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🕮 CHAPTER-1
INTRODUCTION

Freshwater is one of the most essential natural resources for life on Earth. It is required for drinking, agriculture, fisheries, and various industrial and domestic activities. In addition to human use, freshwater systems such as rivers, lakes, and ponds play a major role in maintaining ecological balance. Clean water is essential for the survival of aquatic organisms and for the proper functioning of ecosystems. According to J. C. Briggs (2003), a decline in freshwater quality can create serious risks for both biodiversity and ecosystem stability.

In recent decades, human population growth and development activities have increased rapidly. Urbanization, industrialization, and modern agricultural practices have led to the release of large amounts of waste materials into freshwater bodies. These wastes include chemicals, organic matter, and toxic substances that degrade water quality. As a result, aquatic environments are facing increasing levels of stress. S. A. Alrumman et al. (2016) explained that water pollution occurs when harmful substances enter water and make it unsafe for living organisms.

Water becomes polluted when pollution harmful physical , chemical,or biological substances mix with it and change its natural quality. Water bodies are often used as dumping sites for domestic sewage, industrial effluents, and agricultural runoff. Subhendu Kundu (2000) described pollution as an undesirable change in the natural characteristics of an ecosystem due to external factors. When pollutants keep entering water bodies, their natural purification capacity declines, which harms aquatic organisms.

Freshwater ecosystems receive different types of pollutants such as pesticides, fertilizers, heavy metals, petroleum products, and industrial wastes. R. Kaur and A. Dua (2014) reported that agricultural and industrial activities are major sources of water pollution. Similarly, L. Pinto et al. (2015) highlighted that heavy metals released from industries are highly toxic and harmful for aquatic organisms.

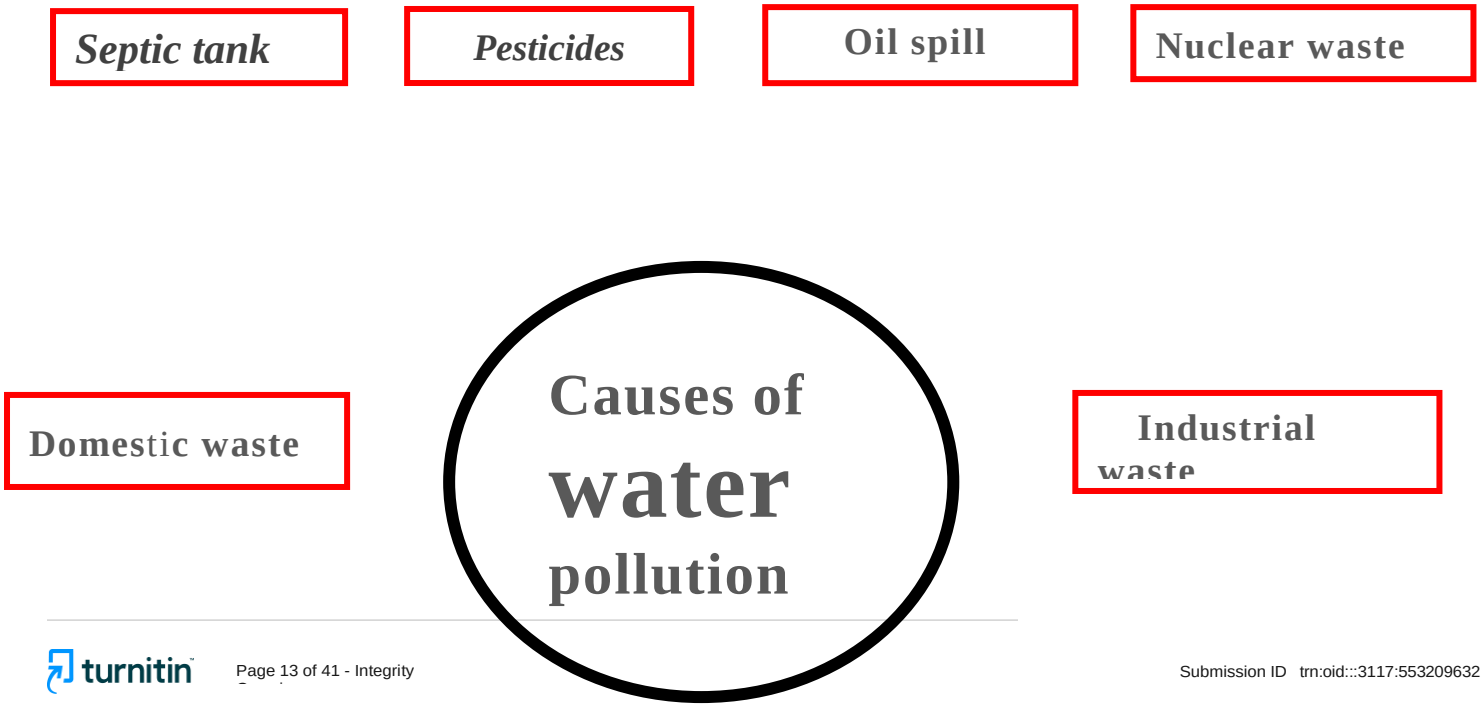
Among these pollutants, heavy metals and pesticides are particularly dangerous because they are non-biodegradable and remain in the environment for a long time. These substances can accumulate in the bodies of aquatic organisms through a process known as bioaccumulation. R. Khoshnood (2016) explained that such accumulation can cause long-term harmful effects. Y. Qiu et al. (2004) observed that pesticides from agricultural fields can enter water bodies through runoff, spray drift, and soil erosion.

Fish are highly sensitive to changes in water quality, making them reliable indicators of pollution in aquatic ecosystem.They absorb toxic substances through their gills, skin, and food. According to S. Ali et al. (2008), polluted water can disturb the physiological and biochemical processes of fish. Continuous exposure to pollutants can lead to accumulation of toxic substances in fish tissues.

The impacts of water pollution on fish can be classified into acute and chronic effects. Acute effects occur when fish are exposed to high concentrations of pollutants for a short time, which may result in sudden death. Chronic effects occur due to long-term exposure to low levels of pollutants, leading to slow damage in the body. N. Aich and S. Walia (2015) reported that chronic exposure can reduce immunity, disturb metabolism, and damage important organs such as gills, liver, and kidneys.

Fish gills are very delicate and play an important role in respiration and maintaining internal balance. Since they are directly exposed to water, they are highly affected by pollutants. Damage to gill tissues reduces oxygen intake and creates respiratory stress. Pollution can also disturb other organs like liver and kidneys, which are involved in detoxification and waste removal.

In addition to physical damage, water pollution also affects fish behavior. Fish living in polluted water may show reduced feeding, slow movement, and abnormal swimming patterns. These behavioral changes are early indicators of poor water quality. Pollution can also affect reproduction, leading to reduced fertility and lower survival of fish populations. Several studies have reported pollution-related diseases in fish such as fin rot, gill damage, and tissue injury. R. Sharma et al. (2018) observed that poor water quality increases the chances of disease and mortality in fish. Some pollutants may also change into more toxic forms inside the body, increasing their harmful effects.
The main aim of this study is to understand the impact of water pollution on freshwater fish, with special focus on structural and physiological changes. This study will help in assessing environmental conditions and will be useful for the conservation and management of freshwater resources and this study also helps in understanding environmental problem.



Sources of Water Pollutants

Water pollution arises from a variety of natural as well as anthropogenic sources, which significantly degrade the quality of aquatic ecosystems. According to **Ellis (1989**), water pollutants are mainly introduced into water bodies through domestic, industrial, and agricultural activities. One of the major sources of water pollution is domestic sewage, which contains organic waste, detergents, human excreta, and pathogenic microorganisms**. Metcalf and Eddy (2003)** reported that untreated

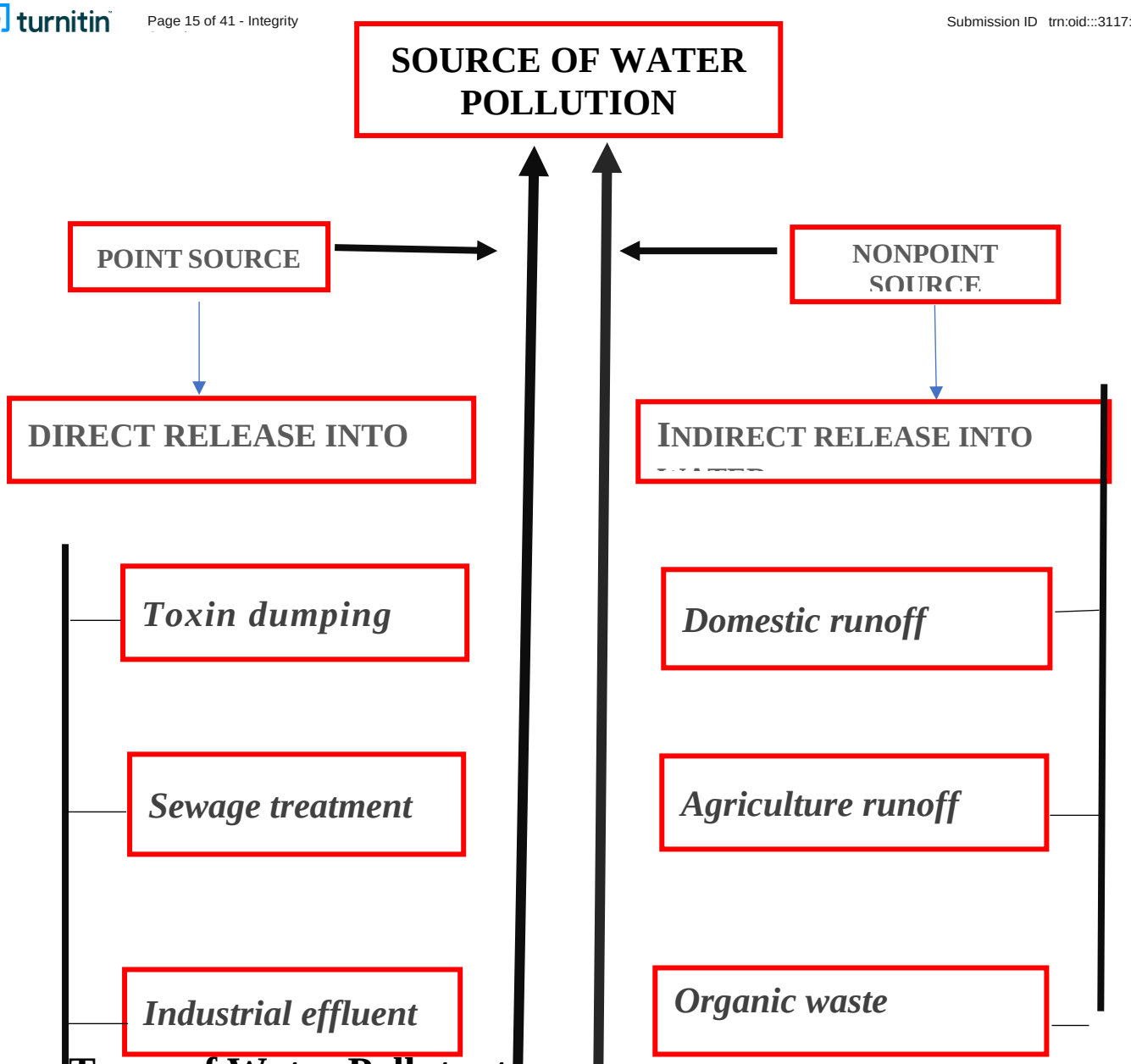
Domestic wastewater increases biological oxygen demand (BOD), leading to oxygen depletion in freshwater bodies.

