Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 945 – 958 (2024)
www.ejabf.journals.ekb.eg

Histopathological and Osmoregulation Aspects of Freshwater Fish Gills as Biological Indicators of Contamination in the Shatt al-Arab River in Basra Governorate, Iraq

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ARTICLE INFO

Article History:
Received: Aug. 21, 2024
Accepted: Aug. 23, 2024
Online: Dec. 4, 2024

Keywords:
Fish,
Gill,
Histology,
Ions,
Bio- indicator,
Aquatic pollution

ABSTRACT

The histological and functional characteristics of fish gills are crucial indicators of environmental quality and are widely utilized as bioindicators for ecological monitoring. This study examined the gill histology of *Cyprinus carpio* and *Liza abu* to assess the ecological health of the Shatt al-Arab River across four sites: Site 1 (Haratha Electric Power); Site 2 (Najebia Electric Power); Site 3 (Kandac Canal); and Site 4 (North Shatt Al Arab). Site 4, considered as a control station, exhibited good environmental conditions, while Sites 1, 2, and 3 were contaminated with human and industrial wastes. At Sites 1, 2, and 3, the gills of both fish species showed significant damage, including epithelial lifting, necrosis, degeneration, and congested blood vessels. Furthermore, there were disturbances in osmoregulation, as indicated by altered concentrations of sodium (Na+), potassium (K+), and calcium (Ca2+) ions in the gills, as well as an increase in gill chloride cells. Genetic mutations were also observed, evident from the presence of micronuclei in the red blood cells. In contrast, no apparent gill damage was observed at Site 4. Our results confirmed that *L. abu* was more sensitive to aquatic pollution than *C. carpio*. Overall, the findings suggest that gill histological markers can be effectively used as bioindicators for evaluating the environmental impact on river ecosystems.

INTRODUCTION

The release of waste from industrial, urban, and agricultural operations into aquatic systems leads to the production of a wide range of pollutants, including inorganic and organic substances; nevertheless, aquatic ecosystems near industrial units are particularly vulnerable to contaminants by different chemicals such as solvents, oils, petrochemicals, and heavy metals **(Rajamanickam & Narayanan, 2009)**. High levels of pollution in aquatic systems frequently result in significant histological and physiological alterations in aquatic animals **(Zhou *et al.*, 2008)**. However, the most commonly used methods for assessing water quality in aquatic environments are measures of contamination levels in sediment and water **(Vasanthi *et al.*, 2013)**. Since these

procedures are not cost-effective and do not provide specific information on the biological reactions of aquatic species, a broad range of biological indicators has been developed to measure and qualitatively evaluate the physiological reactions of aquatic organisms to water contamination **(Van der Oost *et al.*, 2003)**. Aquatic ecosystems are highly vulnerable due to the accumulation of large amounts of toxins from their surrounding environments. As a consequence, water quality parameters such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), and total suspended solids (TSS) are often compromised. Water bodies are commonly used as reservoirs for a variety of xenobiotics, which can lead to significant histological and biochemical changes in fish **(Reddy, 2012)**. Water contamination can be measured using chemical monitoring, and analyses of several biochemical and molecular indicators are used to assess the impact of pollutants. Histopathology should not be overlooked in the assessment of pollutant impacts, as it allows for the rapid diagnosis of contamination effects by localizing, describing, and even quantifying lesions that appear in specific organs **(Reddy & Rawat, 2013)**. In general, biomarker responses can be used to evaluate and analyze the impacts of pollution on numerous aquatic animals, including fish; however, most biomarkers are narrow in their expression, whereas histopathology is broad in its evaluation **(Medja & Golemi, 2011)**. But it's important to choose the bio-indicator and the species carefully. For this investigation, the fish species *C. carpio* was chosen for two reasons: the first is that they occupy the top position in the aquatic food chain, and they reflect both the biotic and abiotic factors present in the specific aquatic environment **(Dragun *et al.*, 2007)**. Second, as a member of the genus *Carpio*, which is common in Iraqi rivers, this specific species allows for comparison across distant geographical regions. The selection of acceptable organs for histological investigations, on the other hand, was motivated by a desire to detect the repercussions of contamination events as soon as possible. Although exposure of fish to chemical and microbiological pollutants may produce lesions in numerous organs, we chose gills, which are a good indicator of present environmental conditions **(Amiri *et al.*, 2011)**. In addition, histological alterations are commonly used as indicators for evaluating the health of fish exposed to pollutants and to clarify the mechanism that regulates of various stress factors **(Nascimento *et al.*, 2012)**. These histological changes quickly assess how irritants, affect various tissues and organs **(Velmurugan *et al.*, 2009)**. Early detection of respiratory organ damage is environmentally important. It is an inexpensive, fast, and easy method, and can also be employed as a biological indicator **(Sousa *et al.*, 2013)**. According to **Gomes *et al.* (2012)**, exposure to various pollutants can cause alteration in the gill epithelium, and the extent of these changes depends on the percentage of contaminants and the duration of exposure. Furthermore, they can also act as early warning signs for illnesses that impact the health of fish **(Sorour, 2001)**. Research suggests that histopathological lesions in fish gills can indicate environmental pollution levels **(Ogundiran *et al.*, 2009; Flores-Lopes & Thomaz, 2011)**. Disruptions

