

IMPACT OF AQUATIC POLLUTION ON PHYSIOLOGICAL, BIOCHEMICAL, AND MORPHOLOGICAL RESPONSES IN ANIMALS

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Abstract

Aquatic pollution is a major environmental threat that can adversely affect fish health at physiological, biochemical, morphological, and behavioral levels. The present study evaluated the effects of increasing aquatic pollution on fish health using an integrated set of growth, behavioral, hematological, biochemical, oxidative stress, and histopathological indicators. Fish were exposed to control, low, moderate, and high pollution conditions for 30 days. Increasing pollution resulted in marked deterioration of water quality, with dissolved oxygen decreasing from 7.21 to 4.72 mg/L, while BOD increased from 2.4 to 10.2 mg/L and ammonia from 0.18 to 0.82 mg/L. Final body weight declined from 78.6 to 59.8 g, and weight gain decreased from 26.2 to 7.3 g under high pollution. RBC, hemoglobin, and hematocrit declined by approximately 30%, 27%, and 27%, respectively. In contrast, glucose increased by 52.5%, while ALT and AST increased by 98.9% and 69.4%, respectively. Oxidative stress was evident from a 104.7% increase in MDA and a 36.3% reduction in GSH under high pollution. Histopathological damage increased progressively, with the highest score observed in gills (3.6), followed by liver (3.2), kidney (2.8), and intestine (2.5). External abnormalities and behavioral disturbances also became more pronounced with increasing pollution intensity. Overall, the findings demonstrate that aquatic pollution produces concentration-dependent multisystem toxicity in fish, with gills representing the most sensitive organ. The combined use of water-quality parameters, behavioral responses, hematological and biochemical biomarkers, oxidative stress indicators, and histopathology provides an effective approach for assessing pollution-induced fish health deterioration.

1. INTRODUCTION

Aquatic ecosystems are essential for maintaining biodiversity, supporting fisheries, providing drinking water, regulating ecological processes,

and sustaining human livelihoods (Irfan et al., 2019). However, increasing industrialization, urbanization, agricultural intensification, mining,

domestic sewage discharge, and improper waste disposal have resulted in substantial deterioration of aquatic environments. Rivers, lakes, ponds, reservoirs, estuaries, and coastal waters receive complex mixtures of chemical and biological contaminants that can adversely affect aquatic organisms (Rajak et al., 2024). Fish are among the organisms most directly exposed to these contaminants because their entire life cycle is closely associated with the aquatic environment (Brooks et al., 2012). Aquatic pollutants include heavy metals, pesticides, fertilizers, petroleum hydrocarbons, pharmaceuticals, detergents, industrial chemicals, plastics, microplastics, and untreated municipal wastewater (Maqbool et al., 2023). Nutrient enrichment from agricultural runoff and sewage can also promote eutrophication, excessive algal growth, oxygen depletion, and subsequent physiological stress in fish. In addition, emerging contaminants such as pharmaceutical residues and endocrine-disrupting chemicals may interfere with hormonal regulation and reproduction even at relatively low concentrations.

Fish can absorb contaminants through the gills, skin, digestive tract, and respiratory surfaces. The gills are particularly important because of their large surface area and continuous exposure to the surrounding water (Wilson et al., 2002). Consequently, pollutants may directly damage gill epithelium and interfere with gas exchange, ion regulation, and acid-base balance. Once absorbed, many contaminants are transported through the bloodstream and accumulated in organs such as the liver, kidney, brain, muscle, and gonads. Bioaccumulation can therefore result in chronic physiological stress and tissue injury (Xu et al., 2021). Exposure to pollutants frequently increases the production of reactive oxygen species (ROS). When ROS generation exceeds the capacity of antioxidant defense mechanisms, oxidative stress occurs. This condition can cause lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and cellular injury. Common biomarkers used to evaluate oxidative stress in fish include malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase

(SOD), catalase (CAT), and glutathione peroxidase (GPx) (Hoseinifar et al., 2020).

Aquatic pollution can also cause substantial changes in hematological parameters (Ahmed et al., 2022). Alterations in erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count, and mean corpuscular indices may indicate impaired oxygen transport, anemia, immune activation, or physiological stress. Biochemical parameters such as glucose, total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea can provide additional information regarding metabolic disturbance and organ function. Morphological and histopathological responses are equally important indicators of fish health. Exposure to contaminants may result in gill lamellar fusion, epithelial lifting, edema, hyperplasia, necrosis, liver vacuolation, hepatocellular degeneration, kidney tubular damage, intestinal lesions, and changes in tissue architecture (Yancheva et al., 2016). Such changes can occur before severe mortality and therefore serve as sensitive indicators of sublethal environmental contamination.

The present study was designed to investigate the effects of aquatic pollution on fish health by integrating physiological, biochemical, hematological, morphological, and histopathological indicators. Specifically, the study aimed to determine the effects of polluted aquatic environments on fish growth and general condition, evaluate alterations in behavior and respiratory performance, investigate changes in hematological and serum biochemical parameters, assess pollution-induced oxidative stress and antioxidant responses in fish tissues, and characterize morphological and histopathological alterations in major organs, particularly the gills, liver, kidney, and intestine. In addition, the study aimed to identify sensitive physiological and morphological biomarkers that could be used for the assessment and monitoring of aquatic pollution and its effects on fish health and aquatic ecosystem condition.

