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Histopathological Assessment of Goldfish *(Carassius auratus)* Gills Under Aquatic Pollution Conditions

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Abstract Gills consist of specialized structures including primary and secondary lamellae, chloride cells, epithelial cells, mucous cells, and venous sinuses, which are crucial for breathing and osmotic pressure management. The findings underscore substantial histological alterations caused by pollution exposure. *Carassius auratus* collected from ornamental fish shops in five different locations of Wasit Governorate, at a rate of four goldfish from each site. In addition, five water samples were collected from the same ponds from which the fish were obtained, the necessary chemical and physical analyses were conducted on them to determine the levels of pollutants in those ponds. The physicochemical analyses of the water revealed a clear, significant difference regarding the level of contamination in that water. On the other hand, histological results of the gills after they were stained with routine dye H&E observed the primary and secondary lamellae in addition to, appearance of oedema and epithelial lifting in the tissue. Meanwhile, the histochemical results of the samples revealed a strong positive in mucous cell, and hyper secretion inter lamellae positive mucous cell hyperplasia with PAS stain. In addition, Mason's trichrome stain was used in present work to visualize collagen fibers; here, we note moderate fibrosis with lamellae fusion, hyperplasia and vascular congestion.

Keywords: Histopathological, Goldfish, Gills, Water pollution, *Carassius auratus.*

Introduction Due to the increasing need of ornamental fish as pets and the use of ornamental fish as decorations, ornamental fish farming is considered as one of the lucrative sectors in the interim and one of the major alternative industry. Owing to their shape, color, and action, those fish are often called living gems and amusements. They could also grow up in small spaces (1). It is regarded as a high-income industry. Global ornamental fish exports brought approximately US $283 million in revenue in 2006 (2). The United States, Europe, and Japan make up most of the market (3). Over half of the world's ornamental fish come from South East Asian nations (4). Many nations profit from the pet fish trade. However, the proliferation of different illnesses brought on by the importation of non-native species might have unfavorable effects. The most popular ornamental fish kept as pets are varieties of the freshwater goldfish *Carassius auratus,* which belongs to the Cyprinidae family. Goldfish come in eleven different types, including fantail (5). Elevated pollution levels in aquatic ecosystems result in significant morphological and physiological alterations in aquatic creatures (6

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There has been significant goldfish culture throughout the last few decades. Every year, more over 2.9 million tons are produced (7). Infection by bacteria and other infectious diseases are a major cause of concern to the ornamental fish reared in overcrowded tanks with poor water quality and poor management (8), viral diseases (9), parasitic diseases (10). Biomarkers based on histological alterations have found widespread use in assessing the health of polluted fish and in understanding the action mechanisms of various stress agents (11). These alterations provide a rapid way to identify the ways in which various tissues and organs are affected by irritants, particularly long-term ones (12). Because of their huge surface area, which comes into direct and ongoing touch with certain irritants, fish gills are effective bio monitoring instruments. In addition to regulating osmoregulation and acid-base balance, the gills are in charge of respiratory gas exchange (13).

When faced with unsavory changes in their environment, these organs are the first to react (14). Exposure to different contaminants may cause alterations in gill epithelium, the extent of these alterations contingent on the concentration and period of exposure to the pollutant, so they are therefore useful as early warning of the health state of fish (15). Other major impacts of pollution include the occurrence of disorders in gas exchange and ion regulation which is a role of gills in part. As well, the capacity of fish to resist injurious substances greatly relies on the mucous cells on their gills (16). The aim of this study was to study the histopathological variations in the gillsof fish when the water of the ponds in which they live is polluted. And determine their suitability as vital indicators of the effects of water pollution.

Materials and Methods

Ethical approval

The current investigation was performed in agreement with the authorized norms for animal care and use by the Ethical Committee at the University of Wasit (No. 87 in 1-9-2025), Al-Kut City, Iraq.