in ion regulation and gas exchange, functions partially carried out by the gills **(Ledy *et al.,* 2003)**, are among the primary bio-indicators of contamination. As a biomarker, mucous cells in the fish gills also play an important role in fish resistance to poisonous compounds **(Bernet *et al.,* 1999)**, abrasive damage caused by solids suspended in water (**Dezfuli *et al.,* 2003)**, and pollutants **(Ledy *et al.,* 2003; Roberts & Powell, 2003)**. This paper aimed to examine the effects of several persistent pollutants on the fish gills, which are used as bioindicators of environmental contamination. Fish are effective indicators of pollution in aquatic environments because they occupy various trophic levels, come in different sizes and ages, and are more sensitive to numerous toxic substances than invertebrates **(Mendil *et al.,* 2010)**.

MATERIALS AND METHODS

Sampling site and water quality data

The Shatt al-Arab River is considered the main freshwater source in southern Iraq. It extends approximately 190 kilometers before emptying into the Gulf of Arabia. The river's breadth varies from 250 meters in Basrah City to around 1500 meters before draining into the Arabian Gulf. The maximum depth of this river is between 5 and 12m **(Abdullah, 1990)**. All fish samples were collected in December 2021 from four sites from Shatt aL- Arab River, (St1: electric power of the Haratha); (St2: electric power of the Najebia), (St3: Kandac canal); (St4: south shat AL Arab) (Fig. 1.). The physical and chemical parameters of the water column were estimated using the following methods: water temperature measured with a digital thermometer; electrical conductivity (EC) determined with a WTW COND 3151 (Germany); concentrations of nitrite (NO2) and nitrate (NO3) assessed using a UV-1200 spectrophotometer (Germany) as per **APHA (2005)**; total hardness measured using the titrimetric method (Lind, 1979); and heavy metals analyzed using an atomic absorption spectrophotometer AA-7000 (Japan) according to **APHA (1998)**.

Sample collection and processing

Thirty fish from each species were collected using a cast net. The average weights of *Cyprinus carpio* and *Liza abu* were approximately 340g ± 20 and 85.7g ± 4.2, respectively. The fish were then transported to the laboratory, where they were dissected, and histological and physiological parameters were measured.

Histological observations

Each fish specimen was killed with a quick blow to the head, and the mid-section of the second-gill arch on the left side was sampled and fixed in 10% formalin. Following decalcification, the specimens were dehydrated in a graded ethanol series, washed with

xylene, and embedded in paraffin blocks **(Luna, 1968)**. Six micron-thick sections were cut, and three sections were stained with the usual hematoxylin and eosin (H and E).

