseph, K. O., Muralidhar, M., et al., (2003) Acute toxicity of some heavy metals to lates calcarifer fry with a note on its histopathological manifestations. Journal of Environmental Science and Health, Part C, 38(4),645-655.
Kumar, A., Prakash, S., Parmar, A. and Bajpeyee A. K. (2019) Effect of cadmium on fresh water teleost, Heteropneustes fossilis (Bloch). International J. of Biological Innovations. 1 (1):14-17. https://doi.org/10.46505/IJBI.2019.1103.
Kumar,B. ,Mukherjee,D.P., Kumar,S., Mishra,M., Prakash,D., Singh,S.K. and Sharma,C.S.(2011)Bioaccumulationofheavymetalsinmuscletissueoffishesfrom selectedaquaculturepondsineastKolkatawetlands,AnnalsofBiologicalResearch,2 (5) :125-134.
Kumari, N. , Kumari, ,R. Yadav, B. N. and Singh. R.P (1989) Effect of cadmium chloride on certain tissues and mortality trend in channa punctatus; Advances in Zoology:editedbyDr.A.K.Sharma,(BulleteinsZoologicalSeries,No:1).
Kumda, H.S., Kimura, S. and Matida,Y. (1972) Acute and chronic toxicity, uptake,andretentionofcadmiuminfreshwaterorganisms.BulletinofFreshwaterFish Research Laboratory Tokyo,

22:157-165.
Li,Z.H,Zlabek,V.,Grabic,R.,Velisek,J.,Machova,J.,Randak,T.(2010)Enzymatic alterations and RNA/DNA ratio in intestine of rainbow trout, Oncorhynchusmykiss, inducedbychronicexposuretocarbamazepine.Ecotoxicology19:872–878.
Llyod, H.G.(1992) In: Aquatic Toxicology 2nd Ed. 110-112, 410 Pp, CabridgePub. Uk.
Marr, J.C.A., Hansen, J.A., Meyer, J.S., Cacela, D.,Podrabsky, T., Lipton, J. And Bergman,H.L.,(1998)Toxicityofcobaltandcoppertorainbowtrout:Applicationof amechanisticmodelforpredictingsurvival.Aquat.Toxicol.,43:225-238.
Mason,C.F.(1996):Biologyoffreshwaterpollution,3rdedn.,Longman,U.K.pp1-4.
McKim, J. M. and Lien, G. J. (2001) Toxic responses of the skin. In: Target organ toxicityinmarineandfreshwaterteleostsorgans.Eds.,SchlenkD.andBenson,W.H. 1, 225-268.
Mohanty,B.P.,Mahananda,M.P.,Pradhan,S.(2013)CadmiumInducedToxicityand Antioxidant Activities in Labeo Rohita (Hamilton),Environment and Ecology Research 1(2):41-47.
Moore, J., Ramamorthy, W.(1989) Heavy metals in natural applied monitoring and impact assessment.Spring-Verlang, New York, NY, USA.
Nammalwar,P.(1985)HeavyMetalPollutionInAdyarEstuary,Madras.IndianProc. Symp. Assess. Environ. Poll.,235-2382.
Nanda, P.(2014),Bioaccumulation of Heavy Metals and Physiological Response in Anabas testudineus on Exposure to Paper Mill Effluent, Nanda, J Environ Anal Toxicol 2014,5:1.
Susanta1, N. , Madhumita, B. (2013)Levels of toxic metals in edible fish from a wetland of India, International Journal of Environmental Sciences, Volume : 3, Issue : 5
Okada, I.A, Sakuma, A.M, Maio, F.D., Dovidemskas, S. and Zenebon, O. (1997) Evaluation of lead and cadmium in milk due to environmental contamination in ParaibaValleyregionofSouthEasternBrazil.Revista-de-Saude-Publication.31:140- 143.
Okocha, R.C. and Adedeji, O.B. (2011) Overview of cadmium toxicity in fish. J. AppliedSci.Res.,7:1195-1207.
Olsson, P., Kling, P., and Hogstrand, C. (1998). Mechanism of heavy metal accumulation and toxicity in fish. Metal Metabolism in Aquatic Environments, Langston, W.J. and M.J. Bebianno (Eds.). Chapman and Hall, London, UK., ISBN- 13: 978-0412803703, pp:321-341.
Palaniappan, P.R., Karthikeyan, S. (2009) Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fishCirrhinus mrigala individually and in binary solutions with nickel, Journal of environmental Sciences, 2009;21:229-236.
Pärt, P. and Lock, R. A. C. (1983) Diffusion of calcium, cadmium and mercury in a mucous solution from Rainbow trout. Comp. Biochem. Physiol., 76,259-263.
Part,PandSvanberg,O.(1981)Uptakeofcadmiuminperfusedgillsofrainbowtrout (Salmogairdneri).JournalofFisheriesandAquaticSciences,38:917-924.
