

## **Histological Evidence of Pollution-Induced Liver Damage in Fish: Comparative Analysis of Shatt Al-Basrah Canal and East Hammar Marsh Ecosystems**

**Sura K. Abduljabbar<sup>1</sup>**

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq.

**Habeeb M. Alsudani**

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq.

habeebalsudani@gmail.com

**Jassim M. Abed**

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq.

**Daniel J. Larkin**

Department of Fisheries, Wildlife and Conservation Biology, University of Minnesota, St Paul, MN, USA.

Received Date: 15-8-2025

Publication accepted date : 21-9-2025

### **ABSTRACT**

Comparative study of liver tissue from two fish species (*Oreochromis niloticus* [Nile tilapia] and *Planiliza suviridis* [greenback mullet]) in Shatt Al-Basrah Canal and East Hammar Marsh in southern Iraq revealed striking differences in liver health. Species from the environmentally degraded Shatt Al-Basrah Canal exhibited pronounced histological alterations, including cellular damage, inflammation, and necrosis, likely attributable to pollution and poor water quality in this aquatic system. In addition, fish hepatocytes from Shatt Al-Basrah Canal showed degenerative changes and pronounced nuclear damage, indicating impaired liver function. In contrast, fish from East Hammar Marsh, a more stable environment with better water quality, displayed healthy liver tissue, characterized by normal structural architecture. This comparative analysis highlights the influence of environmental factors on liver health and shows the utility of this multi-species histological approach for assessing environmental pollution.

Keywords: Liver histology, environmental pollution, *Oreochromis niloticus*, *Planiliza suviridis*, Shatt Al-Basrah Canal, East Hammar Marsh

### **INTRODUCTION**

Fish are a vital source of protein for billions of people worldwide<sup>1</sup>. However, the escalating demand for fish protein poses significant pressure on global fish stocks<sup>2</sup>, underscoring the need for sustainable fisheries management<sup>3</sup>.

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The liver plays a pivotal role in fish physiology, regulating key functions and processes, including anabolism and catabolism<sup>4</sup>. As a sensitive organ in teleosts, it responds to environmental pollutants through changes in histology, biochemistry, and physiology<sup>5-6</sup>. In fish, the liver is the primary organ for detoxification, metabolism, and excretion of harmful substances<sup>7</sup>. Its unique size and shape adapt to accommodate surrounding organs within the body cavity<sup>8</sup>. Monitoring histological changes in fish liver tissue provides a sensitive and accurate method for assessing the impact of foreign compounds in both field and laboratory studies<sup>9</sup>. Research has shown that exposure to heavy metals, such as mercury and cadmium, causes significant histological alterations in freshwater fish, including hepatocyte degeneration, necrosis, and sinusoidal lesions<sup>10</sup>. Similar findings have been reported in other studies, where heavy metal exposure resulted in hepatocyte vacuolization, cellular expansion, and blood canal congestion in various fish species<sup>11-12</sup>. Nile tilapia (*Oreochromis niloticus*) and greenback mullet (*Planiliza subviridis*) are two ecologically and nutritionally significant freshwater fish species. Nile tilapia (Linnaeus, 1758) is widely distributed in tropical and subtropical environments and is one of Africa's most common and important fishes<sup>13-15</sup>. Greenback mullet *P. subviridis* (Valenciennes, 1836), found in shallow coastal waters, estuaries, and freshwater environments across the Indian and Pacific Oceans, has undergone taxonomic reclassification from the genus *Liza* to *Planiliza*<sup>16</sup>. This research aims to investigate how variations in aquatic environmental quality influence fish tissues, through a comparative analysis of liver tissue from Nile tilapia and greenback mullet.

## Results

### Histological comparison of Nile Tilapia (*O. niloticus*) liver tissue

Comparative Histological analysis of liver tissue from Nile tilapia inhabiting East Hammar Marsh and Shatt Al-Basrah Canal revealed significant differences in tissue structure and overall liver health (Figure 2, 8). The examination focused on hepatocytes, hepatocyte nuclei, pancreatic tissue, cytoplasm, bile duct and blood vessels.

Hepatocytes (He): Nile tilapia from Shatt Al-Basrah Canal showed irregularly shaped hepatocytes with distorted cellular architecture, necrosis and degeneration, indicating poor liver health. In contrast, hepatocytes from East Hammar marsh were polygonal and well-organized, suggesting healthy liver function.

Hepatocyte nuclei in Shatt Al-Basrah Canal fish were swollen, displaced, or fragmented, whereas those in East Hammar marsh fish were intact and centrally located, indicating normal cellular function. Pancreas cells (Pa): Pancreatic tissue in Shatt Al-Basrah Canal Nile tilapia showed degeneration and necrotic changes, whereas East Hammar marsh Nile tilapia exhibited no significant abnormalities. Cytoplasm of hepatocytes (Cp): cytoplasmic vacuolation in Shatt Al-Basrah Canal Nile tilapia indicated cellular injury, likely due to lipid accumulation or hydropic degeneration from environmental pollution. In contrast, the same species from East Hammar marsh showed homogeneous and granular cytoplasm, indicating normal metabolic activity.