Osmoregulation and cytological observations

The middle sections of the secondary left gill arches were isolated from both fish species and placed in a sodium chloride solution at a concentration of 0.98mg/ L. The concentrations of Na^{2+}, K^{2+}, and Ca^{2+} ions were measured using a flame photometer (ANA-10AL) **(Al Sudany, 1999)**. Additionally, chloride cells in the gills were identified according to the methods described by **Sargent *et al.* (1978)**, while the respiratory diffusion distance in the gills was measured using a microocular measurement technique **(Nowak, 1991)**. Blood samples were collected from the caudal vasculature using a heparinized syringe, and a blood smear was prepared on a glass slide and stained with Giemsa dye **(AL-Sabti, 1985)**.

Statistical analysis

All measurements were expressed as the mean ± standard deviation (SD). The one-way analysis of variance (ANOVA) was conducted to check for normality of variance, and the least significant difference (LSD) test was used to determine whether the differences in change indices among locations were statistically significant ($P \leq 0.05$).

RESULTS

Water quality

Investigation of the water quality revealed some variations between the sites in several parameters (Table 1). Site differences were mostly accounted for by the water temperature degree, conductivity (Ec), pH, total hardness (TH), No2, No3, Pb+2, Cu+2, Cd+2, and Zn+2, according to the guidelines of the **WHO (2017)**. The results showed different significance among sites. However, Sites 2 and 3 were the ones that recorded the most extreme and abnormal values compared to the other sites (1 and 4), as well as guidelines and limit values declared by the World Health Organization.

Table 1. Water quality indicators for the Shatt aL- Arab River are in various locations based on WHO criteria

Parameter	Site1	Site2	Site3	Site4	WHO (2004 and 2011)
Temperature (C^{o})	17.33±1.22 b	18.44± 1.42 b	12.56±1.11 a	10.32± 1.01 a	-
Ec	0.21±0.003 **a**	4.43± 0.87 b	25.54±1.32 b	0.11± 0.00021 a	-
pH	7.8± 0.056 **ac**	8.01± 0.087 a	8.43±0.045 b	7.64± 0.055c	6.5 – 8.6
TH	445± 44.6 **d**	787± 63.6 c	3451±105.54 b	213± 30.5 a	-
No$_2$	0.58±0.055 **a**	1.25± 0.067 c	1.99±0.087 b	0.464±0.099 a	< 3
No$_3$	6.21±0.321**b**	6.01±0.0215ba	6.28±0.0311b	5.46± 0.0153 a	< 50
Pb^{+2}	0.143±0.004**d**	0.342±0.010 c	0.87±0.021b	0.054±0.002 a	0.1
Cu^{+2}	0.057±0.005 **a**	0.078±0.003 c	0.104±0.003b	0.055±0.0013 a	2
Cd^{+2}	0.0051±0.00**d**	0.017±0.002 c	0.031±.0021b	0.0032±.00001a	0
Zn^{+2}	0.29±0.003 **d**	0.33±0.004 c	0.39±0.002 b	0.22±0.0013 a	3

Histological observations

The microscopic examination of gill filaments shows similarities between *C. carpio* and *L. abu* fish. The histological structure of normal gills consists of primary and secondary respiratory lamellae, which are covered by an epithelial layer. This layer contains chloride cells, pillar cells, mucous cells, and blood sinuses (Fig. 2a, b). Significant histopathological changes in the morphological structure of the gills, specifically in the primary and secondary lamellae, were the most severe modifications observed in this study. As a bio-indicator of fish gill histology, gill tissue damage was significantly higher in fish collected off Sites 2 and 3 than those from Sites 1 and 2. The histopathological results indicated that *Liza abu* exhibited more severe morphological and structural alterations in the gill epithelium compared to *Cyprinus carpio*. Histological changes were observed in both species, with the most frequent and severe alterations occurring at Sites 2 and 3, particularly in the primary and secondary lamellae tissue. These alterations included necrosis, hyperplasia, hypertrophy, edema, hemorrhage, congestion, inflammation, fusion of neighboring secondary lamellae, and clubbing of lamellae (Fig. 2b, c, d, and e). In contrast, slight histological changes were noted at Sites 1 and 4 for both species, including hyperplasia, hypertrophy, edema, and fusion of neighboring secondary lamellae (Fig. 2f, g, h, i, and j). Notably, necrosis, which causes irreversible damage to the gill tissue, was observed only at Sites 2 and 3.