Industrial effluents are another important source of water pollutants. Industries such as textile, paper, fertilizer, tannery, and chemical industries discharge large quantities of toxic substances, heavy metals, acids, and alkalis into nearby rivers and lakes**. Forstner and Wittmann (1981)** emphasized that heavy metals like mercury, lead, and cadmium released from industrial wastes are highly persistent and bioaccumulative, posing serious threats to aquatic organisms.

Agricultural activities also contribute significantly to water pollution. The excessive use of chemical fertilizers, pesticides, and herbicides leads to runoff during rainfall, which carries these pollutants into surface and groundwater . According to **Carpenter et al. (1998**), nutrient-rich agricultural runoff causes eutrophication, resulting in excessive algal growth and deterioration of water quality. Intensive farming practices further enhance nitrate and phosphate contamination of water bodies.

Another source of water pollution is oil spills, which occur during oil extraction, transportation, and accidental leakage from ships and refineries**. GESAMP (1993**) reported that oil pollution forms a thin layer on the water surface, reducing oxygen exchange and affecting aquatic life. In addition, radioactive wastes released from nuclear power plants and research laboratories contaminate water bodies with harmful radionuclides, as explained by **Odum (1971**).

Thus, water pollution is caused by multiple interconnected sources, and their combined effects severely disturb aquatic ecosystems and human health.



Types of Water Pollutants

Water pollutants can be broadly classified into three major categories: physical pollutants, chemical pollutants, and biological pollutants. These pollutants enter freshwater bodies through different natural and human-induced activities and significantly affect the physical, chemical, and biological characteristics of water. The presence of these contaminants alters the natural balance of aquatic ecosystems and creates harmful conditions for aquatic organisms, particularly freshwater fish.

Physical pollutants are substances that mainly disturb the physical properties of water such as temperature, turbidity, and light penetration. Suspended solids, sediments, and particulate matter are common examples of physical pollutants. These materials often originate from soil erosion, construction activities, mining operations, and agricultural runoff. When large amounts of sediments enter water bodies, they increase turbidity and reduce water clarity. This reduction in transparency prevents sunlight from penetrating into deeper layers of water, which in turn affects photosynthesis in aquatic plants and phytoplankton. Since these organisms form the base of the aquatic food chain, any disturbance in their growth can indirectly affect fish populations. Increased sediment deposition may also clog fish gills, impair respiration, and reduce the availability of dissolved oxygen in water.

Another important physical pollutant is **thermal pollution**, which occurs when industries release heated water into rivers or lakes. A sudden increase in water

temperature can disturb the metabolic activities of fish and other aquatic organisms. Higher temperatures reduce the solubility of oxygen in water, leading to lower dissolved oxygen levels that can cause respiratory stress in fish. Prolonged exposure to elevated temperatures may also disrupt reproductive cycles and reduce the survival rate of fish populations.

Chemical pollutants represent one of the most harmful categories of water contaminants. These pollutants include heavy metals, pesticides, fertilizers, industrial chemicals, petroleum products, and various toxic compounds. Heavy metals such as mercury, lead, cadmium, and arsenic are particularly dangerous because they are non-biodegradable and can accumulate in aquatic organisms over time. When these metals enter freshwater systems through industrial discharge, mining activities, or urban runoff, they may be absorbed by fish tissues and organs.

The accumulation of heavy metals in fish can interfere with enzyme activity, damage cellular structures, and disrupt normal physiological processes. In addition, agricultural chemicals such as pesticides and fertilizers often enter water bodies through surface runoff . Excess nutrients from fertilizers can cause **eutrophication**, a process in which rapid growth of algae depletes oxygen levels in water. This condition creates hypoxic environments that are unsuitable for fish survival.

Biological pollutants include microorganisms such as bacteria, viruses, fungi, and parasites that contaminate water bodies. These organisms usually originate from domestic sewage, animal waste, and untreated wastewater. When pathogenic microorganisms enter freshwater ecosystems, they may cause diseases in fish and other aquatic organisms. Certain bacteria and parasites can infect fish tissues, weaken their immune system, and reduce their ability to survive in polluted environments.

The combined presence of physical, chemical, and biological pollutants can severely degrade water quality and create complex environmental stress for freshwater fish. Understanding the different types of pollutants and their sources is essential for developing effective strategies to control water pollution and protect aquatic ecosystems.

Effects of Water Pollution on Fish

When physical, chemical, and biological contaminants occur together, they drastically lower water quality and place multiple stresses on freshwater fish. Since fish live entirely within aquatic environments, they are constantly exposed to pollutants present in the water. Pollutants can enter the fish body through various routes including the gills, skin, and digestive system. As a result, fish are particularly vulnerable to changes in water quality and chemical contamination.

One of the most immediate effects of water pollution is **damage to the respiratory system of fish.** The gills are delicate organs responsible for gas exchange, allowing fish to absorb oxygen from water and release carbon dioxide. When polluted water contains suspended particles, toxic chemicals, or heavy metals, these substances may accumulate on the gill surface and interfere with normal respiration. In severe cases, pollutants may cause structural damage to gill tissues, reducing their efficiency in oxygen absorption. This can lead to respiratory stress, reduced activity, and even death in extreme conditions.

Another important organ affected by water pollution is the liver, which plays a central role in metabolism and detoxification. The liver helps break down harmful substances that enter the fish body. However, when fish are exposed to high concentrations of toxic chemicals, the liver may become overloaded with pollutants. This can lead to cellular damage, inflammation, and impaired metabolic function.

Long-term exposure to pollutants may also cause liver enlargement, tissue degeneration, and reduced ability to detoxify harmful substances.

The kidneys of fish are also highly sensitive to water pollutants. Kidneys regulate the balance of salts and water in the body and help remove metabolic waste products. Toxic substances present in polluted water can damage kidney tissues and disrupt excretory functions. This may result in accumulation of harmful substances within the fish body and lead to physiological stress.

Water pollution can also have severe impacts on the **reproductive system of fish**. Exposure to toxic chemicals such as pesticides and heavy metals may interfere with hormone regulation and reproductive processes. Pollutants can reduce fertility, affect egg development, and decrease the hatching success of fish larvae. Over time, these reproductive disturbances may lead to a decline in fish populations within polluted habitats.

In addition to physiological damage, polluted water often causes noticeable **behavioral changes** in fish. Fish exposed to toxic environments may display abnormal swimming patterns, reduced feeding behavior, loss of equilibrium, and increased lethargy. Such behavioral alterations are often early indicators of environmental stress and may signal deteriorating water quality.

Water pollution can also weaken the **immune system of fish**, making them more susceptible to infections and diseases caused by pathogens. Pollutants may suppress immune responses and reduce the ability of fish to fight against bacterial, viral, and parasitic infections. As a result, fish living in polluted environments often show higher rates of disease and mortality.

In severe cases, prolonged exposure to polluted water may result in **bioaccumulation and biomagnification** of toxic substances in fish tissues. These processes allow harmful chemicals to accumulate within the bodies of aquatic organisms and become more concentrated at higher levels of the food chain. This not only threatens fish survival but also poses health risks to humans who consume contaminated fish.

Overall, the presence of pollutants in freshwater ecosystems can disrupt normal physiological functions, impair growth and reproduction, alter behavior, and increase mortality rates in fish populations. Therefore, controlling water pollution and maintaining good water quality are essential for protecting freshwater fish and ensuring the long-term sustainability of aquatic ecosystems.

CHAPTER-2
REVIEW OF LITERATURE

Water pollution has been recognized as one of the most serious environmental problems affecting aquatic ecosystems across the world. Freshwater bodies such as rivers, lakes, and ponds are increasingly exposed to pollutants released from industrial, agricultural, and domestic sources. These pollutants alter the physical, chemical, and biological characteristics of water and create harmful conditions for aquatic organisms. Among aquatic organisms, freshwater fish are particularly vulnerable to water pollution because they live in direct contact with the aquatic environment and absorb pollutants through their gills, skin, and digestive system. Therefore, many researchers have studied the effects of water pollution on fish health and survival.

Several studies have focused on the impact of heavy metal contamination on freshwater fish. Heavy metals such as mercury, cadmium, lead, and arsenic are considered highly toxic pollutants because they are non-biodegradable and tend to accumulate in aquatic organisms. **Gupta (2017)** highlighted that heavy metals from industrial sources often enter freshwater systems and gradually build up in fish tissues through bioaccumulation. This buildup interferes with enzyme function, damages cell structures, and ultimately disrupts the metabolic processes of fish.Similarly, **Kumar and Sharma (2019)** investigated the concentration of heavy metals in freshwater fish collected from polluted rivers. Their study revealed that toxic metals were mainly concentrated in the liver and kidney tissues of fish. These organs are responsible for detoxification and excretion, and the accumulation of toxic substances in these tissues may cause severe physiological stress. The researchers concluded that long-term exposure to heavy metals may lead to reduced growth, impaired organ function, and increased mortality in fish populations.

Research conducted by **Singh et al. (2018)** examined the toxic effects of cadmium exposure on freshwater fish species. The study reported significant histological changes in the gill tissues of fish exposed to cadmium. Degeneration of gill lamellae and swelling of epithelial cells were observed, which reduced the efficiency of oxygen exchange. As a result, fish experienced respiratory stress and decreased survival rates in polluted environments.

Industrial pollution is another important factor contributing to water contamination. **Patel and Desai (2018**) studied the impact of industrial effluents on freshwater ecosystems and found that wastewater discharged from factories contains toxic chemicals, dyes, acids, and heavy metals. These pollutants alter important water quality parameters such as pH, temperature, and dissolved oxygen concentration. According to their study, fish living in water bodies receiving industrial effluents showed reduced activity, abnormal swimming behavior, and signs of physiological stress.

Agricultural runoff has also been identified as a significant source of water pollution. The excessive use of fertilizers, pesticides, and herbicides in agricultural fields leads to the contamination of nearby rivers and lakes. According **to Sharma et al. (2018),** pesticide exposure in freshwater habitats leads to noticeable behavioral changes in fish. Affected individuals often show irregular swimming, difficulty maintaining balance, and reduced feeding activity, which serve as early warning signs of toxic stress. These behavioral changes were considered early indicators of toxic stress in fish.