Fish sampling

20 healthy female Goldfish (*Carassius auratus*) with average body weight of (25-30 g) and body length ranged between (11-15 cm.) were purchased from ornamental fish shops in five different locations of Wasit Governorate from period September to October 2025. Four goldfish were purchased from each shop, and a water sample was taken from the same tank from which the fish were taken.

Water data

Five water samples were collected from different sites and from the same tanks which the fish were collected in special opaque plastic containers at a depth of 30 cm from inside the tank. The size of each tank was 80 x 40 x 30 cm. The water samples were transferred directly to the Environment Department's laboratory to determine the following physical and chemical parameters: Temperature, PH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS) , Ammonia (NH_3), Nitrate (NO_3).

Histopathological examination

Goldfish were transported to the Animal House affiliated with the College of Science dissected, and the gills were carefully separated as shown in Figure1. After that samples fixed in 10% buffered formalin. Next, the samples were transferred to the histology laboratory and subsequent histological techniques were then performed on the samples. The preserved tissues were dehydrated using increasing ethyl alcohol concentrations, cleaned in xylene, embedded in paraffin wax, and then blocked. To find the collagen fiber, sagittal slices (4 µm thick) were cut, placed on glass slides, and dyed with Masson Trichrome. Periodic acid Schiff (PAS) to see the mucus cells, and H&E for histological description (17). A Nikon Coolpix 4300 digital camera and an Olympus B×41 microscope were

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used to study and take pictures of sections of each fish gill. Gill tissue histological alterations were categorized using a scale of 0- 3, with 0 denoting no alterations, 1 denoting minor alterations, 2 denoting moderate alterations, and 3 denoting severe alterations (18).

Figure 1: An image showing the removal of gills from a fish.

Statistical analysis

The data was collected, compiled, analyzed, and presented using Microsoft Office Excel 2010 and the statistical program for social sciences (SPSS) version 26. Numerical data were presented as mean and standard deviation following the Kolmogorov-Smirnov normality test, which identified whether variables were normally and non-normally distributed. If the variable is normally distributed, significant differences between more than two groups were examined using the one-way ANOVA test. P-value of less than 0.05 was deemed significant, while 0.01 or less was deemed highly significant.

Results

Water analysis

According to the statistical findings, the water samples had a significant difference ($p<0.05$). After conducting chemical and physical tests on them, and the results percentages were specified in Figure 2-7 and Table 1 below. On the other hand, the tested water quality standards showed that some samples were above the permitted limits established by WHO in 2013 (19).

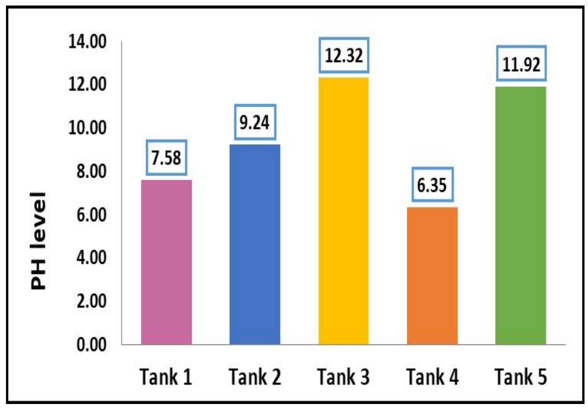

Figure 2: Mean of pH levels for samples.

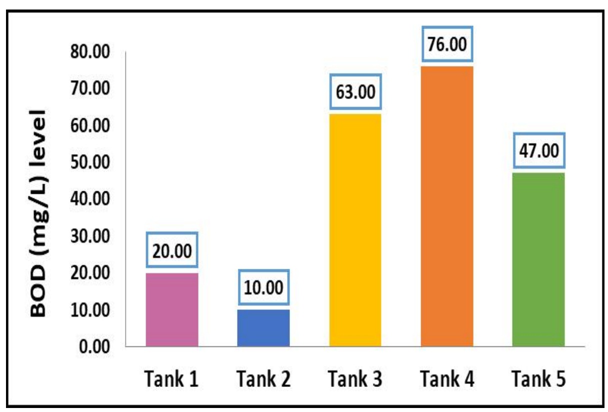

Figure 3: Mean of BOD levels for samples.

