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# Histopathological and Hematological Alterations in Red Mullet (*Mullus barbatus*) Induced by Heavy Metal Pollution in Sousa Port, Libya


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**Abstract:**

Heavy metals pollution of seawater is a major environmental concern, affecting the aquatic environment and fish health. The current study focused on the levels of selected heavy metals (iron, copper, lead, and cadmium) in the water, gill, liver, and kidney tissues of (*Mullus barbatus*) fish collected from the port of Sousa, Libya, during years 2024-2025, and recorded the associated histopathological and hematological changes. Water analysis revealed elevated concentrations of lead (0.67 mg/L) and iron (5.09 mg/L), exceeding the permissible limits set by the world health organization (WHO). These concentrations indicate significant contamination. The results also showed higher levels of iron and copper in the liver, while lead and cadmium were more concentrated in the kidney. The histopathological screening of gills revealed hyperplasia of secondary lamellae and dense inflammatory cell infiltration, the liver showing hepatocyte vacuoles, cytoplasmic degeneration, and lipid accumulation in the liver, as well as tubular degeneration and an increase in hematopoietic cells in the kidney, reflecting heavy metal-induced organ toxicity. Blood analysis appeared elevated hemoglobin (Hb) and hematocrit (Ht) levels, along with a severe decrease in Thrombocytes count (TCs), denoting physiological stress and impaired hematopoiesis. The results confirm that the M. barbatus fish is an effective biomarker for heavy metal pollution and highlight the environmental and potential public health risks associated with environmental pollution in Libyan coastal waters.



**Keywords**: Heavy Metals, Sousa, *Mullus Barbatus*, Histopathology, Blood Analysis.


# التغيرات النسيجية والدموية في سمك البوري الأحمر (Mullus barbatus) الناجمة عن تلوث المعادن الثقيلة في ميناء سوسة، ليبيا


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**الملخص**
يُعد تلوث مياه البحر بالمعادن الثقيلة مصدر قلق بيئي رئيسي، إذ يؤثر على البيئة المائية وصحة الأسماك. ركزت الدراسة الحالية على مستويات المعادن الثقيلة (الحديد والنحاس والرصاص والكادميوم) في أنسجة الماء والكبد والكلى لدى سمك التريليا (Mullus barbatus) التي جُمعت من ميناء سوسة بليبيا خلال عامي 2024 و2025، وسُجلت التغيرات النسيجية والدموية المصاحبة لها. كشف تحليل المياه عن تركيزات مرتفعة من الرصاص (0.67 ملغم/لتر) والحديد (5.09 ملغم/لتر)، متجاوزةً الحدود المسموح بها التي وضعتها منظمة الصحة العالمية. تشير هذه التركيزات إلى تلوث كبير. كما أظهرت النتائج ارتفاع مستويات الحديد والنحاس في الكبد، بينما كان الرصاص والكادميوم أكثر تركيزًا في الكلى. كشف الفحص النسيجي للخياشيم عن وجود فرط تنسج وارتشاح للخلايا الالتهابية، كما تم ملاحظة وجود فجوات كبدية، وتنكس سيتوبلازمي، وتراكم للدهون في الكبد، بالإضافة إلى تنكس أنبوبي وزيادة في الخلايا المكونة للدم في الكلى، مما يعكس تأثير سمية المعادن الثقيلة. أظهر تحليل الدم ارتفاعًا في مستويات الهيموغلوبين (Hb) والهيماتوكريت (Ht)، إلى جانب انخفاض حاد في عدد الخلايا الخثارية، مما يشير إلى إجهاد فسيولوجي وضعف في تكوين الدم. تؤكد النتائج أن سمكة التريليا تُعدّ مؤشرًا حيويًا فعالًا لتلوث المعادن الثقيلة، وتُسلّط الضوء على المخاطر البيئية والمخاطر المحتملة على الصحة العامة المرتبطة بالتلوث البيئي في المياه الساحلية الليبية.