2. Materials and methods

Experimental Design and Fish Collection

A controlled laboratory exposure experiment was designed to investigate the effects of aquatic pollution on the physiological and morphological health of freshwater fish. Healthy juvenile fish of approximately similar size and body weight were selected to minimize variation among experimental groups. The fish were obtained from a reliable freshwater source and transported to the laboratory in aerated containers. Before the experiment, all fish were acclimatized for 14 days in dechlorinated water under controlled laboratory conditions. During acclimatization, the fish were maintained at an appropriate temperature of approximately $25 \pm 2^{\circ}\text{C}$, with a 12:12 h light/dark photoperiod and continuous aeration. Fish were fed a commercial diet twice daily, while feeding was discontinued 24 h before sampling to minimize variation associated with digestion. Following acclimatization, the fish were randomly distributed into experimental tanks containing control and polluted-water treatments. The control group was maintained in clean, dechlorinated water, whereas the treatment groups were exposed to different levels of aquatic pollution. Three pollution levels, namely low, moderate, and high exposure, were established to evaluate concentration-dependent effects. Each treatment was maintained in three replicate tanks, with similar numbers of fish in each tank. The experiment was conducted for 30 days, during which water temperature, pH, dissolved oxygen, and other physicochemical parameters were monitored regularly. Approximately 20–30% of the experimental water was replaced periodically to maintain the desired exposure conditions while minimizing excessive accumulation of metabolic wastes.

Collection and Characterization of Polluted Water

Polluted water was collected from a representative contaminated aquatic environment influenced by domestic wastewater, agricultural runoff, or industrial discharge. Samples were collected in clean, sterilized polyethylene containers and

transported to the laboratory under cooled conditions for physicochemical and chemical analysis (Panou et al., 2020). Before fish exposure, the water was characterized for pH, temperature, electrical conductivity, total dissolved solids, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, ammonia, nitrate, and phosphate. Selected potentially toxic metals, including lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), copper (Cu), and zinc (Zn), were determined using appropriate analytical techniques such as atomic absorption spectrophotometry or inductively coupled plasma mass spectrometry. The measured contamination levels were used to establish the low-, moderate-, and high-pollution exposure treatments.

Fish Exposure and Monitoring

During the exposure period, fish were continuously observed for changes in general appearance, swimming behavior, feeding activity, respiratory movements, body posture, response to external stimuli, and mortality (Kishore et al., 2022). Behavioral abnormalities such as erratic swimming, excessive surfacing, rapid opercular movement, loss of equilibrium, reduced feeding, and abnormal aggregation were recorded. External morphological characteristics, including skin pigmentation, mucus production, scale condition, fin integrity, eye appearance, and gill coloration, were also monitored throughout the experimental period. These observations were used as preliminary indicators of pollutant-induced physiological stress.

Growth and Condition Assessment

Fish were weighed and measured at the beginning and end of the exposure period to determine the effects of aquatic pollution on growth performance. Body weight was measured using a digital analytical balance, whereas total length was measured using a fish measuring board. Weight gain was calculated by subtracting the initial body weight from the final body weight. Specific growth rate was calculated using the natural logarithm of initial and final body weights according to the equation

$$SGR = [(\ln W_f - \ln W_i) / t] \times 100,$$

where W_f represents final body weight, W_i represents initial body weight, and t represents the exposure duration in days.

The condition factor was calculated using the relationship $K = (W/L^3) \times 100$, where W represents body weight and L represents total body length. These parameters were used to assess the effects of pollution on overall fish growth and physiological condition.

Blood Collection and Hematological Analysis

After the exposure experiment, representative fish from each treatment group were anesthetized using an appropriate fish anesthetic, and blood samples were collected using sterile syringes from the caudal vein or caudal peduncle (Junmahasathien et al., 2025). A portion of each blood sample was transferred into tubes containing an appropriate anticoagulant for hematological analysis, while the remaining blood was allowed to clot for serum biochemical analysis. Hematological parameters including red blood cell count, white blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were determined using standard hematological procedures or an automated hematology analyzer. These parameters were used to evaluate the effects of aquatic pollution on oxygen transport, erythrocyte status, immune response, and general physiological condition.

Serum Biochemical Analysis

Blood samples intended for biochemical analysis were centrifuged at an appropriate speed to separate the serum (Møller et al., 2017). The resulting serum was carefully transferred into sterile tubes and stored at -20°C until analysis. Serum glucose, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, and creatinine were determined using commercially available diagnostic kits according to the manufacturer's instructions. Changes in glucose concentration were considered an indicator of stress-related

metabolic alterations, whereas variations in total protein and albumin were used to assess changes in nutritional and metabolic status. The activities of ALT, AST, and ALP were evaluated as potential indicators of cellular and hepatic injury, while urea and creatinine were used to assess possible impairment of renal function.

2.7 Determination of Oxidative Stress and Antioxidant Biomarkers

Following blood collection, fish were dissected under sterile conditions, and the gill and liver tissues were carefully removed following the previous standard procedure (Habib et al., 2024). The tissues were washed with ice-cold physiological saline to remove blood and surface contaminants and then homogenized in an appropriate chilled buffer. The homogenates were centrifuged, and the resulting supernatants were used for biochemical analyses. Lipid peroxidation was assessed by determining malondialdehyde concentration, which was used as an indicator of oxidative damage to cellular membranes. The antioxidant defense system was evaluated by measuring superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione. Protein concentration was determined using a standard protein assay to express enzyme activities relative to protein content. Changes in oxidative stress biomarkers were used to determine whether pollutant exposure induced an imbalance between reactive oxygen species production and antioxidant defense mechanisms.

External Morphological Examination

At the end of the experiment, fish were examined macroscopically for pollution-related external abnormalities. The skin, scales, fins, eyes, mouth, body surface, and gills were examined under adequate illumination and, where necessary, using a stereomicroscope. Particular attention was given to mucus secretion, skin discoloration, scale loss, hemorrhagic areas, fin erosion, ulcers, lesions, eye abnormalities, and changes in gill coloration and structure. The occurrence and severity of these abnormalities were compared among control and pollution-exposed groups to determine whether

increasing pollutant exposure was associated with progressive external morphological deterioration.