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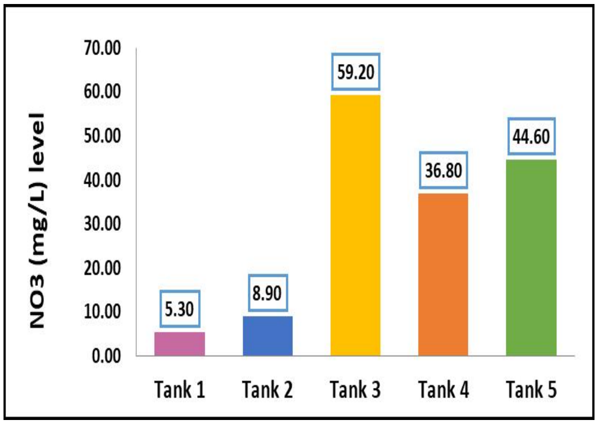

Figure 5: Mean of NH_3 levels for samples..

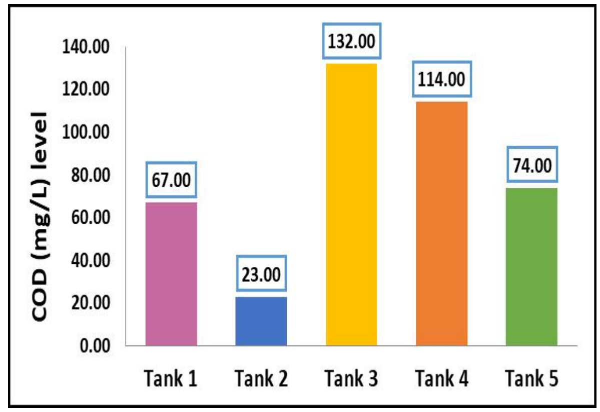

Figure 4: Mean of COD levels for samples.