Bile duct (BD) of Nile tilapia in Shatt Al- Basrah canal were dilated and irregular in structure, suggesting obstruction or weakness in secretion flow. Furthermore, Blood vessels (BV) were congested with erythrocytes, while endothelial cells showed a state of damage, indicating circulatory disturbances and vascular stress. East Hammar marsh fish had regularly shaped and

sized bile ducts without significant dilatation or structural abnormalities, indicating that bile secretion and flow were normal (Figure 2, 8).

Histological examination of Nile tilapia liver from Shatt Al-Basrah Canal exhibited significant cellular changes indicating obvious damage from environmental stressors (Figure 3). Key observations include:

**Leukocytes (Le):** Infiltrated leukocytes (white blood cells) in liver tissue signified an inflammatory response to environmental stressors, pathogens, or tissue damage. **Red Blood Cells (Er) (erythrocytes):** congestion of erythrocytes in blood vessels suggested circulatory problems, potentially leading to oxygen deprivation (hypoxia) and liver cell damage.

Comparison of Nile tilapia liver tissue (Figure 4) from East Hammar marsh (BH) with those from Shatt Al-Basrah Canal (BS), revealed severe histological changes in Shatt Al-Basrah canal samples, indicating cell damage and inflammatory responses.

**Lymphocytic Inflammation (NI):** accumulation of lymphocytes in necrotic areas indicates ongoing or previous inflammatory reactions. **Melanocytes (Melanomacrophage) (Nm):** The presence of melanocytes suggested a defense mechanism against stress inflammation, or cellular debris. **Coagulative Necrosis (Cn):** Observed necrosis indicated ischemic conditions or toxin exposure. In general, these findings indicate that Nile tilapia in the Shatt Al-Basrah canal are exposed to significant environmental stressors, leading to chronic inflammation, immune response activation, and necrotic damage in liver tissue.

#### **Histological comparison of greenback mullet (*P. suviridis*) liver tissue**

Histological examination of liver tissue from greenback mullet inhabiting Shatt Al-Basrah Canal vs. East Hammar Marsh revealed significant differences in tissue structure and overall liver health (Figure 5, 8).

It was noted that hepatocyte (He) cells exhibited cellular degeneration, characterized by cytoplasmic vacuolation and loss of normal cellular structure. Necrotic areas were observed, indicating advanced damage and liver tissue death due to environmental stressors such as pollutants or toxins. At the nuclear (Nu) level, many hepatocyte nuclei were enlarged (karyomegaly), indicating cellular stress. Nuclear thickening (pyknosis) and fragmentation (karyorrhexis) were noted, which signal imminent cell death. In addition, infiltration of lymphocytes and macrophages indicated an immune response to unhealthy environmental conditions. Melano-macrophages (Mm) suggested active inflammation and potential chronic exposure to harmful substances. Pigmentation in macrophages likely resulted from hemosiderin or lipofuscin accumulation, indicating cellular stress or damage. In contrast, a histological study of greenback mullet from the East Hammar Marsh showed normal hepatocyte (He) cells with defined cytoplasmic borders and normal shapes. There were no signs of vacuolation or degeneration, indicating healthy nuclei (Nu), there was a normal chromatin pattern, and no pathological changes (pyknosis or karyomegaly).

Pancreas and bile duct histology (Figure 5) in Shatt Al-Basra Canal greenback mullet liver tissue showed pancreas cells (Pa) with signs of edema characterized by swelling and extracellular fluid accumulation, which lead to cellular dysfunction, and torn acinar cells. Bile duct (BD) showed marked dilation and obstruction, potentially due to bile acid accumulation. Blood vessels (BV) displayed congestion characterized by dilated walls, reduced lumen volume, and clotted blood, which impedes normal circulation and nutrient delivery.

Histological examination of green mullet fish liver tissue (Figures 6 and 7) illustrates significant differences in cellular structure and health between East Hammar Marsh (BH) and Shatt Al-Basrah Canal (BS). In East Hammar marsh, hepatocytes (He) displayed symmetrical appearance, well-organized cytoplasmic boundaries, and normal distribution of cellular organelles. Regular cellular arrangement and normal nuclear characteristics indicated healthy metabolic activity. There was an absence of pathological changes, nuclear abnormalities, or cellular damage.

In Shatt Al-Basrah canal, greenback mullet tissue exhibited loss of communication between hepatic and pancreatic cells (Hp), which leads to impaired metabolic functions and digestive processes. Melano-macrophages (Mm) suggested active inflammatory response and potential chronic to harmful substances. Hepatic cells (Hh) showed significant enlargement, reflecting adaptive response to stress or increased metabolic demands. Nuclear abnormalities, including karyomegaly, indicated cellular stress and potential disruption of gene expression patterns.