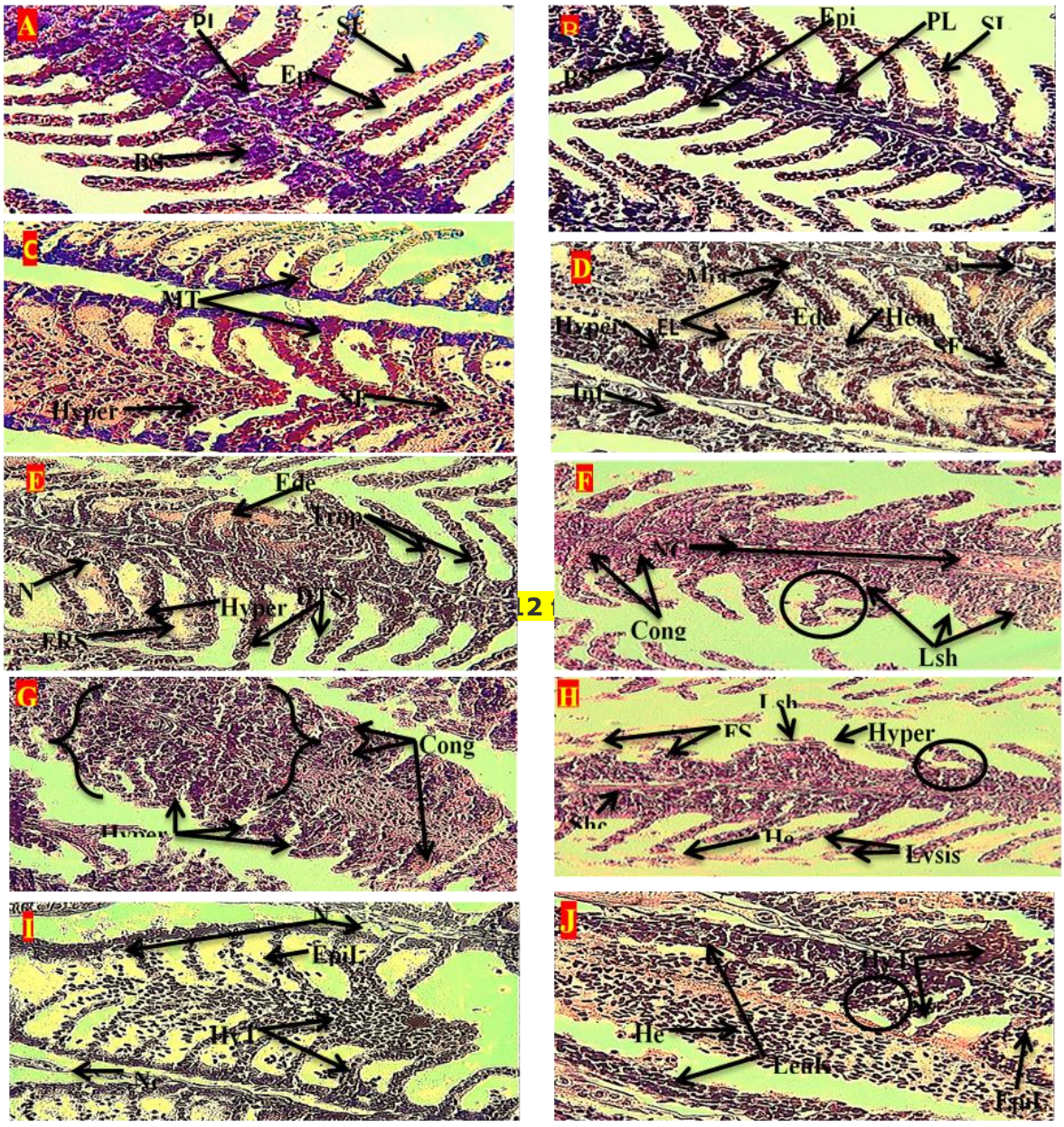

Fig. 2. Histological examination of gills from *Cyprinus carpio* and *Liza abu* at various sites (H and E, 400X magnification)

- **(A, B, Site 4):** Normal gill structure in *C. carpio* and *L. abu* consisting of primary (PL) and secondary (SL) respiratory lamellae, covered by the epithelial layer (Epi) and blood sinuses (BS).