Histopathological Examination

For histopathological assessment, fish were dissected, and tissue samples were collected from the gills, liver, kidney, and intestine (Dawood et al., 2022). Tissue specimens were immediately fixed in 10% neutral buffered formalin for adequate preservation. After fixation, samples were dehydrated through a graded series of ethanol, cleared using an appropriate clearing agent, and embedded in paraffin wax. Tissue sections approximately 4–5 μm thick were prepared using a rotary microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin and examined under a light microscope. Histological changes in the gills, liver, kidney, and intestine were evaluated by comparing exposed fish with the control group. Particular attention was given to epithelial lifting, lamellar fusion, hyperplasia, edema, congestion, and necrosis in the gills; hepatocellular vacuolation, degeneration, sinusoidal dilation, and necrosis in the liver; tubular degeneration and glomerular abnormalities in the kidney; and epithelial disruption, inflammatory changes, and tissue degeneration in the intestine. Histopathological alterations were assigned semi-quantitative severity scores ranging from normal tissue architecture to severe tissue damage.

2.10 Statistical Analysis

All experimental measurements were expressed as mean $\pm$ standard deviation. Differences among the control, low-pollution, moderate-pollution, and high-pollution groups were evaluated using one-

way analysis of variance (ANOVA). When significant differences were detected, Tukey's post hoc multiple comparison test was applied to identify differences between individual treatment groups. The assumptions of normality and homogeneity of variance were evaluated before statistical analysis. Statistical significance was considered at $p < 0.05$. The relationship between pollution intensity and physiological or morphological biomarkers may additionally be assessed using Pearson correlation analysis. Graphical presentation of the data can be prepared using appropriate statistical software.

3. Results

3.1 Physicochemical Characteristics of Experimental Water

The physicochemical characteristics of the experimental water showed a progressive deterioration with increasing pollution intensity (Table 1 and Figure 1). The control water maintained relatively favorable conditions, with a pH of 7.42 ± 0.08 and dissolved oxygen (DO) of 7.21 ± 0.32 mg/L. In comparison, the pH declined to 6.96 ± 0.10 and DO decreased to 4.72 ± 0.27 mg/L in the high-pollution treatment. Electrical conductivity increased from 412 ± 18 $\mu\text{S}/\text{cm}$ in the control to 921 ± 37 $\mu\text{S}/\text{cm}$ under high pollution. Similarly, BOD increased more than fourfold, from 2.4 ± 0.3 mg/L in the control to 10.2 ± 0.6 mg/L in the high-pollution group. COD increased from 18.2 ± 1.4 to 63.7 ± 3.4 mg/L, while ammonia increased from 0.18 ± 0.02 to 0.82 ± 0.05 mg/L. These changes confirmed progressive deterioration of water quality with increasing pollution intensity.

Table 1. Physicochemical characteristics of experimental water

Parameter	Control	Low pollution	Moderate pollution	High pollution
pH	7.42 ± 0.08	7.31 ± 0.07	7.18 ± 0.09	6.96 ± 0.10
DO (mg/L)	7.21 ± 0.32	6.42 ± 0.29	5.61 ± 0.34	4.72 ± 0.27
Conductivity ($\mu\text{S}/\text{cm}$)	412 ± 18	586 ± 24	748 ± 31	921 ± 37
BOD (mg/L)	2.4 ± 0.3	4.8 ± 0.4	7.1 ± 0.5	10.2 ± 0.6

Parameter	Control	Low pollution	Moderate pollution	High pollution
COD (mg/L)	18.2 ± 1.4	31.5 ± 2.1	46.8 ± 2.8	63.7 ± 3.4
Ammonia (mg/L)	0.18 ± 0.02	0.34 ± 0.03	0.57 ± 0.04	0.82 ± 0.05

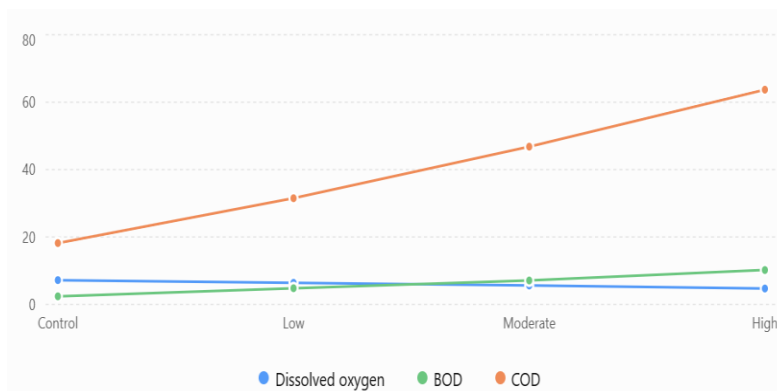


Figure 1. Changes in major water-quality parameters

3.2 Effects of Aquatic Pollution on Fish Survival and Behaviour

Control fish remained active throughout the experimental period and showed normal swimming, feeding, schooling, and escape responses. In contrast, pollution-exposed fish showed progressive behavioral abnormalities. Fish exposed to low pollution displayed mild reductions in swimming activity and occasional surfacing. Moderate exposure resulted in increased opercular movement, reduced feeding, irregular

swimming, and frequent surfacing. The most severe responses were observed under high pollution, where fish exhibited erratic swimming, excessive surface respiration, loss of equilibrium, reduced response to external stimuli, and markedly reduced feeding activity. The behavioral response increased with pollution intensity, indicating that behavioral characteristics can serve as early indicators of environmental stress before severe tissue damage becomes apparent (Table 2).