**الكلمات المفتاحية:** عناصر ثقيلة، سوسة، سمك التريليا، الفحص النسيجي المرضي، فحص الدم.

## Introduction

The contamination of aquatic ecosystems by metals has long been regarded as a significant environmental issue. fish inhabiting waters with high metal concentrations frequently absorb contaminants directly from their environment [1]. Such contamination can greatly disturb the ecological balance and adversely affect the biodiversity of aquatic organisms [2] .Heavy metals are resistant to biodegradation and can remain in aquatic environments for extended periods. Once present in the water, they may accumulate in fish tissues through metabolic activities and bioabsorption. This process result in bioconcentration, particularly in vital organs such as the gills, liver, and kidneys, which are essential for respiration, detoxification, and excretion. The degree of accumulation is influenced by several factors, including the specific type of metal, the fish species involved, the water's pH levels, and the length of exposure. Consequently, fish can serve as carriers of heavy metals within the aquatic food web, creating potential hazards for both aquatic life and human consumers [3]. Heavy metals such as cadmium (Cd), lead (Pb) have minimal roles in normal biological functions, yet they can cause significant damage once they enter living systems [4]. These elements are highly toxic non-essential elements which can accumulate in aquatic ecosystems and pose significant threats to both aquatic life and human health [5]. Sources of metal pollution generally arise a variety of natural and humen activities, including mining, industrial wastewater discharge, sewage effluents, port operations, and application of agrochemicals [6]. Environmental toxins can heighten the vulnerability of aquatic animals to various diseases by interfering with the normal functioning of their immune, reproductive, and developmental systems [7]. Prolonged exposure to aquatic pollutants, even at trace levels, can cause substantial morphological, histological, and biochemical alteration in fish tissues, potentially negatively impacting their health and overall quality [8] .Blood tests, including hemoglobin concentration (Hb), red and white blood cell counts (RBC and WBC), hematocrit (Hct) and Thrombocytes count (TCs) serve as essential diagnostic tools for evaluting the health status of fish at the cellular level [9] . Fish blood parameters have long been utilized as indicators to monitor metal contamination in aquatic environment.[8][10]

**Study objectives:**

1. Determine the concentrations of selected heavy metals (Fe, Cu, Pb, Cd) in seawater and in liver and kidney tissues of oysters collected from the port of Sousse, Libya.
2. Evaluate the histopathological changes in the gill, liver and kidney of Mullus barbatus associated with heavy metal exposure.
3. Evaluate hematological parameters of *Mullus barbatus* as indicators of physiological stress induced by environmental pollution.

## Methods

**Area of study:**

Sousa, situated on the southern coast of the eastern Mediterranean in northern Libya, is one of the country’s most important fishing centers (figure 1). Supplying roughly 25% of the total seafood for the Green Mountain region. The city, with a population of about 40,000, is bordered to the south by a range of rocky hills and has expanded along the coastline. Susa's fisheries serve as a primary source of fish for nearby major cities such as El-Bayda, Shahhat, and other local markets in Libya. However, the quality of its coastal waters has gradually declined, largely due to the discharge of untreated domestic, industrial, and agricultural waste, which has led to a decline in fishing. In this study, fish are considered bioindicators of contamination [11].


**Figure 1**. Map showing the location of Sousa Port, Libya, where water and fish samples were collected

**Data collection procedure**
**Water and fish samples:**
Water and fish samples were obtained from the port of Sousa (Figure 2). The fish species was adult red mullet weighing 200–350 g (Figure 3) during 2024–2025. Kidney and liver samples from five fish were taken immediately after capture for histological examination and fixed in 10% formalin. In the laboratory, the other fish samples were washed with deionized water, individually packed in acid-washed polyethylene bags, and kept frozen at -20°C until analysis.