- **(C, Site 2):** *C. carpio* gills exhibiting hyperplasia of the epithelial lamellae (Hyper), slightly fused secondary lamellae (SF), hyperemia, and telangiectasia (HT).
- **(D, Site 2):** *L. abu* gills showing hyperplasia of the epithelium (Hyper), fusion of secondary lamellae (SF), hemorrhage (Hem), edema (Ede), necrosis of secondary lamellae tips (N), inflammatory reaction (Inf), epithelial removal (EL), and hyperemia (Mia).
- **(E, Site 3):** *C. carpio* gills with necrosis (N) and distended tips of secondary lamellae (DTS), acute hyperplasia leading to overlapping primary and secondary lamellae (Hyper), focal rupture of secondary lamellae (FRS), edema aggregation (Ede), and hypertrophy of epithelial cells (Troph).
- **(F, Site 3):** *L. abu* gills showing blood clots (Cong) in aggregates, lamellar blood congestion, necrosis of cartilage (NC), shortening of secondary lamellae length (Lsh), and localized secondary lamellar rupture resulting in bleeding (Circle).
- **(G, Site 3):** *C. carpio* gills exhibiting acute hyperplasia of the epithelium causing fusion of secondary lamellae (Hyper), agglomerated red blood cells, lamellar blood congestion (Cong), and loss of histological features (The Bow).
- **(H, Site 3):** *L. abu* gills showing acute hyperplasia of epithelial cells (Hyper), excessive fusion of secondary lamellae (FS), severe focal secondary lamellar rupture (Circle) causing hemorrhage (He), acute hypertrophy of epithelial cells, shrinkage of the cartilaginous supporting mass (Shc), epithelial lysis (Lysis), and shortening of secondary lamellae (Lsh).
- **(I, Site 3):** *C. carpio* gills showing acute necrosis of secondary lamellae cells (N), epithelial lifting (EpiL), necrosis of cartilage lamellae (Nc), hyperemia, and telangiectasia (Hyt).
- **(J, Site 3):** *L. abu* gills showing heavy hemorrhage (He), hyperemia, telangiectasia (HyT), epithelial lifting (EpiL), acute necrosis of secondary lamellae cells (Circle), and leukocyte infiltration (Leuk).

The observation of gill function

The results from the observations of gill function can help interpret the histopathological changes noted earlier. Fig. (2) presents the findings related to the regulation of ion concentrations (Na+, K+, and Ca2+), as well as data about the number of chloride cells, respiratory diffusion distance, and RBC micronuclei in the gills of *C. carpio* and *L. abu* collected from the selected sites. Panels A and B of Fig. (2) illustrate the ion concentration levels (Na+, K+, and Ca2+) in the gills of the fish. The results varied significantly across the sites, with Sites 2 and 3 showing the highest ion concentration values ($P \leq 0.05$) in both species compared to the other sites. Furthermore, *L. abu* appeared to be more adversely affected than *C. carpio*. Additionally, there was a

notable increase in the number of chloride cells, respiratory diffusion distance, and RBC micronuclei ($P \leq 0.05$) for both *C. carpio* and *L. abu* at Sites 1, 2, and 3 when compared to Site 4 (as shown in Panels C and D of Fig. (2) and Panels A and B of Fig. (4)).