Similarly, **Verma (2020**) investigated the reproductive toxicity of pesticides in freshwater fish. The study found that certain insecticides interfere with the hormonal

system of fish and disrupt reproductive processes. As a result, exposed fish showed reduced fertility, abnormal egg development, and decreased hatching success of larvae. Such reproductive disturbances may ultimately lead to a decline in fish populations in polluted water bodies.

In addition to chemical pollutants, biological contamination also affects aquatic organisms. **Reddy and Rao (2017) s**tudied the presence of pathogenic microorganisms in polluted freshwater bodies and reported that contaminated water often contains harmful bacteria, viruses, and parasites. These pathogens can infect fish tissues and weaken their immune system, making them more susceptible to diseases.

Histological studies conducted by **Das and Mukherjee (2019)** provided detailed evidence of structural damage in fish organs due to water pollution. Their research revealed significant abnormalities in the gills, liver, and kidney tissues of fish collected from polluted environments. The researchers observed inflammation, tissue degeneration, and necrosis in affected organs, indicating the toxic effects of environmental contaminants.

Another important aspect of water pollution is bioaccumulation and biomagnification of toxic substances. **Khan et al. (2020)** reported that pollutants present in water bodies can accumulate in fish tissues and become more concentrated as they move up the food chain. This process not only affects fish health but also posses potential health risks to humans who consume contaminated fish.

Gupta and Singh (2021) studied the behavioral responses of fish exposed to polluted environments and found that fish exhibited reduced swimming activity, slow reaction to external stimuli, and decreased feeding behavior. These changes indicate that polluted water creates stressful conditions that affect the normal physiological functioning of fish.

Eutrophication is another environmental problem associated with water pollution. **Patel et al. (2019)** studied the effects of nutrient enrichment in freshwater bodies and reported that excessive input of nitrogen and phosphorus from fertilizers leads to rapid growth of algae. This phenomenon, known as algal bloom, reduces dissolved oxygen levels in water and creates hypoxic conditions. Fish living in such environments may suffer from oxygen deficiency and increased mortality.

Research conducted by **Kumar and Patel (2020)** further supported these findings by demonstrating that polluted water can significantly reduce fish growth and overall physiological performance. The study concluded that continuous exposure to contaminated water affects fish metabolism, leading to reduced energy availability for growth and reproduction.

Similarly**,Singh and Yadav (2022**) reported that fish exposed to polluted environments showed weakened immune responses and increased susceptibility to infections. The researchers suggested that environmental stress caused by pollutants may compromise the natural defense mechanisms of fish.

Overall, the studies reviewed above clearly indicate that water pollution has severe impacts on freshwater fish. Pollutants such as heavy metals, pesticides, fertilizers, and industrial chemicals can damage vital organs, disrupt physiological processes, and reduce the survival rate of fish populations. Continuous contamination of freshwater ecosystems may therefore lead to a decline in aquatic biodiversity and disturb ecological balance.

These findings highlight the urgent need for effective water pollution control measures and sustainable environmental management practices to protect freshwater ecosystems and ensure the conservation of fish species.

🕮 CHAPTER-3
MATERIALS AND METHODS

Materials

The following materials and equipment were used during the study to collect and analyze water samples and to observe the health condition of freshwater fish.

Sampling Materials

Clean plastic bottles were used for the collection of water samples from the selected canal site. The bottles were thoroughly washed and rinsed with distilled water before sampling to avoid contamination. Labels and markers were used to record the sampling date, time, and location on each bottle.

Fish Collection Equipment

Fish samples were collected using hand nets and simple fishing nets. These nets were used carefully to avoid injury to the fish during the sampling process. Plastic containers filled with canal water were used to temporarily store fish samples before transporting them to the laboratory.

Measuring Instruments

Several laboratory instruments were used to measure different water quality parameters . A thermometer was used to measure water temperature. A digital pH meter was used to determine the acidity or alkalinity of water samples. Dissolved oxygen levels were measured using standard laboratory procedures.

Laboratory Materials

Glass beakers, measuring cylinders, and test tubes were used for the analysis of water samples in the laboratory. Distilled water was used for cleaning and dilution purposes during the analysis. A laboratory notebook was used to record all observations and experimental data during the study.

Methods

-Study Area

The present study was conducted in a freshwater pond located in [Baraut ,Uttar Pradesh]. The pond is surrounded by agricultural fields and nearby residential areas. Due to agricultural runoff and domestic activities, the pond water may receive various pollutants that can affect the health of aquatic organisms.

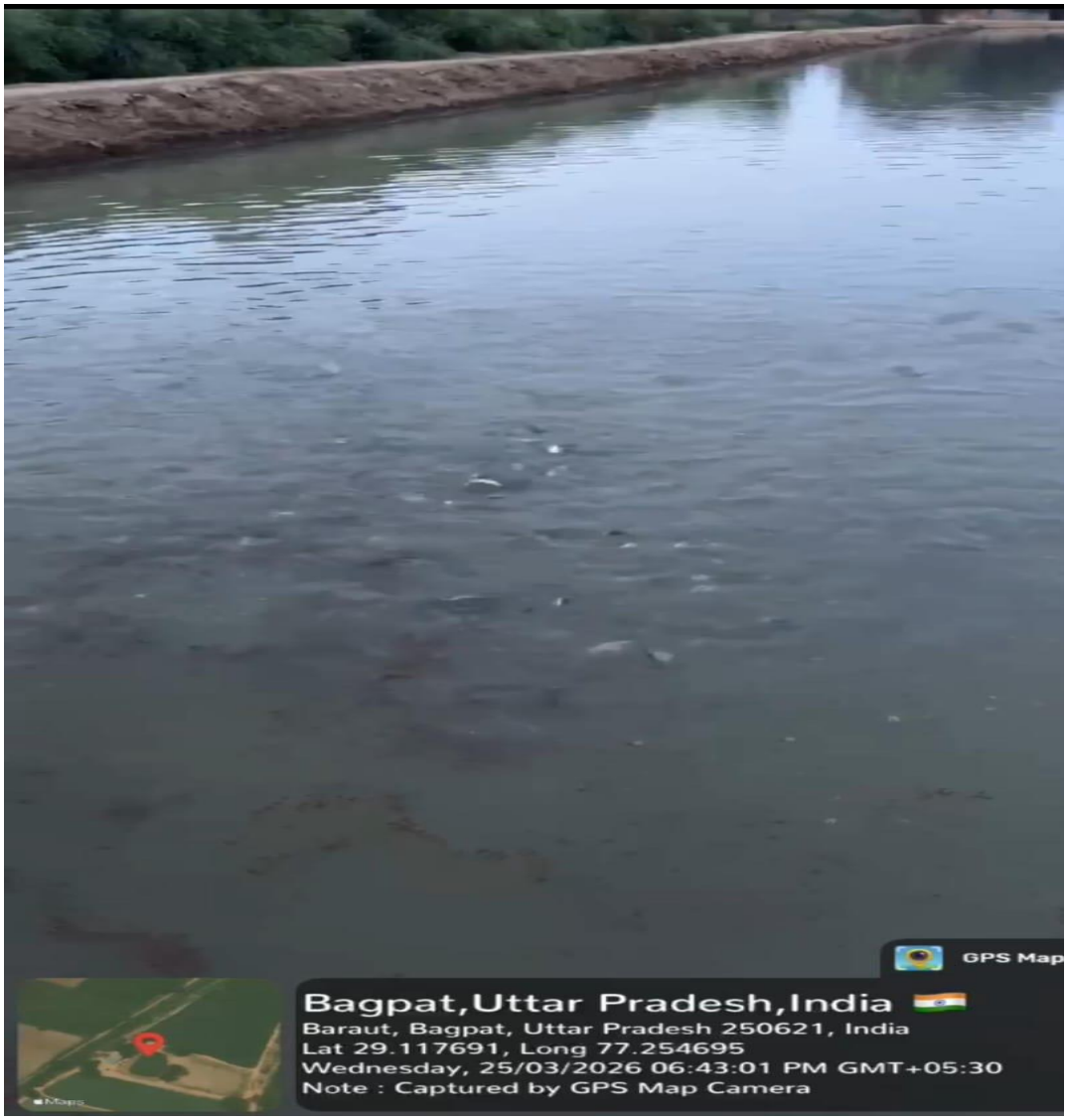
Freshwater ponds provide a suitable habitat for fish and other aquatic organisms. However, the entry of pollutants into such ecosystems may alter water quality and create stressful conditions for aquatic life. The selected pond was therefore chosen as the sampling site for studying the impact of water pollution on freshwater fish.

[Photograph of the pond showing the study area]

-Description of Sampling Site

The sampling site was selected based on the presence of fish and the possible exposure of the canal to pollutants. The surrounding agricultural fields frequently use fertilizers and pesticides, which may enter the canal through surface runoff during rainfall or irrigation.

Domestic wastewater from nearby settlements may also contribute to water contamination. These factors make the pond an appropriate site for studying the effects of polluted water on fish health.



Collection of Water Samples

Water samples were collected from the pond using clean plastic bottles. Before sampling, the bottles were rinsed with canal water to ensure that the samples were not contaminated.

Samples were collected from approximately 20–30 cm below the surface of the water

[Water sampling from the pond]

-Collection of Fish Samples

Fish samples were collected from the pond using hand nets. The fish were handled carefully to avoid physical damage during sampling. After collection, the fish were placed in containers containing pond water and transported to the laboratory.

The collected fish were observed for physical appearance, behavior, and signs of stress or disease. Observations were made regarding swimming activity, body coloration, and feeding behavior.

[Fish samples collected from the pond]

-Analysis of Water Quality Parameters

Several water quality parameters were analyzed to evaluate the level of pollution in the pond water.

Temperature

Water temperature was measured using a laboratory thermometer. Temperature plays an important role in regulating metabolic activities and oxygen availability in aquatic environments.

pH

The pH of the water sample was measured using a pH meter. The pH level indicates whether the water is acidic, neutral, or alkaline.

Dissolved Oxygen (DO)

Dissolved oxygen concentration was measured because it is essential for the respiration of fish and other aquatic organisms.

Turbidity

Turbidity was observed to determine the presence of suspended particles in the water. High turbidity indicates increased sediment or organic matter in water.

Biological Oxygen Demand (BOD)

Biological oxygen demand was estimated to understand the amount of oxygen required by microorganisms to decompose organic matter present in water

Observation of Fish Health

The health condition of fish was examined through visual observation. Fish behavior was monitored to detect abnormal swimming patterns, reduced activity, or loss of equilibrium.

External body parts such as fins, skin, and gills were examined for signs of discoloration, lesions, or damage. Special attention was given to the gills because they are highly sensitive to pollutants present in water.

Data Recording and Analysis

All observations and measurements obtained during the study were recorded systematically. Water quality parameters and fish health observations were noted carefully for each sampling event.

The collected data were analyzed to understand the relationship between water pollution and fish health. Comparisons were made between water quality conditions and the physiological responses observed in fish.