**Figure 2**. Sousa port where water and fish samples were collected

**Figure 3.** Red mullet (*M. barbatus*) fish

**Preparation and analysis of water samples:**
Water samples were collected from the seawater of the port (30 cm depth) in polyvinyl chloride Van Dorn bottles. Water samples were stored in sealed containers. The analysis of water samples was carried out

according to[12] . part from the water samples were preserved by the addition of one ml of concentrated nitric acid per liter until the time of analysis. The water samples were filtered through 0.45µl membrane filter. The required volume (100 ml) of the filtrate was collected to measure lead, cadmium, iron and copper levels in water samples by using Atomic Absorption Spectrophotometer (AA Spectrometer Thermo Company) (figure 4). another part of water samples was for Physical and chemical analyses of water samples were performed according to [13]. Temperature, pH, DO (dissolved oxygen), chemical oxygen demand (COD) and biological oxygen demand (BOD).


**Figure 4.** AA Spectrometer Thermo Company

**Preparation and analysis of *M.barbatus* fish samples:**
Procedure (A): Each sample was represented by one gram of tissues dissected from the liver, kidney then placed in a clean screw-capped tube and digested according to, the method described by [14]. The obtained solutions were then analyzed by using Atomic Absorption Spectrophotometer (AA Spectrometer) for determination of copper (Cu), lead (Pb), cadmium (Cd) and Iron (Fe) levels in examined samples.

**Histopathological examination of *M.barbatus* fish:**
Tissue specimens from red mullet were taken (Gill and liver and kidney) and fixed in 15 % buffered neutral formalin. They were processed to obtain five-micron thick paraffin sections then stained with Hematoxylin and Eosin and examined under light microscope [15].

**Hematological Analysis of *M.barbatus* Fish:**
Blood samples were collected from the caudal vein of the fish using a heparinized syringe and transferred into sterile plastic tubes containing heparin as an anticoagulant. The fresh whole blood was immediately used for the determination of red blood cell (RBC) count, total white blood cell (WBC) count, Thrombocytes count (TCs) count, hemoglobin concentration (Hb), and hematocrit value (Ht). To ensure accuracy, five live specimens were transported to the laboratory and analyzed promptly for hematological parameters.

**Results**
**Physicochemical variables of sea water of sousa port:**
The values of the physical and chemical parameters are recorded in Table 1. The values of these parameters at three sites in the port of sosua show a slight increase in temperature, pH, and dissolved oxygen as the sampling sites approached the seaport dock, implying an increased toxicity.

**Table 1**. physical and chemical parameters of water

| Variables | Sea water (mean value) | Site 1 | Site 2 | Site 3 |
|---|---|---|---|---|
| **Temperature** | **25 C** | **27 C** | **26C** | **25C** |
| **PH** | **7.8 – 8.3** | **8.2** | **7.1** | **7** |
| **DO** | **5 – 8 mg/L** | **8.1** | **6** | **6** |
| **COD** | **2 – 20 mg/L** | **25** | **22** | **20** |
| **BOD** | **< 2 mg/L** | **3** | **1.5** | **1.7** |

DO: dissolved oxygen; BOD: biological oxygen demand; COD: chemical oxygen demand

**Heavy metals in water:**

The concentrations of selected metals in the studied samples were illustrated in Tables (2), the concentration of iron (Fe) were at 5.091 mg/L, The lowest concentration of cadimum (Cd) was 0.011 mg/L which is higher than some environmental standards and may indicate contamination, The concentration of lead (Pb) in the water of sousa Harbor was 0.67 mg/L, Lead (Pb) concentrations in the tested samples were relatively high compared to the permissible limits in seawater, which are usually less than 0.01 mg/L WHO [16]. which is higher than some environmental standards and may indicate contamination, the concentration of copper (Cu) was in normal limits.