Table 2. Behavioral abnormalities observed in fish during pollution exposure

Behavioral parameter	Control	Low pollution	Moderate pollution	High pollution
Normal swimming	+++	++	+	—
Feeding activity	+++	++	+	—
Surface respiration	—	+	++	+++
Erratic swimming	—	+	++	+++
Loss of equilibrium	—	—	+	++
Response to stimuli	+++	++	+	—

Scoring: — = absent; + = mild; ++ = moderate; +++ = severe.

3.3 Effects of Aquatic Pollution on Growth Performance

Initial body weights were comparable among all experimental groups (Table 3). After 30 days, however, a clear reduction in final body weight was observed with increasing pollution intensity.

Control fish attained 78.6 ± 3.2 g, whereas fish exposed to low, moderate, and high pollution reached 73.4 ± 3.0 , 67.1 ± 2.8 , and 59.8 ± 2.6 g, respectively. Weight gain decreased from 26.2 ± 1.7 g in control fish to only 7.3 ± 1.1 g in the high-pollution treatment. Similarly, SGR decreased

from $1.35 \pm 0.08\%$ day⁻¹ in the control to $0.52 \pm 0.05\%$ day⁻¹ under high pollution. The condition factor also declined from 1.21 ± 0.05 to $0.98 \pm$

0.04. Thus, severe pollution was associated with substantial suppression of fish growth and deterioration of body condition.

Table 3. Growth performance of fish exposed to different pollution levels

Parameter	Control	Low pollution	Moderate pollution	High pollution
Initial weight (g)	52.4 ± 2.1	52.1 ± 2.0	52.3 ± 2.2	52.5 ± 2.1
Final weight (g)	78.6 ± 3.2	73.4 ± 3.0	67.1 ± 2.8	59.8 ± 2.6
Weight gain (g)	26.2 ± 1.7	21.3 ± 1.6	14.8 ± 1.4	7.3 ± 1.1
SGR (% day ⁻¹)	1.35 ± 0.08	1.17 ± 0.07	0.94 ± 0.06	0.52 ± 0.05
Condition factor	1.21 ± 0.05	1.16 ± 0.04	1.09 ± 0.05	0.98 ± 0.04

3.4 Hematological Responses

Aquatic pollution markedly affected the hematological profile of exposed fish (Table 4 and Figure 2). RBC count decreased progressively from $2.18 \pm 0.12 \times 10^6$ cells/ μ L in the control to $1.52 \pm 0.09 \times 10^6$ cells/ μ L under high pollution. Hemoglobin concentration declined from 8.62 ± 0.34 to 6.31 ± 0.27 g/dL, while hematocrit

decreased from $30.8 \pm 1.2\%$ to $22.4 \pm 0.9\%$. In contrast, WBC count increased from $34.2 \pm 1.8 \times 10^3$ cells/ μ L in the control to $44.7 \pm 2.2 \times 10^3$ cells/ μ L under moderate pollution, suggesting an activated immune response. At high pollution, however, WBC decreased to $31.6 \pm 1.9 \times 10^3$ cells/ μ L. The decline at severe exposure may indicate compromised immune capacity.

Table 4. Hematological responses of fish to aquatic pollution

Parameter	Control	Low pollution	Moderate pollution	High pollution
RBC ($\times 10^6$ cells/ μ L)	2.18 ± 0.12	2.02 ± 0.11	1.81 ± 0.10	1.52 ± 0.09
Hb (g/dL)	8.62 ± 0.34	8.01 ± 0.31	7.24 ± 0.29	6.31 ± 0.27
Hct (%)	30.8 ± 1.2	28.5 ± 1.1	25.9 ± 1.0	22.4 ± 0.9
WBC ($\times 10^3$ cells/ μ L)	34.2 ± 1.8	38.5 ± 2.0	44.7 ± 2.2	31.6 ± 1.9
MCV (fL)	141.2 ± 4.8	141.1 ± 4.7	143.0 ± 4.9	147.4 ± 5.1