Degenerative changes in Shatt Al-Basrah Canal (Figure 7) greenback mullet liver tissue included hepatocytes exhibiting cellular degeneration (Cd), these were characterized by loss of structural integrity and vacuole formation. Intravascular hemolysis (Ih) indicated circulatory system stress due to environmental pressures. Pancreatic cells (Dp) showed degeneration, swelling, and loss of the normal structure of acinar cells. Cytoplasmic vacuoles in hepatocytes (Cv) would have disrupted cellular functions. Dilation and congestion (Ds) would lead to poor blood flow, increased pressure, and potential liver dysfunction.

## Discussion

### Tilapia liver

Fish are susceptible to environmental changes and respond significantly to pollution, making their liver an interesting model for studying interactions between environmental factors and liver structure and function<sup>4</sup>. Liver phenotypic characteristics reflect fish physiological state<sup>30</sup> and environmental quality<sup>31-33</sup>. Fish liver is among the most sensitive organs<sup>34-35</sup>. Histological examination of fish liver tissue provides a useful indicator of chemical toxicity and a method for assessing the effects of exposure to aquatic toxins<sup>17-18</sup>. The findings of this study reveal that Nile tilapia in the Shatt Al-Basrah canal exhibited irregularly shaped liver cells with distorted structure, necrosis, and degeneration, indicative of poor liver health due to environmental factors, pollutants and toxins. These results align with previous studies demonstrating necrosis and inflammation in infected *Cyprinus carpio* (common carp)<sup>19</sup> and degenerative, necrotic, hyperinflammatory and coagulative changes in male *Coptodon zillii* (redbelly tilapia) exposed to heavy metal pollution<sup>20</sup>. Studies on the Shatt al-Basrah Canal's environment have revealed significant pollution, particularly with organic pollutants. Notably, Al-Mahmoud et al.<sup>20</sup> classified the waters of the Shatt al-Basrah Canal as severely degraded, the lowest category of water quality, based on an assessment using the Canadian Water Quality Index (WQI) model.

Liver necrosis can result from biological factors (viruses, fungi, bacteria and parasites) and blood transport disturbances affecting tissue blood flow<sup>21</sup>. In contrast, liver cells from Nile tilapia in East Hammar marsh displayed normal polygonal shape and organization with no signs of tissue damage, suggesting a more stable and less polluted environment. Nuclear abnormalities, including swollen, distorted, and fragmentation were observed in hepatocytes of the Nile tilapia

liver tissue from the Shatt Al-Basra Canal, indicating cellular stress, nuclear damage and programmed cell death, often caused by toxic substances or inflammatory responses<sup>22</sup>. Conversely, hepatocyte nuclei in East Hammar Marsh remained intact and centrally located without any abnormalities, indicating normal cellular function.

Microscopic observations revealed exocrine pancreatic tissue within the liver, characterized by acinar arrangement and diffuse distribution<sup>23</sup>. Cytoplasmic vacuoles in hepatocytes of the Nile tilapia from the Shatt Al-Basrah channel showed cellular damage, potentially resulting from lipid accumulation or hydrolytic degeneration as a result of environmental pollution<sup>24-25</sup>. In contrast, hepatocytes of Nile tilapia liver tissue from East Hammar marsh displayed homogeneous and granular cytoplasm, indicating normal metabolic activity without signs of cellular stress or injury, consistent with a previous study<sup>17</sup>. The liver's normal structure and the absence of pathological abnormalities in Nile tilapia liver in East Hammar marsh suggest a healthy environment. However, bile duct dilation and irregularity of Nile tilapia fish living in the Shatt Al-Basrah Canal indicate obstruction or weak secretion flow, pointing to liver dysfunction. This finding supports the concept of vacuole formation as a cellular defiance mechanism against harmful substances<sup>26</sup>.

The present study observed crowded blood vessels with red blood cells and damaged epithelial cells in Nile tilapia from Shatt Al-Basrah canal, indicating circulatory disorders and vascular stress. Environmental toxins likely caused poor blood flow and oxygen delivery to liver tissue<sup>27</sup>. Liver cell analysis revealed red blood spots outside blood vessels, loss of distinctive structure, increased vacuolation, and rough, pink and dark-stained granules and vacuoles in the cytoplasm. Infiltration of ocular cells and red blood cells and leukocytes into the sinuses indicated increased inflammation due to pollutant and heavy metal exposure. These findings align with<sup>9</sup>, who reported significant liver changes after aluminum exposure including hepatomegaly, nuclear enlargement, congestion, and melanocyte macrophage spread. Aluminum-induced cytoplasmic vacuolization, cellular degeneration, nuclear damage, and cholestatic stasis were also observed<sup>28-29</sup>. Increased presence of leukocyte infiltration indicated an inflammatory response to environmental stressors, or pathogens or tissue damage. Congestion and red blood cell accumulation in blood vessels suggested circulatory problems, potentially leading to hypoxia and liver cell damage. This is consistent with<sup>25</sup>, who found histological changes observed in fish livers responding to pollutant effects.