**Table 2.** Comparison of Heavy Metals Concentrations with Standards

| Metal | Measured Concentration (mg/L) | Standard Limit in Seawater (mg/L) | Evaluation |
|---|---|---|---|
| **Iron (Fe)** | 5.091 | 0.3 – 1.0 (WHO guideline) | Higher than limit → Possible contamination |
| **Cadmium (Cd)** | 0.011 | 0.001 (WHO guideline) | Higher than limit → Contamination risk |
| **Lead (Pb)** | 0.67 | 0.01 (WHO guideline) | Much higher → Serious contamination |
| **Copper (Cu)** | 1.5 | 1.0 – 2.0 (WHO guideline) | Normal |

**Heavy metals in gill and liver and kideny:**

Iron concentrations in the liver and kidney organs of fish from Sousa are shown in Table 3. Iron concentrations were 46.18 µg/g in the kidney, and 60.55 µg/g in the liver, and 24.2 ug/g in gill, copper concentrations in the liver and kidney organs of fish from Sousa are shown in Table 3. Copper concentrations were 9.96 µg/g in the kidney, and 40.55 µg/g in the liver, and 16.7 ug/g in gill, lead concentrations in the liver and kidney organs of fish from Sousa are shown in Table 3. Lead concentrations were 36.18 µg/g in the kidney, and 18.55 µg/g in the liver, and 7.4 ug/g in gill, cadmium concentrations in the liver and kidney organs of fish from Sousa are shown in Table 3. cadimum concentrations were 2.18 µg/g in the kidney, and 1.55 µg/g in the liver and 0.62 ug/g in gill.

**Table 3.** Heavy Metals Concentrations in Liver and Kidney and gill of *M.barbatus* Fish from Sousa

| Metal | Kidney (µg/g) | Liver (µg/g) | Gill (ug/g) |
|---|---|---|---|
| **Iron (Fe)** | 46.18 | 60.55 | 24.2 |
| **Copper (Cu)** | 9.96 | 40.55 | 16.7 |
| **Lead (Pb)** | 36.18 | 18.55 | 7.4 |
| **Cadmium (Cd)** | 2.18 | 1.55 | 0.62 |

**Histopathological examination of *Mullus barbatus* fish:**

Histological examination of fish gills revealed progressive pathological changes. In Figure (5), epithelial hyperplasia with partial fusion of secondary lamellae was observed. Figure (6) showed pronounced lamellar hyperplasia, fusion, severe vascular congestion, and intercellular edema.

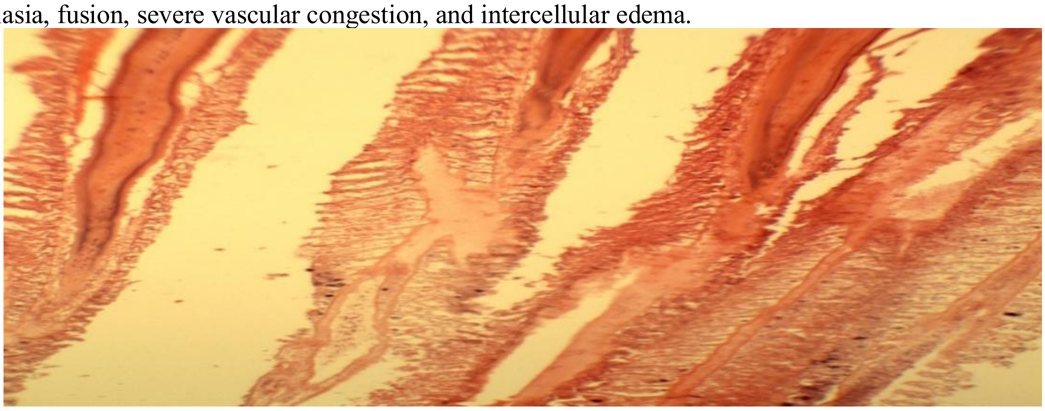

**Figure 5.** Histological section of fish gills showing marked epithelial hyperplasia and partial fusion of secondary lamellae, indicating pathological alterations possibly related to environmental stress. (H&E stain, ×100)