🕮 CHAPTER-4
RESULTS AND DISCUSSION

The present study was conducted to evaluate the impact of water pollution on the health and survival of freshwater fish inhabiting the selected pond. Water samples were collected from the study site and analyzed for several important physicochemical parameters that influence the quality of aquatic environments. These parameters included temperature, pH, dissolved oxygen, turbidity, and biological oxygen demand. The results obtained from the analysis were compared with the standard ranges considered suitable for freshwater fish survival.

The observations indicated that some parameters of the pond water were within acceptable limits, while others showed deviations that may indicate the presence of pollution. The recorded data are summarized in the following table.

Table 1: Water Quality Parameters Recorded from Pond Water

Parameter	Observed Value	Standard Range for freshwater fish	Interpretation
Temperature	28°C	20–30°C	Within acceptable range
pH	8.1	6.5-8.5	Slightly alkaline but tolerable
Dissolved Oxygen(DO)	4.0mg/l	5-8 mg/l	Lower than recommended level
Turbidity	High	Low	Indicates suspended particles in water
Biological Oxygen Demand(BOD)	6 mg/l	<3 mg/L	Suggests organic pollution

The temperature of the canal water was recorded at approximately 28°C, which falls within the acceptable range for most freshwater fish species. Temperature plays an important role in regulating the metabolic processes of aquatic organisms. Although the recorded temperature was suitable, slight increases in temperature may reduce the solubility of oxygen in water, which can negatively affect fish respiration.

The pH value of the canal water was measured at 8.1, indicating that the water was slightly alkaline. Freshwater fish generally tolerate a pH range between 6.5 and 8.5, and therefore the recorded value was still within the acceptable limit. However,

prolonged exposure to highly alkaline or acidic conditions may cause physiological stress in fish and can affect their growth and survival.

Dissolved oxygen is one of the most critical parameters for aquatic life. The dissolved oxygen level recorded during the study was 4.0 mg/L, which is lower than the recommended level required for healthy fish survival. Low oxygen levels can cause respiratory stress in fish and may reduce their activity levels.

Turbidity was observed to be high in the pond water. High turbidity indicates the presence of suspended particles such as soil sediments, organic matter, and other pollutants. These particles may enter the canal through soil erosion, agricultural runoff, and domestic waste **discharge.**

The biological oxygen demand value recorded during the study was higher than the acceptable limit. A high BOD value indicates that microorganisms present in the water are actively decomposing organic matter , which consumes a significant amount of dissolved oxygen. As a result, less oxygen becomes available for aquatic organisms such as fish.

Table 2: Observed Behavioral and Physiological Effects on Freshwater Fish

Observed Change in fish	Possible Cause
Slow swimming behavior	Reduced dissolved oxygen
Frequent movement near water surface	Oxygen deficiency
Reduced feeding activity	Stress due to polluted water
Gill irritation and discoloration	Suspended particles and chemicals
Reduced fish population	Long-term exposure to pollution

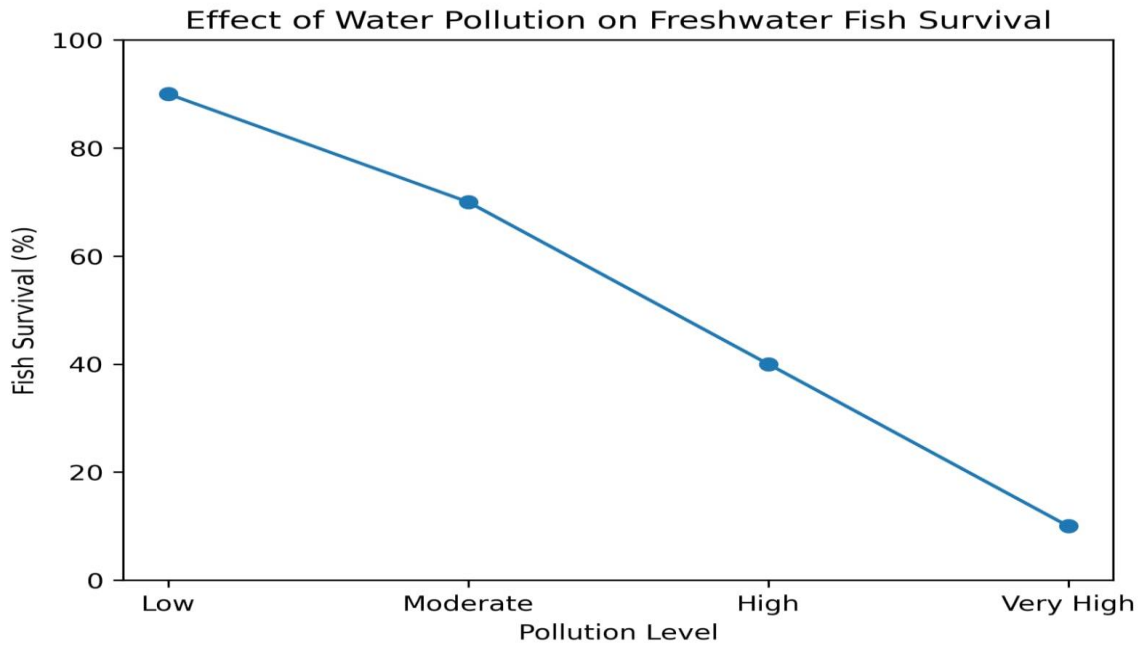
Field observations also revealed noticeable behavioral changes in the fish inhabiting the canal. Fish were often seen swimming slowly and appeared less active than usual. In several instances, fish were observed moving toward the surface of the water and remaining there for longer periods of time.

Such behavior is often associated with low dissolved oxygen levels in the water. Fish tend to move toward the surface because oxygen concentration near the surface is slightly higher due to contact with atmospheric air.

Reduced feeding activity was another important observation recorded during the study. Fish exposed to polluted environments often show decreased appetite and reduced growth rates. This may be due to physiological stress caused by poor water quality.

Some fish also showed signs of irritation in the gill region. The gills of fish are highly sensitive organs responsible for respiration. Suspended particles and chemical pollutants present in polluted water may damage the delicate gill tissues and reduce their efficiency in oxygen exchange.

The combined observations from water quality analysis and fish behavior suggest that the canal water may be experiencing moderate levels of pollution that could potentially affect the health and survival of freshwater fish.



Effect of water pollution levels on the survival of freshwater fish.

-The graph illustrates the relationship between pollution levels and fish survival. As pollution increases from low to very high levels, the survival rate of freshwater fish decreases significantly. This indicates that poor water quality can negatively affect fish health and increase the risk of fish mortality

DISCUSSION

Water pollution has become a major environmental concern affecting freshwater ecosystems across the world. Freshwater fish are particularly vulnerable to pollution because they live entirely within the aquatic environment and are constantly exposed to the surrounding water. Pollutants present in water may enter the fish body through gills, skin, and digestive system, leading to various physiological and behavioral changes.

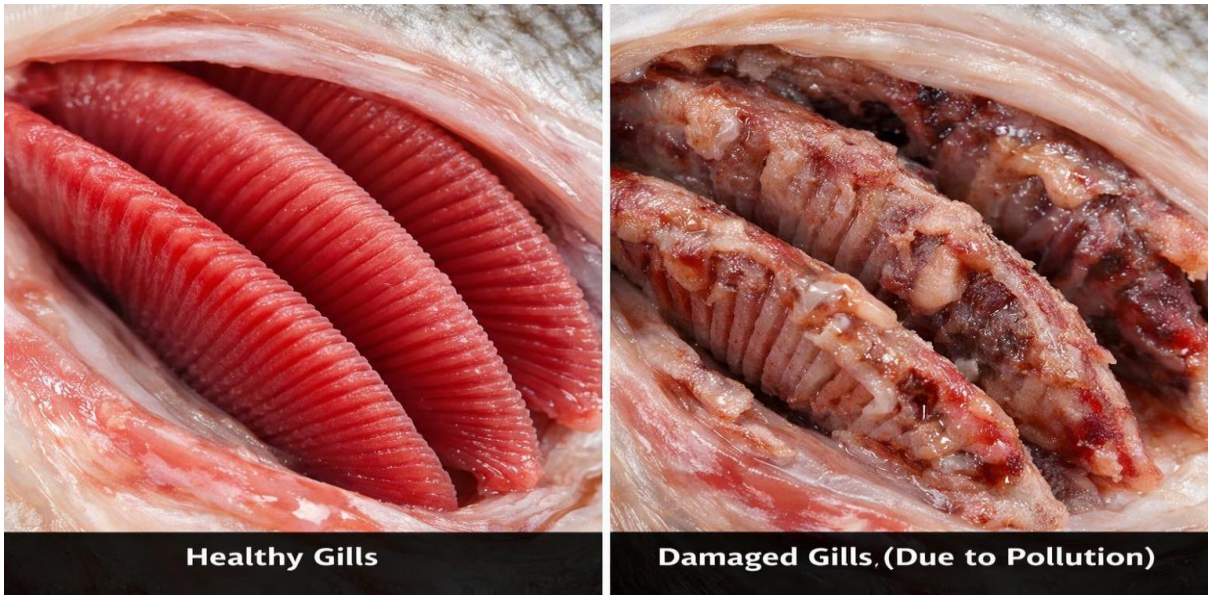
The results obtained from the present study indicate that the water quality of the selected canal shows certain signs of pollution. Although some parameters such as temperature and pH were within acceptable limits , other parameters such as dissolved oxygen, turbidity, and biological oxygen demand indicated possible contamination of the water body.

Temperature is an important environmental factor that regulates the metabolic activity of fish. Fish are ectothermic organisms, meaning their body temperature depends on the surrounding environment. Slight increases in water temperature can increase the metabolic rate of fish and reduce the availability of dissolved oxygen in water. Therefore, even moderate changes in temperature may indirectly affect fish health.

The pH value recorded during the study indicated that the canal water was slightly alkaline. While most freshwater fish species can tolerate small variations in pH, extreme pH conditions may cause severe physiological stress. Changes in pH can affect ion balance in fish and may damage sensitive tissues such as gills and skin.

Dissolved oxygen is considered one of the most essential factors for the survival of aquatic organisms. Fish rely on dissolved oxygen for respiration, and insufficient oxygen levels can cause severe stress. In the present study, the dissolved oxygen concentration was lower than the recommended level for freshwater fish survival. Low dissolved oxygen conditions may force fish to migrate to areas where oxygen

levels are higher. Fish may also exhibit behavioral changes such as rapid gill movement, frequent surfacing , and reduced swimming activity. If the oxygen level continues to decrease, fish may eventually die due to respiratory failure.
High turbidity observed in the pond water suggests the presence of suspended particles such as soil sediments and organic matter. These particles may enter the canal through soil erosion, agricultural runoff, and domestic waste discharge. Suspended particles can clog fish gills and reduce their ability to extract oxygen from water.



[Healthy fish gills compared with damaged gills exposed to polluted water]
-Fish gills are very delicate respiratory organs responsible for oxygen exchange. Damage to gills can reduce oxygen absorption and cause respiratory stress in fish. Continuous exposure to polluted water may also lead to inflammation, tissue damage, and reduced survival of freshwater fish.