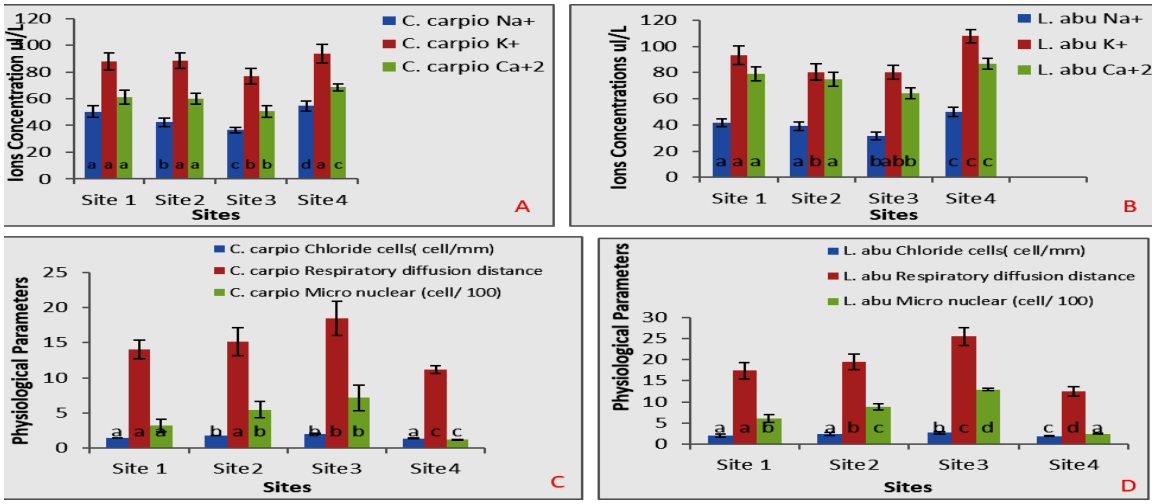

Fig. 3. Ion concentrations level for Na+, K+, and Ca+2 of fish gills of **(A)** *C. carpio*, and **(B)** *L. abu*. Chloride cells and respiratory diffuse distance and RBC micro nuclear of **(C)** *C. carpio* gills and **(D)** *L. abu*. $P \leq 0.05$

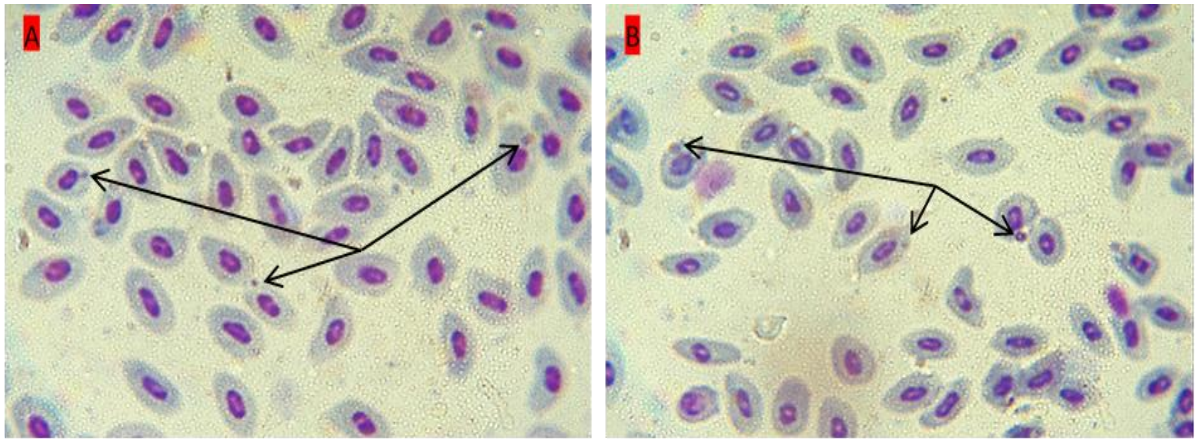

Fig. 4. Micronuclei in red blood cells (RBCs) from *C. carpio* and *L. abu* at Site 3. (A): Micronuclei (arrows) observed in red blood cells of *C. carpio* at Site 3. (B): Micronuclei (arrows) observed in red blood cells of *L. abu* at Site 3

DISCUSSION

The gills of fish serve as the primary organ for osmoregulation, making them highly sensitive to various factors, such as aquatic pollution (including heavy metals), environmental stress, and changes in water chemistry (e.g., salinity, pH, BOD) **(Uchida *et al.*, 2000)**. Chemical analysis of the water column, along with the pollutant levels, can provide valuable insights into the histological changes observed in fish gills, helping to

interpret the impact of contaminants **(Lujic *et al.*, 2015)**. In this study, significant differences ($P \leq 0.05$) were found in the frequency of histological changes, ion concentrations, chloride cells, and respiratory diffusion distances in the gills, as well as cytogenetic alterations (e.g., micronuclei in red blood cells), between fish specimens collected from different sites.