#### Greenback mullet liver

In the present study, histological examination of greenback mullet liver cells from Shatt Al-Basrah canal revealed cellular degeneration, cytoplasmic vacuoles, and necrosis in liver cells, indicating advanced damage from environmental stressors.

In contrast, greenback mullet in East Hammar marsh showed normal, healthy liver cells with defined cytoplasmic borders and regular shape, indicating a healthy metabolic state<sup>38</sup>. However, pancreatic cell tissues exhibited edema and bile duct showed dilatation and obstruction, potentially leading to liver dysfunction. Congestion, blood clotting, and nuclear abnormalities were also observed. A first-level screening can identify potential exposure to contaminants and their effects on fish<sup>36</sup>. Fish have a buffering factor against trace element bioaccumulation in liver tissue<sup>37</sup>, but excessive accumulation leads to liver degeneration.

Hepatocyte hypertrophy is an indication of adaptive change to environmental stressors or heightened metabolic demands resulting from exposure to pollutants<sup>17</sup>. Cellular enlargement may reflect impaired lipid metabolism or detoxification. The liver is an important organ involved in

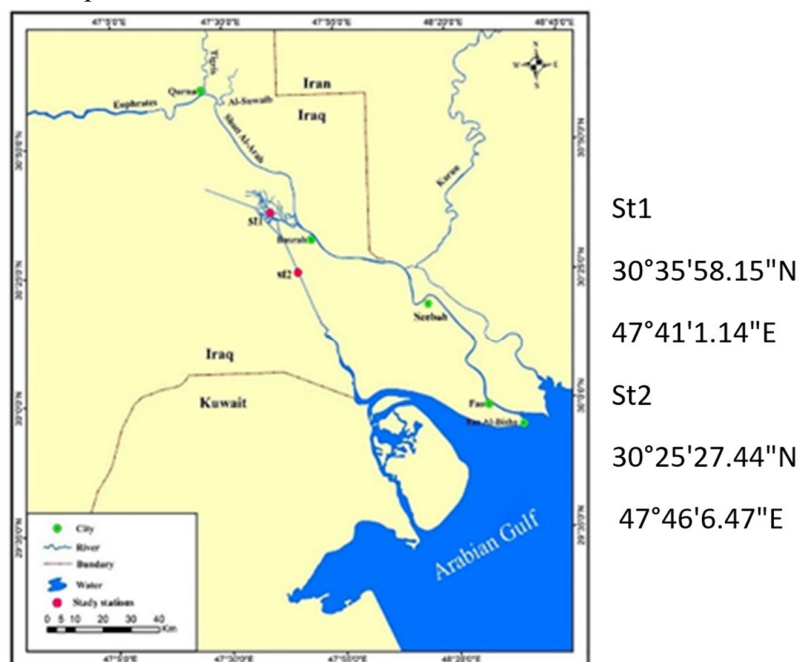
the detoxification, synthesis, storage, and production of energy, assisting in the maintenance of physiological homeostasis<sup>39</sup>. Its unique blood supply renders it susceptible to toxicants, thereby making hepatic health paramount in environmental pollution<sup>40</sup>.

Histological examination confirms a clear difference in the health status of fish living in unpolluted versus polluted environments. Fish from East Hamar Marsh showed a healthy liver structure with normal cellular characteristics. In contrast, fish from Shatt Al-Basrah Canal displayed significant pathological changes, highlighting the detrimental effects of environmental pollution on aquatic life. These results underscore the urgent need for continued monitoring and remediation in polluted aquatic ecosystems to protect fish health and conserve biodiversity.

## Materials and Methods

### Study sites

The East Hammar Marsh, influenced by tidal flow, supports a diverse range of fish species due to the influx of marine species alongside resident riverine species. The Shatt Al Basrah canal is 29 km in length<sup>41</sup> and serves as a flood control measure mitigating tidal impacts on surrounding areas. A severe lack of dissolved oxygen in the canal is due to large amounts of organic wastewater discharged into the canal from adjacent estuaries<sup>42-43</sup>.<sup>44</sup> studied the concentrations of organic pollutants in the Shatt Al-Basrah Canal, and found turbidity exceeding water quality standards (32.3 NTU), as well as high BOD (5.5 mg/l) and total hardness (2479 mg/l) and low dissolved oxygen (3.8 mg/l) (Figure 1). The waters of the Shatt al-Basrah Canal are classified as severely degraded, the poorest regulatory classification<sup>45</sup>. Sewage is discharged into Shatt Al-Basrah Canal through pumps without any pre-treatment. As a result of this continuous, severe pollution, the canal is characterized by high organic pollutants that are harmful to the environment and public health<sup>44,46</sup>.