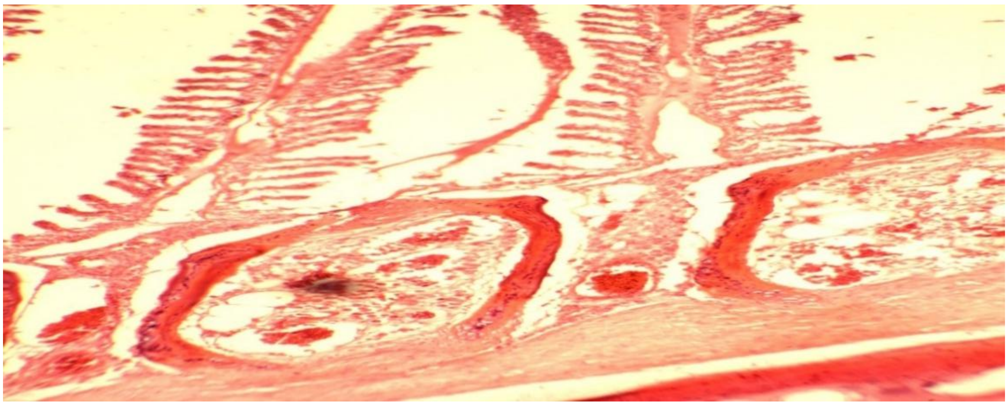

**Figure 6.** Histological section of fish gills showing marked hyperplasia of the secondary lamellae, lamellar fusion, severe vascular congestion, and intercellular edema. Note the deformation of gill architecture, which may impair respiratory efficiency. (H&E stain, magnification ×100)

Figure (7) exhibited marked capillary congestion, dense inflammatory infiltration, lamellar fusion, intercellular edema, and structural distortion of secondary lamellae, resulting in narrowed respiratory spaces. Figure (8) demonstrated normal gill architecture.

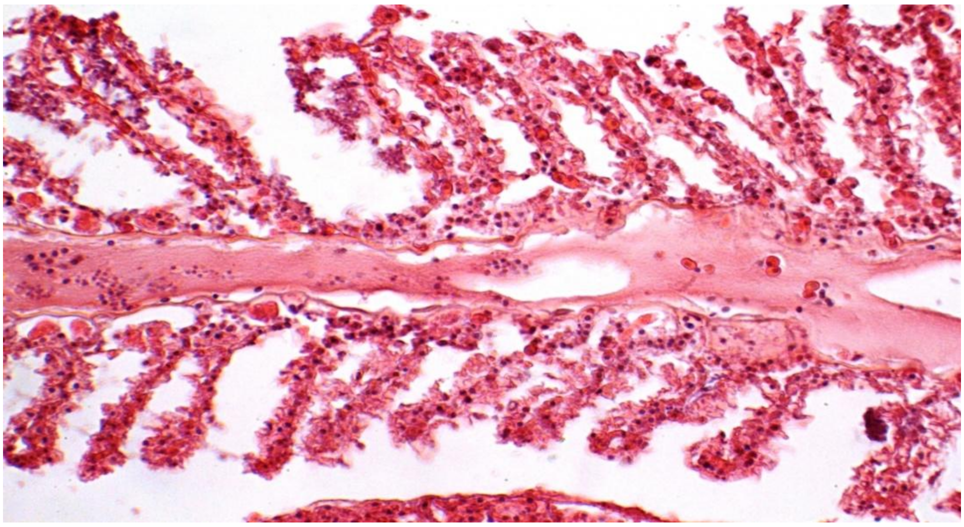

**Figure 7.** The examined gill sections exhibit pathological alterations characterized by congestion, edema, inflammatory cell infiltration, hyperplasia, and fusion of the secondary lamellae.