The data revealed that fish from Sites 3 and 2 exhibited higher frequencies of histological, physiological, and RBC micronucleus biomarkers, with statistical differences observed between the sites. Moreover, our results confirmed that *L. abu* was more sensitive to water pollutants than *C. carpio*. The gill tissue biomarkers of both species showed a spectrum of damage, ranging from minor alterations to irreversible harm (Fig. 2A, B, C, D, E, F, G, I, and J).

Mallatt (1985), **Wood (2001)** and **Au (2004)** synthesized data from over 100 ecological and toxicological studies that examined structural and histological changes in fish gills using light and electron microscopy. Their findings provided significant insights into gill histological alterations caused by exposure to aquatic toxicants and environmental stress. Among the most frequent lesions is the lifting of the lamellar epithelial cells from the basement membrane, caused by fluid penetration. This increases the diffusion distance and shortens the interlamellar distance, which could impair respiratory gas exchange. The lifting of pavement cells can also lead to lamellar fusion and epithelial rupture, resulting in more severe gill damage **(Au, 2004)**.

Necrosis of primary and secondary lamellae cells is another common reaction, especially in environments contaminated with heavy metals. Heavy metals may interact with ion-transport proteins, blocking their function and leading to necrosis, which disrupts ionic and water regulation in the gills **(Salamat & Zarie, 2012)**. Additionally, the infiltration of inflammatory cells is a typical adaptive response to water contaminants **(Bury *et al.*, 1998)**. The proliferation and hyperplasia of pavement, mucous, and chloride cells are also observed in response to pollution. While these processes help prevent chemical access to the gill surface, they can obstruct gas exchange, further compromising the fish's health **(McDonald & Wood, 1993)**.

The study by **Olojo *et al.* (2005)** on *Clarias gariepinus* and **Aldoghachia *et al.* (2015)** on *Oreochromis sp.* exposed to toxins like heavy metals confirmed that gill pillar cell system disintegration leads to capillary congestion. The gills' defense mechanisms, such as partial fusion of lamellae and hypertrophy and hyperplasia of epithelial cells, act as barriers to contaminants by increasing the distance between the blood and external water, thus reducing the entry of harmful substances **(Fernandes & Mazon, 2003)**.

Gill histological alterations thus hold promise as biomarkers for monitoring environmental contamination. Additionally, the presence of micronuclei in the fish's red blood cells indicates mutagenic activity, suggesting that pollutants in the environment may cause genetic damage. As noted by **Palhares and Grisolia (2002)**, the frequency of micronuclei can be influenced by various factors, including the species, degree, and

concentration of water contamination, the types of pollutants, and the biological niches. This study highlights the importance of geographic variations in environmental contamination, demonstrating the need for localized monitoring efforts.

CONCLUSION

The findings of this study, which highlight cytogenetic mutations (indicated by micronuclei in red blood cells) and histological and physiological alterations in the gills of two freshwater fish species (*C. carpio* and *L. abu*), provide valuable insights into the differential water quality across various sites within the same aquatic ecosystem. These results contribute to a deeper understanding of the biological responses to fluctuations in water quality in the Shatt al-Arab River and underscore the importance of cytogenetic and histophysiological mutation biomarkers for environmental monitoring. Furthermore, the study revealed that *L. abu* is more vulnerable to water pollutants than *C. carpio*, demonstrating species-specific sensitivity to contamination. Given their significance, we recommend that these biomarkers, alongside traditional physical and chemical analyses, be incorporated as standard techniques in environmental monitoring to assess the impact of pollutants on aquatic ecosystems.

Acknowledgments

The authors express gratitude to the Anatomy and Histology department of the University of Basrah College of Veterinary Medicine for providing the necessary facilities for this experiment.

Funding

This study did not receive any specific funding from public, private, or nonprofit organizations.

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