Turbidity also reduces the penetration of sunlight into water. Reduced sunlight availability may affect the growth of aquatic plants and phytoplankton, which are primary producers in aquatic ecosystems. A decline in primary producers can disrupt the aquatic food chain and affect fish populations.
The biological oxygen demand recorded during the study was higher than the standard limit. High BOD values indicate that large amounts of organic matter are present in the water and are being decomposed by microorganisms. During this process, microorganisms consume dissolved oxygen, leaving less oxygen available for fish and other aquatic organisms.
Behavioral observations made during the study further support the presence of environmental stress in fish. Reduced feeding activity, slow swimming behavior, and frequent surfacing are commonly observed symptoms of pollution stress in fish.
Long-term exposure to polluted water may also lead to the accumulation of toxic substances in fish tissues through a process known as bioaccumulation. Heavy metals and other pollutants may accumulate in vital organs such as the liver, kidneys, and gills. Over time, this accumulation can damage these organs and impair important physiological functions.
Pollution can also affect the reproductive capacity of fish. Exposure to toxic chemicals may interfere with hormone regulation and reduce fertility rates. This may

eventually lead to a decline in fish populations within polluted water bodies.
In addition to affecting fish health, water pollution may also reduce the overall biodiversity of aquatic ecosystems. Sensitive fish species may disappear from polluted habitats, while only pollution-tolerant species may survive. Such changes can disturb the ecological balance of the ecosystem.
Therefore, regular monitoring of water quality parameters is essential for maintaining healthy aquatic environments. Preventing the entry of pollutants such as agricultural chemicals, domestic waste, and industrial effluents into freshwater systems can help protect aquatic biodiversity and ensure the survival of freshwater fish populations.

1.Bioaccumulation of Pollutants in Fish

Bioaccumulation refers to the gradual accumulation of toxic substances such as heavy metals and pesticides in the tissues of aquatic organisms. Freshwater fish can absorb pollutants from contaminated water through their gills, skin, and digestive system. Over time, these substances accumulate in vital organs such as the liver, kidneys, and muscles. High concentrations of pollutants may damage these organs and interfere with normal physiological processes. Bioaccumulation not only affects fish health but may also pose risks to humans who consume contaminated fish.

2. Effect of Pollution on Fish Growth

Water pollution can significantly affect the growth and development of freshwater fish. Polluted water often contains toxic chemicals that may interfere with metabolic activities and nutrient absorption. As a result, fish exposed to polluted environments may show reduced growth rates and poor body condition. Chronic exposure to pollutants may also cause deformities and developmental abnormalities in fish.

3. Impact on Fish Reproduction

Pollutants present in water may affect the reproductive system of fish. Certain chemicals such as pesticides and heavy metals may disrupt hormonal balance in fish and reduce their reproductive capacity. Exposure to polluted water may lead to reduced fertility, lower egg production, and decreased survival of fish larvae. Over time, these effects may contribute to a decline in fish populations in polluted water bodies.

4. Changes in Fish Behavior

Behavioral changes are often early indicators of environmental stress in fish. Fish exposed to polluted water may exhibit abnormal swimming patterns, reduced feeding activity, and increased stress responses. Some fish may frequently move toward the water surface due to oxygen deficiency. These behavioral changes indicate that fish are experiencing unfavorable environmental conditions.

5. Impact on Aquatic Food Chain

Water pollution can disrupt the balance of aquatic food chains. Pollutants may reduce the population of phytoplankton and zooplankton, which serve as primary food sources for many fish species. When these organisms decline, fish may experience food shortages. This can lead to reduced growth, poor reproduction, and decreased fish populations.

6.Physiological Stress in Fish

Polluted water may cause physiological stress in freshwater fish. Toxic substances present in water can disturb normal metabolic activities and increase the stress level in fish. Continuous exposure to pollutants may weaken the immune system of fish, making them more vulnerable to diseases and infections.

[Impact of polluted water on freshwater fish showing damaged gills, liver, kidney, lesions, and fin rot]

7.Damage to Fish Organs

Pollutants present in contaminated water may damage internal organs of fish such as liver, kidneys, and digestive system. These organs play an important role in detoxification and metabolism. Damage to these organs may reduce the ability of fish to survive in polluted environments.

8. Long-Term Ecological Consequences

Continuous water pollution can have serious long-term impacts on aquatic ecosystems. Sensitive fish species may disappear from polluted habitats, while only pollution-tolerant species may survive. This can reduce biodiversity and disturb the ecological balance of the aquatic environment. Over time, polluted ecosystems may lose their ability to support healthy fish populations.

9. Importance of Water Quality Monitoring

Regular monitoring of water quality is essential for protecting aquatic ecosystems. Parameters such as pH, dissolved oxygen, temperature, turbidity, and biological oxygen demand should be measured periodically. Monitoring these parameters helps identify pollution sources and allows authorities to take preventive measures before serious damage occurs to aquatic life.

10. Need for Pollution Control Measures

To protect freshwater ecosystems, it is important to control the discharge of pollutants

into water bodies. Proper waste management, treatment of industrial effluents, and responsible use of fertilizers and pesticides in agriculture can help reduce water pollution. Public awareness and environmental conservation efforts are also important for maintaining healthy aquatic environments.

Fish Mortality and Migration as Indicators of Water Pollution

Water pollution can significantly influence the survival and distribution of freshwater fish. When water quality deteriorates beyond the tolerance limits of fish, they may respond either by migrating to a different habitat or by experiencing mortality. Therefore, fish migration and fish death are considered important biological indicators of water pollution.

Fish migration can occur when environmental conditions in the water body become unfavorable. For example, low dissolved oxygen levels, high turbidity, or the presence of toxic substances may force fish to move toward areas with better water quality. If fish are observed frequently moving toward the surface of the water or attempting to leave the polluted area, it may indicate that they are searching for oxygen-rich environments.

On the other hand, if the level of pollution becomes too high and fish are unable to escape from the contaminated water, mortality may occur**. Dead fish floating on the water surface** or found along the banks of the canal may indicate severe water pollution. Fish mortality is often associated with extremely low dissolved oxygen levels, high concentrations of toxic chemicals, or sudden changes in water quality.

Changes in fish population can also help identify whether migration or mortality has occurred. A sudden decrease in fish numbers in a particular water body may indicate that fish have either migrated to cleaner areas or died due to unfavorable environmental conditions.

Therefore, regular monitoring of fish behavior, fish population, and water quality parameters is essential to understand the effects of water pollution on freshwater fish survival and distribution

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🕮 CHAPTER-5
CONCLUSION

Water pollution is an important environmental issue that can significantly affect freshwater ecosystems and aquatic organisms. Freshwater fish are particularly sensitive to changes in water quality because they live continuously in direct contact with the surrounding water. The present study aimed to observe the possible effects of canal

water pollution on the behavior, health, and survival of freshwater fish.

The observations recorded during the study indicate that changes in water quality parameters such as pH, turbidity, dissolved oxygen, and the presence of suspended particles may influence fish health and activity. When water quality becomes poor, fish may experience stressful environmental conditions that disturb their normal physiological processes. Such stress can affect their respiration, feeding behavior, growth, and overall survival.

During field observations, several behavioral changes were noticed in fish inhabiting the canal water. Fish were sometimes observed swimming slowly and appeared less active than usual. In some cases, fish were seen moving toward the surface of the water and remaining there for longer periods of time. This behavior is commonly associated with low dissolved oxygen levels in water. When oxygen concentration in deeper water decreases, fish tend to move toward the surface where oxygen levels are relatively higher.

Reduced feeding activity was another important observation during the study. Fish living in polluted environments often show decreased appetite and slower growth rates. Such changes may occur because pollutants present in water create physiological stress and interfere with normal metabolic processes. Continuous exposure to polluted water may therefore reduce the overall health and productivity of fish populations.

Polluted water may also affect important organs of fish, particularly the gills, which are delicate respiratory structures responsible for oxygen exchange. Suspended particles and chemical pollutants present in contaminated water can damage gill tissues and reduce their efficiency in absorbing oxygen. This may lead to respiratory stress and weaken the fish over time. If exposure to polluted conditions continues for a long period, it may eventually lead to fish mortality.

Overall, the observations from water quality analysis and fish behavior suggest that the canal water may be experiencing moderate levels of pollution. Such pollution can negatively affect freshwater fish and disturb the natural balance of aquatic ecosystems. Therefore, proper monitoring of water quality and effective management of pollution sources are essential to protect freshwater biodiversity and maintain healthy aquatic environments.

In addition, the study highlights the importance of maintaining good water quality in freshwater habitats such as canals, rivers, and ponds. Aquatic ecosystems depend on balanced physical , chemical, and biological conditions for the proper functioning of organisms living in water. Freshwater fish require suitable environmental conditions including adequate dissolved oxygen, appropriate pH levels, and low concentrations of harmful substances for their normal growth and survival . When pollutants enter water bodies through domestic waste, agricultural runoff , or other human activities, they disturb this natural balance and create unfavorable conditions for aquatic life. As a result, fish may experience physiological stress, reduced growth, behavioral abnormalities , and in severe cases increased mortality.

Continuous monitoring of water quality parameters is therefore essential to identify early signs of pollution and prevent further damage to aquatic ecosystems. Regular assessment of parameters such as pH, dissolved oxygen, temperature, and turbidity can help in understanding the health status of freshwater environments. Such monitoring can also assist researchers and environmental authorities in taking necessary steps to control pollution and improve water quality.

Public awareness and responsible human activities also play an important role in protecting freshwater ecosystems. Proper waste management practices, reduction in the excessive use of agricultural fertilizers and pesticides, and prevention of untreated

waste discharge into water bodies can significantly reduce water pollution. By adopting sustainable environmental practices and protecting freshwater resources, it is possible to maintain healthy aquatic habitats and ensure the long-term survival of freshwater fish and other aquatic organisms.

Furthermore, protecting freshwater ecosystems is essential not only for fish survival but also for maintaining the overall ecological balance of aquatic environments. Freshwater fish play an important role in the food chain and contribute to the stability of aquatic biodiversity. When water pollution affects fish health and reduces their population, it may also disturb the balance of other organisms living in the same habitat. Therefore, maintaining clean and healthy water bodies is important for sustaining aquatic life and preserving biodiversity.

The findings of the present study emphasize that even moderate levels of pollution in canal water can have noticeable effects on fish behavior and survival. Although the study was based on limited observations and water quality parameters, it clearly indicates that increasing pollution levels may pose a serious threat to freshwater fish in the long term. Further detailed studies involving advanced water quality analysis and biological assessment of fish populations would provide a better understanding of the extent of pollution and its ecological impact.

In conclusion, reducing water pollution and maintaining good water quality are essential steps for protecting freshwater fish and sustaining aquatic ecosystems. Effective environmental management practices and responsible human behavior can help in preventing further degradation of water resources and ensure the long-term health and survival of freshwater organisms.