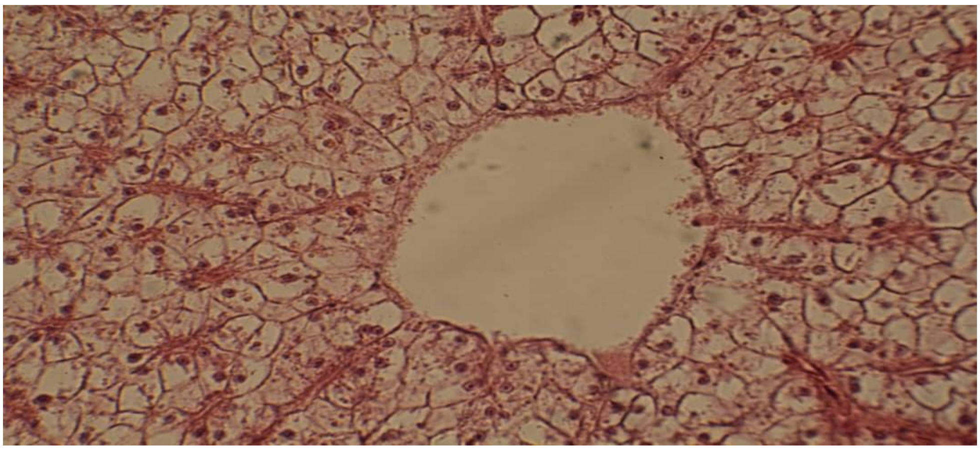

**Figure** 8. Normal liver of M.barbatus slide stained with hematoxylin and eosin (H&E stain)

Liver sections of Mullus barbatus exposed to heavy metals (Figure 9) displayed vacuolated hepatocytes with enlarged cytoplasmic spaces, indicating cellular degeneration. Large white vacuoles suggested lipid accumulation (fatty liver), likely due to heavy-metal–induced oxidative stress disrupting lipid metabolism and inhibiting fatty acid oxidation.

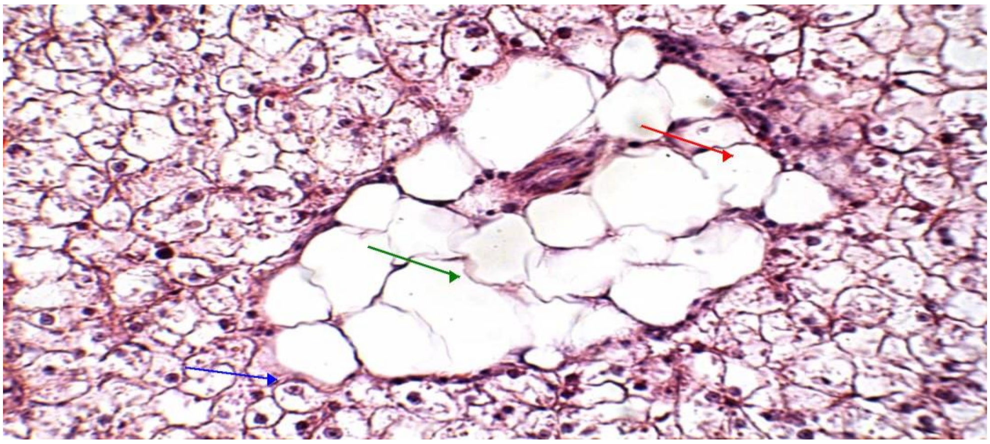

**Figure 9.** Hematoxylin & Eosin (H&E) stained of M.barbatus tissue slide of a liver with fatty changes (Fatty liver / Hepatic steatosis).

**Blue arrow**: refers to cell wall
**Red arrow**: refers to pool of hepatic cells due to fat pressure.
**Green arrow**: refers to fat vacuole
In Figure (10) the kidney tissue shows mild inflammatory changes characterized by cellular congestion and slight degeneration of the renal tubules. There is an increase in hematopoietic cells and occasional nuclear enlargement, suggesting early toxic effects of heavy metal exposure. The overall architecture of the kidney is slightly disturbed, reflecting mild nephrotoxicity caused by lead and cadmium contamination. Figure (8) shows degeneration of tubular epithelial cells with prominent vacuoles and fatty inclusions. These changes indicate fatty degeneration and impaired renal function. These lesions are consistent with heavy metal toxicity in fish.