**Figure 1: Location and Map of Sample Collection Sites in East Hammar Marsh and Shatt Al-Basrah Canal, Southern Iraq.**

### Fish sampling

**Permission to collect fish samples was granted by the Municipality of Basrah governorate.** Nile tilapia and green mullet fish samples were collected from the Shatt Al-Basra Canal and East Hammar Marsh during winter (December 2023) and summer (May 2024) seasons. Fishing was conducted using a cast net (9m diameter, 15 × 15-mm mesh size) from a fishing boat (Table 1). Species identification was verified using<sup>47-48</sup>.

**Table 1: Biometric Data (Length and Weight) of Fish Samples from East Hammar Marsh and Shatt Al-Basrah Canal**

Species	East Hammar Marsh		Shatt Al-Basra Canal	
	Length (mm)	Weight (gm)	Length (mm)	Weight (gm)
<i>Oreochromis niloticus</i>	170-220	100-171	135-155	42-62
<i>Planiliza suviridis</i>	160-170	79-90	160-210	60-129

### Laboratory Procedures

**Euthanasia method:** The collected fish were humanely euthanized using the Clove Oil protocol, which involved mixing 10-15 drops of clove oil per litre of warm water to induce rapid and painless euthanasia. Transfer fish to secondary container with dechlorinated water. The clove oil solution was slowly added to the container to ensure even distribution. A few minutes later, the fish became sedated and lost balance. With time entered deep sedation, eventually led to death. Wait about 10-15 minutes after the fish stops breathing to confirm death. Then immediately dissected post-euthanasia, and the target organs (livers) were removed and labelled. Samples were fixed in 10% formalin for 24 hours, followed by a 1-hour wash using distilled water to remove fixative residues.

### Histological Preparation

Microscopic slides were prepared using standard optical preparation techniques<sup>49</sup> and Bancroft and Stevens as follows: Dehydration: Samples were dehydrated using an ascending series of ethyl alcohol concentrations (35%, 50%, 70%, 80%, 90%, 100%) for 1 hour at each concentration. Clarification: Samples were cleared with xylene in gradual steps. Infiltration: Samples were passed with a mixture of Paraffin (melting point 60°C) and xylene. Embedding: Samples were embedded in the same type of paraffin used for infiltration in special mould. Sectioning: Samples were sectioned using a rotary microtome at a thickness of 7 micrometers. Staining: Samples underwent a series of staining steps. Mounting: Slides were mounted using D.P.X. (Hematoxylin and Eosin (H&E) staining with a scale of 0.2 µm used to imaging the slides).

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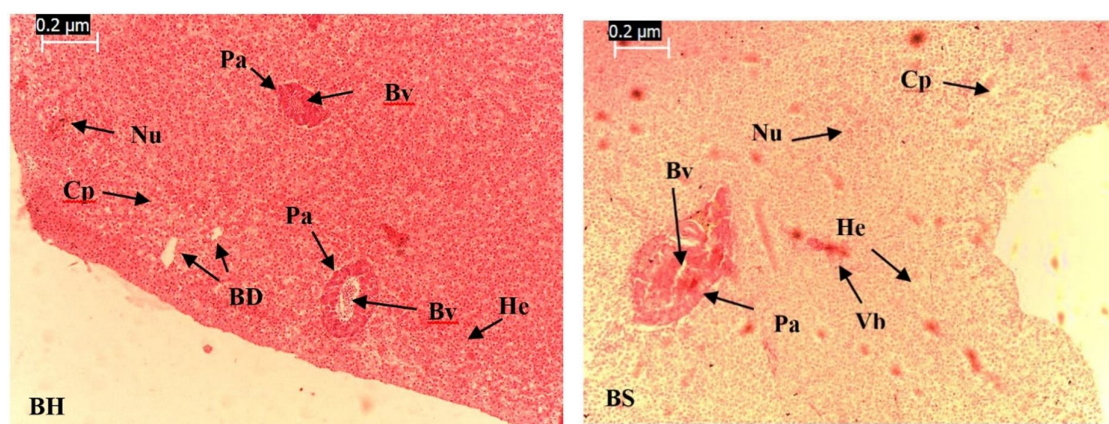
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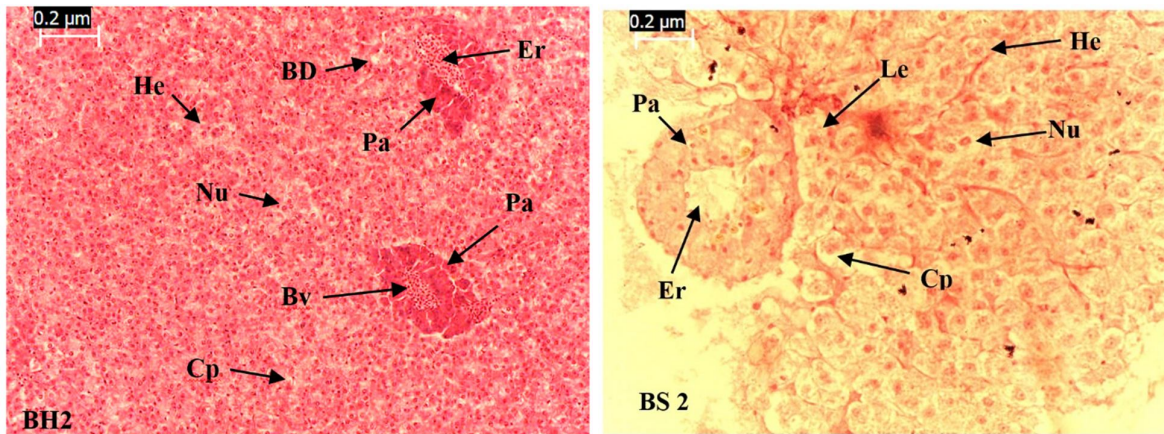
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#### Author contributions statement