The present study provides a basic understanding of how water pollution can influence **the health and survival of freshwater fish**. Although the observations were limited to a specific canal area, the findings highlight the importance of maintaining good water quality for the protection of aquatic organisms. Further research involving detailed chemical analysis of water and long-term monitoring of fish populations would help in better understanding the extent of pollution and its ecological consequences.

🕮 CHAPTER-6
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Chapter -1
Introduction

Aquatic ecosystems represent one of the most important components of the natural environment. These ecosystems include rivers, lakes, ponds, reservoirs, and oceans that support a wide variety of living organisms. Among aquatic vertebrates, fish form the largest and most diverse group and play a crucial role in maintaining ecological balance. Fish are an integral part of aquatic food chains and contribute significantly to nutrient cycling and energy transfer within aquatic environments. In addition to their ecological importance, fish are also economically valuable as they provide an important source of protein and support the livelihood of millions of people worldwide through fisheries and aquaculture.

Water quality is a critical factor that determines the health and survival of aquatic organisms. However, in recent decades, rapid industrialization, urbanization, and agricultural development have led to the deterioration of water quality in many parts of the world. Large quantities of pollutants are continuously released into water bodies through industrial discharge, domestic sewage, agricultural runoff, and other human activities. These pollutants include heavy metals, pesticides, fertilizers, detergents, pharmaceuticals, and various organic and inorganic chemicals. The accumulation of these substances in aquatic environments significantly alters the physical and chemical properties of water and creates unfavorable conditions for aquatic life.

Fish are particularly sensitive to environmental changes because they live entirely within the aquatic environment and depend on water for respiration, feeding, reproduction, and other physiological functions. Pollutants present in water can easily enter the body of fish through different routes such as gills, skin, and digestive tract. Once absorbed, these toxic substances may interfere with normal physiological and biochemical processes. Continuous exposure to polluted water may therefore lead to various adverse effects on fish including impaired growth, reduced reproductive capacity, behavioral abnormalities, and increased susceptibility to diseases.

Environmental pollution acts as a major stressor for aquatic organisms. Stress can be defined as a condition in which an organism experiences disturbances in its internal equilibrium due to external environmental factors. In fish, stress responses are triggered when environmental conditions deviate from the optimal range required for normal physiological functioning. Polluted water represents a significant environmental stress that can disrupt the internal balance of fish and induce a variety of physiological responses aimed at maintaining homeostasis.

The physiological stress response in fish is regulated by complex neuroendocrine mechanisms. One of the most important systems involved in stress regulation is the hypothalamus–pituitary–interrenal (HPI) axis. When fish are exposed to stressful environmental conditions such as pollution, the HPI axis becomes activated and stimulates the release of stress hormones, particularly cortisol and catecholamines. These hormones play an essential role in helping the organism cope with adverse environmental conditions by regulating metabolism, energy utilization, ion balance, and immune responses.

One of the primary physiological responses to stress in fish is the alteration of metabolic processes. Stress hormones stimulate the mobilization of energy reserves in order to provide sufficient energy for survival under unfavorable conditions. As a result, biochemical parameters such as blood glucose levels often increase during stress. Elevated glucose levels provide an immediate energy source for tissues and organs that require additional energy to maintain physiological stability. Similarly, changes in protein metabolism and enzyme activity may also occur as part of the stress response.

The respiratory system of fish is also highly affected by water pollution. Fish obtain oxygen from water through their gills, which are delicate structures responsible for gas exchange and ionic regulation. Pollutants present in water may damage the gill tissues, reduce the efficiency of oxygen uptake, and disturb the osmotic balance of fish. In polluted environments, fish may exhibit increased respiratory movements or structural changes in gill tissues as an adaptive response to reduced oxygen availability or toxic substances.

Hematological parameters are widely used as important indicators of physiological stress in fish. Blood plays a vital role in transporting oxygen, nutrients, and metabolic wastes throughout the body. Changes in hematological characteristics such as red blood cell count, white blood cell count,

hemoglobin concentration, and hematocrit value can reflect the health status of fish. Exposure to polluted water may lead to significant alterations in these parameters, indicating physiological disturbances and stress conditions.

Heavy metals are among the most dangerous pollutants found in aquatic environments. Metals such as lead, mercury, cadmium, and arsenic are released into water bodies through industrial activities, mining operations, and agricultural practices. Unlike many organic pollutants, heavy metals are not biodegradable and can persist in the environment for long periods. These metals accumulate in the tissues of aquatic organisms through a process known as bioaccumulation. Long-term exposure to heavy metals can cause oxidative stress, enzyme inhibition, tissue damage, and disruption of normal physiological functions in fish.

Agricultural chemicals such as pesticides and fertilizers also contribute significantly to water pollution. Runoff from agricultural fields often carries these chemicals into nearby rivers, lakes, and ponds. Many pesticides are toxic to aquatic organisms and can interfere with important biochemical pathways in fish. Prolonged exposure to such chemicals may affect growth, reproduction, and survival of fish populations.

Fish possess certain physiological and biochemical mechanisms that help them adapt to moderate levels of environmental stress. These adaptive responses include changes in metabolic activity, activation of antioxidant defense systems, and behavioral adjustments. However, when the concentration of pollutants exceeds the tolerance capacity of fish, these protective mechanisms may become insufficient. As a result, severe physiological disturbances may occur, ultimately leading to reduced survival and population decline.

Because of their sensitivity to environmental changes, fish are widely used as biological indicators of aquatic pollution. The study of physiological and biochemical responses in fish provides valuable information about the health of aquatic ecosystems. Monitoring these responses can help detect early signs of environmental contamination and provide important insights into the effects of pollutants on aquatic organisms.

The investigation of physiological stress responses in fish exposed to polluted water is therefore essential for understanding the impact of environmental pollution on aquatic life. Such studies contribute to the development of effective strategies for pollution monitoring, environmental protection, and sustainable management of aquatic resources. In addition, knowledge of stress physiology in fish is also useful for improving aquaculture practices and ensuring the health and productivity of cultured fish species

Chapter- 2
Review of Literature

Water pollution has become one of the major environmental concerns affecting aquatic ecosystems worldwide. Numerous studies have been conducted to understand how pollutants influence the physiology and health of aquatic organisms, particularly fish. Since fish live in close contact with their aquatic environment, they are highly sensitive to changes in water quality and are therefore widely used as biological indicators of environmental pollution. Many researchers have investigated the physiological, biochemical, and hematological responses of fish exposed to polluted water.

According **to Wedemeyer (1996), environmental stress in fish occurs when external factors such**

as temperature fluctuations, low oxygen levels, or toxic pollutants disturb the normal physiological balance of the organism. Stress responses in fish involve a series of neuroendocrine and metabolic changes that help the organism adapt to unfavorable environmental conditions. The author emphasized that physiological stress responses are important indicators for assessing the impact of environmental disturbances on aquatic organisms.

Barton and Iwama (1991) reported that stress responses in fish are primarily regulated through the activation of the hypothalamus–pituitary–interrenal (HPI) axis. Activation of this system leads to the secretion of stress hormones such as cortisol and catecholamines, which play an important role in regulating metabolic processes and energy mobilization. Elevated cortisol levels are considered a key indicator of stress in fish exposed to environmental pollutants.

Fry (1971) explained that environmental stressors such as water pollution may influence several physiological functions in fish, including respiration, metabolism, and growth. The author described how fish respond to environmental stress through adaptive physiological mechanisms that allow them to maintain internal stability. However, prolonged exposure to stressful conditions may exceed the adaptive capacity of fish and result in physiological damage.

Heath (1995) reported that water pollution caused by industrial discharge and agricultural runoff can significantly affect fish physiology. Pollutants such as heavy metals, pesticides, and organic contaminants may interfere with normal metabolic activities and disrupt enzyme systems in fish. These toxic substances may accumulate in fish tissues and lead to various physiological disorders.

Studies conducted by **Kori-Siakpere and Ubogu (2008)** demonstrated that exposure to polluted water results in significant changes in hematological parameters of fish. The researchers observed alterations in hemoglobin concentration, red blood cell count, and white blood cell count in fish exposed to contaminated environments. Such hematological changes are considered reliable indicators of physiological stress and health status in fish.

Lushchak (2011) highlighted the role of oxidative stress in fish exposed to environmental pollutants. According to the author, toxic substances present in polluted water can increase the production of reactive oxygen species (ROS) in fish tissues. Excessive accumulation of these reactive molecules may cause damage to cellular structures such as lipids, proteins, and DNA. Fish respond to oxidative stress by activating antioxidant defense mechanisms, including enzymes such as catalase, superoxide dismutase, and glutathione peroxidase.

Heavy metal contamination in aquatic environments has been extensively studied due to its harmful effects on aquatic organisms. **According to Jezierska and Witeska (2006),** metals such as lead, cadmium, mercury, and arsenic can accumulate in fish tissues through bioaccumulation and biomagnification processes. These metals may disrupt normal physiological functions by interfering with enzyme activity, protein synthesis, and ion regulation.

Vinodhini and Narayanan (2008) studied the accumulation of heavy metals in different tissues of freshwater fish and reported that gills, liver, and kidneys are particularly vulnerable to metal toxicity. The accumulation of metals in these organs may lead to structural damage and impaired physiological functioning. The researchers also suggested that fish can serve as effective bioindicators for monitoring heavy metal pollution in aquatic environments.

The gills of fish are highly sensitive to environmental pollutants because they are directly exposed to water and play an essential role in respiration and ion regulation. According to **Evans et al. (2005**), toxic substances present in water can damage gill tissues and reduce the efficiency of gas exchange. Structural changes in gill lamellae and epithelial tissues have been observed in fish exposed to contaminated water.

Changes in biochemical parameters are also widely used to assess stress responses in fish. **According to Wendelaar Bonga (1997),** stress hormones such as cortisol stimulate metabolic processes that lead to increased glucose levels in fish blood. Elevated glucose concentration is considered a common physiological response to environmental stress. The author emphasized that biochemical indicators such as glucose, proteins, and enzyme activities are useful for evaluating stress conditions in fish.

Hematological studies have also been widely used to assess the health condition of fish exposed to pollutants. According to **Blaxhall and Daisley (1973**), blood parameters such as hemoglobin content, hematocrit value, and erythrocyte count can provide valuable information about the physiological condition of fish. Changes in these parameters often occur when fish are exposed to environmental stress or toxic substances.