Patil, P.N., Sawant, D.V, Deshmukh, R.N,(2012)Physico-chemical parameters for testing of water – A review ,International Journal Of Environmental Sciences Volume 3, No3.
Perera, P.A.C.T., Suranga, P., Kodithuwakku, T.V., Sundarabarathy andEdirisinghe,U. (2015). Bioaccumulation of Cadmium in Freshwater Fish: An Environmental Perspective. Insight Technology. DOI: 10.5567/ECOLOGY-IK.2015.1.12.
Power,M.A.andThomas,S.1999.HeavymetalsconcentrationtrendsintheThames estuary. Wat. Res. 33(7):1672-1680.
Prabhahar , C. ,Saleshrani, K. et al (2012)Studies on the Effect of Cadmium CompoundontheBiochemicalParametersofFreshWaterFishinCirrhinusmrigala, InternationalJournalofPharmaceutical&BiologicalArchives2012;3(1):69-73.
Pradhan, S. C. &Patra, A. K. ,Sarkar. P. &Pal, A. (2012) Seasonal changes in hematologicalparametersofCatlacatla(Hamilton1822),CompClinPathol21:1473– 1481 DOI10.1007/s00580-011-1316-2.
Qadir A , Malik, R.N. (2011)Heavy Metals in Eight Edible Fish Species from Two PollutedTributaries(AikandPalkhu)oftheRiverChenab,Pakistan.BiologicalTrace Element Research, vol. 143, no. 3, pp.1524–1540.
Rahman, Z., Ahmad, K. and Rashid,I. (2018) Effects of Cadmium Exposure on Growth and Survival and Accumulation in Various Organs of Nile Tilapia (Oreochromisniloticus,Linnaeus),ResearchArticleVolume1Issue1.
Rajkowska, M., Protasowicki, M. (2011)Distribution of selected metals in bottom sediments of lakes in sko and Wiola Poland Ecological Chemistry and Engineering, 18:805-812.
Ramesh, F. and Nagaranjan, K. (2007). Histopathological changes in gills of Clarias batrachustreatedwithsagoeffluent.J.Exp.Zool.10(1):169-171.
Rao, L. and Padmaja, G. (2000) Bioaccumulation of Heavy Metals in M. cyprinoids from the Harbor Waters of Visakhapatnamu. Bulletin Applied Science, 19, 77-85.
Regan,L. (1993) “The design of metal-binding in proteins:Review”Annu. Rev. Biophys. Biomol Structure. vol.2, pp.257-281.
Rurangwa,E.,Roelants,I.,(1998).Theminimumacceptablespermatozoatoeggratio forartificialinseminationandtheeffectsofheavymetalpollutantsonspermmotility and fertilization ability in the African catfish (Clarias gariepinus, Burchell 1822). Journal of Fish Biology, 53,402–13.
Salman, N.M. , AbdAlmotalib, J., Al-Rudainy, Anaam, B.

Faleh(2015)HistopathologicalEffectsofCadmiumChlorideonBarbussharpeyi,Iraqi Journal of Science, 2015, Vol 56, No.1C, pp:713-722.
Sastry, K.V. and Subhadra, K. (1982) Effect of cadmium on some aspects of carbohydrate metabolism in a freshwater catfish, Heteropneustes fossilis. Toxicol. Lett. 14:45-55.
Scott, G.R. and Sloman, K.A. (2004) The Effects of Environmental Pollutants on Complex Fish Behavior: Integrating Behavioral and Physiological Indicators of Toxicity. Aquatic Toxicology, 68, 369-392.
Selvi, R. T. and Ilavazhahan, M. (2012) Histopathological Changes in Gill Tissue of theFishCatlacatlaExposedtoSublethalConcentrationofPesticideMethylParathion and a Heavy Metal Ferous Sulphate ,Biomedical& Pharmacology JournalVol. 5(2), 305-312.
Sengar, N. S. (2016) Water Pollution:-Toxic EffectofCadmiumChloride On Water Resources And Fish Specie, International Journal Of Scientific Research In Chemical Sciences ,Volume-3, Issue-4 Issn:2455-3174.
Shivakumar, C.K., Thippeswamy, B., Tejaswikumar, M. and Prashanthakumara, S.M.(2014)Bioaccumulation of Heavy Metals and its Effect on Organs of Edible FishesLocated in Bhadra River, Karnataka ,International Journal of Research in Fisheries and Aquaculture 2014; 4(2):90-98.
Silene, D.C.,Sandra, M.H. (2009) "Evaluation of trace metals (Cd,Cr, Cu, Zn) in tissues of commercially important Leporinusobstusidens from Guiba Lake,Southern Brazil, "Brazarch.Biol. Technol, vol. 52, pp.247-250.
Sinha, M.(2019)Acute Toxicity of Heavy Metal, Zinc in Fresh Water Fish, Catla Catla,©JAN2019|IREJournals|Volume2Issue7|ISSN:2456-8880.