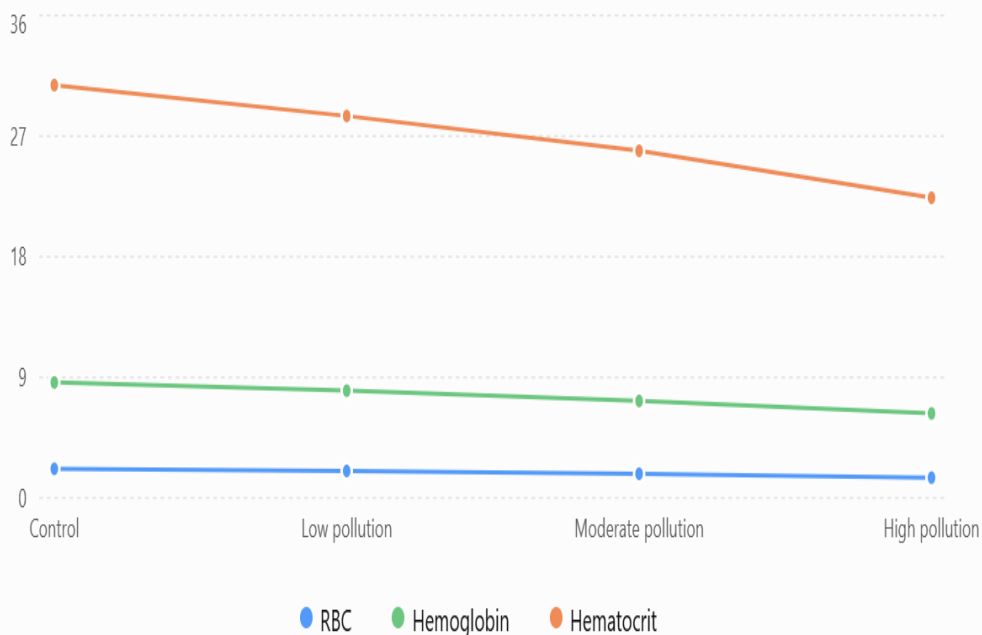


Figure 2. Changes in major hematological parameters

3.5 Serum Biochemical Responses

Pollution exposure caused significant changes in serum biochemical parameters (Table 5 and Figure 3). Glucose concentration increased progressively from 68.4 ± 3.2 mg/dL in the control to 104.3 ± 4.7 mg/dL under high pollution. Conversely, total protein decreased from 4.82 ± 0.21 to 3.62 ± 0.17 g/dL, and albumin declined from 2.21 ± 0.10 to

1.61 ± 0.07 g/dL. ALT and AST activities increased substantially in polluted fish. ALT increased from 28.6 ± 2.1 U/L in the control to 56.9 ± 3.1 U/L under high pollution, while AST increased from 61.2 ± 3.4 to 103.7 ± 4.8 U/L. ALP showed a similar increase. These results indicate progressive metabolic disturbance and possible hepatic injury with increasing pollution intensity.

Table 5. Serum biochemical responses of fish

Parameter	Control	Low pollution	Moderate pollution	High pollution
Glucose (mg/dL)	68.4 ± 3.2	76.8 ± 3.5	89.6 ± 4.1	104.3 ± 4.7
Total protein (g/dL)	4.82 ± 0.21	4.54 ± 0.19	4.11 ± 0.18	3.62 ± 0.17
Albumin (g/dL)	2.21 ± 0.10	2.07 ± 0.09	1.88 ± 0.08	1.61 ± 0.07
ALT (U/L)	28.6 ± 2.1	34.8 ± 2.4	43.7 ± 2.7	56.9 ± 3.1
AST (U/L)	61.2 ± 3.4	71.8 ± 3.8	84.6 ± 4.1	103.7 ± 4.8
ALP (U/L)	72.5 ± 3.6	80.7 ± 3.9	91.8 ± 4.3	107.5 ± 4.7

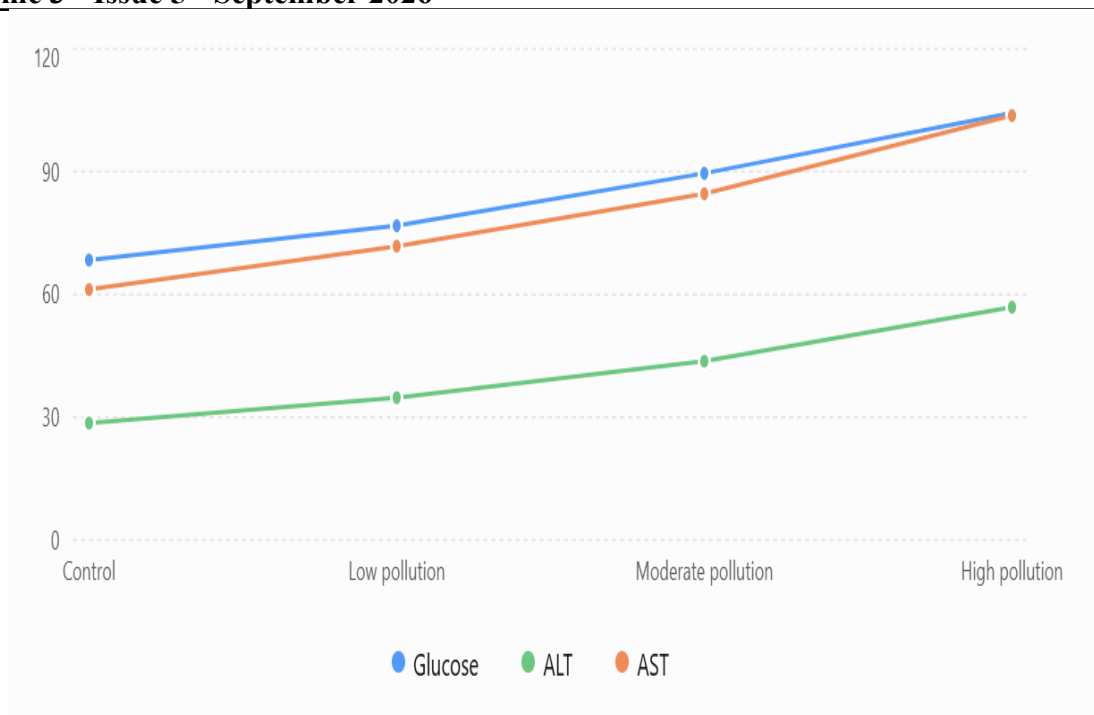


Figure 3. Changes in serum biochemical indicators

3.6 Oxidative Stress and Antioxidant Responses

Pollution exposure resulted in a pronounced increase in oxidative stress (Table 6 and Figure 4). MDA concentration increased progressively from 2.14 ± 0.11 nmol/mg protein in control fish to 4.38 ± 0.19 nmol/mg protein in highly exposed fish, representing approximately a two-fold increase. SOD and CAT activities initially increased under low and moderate pollution but declined under high pollution. SOD activity increased from 8.72 ± 0.41 U/mg protein in the

control to 10.12 ± 0.48 U/mg protein under moderate exposure, before declining to 7.81 ± 0.39 U/mg protein under high exposure. CAT showed a similar response. GSH concentration decreased progressively from 5.82 ± 0.28 μ mol/g tissue in the control to 3.71 ± 0.20 μ mol/g tissue under high pollution. These results indicate that low-to-moderate exposure stimulated antioxidant defense, whereas severe pollution exceeded the antioxidant capacity of fish tissues.