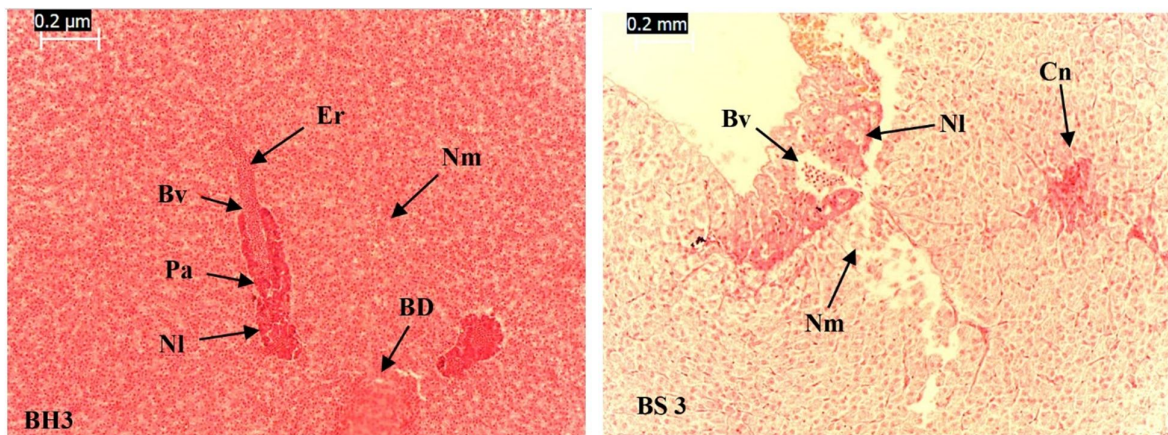
Habeeb M. Alsudani and Jassim M. Abed conceived the experiment, and Sura K. Abduljabbar conducted the experiment, Habeeb M. Alsudani and Daniel J. Larkin analyzed the results. All authors reviewed the manuscript.



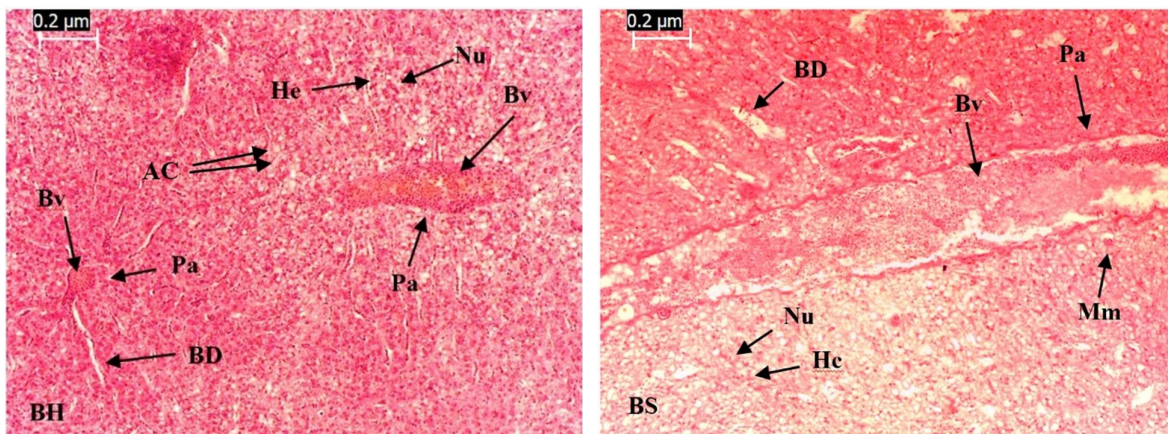
**Figure 2.** Comparison of histological examination of liver tissue of *Oreochromis niloticus* fish from the Eastern Hammar Marsh (BH) and the Shatt al-Basra Canal (BS). (Scale: 0.2  $\mu$ m). (Key: Hepatocyte, Nu: nucleus of hepatocytes, Pa: Pancreas tissue, Cp: cytoplasm, BD: Bile Duct, BV: Blood Vessel).