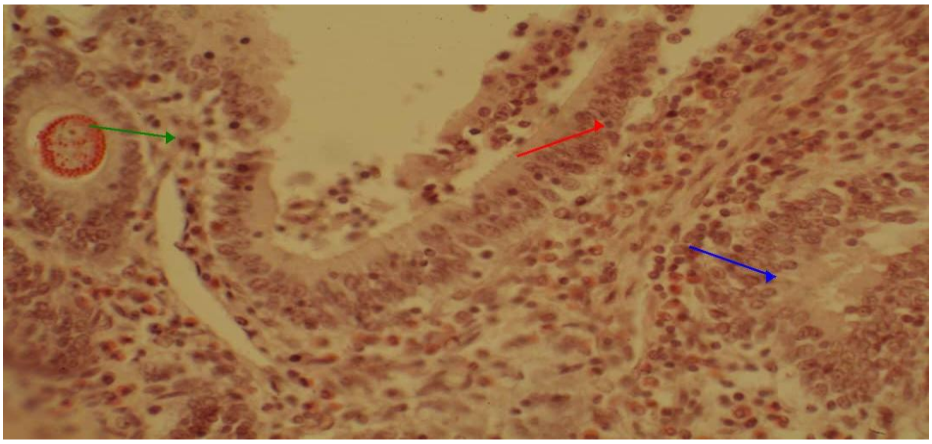

**Figure 10.** kidney tissue stained by (H&E) of *M.barbatus* shows mild inflammatory changes
**Blue arrow:** refers to inflammatory cells (WBCs).
**Red arrow:** refers to renal tubule
**Green arrow:** refers to lumen

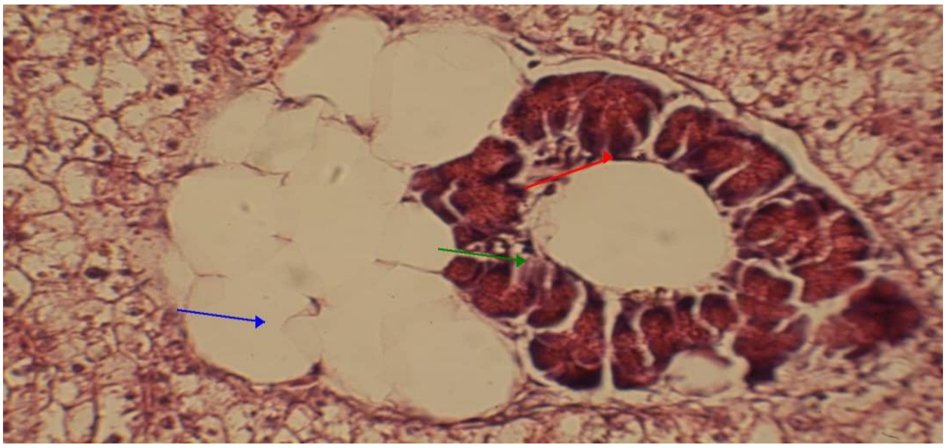

**Figure 11.** kidney tissue of *M.barbatus* shows prominent vacuolation and lipids
**Blue arrow:** refers to vacuolation (lipids).
**Red arrow:** refers to renal tubule
**Green arrow:** refers to lumen

***Explanation of Color Differences Between Slides:**
Although both sections were stained with hematoxylin and eosin (H&E), the intensity of the staining appears different due to several technical and histological factors. Variations in section thickness, staining duration, and microscope light adjustment can affect the final appearance of the tissue. Additionally, liver cells with steatosis exhibit large fatty vacuoles that appear as empty spaces after processing, reducing the intensity of the cytoplasmic staining and making the tissue appear paler compared to normal liver sections. Therefore, the observed color differences are related to technical preparation and the presence of fatty changes, not to the use of different stains.

**Hematological Analysis:**
The measured physiological parameters were significantly higher in fish from the polluted site compared to those from the reference site (Far from the beach). This suggests that the fish are experiencing physiological stress likely due to environmental pollution (table 4).