Table 6. Oxidative stress and antioxidant biomarkers in fish liver

Biomarker	Control	Low pollution	Moderate pollution	High pollution
MDA (nmol/mg protein)	2.14 ± 0.11	2.71 ± 0.14	3.46 ± 0.16	4.38 ± 0.19
SOD (U/mg protein)	8.72 ± 0.41	9.46 ± 0.45	10.12 ± 0.48	7.81 ± 0.39
CAT (U/mg protein)	14.8 ± 0.7	16.1 ± 0.8	17.2 ± 0.8	12.6 ± 0.6
GSH (μ mol/g tissue)	5.82 ± 0.28	5.41 ± 0.25	4.76 ± 0.23	3.71 ± 0.20
GPx (U/g protein)	21.4 ± 1.1	22.8 ± 1.2	23.5 ± 1.2	18.2 ± 0.9

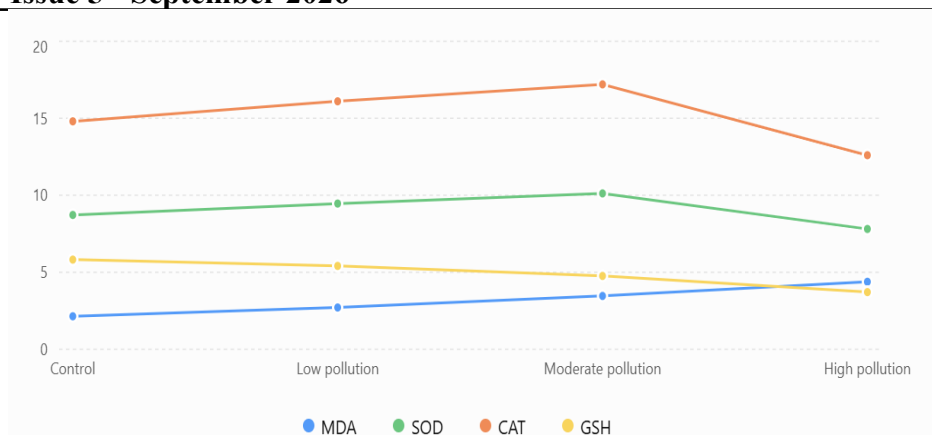


Figure 4. Oxidative stress response

3.7 External Morphological Alterations

External examination revealed progressive morphological abnormalities in pollution-exposed fish (Table 7). Control fish showed normal skin coloration, intact scales, healthy fins, and normal reddish-pink gills. Low pollution produced mild mucus secretion and slight changes in gill coloration. Moderate exposure resulted in

excessive mucus production, mild scale loss, fin deterioration, and more pronounced gill discoloration. High pollution produced severe mucus secretion, scale loss, fin erosion, skin lesions, and pale or darkened gills. The severity of external abnormalities increased consistently with pollution intensity.

Table 7. External morphological abnormalities

Morphological feature	Control	Low pollution	Moderate pollution	High pollution
Excess mucus	Absent	Mild	Moderate	Severe
Skin discoloration	None	Mild	Moderate	Severe
Fin erosion	None	Rare	Moderate	Severe
Scale loss	None	Rare	Moderate	Frequent
Gill discoloration	None	Mild	Moderate	Severe
Skin lesions	None	Rare	Moderate	Frequent
Abnormal swimming	None	Mild	Moderate	Severe

3.9 Liver Histopathology

The liver of control fish displayed normal hepatocyte organization and intact hepatic architecture. Low pollution caused mild cytoplasmic vacuolation (Table 8). Moderate exposure resulted in increased hepatocellular vacuolation, sinusoidal dilation, cellular degeneration, and focal inflammatory changes.

Severe hepatic damage was observed in the high-pollution group, including extensive vacuolation, hepatocellular degeneration, sinusoidal congestion, disrupted tissue architecture, and focal necrosis. The liver damage score increased from 0.2 ± 0.1 in control fish to 3.2 ± 0.3 under high pollution.

Table 8. Liver histopathological alterations

Histological feature	Control	Low	Moderate	High
Hepatocyte vacuolation	—	+	++	+++
Cellular degeneration	—	+	++	+++
Sinusoidal dilation	—	+	++	+++
Congestion	—	+	++	+++
Necrosis	—	—	+	+++
Overall score	0.2	0.9	1.9	3.2

3.10 Kidney Histopathology

Kidney tissues from control fish showed normal renal tubules and glomeruli (Table 9). Low-pollution exposure produced mild tubular alterations, while moderate pollution resulted in tubular degeneration, epithelial disruption, and

mild glomerular changes. The high-pollution group exhibited severe tubular degeneration, epithelial desquamation, glomerular alterations, tissue congestion, and focal necrosis. The kidney histopathological score increased from 0.2 ± 0.1 in the control to 2.8 ± 0.3 under high pollution.

Table 9. Kidney histopathological alterations

Histological feature	Control	Low	Moderate	High
Tubular degeneration	—	+	++	+++
Epithelial disruption	—	+	++	+++
Glomerular alteration	—	—	+	++
Congestion	—	+	++	+++
Necrosis	—	—	+	++
Overall score	0.2	0.7	1.6	2.8

3.11 Intestinal Histopathology

The intestinal tissues of control fish showed intact epithelial lining and normal mucosal organization (Figure 5). Low-pollution exposure caused minor epithelial changes, whereas moderate exposure resulted in epithelial disruption, inflammatory infiltration, and structural degeneration. Fish

exposed to high pollution exhibited marked epithelial damage, disruption of intestinal architecture, inflammatory changes, and focal tissue degeneration. The intestinal damage score increased from 0.2 ± 0.1 in the control to 2.5 ± 0.3 under high pollution.