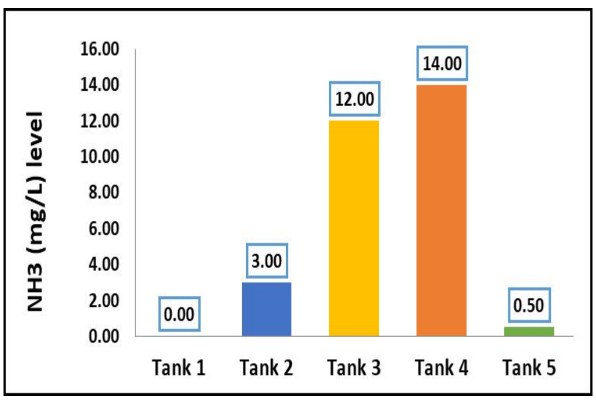

Figure 6: Mean of TDS levels for samples

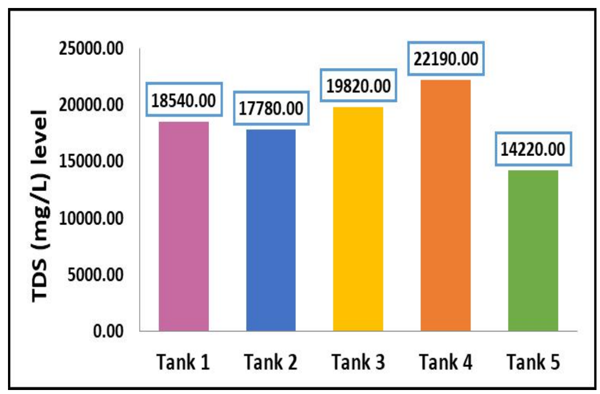

Figure 7: Mean of NO_3 levels for samples

Table 1: Mean and standard deviation of the physicochemical tests of pond water

No.	Physiochemical parameter	Tank1	Tank 2	Tank 3	Tank 4	Tank 5	Acceptable range	P value
1	Temperature (°C)	20 ±2.0	22 ±2.4	18 ±1.98	22 ± 2.2	19 ±3.1	_	0.119
2	pH	7.58 ± 1.2	9.24 ± 2.13	12.32 ± 3.21	6.35 ± 1.28	11.92 ± 3.34	6-9.5	0.001*
3	BOD (mg/L)	20 ± 4.32	10 ± 2.34	63 ± 7.64	76 ± 8.98	47 ± 7.43	40	0.001*
4	COD (mg/L)	67 ± 7.68	23 ± 4.56	132 ± 12.34	114 ± 14.34	74 ± 9.86	100	0.001*
5	TDS (mg/L)	18540 ± 165.4	17780 ± 201.3	19820 ± 255.4	22190 ± 254.6	14220 ± 198.4	_	0.001*
6	NH_3 (mg/L)	0	3 ± 0.03	12 ± 2.3	14 ± 3.88	0.5 ± 0.04	10	0.001*
7	NO_3 (mg/L)	5.3 ± 1.3	8.9 ± 2.61	59.2 ± 8.99	36.8 ± 6.75	44.6 ± 8.32	50	0.001*

Histopathological changes in gills

Gills are immediately exposed to pollutants as a result of frequent interaction with water. In most of the species examined, gradual histological alterations were the most prevalent results. There were different levels of epithelial hyperplasia. A cross section of goldfish gills stained with the routine dye revealed the presence of oedema and epithelial lifting in the tissue, as well as the primary and secondary lamellae. However, some areas showed signs of lamellar hyperplasia, Figure 8,

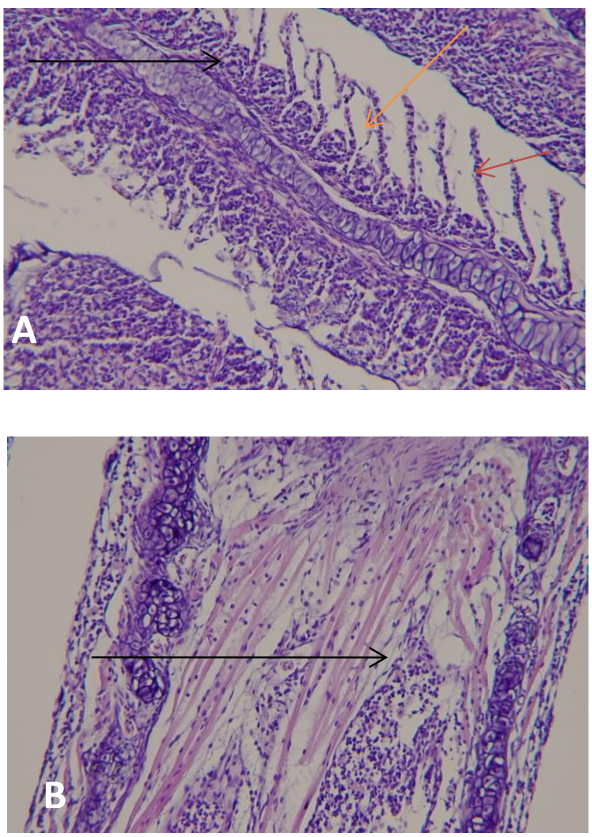

Figure 8A: Cross section of goldfish gills displaying primary lamellae (black arrow), secondary lamellae (red arrow) and epithelial lifting (orange arrow).**B:** Lamellar hyperplasia (black arrow), oedema (red arrow) are mild alterations. Grade 2-3, H&E stain, 40X.

On the other hand, to detect mucus cell and exudates, the samples were treated with Periodic acid Schiff (PAS) stain. The results shows a strong and clear reaction with the PAS stain, mucous cell hyperplasia and interlaminar hypersecretion (black arrow), as seen in Figure 9A. Instead, gills showed a slight positive reaction with the PAS stain as Figure 9B, where cell secretions of mucus between the lamellae appear in a slight and simple manner.