Research conducted by **Pandey et al**. (**2003**) indicated that pesticide contamination in water can significantly affect biochemical and physiological parameters in fish. The study reported alterations in enzyme activity and metabolic processes in fish exposed to pesticide-contaminated water. Such changes may impair normal physiological functioning and reduce the survival rate of fish.
Similarly, **Abdel-Tawwab and Wafeek** (**2014**) investigated the effects of environmental stress on fish physiology and reported that exposure to pollutants may suppress immune responses and increase susceptibility to diseases. The study emphasized that chronic stress caused by environmental pollution may negatively affect fish growth, reproduction, and overall health.
Fish are widely recognized as reliable bioindicators of aquatic pollution because they respond quickly to environmental changes. According to **Van der Oost et al**. (**2003**), physiological and biochemical biomarkers in fish can be used to monitor environmental contamination and evaluate the toxic effects of pollutants. Biomarkers such as enzyme activity, stress hormone levels, and hematological parameters provide valuable insights into the health status of fish populations.
Overall, previous research studies clearly indicate that water pollution has significant effects on the physiological and biochemical functioning of fish. Exposure to polluted water may lead to metabolic disturbances, respiratory problems, hematological changes, and oxidative stress in fish. These physiological responses serve as important indicators for assessing the impact of environmental pollution on aquatic organisms.
Therefore, studying the physiological stress responses in fish exposed to polluted water is essential for understanding how environmental contaminants affect aquatic life. Such investigations contribute to environmental monitoring, pollution management, and conservation of aquatic ecosystems.

Chapter-3
Materials and Methods

1. Study Area

The present study was conducted to evaluate the physiological stress response in fish exposed to polluted water. Water samples were collected from selected polluted water bodies such as rivers, ponds, or lakes located near urban or industrial areas. These sites were chosen because they are frequently exposed to domestic sewage, agricultural runoff, and industrial waste, which may influence water quality and aquatic life.

2. Experimental Fish

Healthy freshwater fish were selected for the present study. Fish of similar size and weight were collected from local fish markets or fish farms to ensure uniformity in the experimental conditions. The selected fish were transported carefully to the laboratory in clean containers filled with fresh water to minimize stress during transportation.
Before starting the experiment, the fish were acclimatized to laboratory conditions for about 7–10 days. During this period, the fish were kept in aerated aquaria containing clean, dechlorinated water. They were fed regularly with a standard fish diet and maintained under controlled environmental conditions such as temperature, pH, and dissolved oxygen levels.

3. Collection of Water Samples

Water samples were collected from polluted water sources using clean and sterilized containers. The samples were transported to the laboratory for analysis. Physical and chemical parameters of the water such as temperature, pH, dissolved oxygen, turbidity, and the presence of pollutants were examined to determine the level of contamination.

4. Experimental Design

After the acclimatization period, fish were divided into two groups:
Control Group:
Fish in this group were maintained in clean and uncontaminated water under normal laboratory conditions.
Experimental Group:

Fish in this group were exposed to polluted water collected from contaminated sources. The exposure period was maintained for a specific duration, such as 7, 14, or 21 days, depending on the experimental design.
Both groups were kept in separate aquaria with proper aeration and environmental conditions. Fish were observed regularly to record any behavioral changes during the experimental period.
5.**Physiological and Biochemical Analysis**
Blood Sample Collection
At the end of the experimental period, blood samples were collected from fish using sterile syringes. Blood was carefully drawn from the caudal vein to avoid injury to the fish. The collected blood samples were stored in anticoagulant tubes for further hematological and biochemical analysis.
Hematological Parameters
Various hematological parameters were analyzed to evaluate physiological stress in fish. These included:
-Red blood cell (RBC) count
-White blood cell (WBC) count
-Hemoglobin concentration
-Hematocrit value
These parameters were determined using standard laboratory procedures and hematological techniques.
Biochemical Parameters
Biochemical analysis was carried out to measure important stress indicators such as:
-Blood glucose levels
-Total protein concentration
Enzyme activity
Changes in these biochemical parameters were used to assess metabolic disturbances in fish exposed to polluted water.

5. **Observation of Behavioral Changes**

During the experiment, fish behavior was carefully observed. Behavioral responses such as abnormal swimming patterns, increased opercular movements, loss of balance, reduced feeding activity, and surface breathing were recorded. These behavioral changes were considered indicators of stress caused by polluted water.

6. **Statistical Analysis**

The obtained data from physiological and biochemical parameters were analyzed using standard statistical methods. Mean values and standard deviations were calculated for each parameter. The results of control and experimental groups were compared to determine the effect of polluted water on fish physiology.

7. **Ethical Considerations**

All experimental procedures involving fish were carried out with proper care to minimize stress and injury. The study was conducted in accordance with standard ethical guidelines for the use of animals in scientific research.

Chapter-4
Results

The present study was conducted to evaluate the physiological stress response in fish exposed to polluted water. Various hematological and biochemical parameters were analyzed in both control and experimental groups. The results obtained from the experiment indicated significant differences between fish kept in clean water (control group) and fish exposed to polluted water (experimental group).
These differences clearly suggest that polluted water induces physiological stress in fish.
During the experimental period, fish exposed to polluted water showed noticeable behavioral changes such as increased opercular movements, irregular swimming patterns, and reduced feeding activity. In contrast, fish maintained in clean water appeared healthy and showed normal swimming behavior and feeding habits.

1. Hematological Parameters

The hematological parameters of fish were analyzed to assess the physiological condition of fish exposed to polluted water. Significant variations were observed in red blood cell count, white blood cell count, hemoglobin concentration, and hematocrit values between the control and experimental groups.
Table 1: Hematological Parameters of Fish in Control and Polluted Water.

- **Parameters. Control group polluted water**

RBC count (×10cells /mm3)	2.8+_0.15	2.1+_0.12
WBC count (×10cells/mm3)	6.5+ _ 0.30	8.2+_0.35
Hemoglobin (g/dl)	10.5+_0.40	8.7+_0.32
Hematocrit (%)	32.4+_1.2	26.8+_1.0

The results show that the RBC count, hemoglobin concentration, and hematocrit values decreased in fish exposed to polluted water compared to the control group. This reduction may indicate physiological stress and possible impairment of oxygen transport in fish. On the other hand, the WBC count increased in the experimental group, which may represent an immune response to environmental stress or exposure to toxic substances.

2. Biochemical Parameters

Biochemical parameters were analyzed to determine metabolic changes in fish exposed to polluted water. The results revealed significant alterations in blood glucose levels and protein concentration.
Table 2: Biochemical Parameters of Fish in Control and Polluted Water

Parameters.	Control group	Polluted water group
Blood / glucose (Mg/dl)	6.54+_2.5	92.8+_3.2
Total protein (g/dl)	5.8+_0.20	4.2+_0.18
Enzyme activity (U/L)	38.5+_1.4	52.3+_1.8

The results indicate that blood glucose levels increased significantly in fish exposed to polluted water. This increase is commonly associated with stress conditions, as stress hormones stimulate energy

mobilization in the body. In contrast, total protein levels decreased in the experimental group, suggesting metabolic disturbances caused by pollutant exposure.
Enzyme activity was also found to increase in fish exposed to polluted water, which may indicate physiological stress and tissue damage caused by toxic substances.

3. Behavioral Observations

Behavioral changes were also observed in fish exposed to polluted water during the experimental period.
Table 3: Behavioral Changes Observed in Fish

Behaviour	control group	Polluted water group
Swimming pattern	Normal	Irregular swimming
Feeding activity	Normal feeding	Reduced feeding
Opercular movement	Normal	Rapid movement
Surface breathing	Absent	Frequently observed

Fish maintained in polluted water showed abnormal swimming behavior, increased opercular movements, and frequent surface breathing. These behavioral responses indicate physiological stress and difficulty in respiration caused by poor water quality.

4. Overall Observations

The results obtained from hematological, biochemical, and behavioral analyses clearly indicate that exposure to polluted water causes significant physiological stress in fish. The decrease in red blood cells and hemoglobin suggests impaired oxygen transport, while the increase in white blood cells indicates activation of immune responses. Similarly, elevated glucose levels and altered enzyme activity reflect metabolic disturbances associated with stress conditions.
These findings demonstrate that polluted water can significantly affect the physiological functioning of fish and may ultimately influence their survival, growth, and overall health.

Chapter- 5
Discussion

The present study was carried out to evaluate the physiological stress response in fish exposed to polluted water. The results obtained from hematological, biochemical, and behavioral observations clearly indicate that polluted water can significantly affect the physiological condition of fish. Environmental pollution acts as a major stressor for aquatic organisms, and fish respond to such stress through various physiological and biochemical changes.
In the present investigation, fish exposed to polluted water exhibited noticeable alterations in

hematological parameters such as red blood cell count, white blood cell count, hemoglobin concentration, and hematocrit values. A decrease in red blood cell count and hemoglobin concentration was observed in fish exposed to polluted water when compared to the control group. These findings suggest that polluted water may interfere with oxygen transport and blood formation processes in fish. Similar observations have been reported in previous studies, where exposure to environmental pollutants resulted in reduced erythrocyte count and hemoglobin levels in fish.

The reduction in hematocrit value observed in the experimental group may also indicate physiological stress and possible damage to blood-forming tissues. Lower hematocrit values can affect the oxygen carrying capacity of blood and may lead to impaired metabolic functions in fish. Such hematological alterations are commonly used as indicators of environmental stress in aquatic organisms.

In contrast, the present study recorded an increase in white blood cell count in fish exposed to polluted water. The increase in leukocyte count may represent an immune response to environmental stress or exposure to toxic substances. White blood cells play an important role in protecting the organism against infections and harmful agents. Therefore, the elevated WBC count observed in the experimental group may reflect the activation of defense mechanisms in fish exposed to polluted environments.

Biochemical parameters also showed significant changes in fish exposed to polluted water. The results of the present study revealed a considerable increase in blood glucose levels in the experimental group. Elevated glucose levels are a common physiological response to stress in fish. Under stressful conditions, stress hormones such as cortisol stimulate the mobilization of energy reserves, leading to increased glucose concentration in the blood. This increase in glucose provides the necessary energy required for the organism to cope with adverse environmental conditions.

The present study also showed a decrease in total protein concentration in fish exposed to polluted water. The reduction in protein levels may be associated with increased protein utilization for energy production during stress conditions. Environmental pollutants may also interfere with protein synthesis and metabolic processes in fish. Such alterations in protein metabolism are commonly observed in fish exposed to toxic substances.

Enzyme activity was also found to increase in fish exposed to polluted water. Increased enzyme activity may indicate tissue damage or metabolic disturbances caused by toxic pollutants. Environmental contaminants often disrupt enzyme systems and interfere with normal biochemical reactions in fish. The increase in enzyme activity observed in the present study may therefore reflect physiological stress and cellular damage resulting from exposure to polluted water.