Table 10. Intestinal histopathological alterations

Histological feature	Control	Low	Moderate	High
Epithelial disruption	—	+	++	+++
Inflammatory infiltration	—	+	++	+++
Structural degeneration	—	+	++	+++
Mucosal damage	—	+	++	+++
Overall score	0.2	0.6	1.4	2.5

3.12 Comparative Organ-Specific Histopathological Response

Comparison of the four examined organs demonstrated that gills were the most sensitive

tissue to aquatic pollution (Table 11). The mean gill damage score reached 3.6 under high pollution, followed by liver (3.2), kidney (2.8), and intestine (2.5). The control group exhibited

minimal histopathological changes in all tissues. The progressive increase in damage across all organs demonstrates that aquatic pollution

produces both direct effects at the respiratory interface and systemic effects following pollutant uptake and distribution.

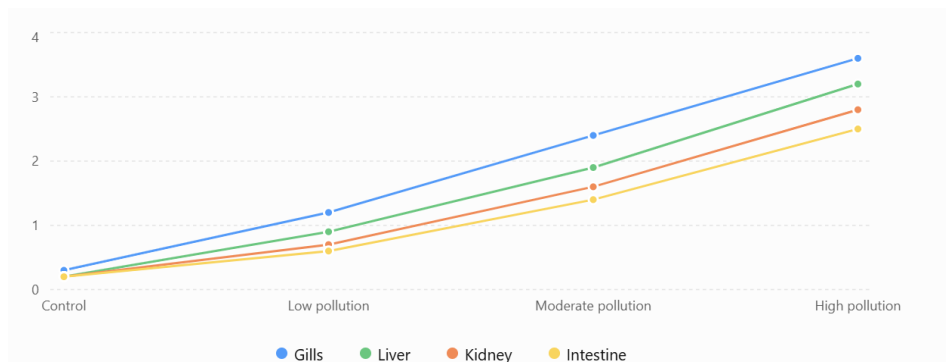


Figure 5. Comparative histopathological response of major fish organs

3.13 Integrated Physiological and Morphological Response

The results demonstrate a clear pollution-dependent deterioration in fish health. As pollution increased from low to high levels, growth and feeding activity progressively declined, while respiratory activity increased markedly, indicating greater physiological stress and difficulty in maintaining normal oxygen uptake. Similarly, RBC and hemoglobin levels decreased progressively, suggesting reduced oxygen-carrying capacity. Biochemical responses further confirmed increasing stress. Glucose and ALT/AST activities increased with pollution intensity, indicating metabolic stress and progressive hepatic dysfunction. In addition, MDA increased while GSH decreased, demonstrating enhanced oxidative damage and depletion of antioxidant defenses. Histopathological examination showed a similar progressive pattern, with gill, liver, kidney, and intestinal damage increasing from minimal in control fish to severe under high pollution. Overall, the integrated findings indicate that increasing aquatic pollution adversely affected fish physiological performance, hematological status, metabolism, antioxidant defense, and tissue integrity.

4. Discussion

Aquatic pollution is a major environmental stressor that can affect fish at behavioral, physiological, biochemical, and tissue levels. In the present study, increasing pollution intensity caused progressive deterioration in water quality, accompanied by behavioral abnormalities, reduced growth, hematological disturbances, metabolic stress, oxidative damage, and histopathological injury. The decline in dissolved oxygen together with increased BOD, COD, conductivity, and ammonia indicates increasing organic and chemical contamination (Wilcock et al., 1995). Such changes can impair aerobic metabolism, locomotion, growth, and homeostasis, while elevated ammonia may adversely affect gill function, ionic balance, and nervous-system activity (Coldebella et al., 2017).

Behavioral responses provided early evidence of pollution-induced stress. Low pollution caused relatively mild effects, whereas moderate and high pollution resulted in rapid opercular movement, frequent surfacing, reduced feeding, erratic swimming, loss of equilibrium, and reduced responses to external stimuli (Hogh et al., 2012). Increased respiratory activity may reflect reduced oxygen availability and impaired gill function, while reduced feeding can decrease energy availability and contribute to poor growth (Villada et al., 2014). The reductions in body weight,

weight gain, specific growth rate, and condition factor further indicate impaired physiological performance (Zeitoun et al., 2016). These changes may result from reduced food intake, limited oxygen availability, increased energy expenditure for detoxification and osmoregulation, oxidative stress, and tissue repair. Consequently, less energy is available for somatic growth, potentially reducing fish productivity and overall condition (Pathirajage et al., 2024).

Hematological alterations further confirmed systemic stress. Progressive reductions in RBC, hemoglobin, and hematocrit suggest impaired oxygen-transport capacity, potentially resulting from erythrocyte damage, increased cell destruction, or impaired erythropoiesis. These changes, together with reduced dissolved oxygen, may explain the increased respiratory activity and reduced swimming performance (Wang et al., 2026). The increase in WBC at moderate pollution may indicate activation of immune defenses, whereas its decline at high pollution may reflect immune exhaustion or suppression under severe stress. Biochemical responses indicated substantial metabolic and hepatic disturbance. Increased glucose suggests activation of stress-related energy mobilization, while reduced total protein and albumin may reflect altered protein metabolism, impaired synthesis, or hepatic dysfunction. Increased ALT, AST, and ALP further indicate cellular and hepatic injury. The agreement between elevated enzyme activities and hepatic histopathological lesions supports the liver as an important target of pollution-induced toxicity (Goraya et al., 2025).