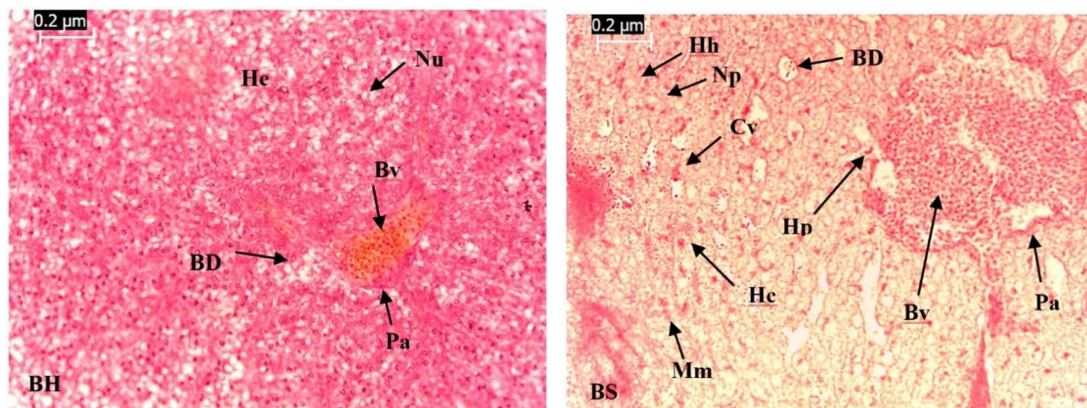
**Figure 3.** Histological examination of the liver of Nile tilapia (*Oreochromis niloticus*) from East Hammar Marsh (BH) and Shatt al-Basra Canal (BS). (Scale: 0.2  $\mu$ m). (Key: Hepatocytes, Nu: Nucleus of hepatocytes, Cp: Cytoplasm, Le: Leukocytes, Er: Red Blood Cells).



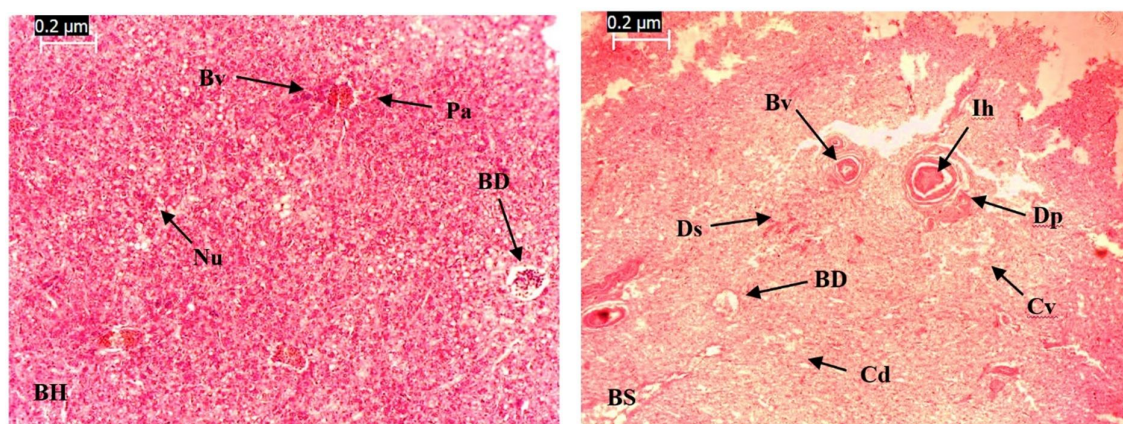
**Figure 4.** Liver sections of Nile tilapia (*Oreochromis niloticus*) from East Hammar Marsh (BH) and Shatt Al-Basrah Canal (BS). (Scale: 0.2  $\mu$ m). (Key: Necrotic pancreatic vesicles (Np) infiltrated with lymphocytic cells (Lc) and melanomacrophages (Nm) are shown, along with coagulative necrosis (Cn), specifically in Shatt Al-Basrah Canal tissues.).