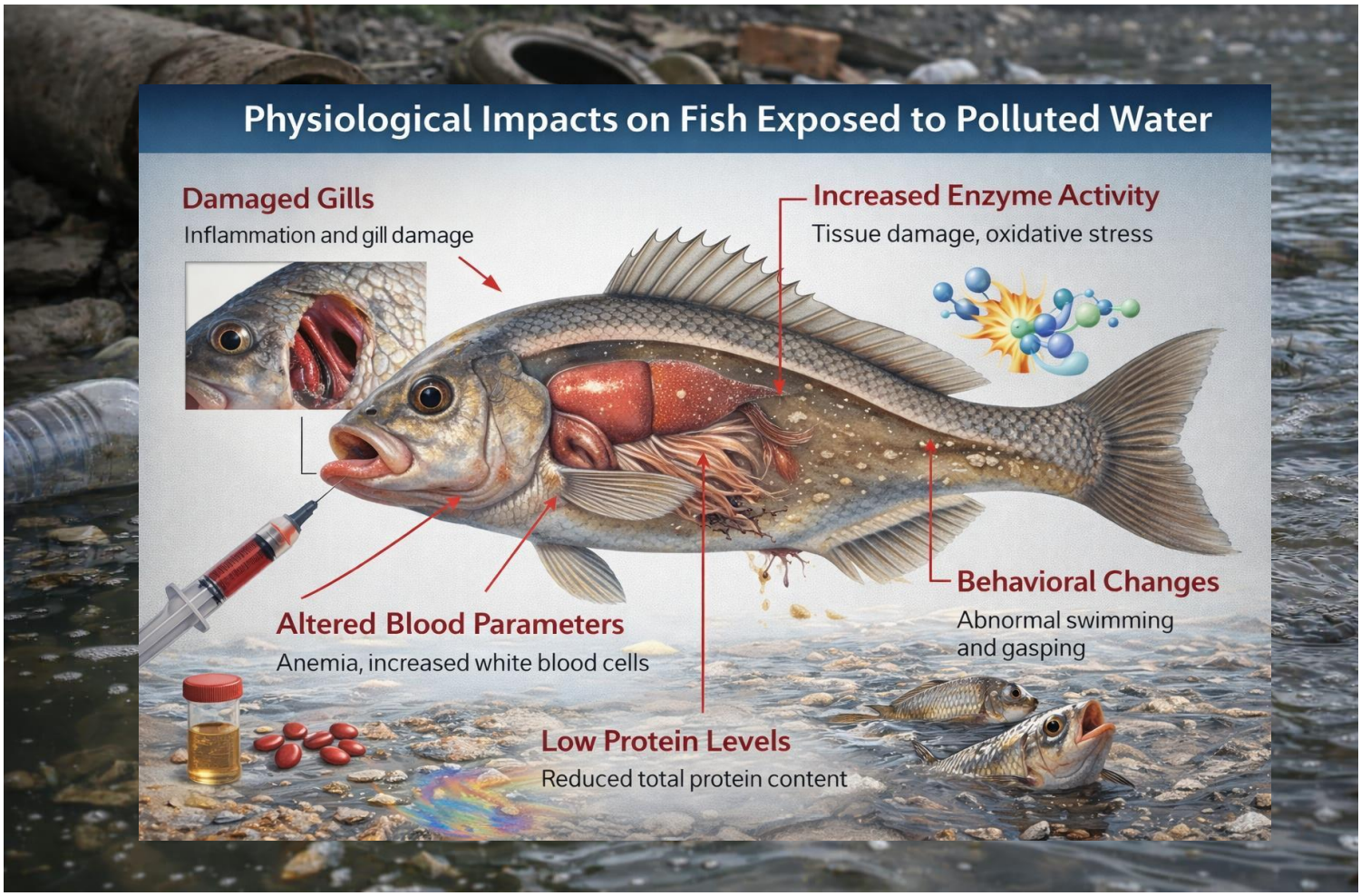
Behavioral observations further supported the presence of stress in fish exposed to polluted water. Fish in the experimental group showed abnormal swimming patterns, increased opercular movements, and frequent surface breathing. These behavioral changes may be associated with respiratory distress caused by poor water quality or reduced oxygen availability. Pollutants present in water may damage gill tissues and reduce the efficiency of gas exchange, forcing fish to increase their respiratory movements.

Behavioral responses are often considered early indicators of environmental stress in fish. Changes in swimming behavior, feeding activity, and respiratory movements may occur before severe physiological damage becomes apparent. Therefore, behavioral observations can provide valuable information about the health status of fish exposed to polluted environments.

The results of the present study are consistent with several previous investigations that have reported physiological disturbances in fish exposed to environmental pollutants. Polluted water containing heavy metals, pesticides, and other toxic substances can disrupt normal physiological processes in fish. These pollutants may accumulate in fish tissues and cause metabolic, hematological, and biochemical alterations.

Overall, the findings of this study indicate that polluted water has a significant impact on the physiological functioning of fish. Changes in hematological and biochemical parameters, along with behavioral abnormalities, clearly demonstrate that fish experience stress when exposed to contaminated environments. These physiological responses may ultimately affect fish growth, reproduction, and survival.

Therefore, monitoring physiological stress responses in fish can provide valuable information about the health of aquatic ecosystems. Fish can serve as effective bioindicators for assessing the level of environmental pollution in water bodies. Understanding how fish respond to polluted water is essential for developing effective strategies for pollution control and for protecting aquatic biodiversity.



Chapter-6
Conclusion

The present study was conducted to investigate the physiological stress response in fish exposed to polluted water. Aquatic ecosystems are increasingly affected by environmental pollution due to rapid industrialization, urbanization, and agricultural activities. The discharge of untreated industrial effluents, domestic sewage, pesticides, fertilizers, and other contaminants into water bodies has significantly deteriorated water quality. Since fish live in direct contact with their aquatic environment, they are particularly sensitive to changes in water quality and are therefore considered excellent indicators of environmental pollution.

The findings of the present study clearly demonstrate that polluted water can induce significant physiological stress in fish. Exposure to contaminated water resulted in noticeable alterations in hematological, biochemical, and behavioral parameters when compared with fish maintained in clean water conditions. These changes indicate that environmental pollutants can disturb the normal physiological functioning of fish and may ultimately affect their health and survival.

One of the major observations of the present study was the alteration in hematological parameters in fish exposed to polluted water. The reduction in red blood cell count and hemoglobin concentration suggests that polluted water may interfere with oxygen transport mechanisms in fish. Hemoglobin plays a vital role in carrying oxygen from the gills to various tissues of the body. Therefore, a decrease in hemoglobin concentration may reduce the oxygen carrying capacity of blood, which may result in physiological stress and metabolic disturbances. Similarly, the reduction in hematocrit value observed in fish exposed to polluted water indicates possible impairment in blood formation processes and may reflect the adverse effects of pollutants on the circulatory system of fish.

In contrast, an increase in white blood cell count was observed in fish exposed to polluted water. This increase may represent an immune response to environmental stress and exposure to toxic substances. White blood cells are an essential component of the immune system and play a critical role in defending the body against pathogens and harmful agents. The elevated leukocyte count observed in the present study suggests that fish exposed to polluted environments activate their immune system in an attempt to protect themselves from the harmful effects of pollutants.

The biochemical parameters analyzed in the present study also revealed significant changes in fish exposed to polluted water. One of the most prominent observations was the increase in blood glucose levels in the experimental group. Elevated glucose concentration is widely recognized as a common physiological response to stress in fish. Under stressful environmental conditions, stress hormones such as cortisol stimulate the mobilization of stored energy reserves, resulting in increased glucose levels in the bloodstream. This increase in glucose provides the necessary energy required for the organism to cope with unfavorable environmental conditions.

A decrease in total protein levels was also observed in fish exposed to polluted water. Proteins play an essential role in various physiological processes including growth, tissue repair, and metabolic regulation. The reduction in protein levels may be attributed to increased protein breakdown and utilization for energy production during stress conditions. Environmental pollutants may also interfere with normal protein synthesis and metabolic processes in fish. Such disturbances in protein metabolism may negatively affect the overall health and physiological functioning of fish.

Another important observation in the present study was the increase in enzyme activity in fish exposed to polluted water. Enzymes are essential biological catalysts that regulate various metabolic processes in living organisms. Increased enzyme activity may indicate physiological stress and tissue damage caused by exposure to toxic substances. Environmental pollutants often disrupt enzyme systems and interfere with normal biochemical reactions within cells. Therefore, changes in enzyme activity may serve as useful indicators of physiological stress in fish exposed to polluted

environments.
Behavioral changes were also observed in fish exposed to polluted water during the course of the experiment. Fish in the experimental group exhibited abnormal swimming patterns, increased opercular movements, reduced feeding activity, and frequent surface breathing. These behavioral responses are commonly associated with environmental stress and poor water quality. Increased opercular movements and surface breathing may indicate respiratory distress caused by reduced dissolved oxygen levels or damage to gill tissues. Since the gills are responsible for gas exchange in fish, any damage to gill structures may significantly affect the respiratory efficiency of fish.
The behavioral changes observed in the present study provide additional evidence that polluted water can create stressful conditions for fish. Behavioral responses are often considered early warning signs of environmental stress and may occur before severe physiological damage becomes evident. Therefore, monitoring behavioral changes in fish can be an effective method for detecting the impact of environmental pollution on aquatic organisms.
The results obtained from the present study are consistent with findings reported by several previous researchers who have demonstrated that environmental pollutants can cause significant physiological disturbances in fish. Pollutants such as heavy metals, pesticides, and industrial chemicals can accumulate in aquatic ecosystems and exert toxic effects on aquatic organisms. These pollutants may interfere with metabolic processes, disrupt enzyme systems, and cause structural damage to tissues and organs.
The study also highlights the importance of using physiological and biochemical parameters as indicators for assessing the impact of environmental pollution on aquatic organisms. Hematological and biochemical changes provide valuable information about the health status of fish and can serve as reliable biomarkers of environmental stress. Monitoring these parameters can help researchers and environmental agencies detect early signs of pollution and evaluate the health of aquatic ecosystems.
Fish are widely used as bioindicators because they respond quickly to environmental changes and pollutants present in water. The physiological stress responses observed in fish exposed to polluted water provide important insights into the level of environmental contamination and its potential effects on aquatic life. Therefore, the study of stress responses in fish can contribute significantly to environmental monitoring and pollution management programs.
The present study also emphasizes the need for effective measures to control water pollution and protect aquatic ecosystems. Preventing the discharge of untreated industrial and domestic waste into water bodies is essential for maintaining water quality and protecting aquatic biodiversity. Proper waste management practices, treatment of industrial effluents, and sustainable agricultural practices can help reduce the entry of harmful pollutants into aquatic environments.
In addition, regular monitoring of water quality and aquatic organisms should be carried out to assess the health of aquatic ecosystems. Scientific studies focusing on physiological stress responses in fish can provide valuable information for developing environmental protection strategies and improving fisheries management practices.
In conclusion, the findings of the present study clearly demonstrate that exposure to polluted water can cause significant physiological stress in fish. Alterations in hematological parameters, biochemical indicators, and behavioral patterns provide clear evidence that pollutants present in water can disrupt normal physiological functioning in fish. These disturbances may ultimately affect fish growth, reproduction, and survival, thereby posing a threat to aquatic biodiversity.
Therefore, understanding the physiological stress responses of fish exposed to polluted water is essential for assessing the ecological impact of environmental pollution. Such studies can contribute to the development of effective strategies for pollution control, environmental conservation, and sustainable management of aquatic resources. Protecting aquatic ecosystems from pollution is crucial not only for the survival of fish and other aquatic organisms but also for maintaining ecological balance and ensuring the availability of clean water resources for future generations

Chapter- 7
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