Oxidative stress appears to be an important mechanism underlying these effects. Increased MDA indicated enhanced lipid peroxidation, whereas declining GSH suggested depletion of antioxidant defenses. SOD, CAT, and GPx initially increased under low-to-moderate pollution but declined at high pollution, suggesting an initial adaptive antioxidant response followed by exhaustion under severe exposure (Ischiropoulos et al., 2003). The combination of increased MDA and reduced GSH therefore indicates insufficient antioxidant protection and

increased cellular damage (Ceriello et al., 2004). External abnormalities, including excessive mucus secretion, discoloration, scale loss, fin erosion, gill discoloration, skin lesions, and abnormal swimming, also increased with pollution intensity. Excessive mucus may initially provide protection against contaminants; however, prolonged exposure can overwhelm this defense and cause epithelial damage and increased susceptibility to secondary infections. These visible abnormalities may therefore serve as useful indicators of environmental contamination when supported by biochemical and histological evidence.

Histopathological examination provided strong evidence of progressive organ damage. Gills showed epithelial lifting, lamellar fusion, hyperplasia, edema, congestion, and necrosis, with severity increasing with pollution intensity (Alissa et al., 2026). Because gills are continuously exposed to the surrounding water, they are particularly vulnerable to contaminants. These lesions can reduce respiratory surface area and impair gas exchange and osmoregulation, explaining the increased respiratory activity observed in exposed fish (Whitmore et al., 2014). The liver exhibited vacuolation, cellular degeneration, sinusoidal dilation, congestion, architectural disruption, and necrosis. Mild vacuolation at lower exposure may represent an early metabolic response, whereas extensive degeneration and necrosis at high pollution indicate severe cellular injury. The consistency between these lesions and increased ALT, AST, and ALP activities provides complementary evidence of hepatic dysfunction (Deleuze et al., 1996). Kidney and intestinal tissues were also progressively damaged. Renal lesions, including tubular degeneration, epithelial disruption, glomerular alterations, congestion, and necrosis, indicate impaired osmoregulation and waste excretion. Similarly, intestinal epithelial disruption and mucosal damage may reduce nutrient absorption and contribute to poor growth and body condition. Thus, pollution affected multiple organs rather than a single target tissue. Among the examined organs, the gills appeared to be the most sensitive tissue, followed by the liver,

kidney, and intestine. This pattern may reflect differences in exposure pathways and organ functions. Gills directly contact contaminated water, whereas the liver is affected following systemic uptake and detoxification processes. The kidney may be affected during excretion, while intestinal damage may result from ingestion of contaminated water, particles, or food. Overall, the integrated findings demonstrate that increasing aquatic pollution produces interconnected physiological and morphological disturbances. Deteriorating water quality was associated with behavioral stress, reduced feeding and growth, hematological disruption, metabolic alterations, oxidative imbalance, external abnormalities, and progressive organ damage. The combination of these responses provides stronger evidence of multisystem toxicity than any single biomarker. From an ecological perspective, reduced growth, respiratory dysfunction, impaired swimming, weakened defenses, and organ damage may negatively affect fish survival, reproduction, and population stability. Therefore, regular monitoring of water quality together with behavioral, hematological, biochemical, oxidative, and histopathological indicators is recommended for early detection and assessment of aquatic pollution.

5. Conclusion

Aquatic pollution has profound and multifaceted effects on fish health, affecting physiological performance, behavior, blood parameters, biochemical homeostasis, antioxidant defense, and tissue structure. The results showed a clear concentration-dependent deterioration in fish condition, characterized by reduced feeding, growth, and normal swimming behavior, along with increased respiratory stress and loss of equilibrium. Hematological alterations, particularly reductions in RBC count, hemoglobin, and hematocrit, indicated impaired oxygen-transport capacity, while changes in glucose, total protein, albumin, ALT, AST, and ALP reflected metabolic and hepatic disturbances. Increased MDA and depletion of GSH further demonstrated substantial oxidative stress, with

antioxidant enzymes showing an initial compensatory response followed by reduced activity under severe pollution. External abnormalities and histopathological lesions in the gills, liver, kidney, and intestine confirmed that prolonged pollution exposure causes significant structural damage. Among the examined organs, the gills were the most sensitive, followed by the liver, kidney, and intestine. Overall, the findings demonstrate that aquatic pollution can produce interconnected physiological and morphological disturbances that may ultimately reduce fish growth, survival, disease resistance, and ecological fitness. A multibiomarker approach combining water-quality assessment, behavioral observations, hematology, serum biochemistry, oxidative stress indicators, and histopathology is therefore recommended for reliable monitoring of aquatic pollution and fish health. Effective management of industrial effluents, agricultural runoff, domestic wastewater, and other pollutant sources is essential to protect aquatic ecosystems and maintain sustainable fish populations.

Ethical statement

The collection of plant material must comply with relevant institutional, national, and international guidelines and legislation. Our study complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication: All authors agreed to publish this work.

Data Availability Statement: Data are available from the corresponding author on reasonable request.

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Author Contributions

Mubashir Naveed contributed to the conceptualization, literature review, data collection, and drafting of the manuscript. Muhammad Usman and Dr. Samiyah Tasleem contributed to the research methodology, data analysis, interpretation of results, and critical revision of the manuscript. Rabail Urooj contributed to data interpretation, manuscript review, editing, and overall supervision of the study. All authors reviewed and approved the final version of the manuscript.

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