**Table 4.** Blood paramaters of *M.barbatus* fish

| Parameter | Our Results | Typical Reference Range | | Interpretation |
|---|---|---|---|---|
| **RBCs ($\times 10^6/\mu L$)** | 2.72 ± 0.11 | 1.5–3.5 | | Normal range, but higher end may indicate early stress (e.g., hypoxia from pollution). |
| **Hematocrit (Ht%)** | 38.90 ± 3.27 | 30–45 | | Slightly elevated, possibly due to dehydration or chronic stress. |
| **Hemoglobin (Hb) (g/dL)** | 13.48 ± 1.4 | 8–12 | | Elevated, suggesting compensatory response to low oxygen (e.g., polluted water). |
| **Thrombocytes count (TCs) ($\times 10^3/\mu L$)** | 12.33 ± 2.51 | 20–100 | | **Critically low**, indicating impaired clotting or bone marrow suppression (common in heavy metal toxicity). |

**Discussion**
In this study, the chemical, histopathological, and hematological changes in the liver and kidney of *Mullus barbatus* fish collected from the port of Sousa (Libya), an area increasingly affected by human pollution, were examined. The chemical results demonstrated a clear link between elevated levels of heavy metals in the aquatic environment and histological changes in the fish, levels of lead, cadmium, copper, and iron recorded near the port of Sousa exceeded the permissible limits set by the (World Health Organization)[17] , indicating significant contamination. Specifically, lead reached levels (0.67 mg/L), well above the maximum limit of 0.01 mg/L. This high lead contamination is concerning, as lead is a non-essential element and highly toxic to aquatic organisms, impairing enzyme activity and disrupting blood formation [4]. while the Cadmium, although recorded at a

relatively lower concentration, still exceeded the safe limit, reflecting the risk of cumulative contamination, Iron levels were normal, but copper was relatively high. This level warrants attention because it may be a sign of chronic environmental copper contamination. Regarding the gills, the main reason for their lower mineral concentrations compared to the liver and kidneys is that the gills are a portal of entry and not a storage site, while the liver is the site of storage and detoxification, and the kidney is the site of excretion and collection. Heavy metal concentrations in the tissues of *Mullus barbatus* exhibited clear organ-specific patterns. Iron (Fe) and copper (Cu) were predominantly concentrated in the liver, reflecting its central role in detoxification and metal storage, whereas lead (Pb) and cadmium (Cd) accumulated mainly in the kidney, consistent with its excretory function. These findings are in agreement with previous studies [18] [19] [8], which reported similar organ-specific accumulation trends in fish inhabiting polluted environments. The observed bioaccumulation confirms *M.Barbatus* as a reliable bio indicator of metal pollution in Libyan coastal waters. Histological examination of both the liver and kidney revealed clear toxic effects. Hepatocyte vacuoles, cytoplasmic degeneration, and lipid accumulation were observed, suggesting fatty liver disease caused by oxidative stress and impaired lipid metabolism. These liver lesions are consistent with the heavy metal-induced oxidative damage reported in catfish and mullet[1][2] . In the kidneys, mild degeneration of the renal tubules, enlarged nuclei, and an increase in hematopoietic cells, and lipid accumulation were observed. These lesions indicate early nephrotoxicity, which impairs the clearance of toxins from the kidneys. These renal changes were largely similar to those observed in fish exposed to environments contaminated with cadmium and lead [7] . The results suggest that Blood parameters were elevated compared to reference values. The increased hemoglobin levels indicate compensatory response to hypoxic stress, likely caused by the reduced oxygen availability in polluted water [10] . The severe decrease in thrombocytes count also reflects inhibited hematopoiesis and impaired clotting capacity, a hallmark of heavy metal toxicity [20]. These results suggest that fish blood is a sensitive biomarker of environmental stress[21]

**Conclusion**

*Mullus barbatus* is an effective bioindicator for monitoring heavy metal pollution in libyan coastal waters, with potential environmental and health risks to consumers.

**Disclaimer**

The article has not been previously presented or published, and is not part of a thesis project.

***Conflict of Interest***

There are no financial, personal, or professional conflicts of interest to declare.

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