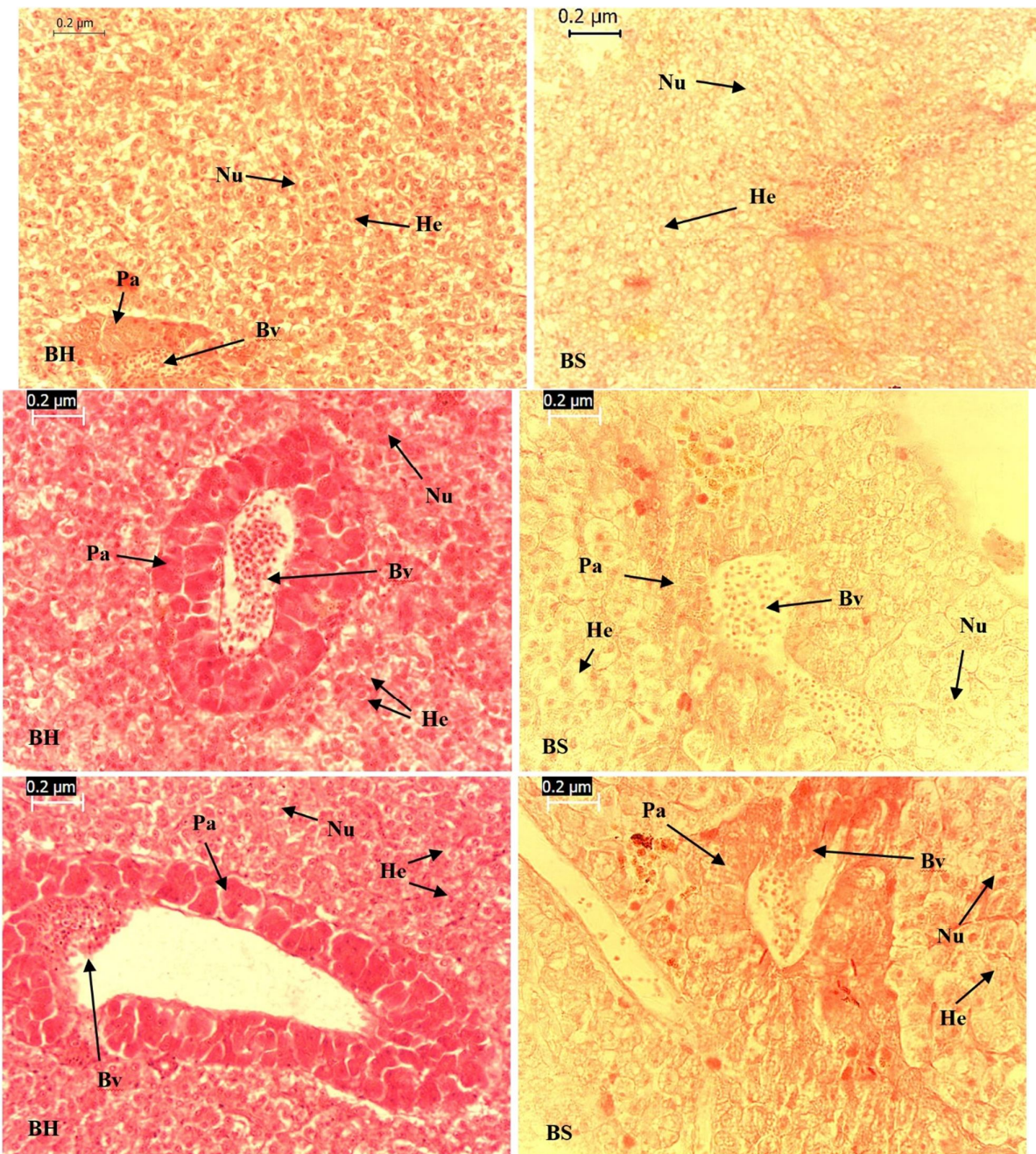
**Figure 5.** Comparison of histological examination of liver tissue of greenback mullet (*Planiliza subviridis*) from the Eastern Harbour of Hammar (BH) and the Shatt al-Basra Canal (BS). (Scale: 0.2  $\mu$ m). (Key: He: Hepatocytes, Nu: Nucleus of hepatocytes, Pa: Pancreas tissue, AC: Acinar cells, BD: Bile duct, BV: Blood vessels, Mm: Melanomacrophages).



**Figure 6.** Histological examination of liver tissue of greenback mullet (*Planiliza subviridis*) from the Eastern Harbour (BH) and the Shatt al-Basra Canal (BS). (Scale: 0.2  $\mu$ m). (Key: He: Hepatocytes, Nu: Nucleus of hepatocytes, Pa: Pancreas tissue, BD: Bile duct, BV: Blood vessels, Hc: Hepatic cords, Hp: Hepatopancreatic junction, Mm: Melanomacrophages, Hh: Hepatocyte hypertrophy, Np: Nuclear enlargement).



**Figure 7.** Histological examination of liver tissue of greenback mullet (*Planiliza subviridis*) from East Hammar Marsh (BH) and Shatt al-Basrah Canal (BS). (Scale: 0.2  $\mu$ m). (Key: He: Hepatocytes, Nu: Nucleus of hepatocytes, Pa: Pancreas, BD: Bile duct, BV: Blood vessels, Cd: Cellular degeneration, Ih: Intravascular hemolysis, Dp: Pancreatic cell degeneration, Cv: Cytoplasmic vacuoles, Ds: Microvascular dilation and congestion).



**Figure 8.** Histological examination with higher magnification of liver tissue of Nile tilapia (*Oreochromis niloticus*) and greenback mullet (*Planiliza subviridis*) from East Hammar Marsh (BH) and Shatt Al-Basrah Canal (BS). (Scale: 0.2  $\mu$ m). (Key: He: Hepatocytes, Nu: Nucleus of hepatocytes, Pa: Pancreas, BV: Blood vessels).