Shah, A. I ,(2017) Heavy metal impact on aquatic life and human health – an over view, IA's Contribution in Addressing Climate Change 37th Annual Conference of the International Association for Impact Assessment .
Sobha, K., Poornima, A. , Harini, P. ,Veeraiah, K.(2007) A Study on Biochemical Changes In The Fresh Water Fish, Catla Catla (Hamilton) Exposed To The Heavy Metal Toxicant Cadmium Chloride , Kathmandu University Journal Of Science, Engineering And Technology Vol.I,No.Iv.
Stebbing, A.R.D. and Fandino, V.J.R.S. (1983)The combined and separate effectsof copper and cadmium on the growth of Campenularia flexuosa colonies (Hydrozoa). Aquatic Toxicology, 3:183-193.
Stohs, S. (1995). Oxidative mechanisms in the toxicity of metal ions. Free Radical BiologyandMedicine,18(2),321–336.https://doi.org/10.1016/0891-5849(94)00159.
Tchobanoglous, G., Burton,F.L., Stensel, H.D. Metcalf & Eddy(2003) Wastewater Engineering:TreatmentandReuse.4thed.NewDelhi:TataMcGraw-HillLimited.
Triebskorn,R.,Telcean,I.,Casper,H.,Farkas,A.,Sandu,C.,Stan,G.,Colărescu,O., Dori,T.,&Köhler,H.R.(2007) MonitoringpollutioninRiverMureş,Romania,part II: Metal accumulation and histopathology in fish. Environmental Monitoring and Assessment, 141(1–3), 177–188.https://doi.org/10.1007/s10661-007-9886-9.
Tyagi, S. ,Sharma, B., Singh,P. , Dobhal, R.(2013)Water Quality Assessment in Terms of Water Quality Index ,American Journal of Water Resources, 2013, Vol. 1, No. 3, 34-38.
Usepa(2001)UpdateofAmbientWaterQualityCriteriaforCadmium,UnitedStates EnvironmentalProtectionAgency(Usepa),Washington,Dc,Usa,Epa-822-R-01001.
Usha, R. (1999) Pathological observations in gills of the freshwater teolost oreochromis mossambicus (Tilapia) with reference to Cadmium toxicity-Indian Journal of comparative Animal Physiology: Vol 17.1, p:18-22.
Vijayan, P.(2017) Concentration Of Cadmium Metal In Water, Sediment And Fish (Catla Catla) Parts From Cauvery River, Mettur, Tamil Nadu, Indi. 2017, Journal OfAcademiaAndIndustrialResearch(Jair)Volume6,Issue7,Issn:2278-5213.
Vinodhini, R. and Narayanan, M. (2008) Bioaccumulation of Heavy Metals in Organs of Fresh Water Fish Cyprinus carpio (Common Carp). International Journal of Environmental Science and Technology, 5, 179-182.
Vosyliene, M.Z. (1999)The effect of heavy metals on haematological indices of fish (survey). Acta. Zool. Litu.9:76-82.
WaisbergM.Joseph,P.,Hale,B.andBeyersmann,D.(2003)TargetforToxicityand DeathduetoExposuretoCadmium.Chloride.Toxicology.192(2-3):95-117.
Wardah,H.,Sajid,A.,Muhammad,A.AndMumtaz,H.(2018)AssessmentOfAcute Metals Toxicity In Catla Catla Through Hematological And Biochemical Blood Markers,Pak.J.Agri.Sci.,Vol.55(2),449-454;ISSN(Print)0552-9034,ISSN (Online)2076-0906
Wong, C.K. and Wong, M.H. (2000) Morphological and biochemical changes in the gills of Tilapia (Oreochromis mossambicus) to ambient cadmium exposure. Aquatic Toxicol. 48(4):517-527.
Wright, D.A. and Frain, J.W. (1981) The effect of calcium on cadmium toxicity in the freshwater amphipod, Gammarus pulex (L.). Arch. Environ. Contam. Toxicol., 10(3):321-328.
Yasmeen, S. and Pathan, T.S. (2021)Behavioural Response in Freshwater Bivalve Mollusk, Lamellidens Marginalis Due to Acute Toxicity of Cadmium Chloride ,International Journal of Biological Innovations ,IJBI 3(1): 148-153 (2021) E-ISSN:2582-1032.
Yousafzai, A.M. And Shakoori, A.R., (2006) Bioaccumulation of chromium, nickel, lead, copper and zinc in the Tor putitora as an indicator of the presence of heavy metals loads in River Kabul. Pakistan J.Zool.4:341-347.
Zhang Z, He L , Li J and Wu Z (2007) Analysis of heavy metals of muscle and intestine tissue in fish- in banan section of chongqing from three gorges reservoir, china. Polish J. of Environ. Stud.,vol 16(6) pp 949-958.