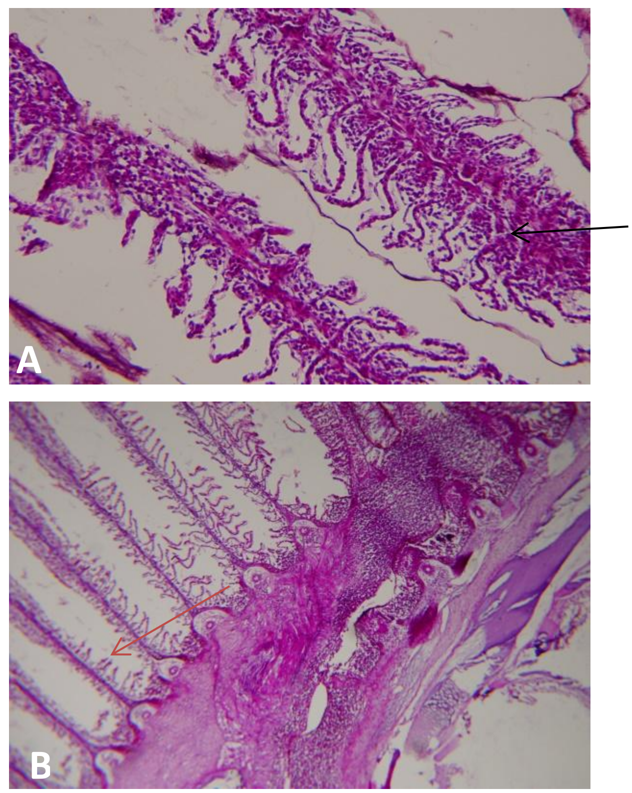

Figure 9A: Cross section in gills showing reaction with stain is strong positive in mucous cell, cell membrane and hyper secretion inter lamellae positive mucous cell hyperplasia, (black arrow). Grade3, 40X.
B: Mild positive reaction in mucous cell, hyper secretion inter-lamellae positive mucous cell, Grade (0-1), PAS stain, 10X. In addition, the sections under the microscope, revealed the presence of fibrosis with lamellae fusion and hyperplasia using Mason's Trichrome stain. Furthermore, the cartilage surrounding minor

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vascular congestion was showed, as in Figure 10 below.

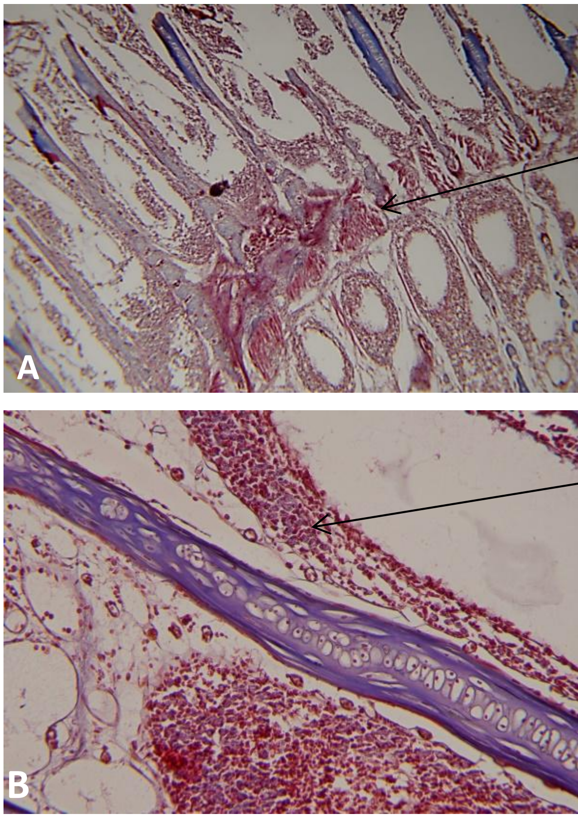

Figure 10 A: Cross section of goldfish gills' reveals moderate degree of fibrosis, secondary lamellae fusion and hyperplasia (black arrow) and fibroblast-like cells, Grade 3, 10X. **B:** Cartilage surrounding slight vascular congestion (black arrow), Grade 1, Masson Trichrome stain, 40X.

Discussion

The production of ornamental fish has a special place in the globe and contributes significantly to both employment and the exchange income of different countries. Numerous studies have concentrated on different elements jeopardizing the health of these aquatics due to their financial significance (20). Meanwhile water pH has a major impact on aquatic creatures' metabolism, it is crucial to evaluate this parameter in order to manage pollution levels. pH is an indicator of the acidity or alkalinity of water (21). We discovered notable variations in pH levels. Turbidity is a crucial assessment for evaluating water quality. Suspended and dissolved substances, such as clay, are distributed (22). Elevated turbidity levels in ponds signify substantial amounts of suspended sediments present at the location. These sediments may obstruct the gills, resulting in oxygen deprivation, which can harm gill tissue. Fish that are exposed to water quality parameters outside of these ranges are stressed, have slower growth, are more susceptible to illness (23), and/or suffer structural tissue damage. This is particularly true of ammonia, whose water level is directly correlated with pH, and to a lesser degree, reduced DO levels and fish water temperature (24).

Ammonia concentrations over 2.0 mg/L are documented to induce gill and tissue damage, profound lethargy, and mortality in affected fish (25). The low-level cellular damage found in the exposed fish's gills may be caused by the ammonia level in the study fish pond water, even if it is within a tolerable range. This is due to the fact that fish exposed to sub lethal ammonia concentrations typically exhibit pathological alterations in their gills (26), whereas fish mortality only happens at toxic levels in fish pond water (27).

Fish respiration is hampered by gill edema lamellae, which decrease the surface area available for gas exchange. Fish that are exposed to contaminants on a regular basis may experience respiratory distress, hypoxia, or even suffocation. Fish may experience a variety of histological changes in their tissues when exposed to sublethal concentrations of certain pesticides or other chemical pollutants in the environment (28).

Even while the study's gill hyperplasia was minor, in severe circumstances, these responses may decrease oxygen transport across the gill epithelium, resulting in hypoxia (29). A shift in blood oxygenation levels is indicated by congestion, which is shown by a change in red hue. Numerous investigations have emphasized the critical function of mucous cells in fish gills (30). The primary physiological functions of mucus production are lubrication of the respiratory tract and defense against harmful microbes (31). Additionally, its secretion is essential for fish ion transport and control. A disruption in the aquatic ecology might be the cause of fish having more mucous cells (32). Fish exposed to acidic environments and different types of contaminants may exhibit a non-specific reaction of hypersecretion of gill mucus (33).

Additionally, a significant rise in epithelial hyperplasia of the epithelial cells lining the gill lamellae was noted in this investigation. In order to shield underlying tissues from more harm, hyperplasia is a protective reaction to persistent irritation or injury brought on by contaminants or pathogens (34). The thickening of the epithelial tissue around the base of the lamella or at the tip of the filament, which resembles baseball bats, is caused by hyperplasia of the cartilage. The gills' surface area may shrink as a result of subsequent lamella cell fusion brought on by hyperplasia. Additionally, erythrocytes will use less oxygen, which will result in necrotic cell lamellae (35).

Water pollution, bacterial infections, parasites, pentatonic acid deficiencies, and low pH levels are examples of external stressors that may have contributed to this discovery (36). Gill enlargement is a typical indicator of long-term toxicity brought on by a variety of chemical contaminants. Such hyperplastic processes have been proposed to thicken the epithelium in order to correct for osmotic imbalance or to block or prevent harmful ions from entering the circulation (37).

Conclusion

The findings indicate that the histological alterations are probably attributable to modifications and contamination in the water. Alterations in composition and an elevation in mucus levels were noted in the gills of goldfish. This result indicates that the sites where these fish lived were contaminated by unidentified xenobiotics. Histopathological changes observed in the gill epithelium indicate that the fish has been exposed to stress. While more research is needed to validate our findings, we suggest that histological changes may serve as effective biomarkers for assessing environmental contamination.

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