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Original Research Article

Effect of Sewage Water on African Catfish (*Clarias gariepinus*) in the Tigris River, Baghdad, Iraq

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Abstract: The dumping of the raw municipal sewage in the Tigris River in Baghdad is also a major danger to the aquatic organisms, especially fish, which forms the source of food and livelihood of the local people. The current research examined the effects of sewage effluent on the African sharptooth catfish, i.e. Clarias gariepinus, by comparing the samples in reference (upstream) and sewage-afflicted (downstream) locations across a 15 kilometre stretch of the Tigris River near the Baghdad Medical City Complex. Water quality analysis revealed significantly elevated biochemical oxygen demand (BOD5 = 48–125 mg/L), chemical oxygen demand (COD = 85–215 mg/L), total suspended solids (TSS = 156–412 mg/L), and heavy metals (Pb: 0.35–0.78 mg/L; Cd: 0.12–0.31 mg/L; Zn: 1.2–3.8 mg/L) at impacted sites compared with reference sites (BOD5 = 4–8 mg/L; COD = 12–18 mg/L; TSS = 22–35 mg/L). The catfish in the sewage affected reaches showed a much lower condition factor (K = 0.85 ± 0.12 versus 1.24 ± 0.14, $p < 0.001$), high liver somatic index (LSI = 2.8 + 0.45 per cent versus 1.6 + 0.38 per cent, $p < 0.001$), and severe histopathological changes in the gills and liver, such as, epithelial hyperplasia, lamellar fusion, gill. Oxidative stress biomarkers indicated elevated lipid peroxidation (MDA: 18.5 ± 3.2 nmol/mg protein vs. 6.8 ± 1.5, $p < 0.001$) and depleted antioxidant defenses (reduced GSH: 15.3 ± 2.8 µmol/mg protein vs. 32.6 ± 4.1, $p < 0.001$) in livers of impacted fish. The indices of gill and liver lesions had positive significant correlations with biochemical oxygen demand (BOD; $r = 0.78$, $p < 0.001$) and heavy-metal concentrations ($r = 0.72–0.81$, $p < 0.001$). Those findings corroborate that continuous exposure to untreated sewage results in multisystemic pathology in catfish, and thus, affects the physiological fitness of catfish, and may lead to the loss of population viability. There should be urgent adoption of efficient wastewater treatment, strict effluent regulation as well as long-term monitoring of fish health in order to protect the Tigris River and human population relying on it.

Keywords: Sewage Pollution, *Clarias gariepinus,* Tigris River, Histopathology, Oxidative Stress, Heavy Metals.

1. INTRODUCTION

The river, the Tigris was the major source of drinking, irrigation and food (especially fish) in Iraq, which has been supporting millions of people since thousands of years. However, urbanization and the fact that the development of the wastewater treatment infrastructure is still insufficient means that the river has been turned into a highly polluted stream with concentrations of coliforms and E.coli reaching 540, 000 per 100 000 liter in the area surrounding Kadhimya. Sewage effluent is a complex mixture of organic matter, nutrients, pathogenic microorganisms, heavy metals, and micropollutants that degrade aquatic water quality and pose severe risks to fish communities [1]. Fish specially In aquatic pollution, fish are generally considered sentinel species due to their ability to integrate over time, respond physiologically and behaviorally in bankable ways, and act as bioindicators of ecosystem health [2, 3]. The African sharptooth catfish (Clarias gariepinus), a common freshwater fish in Iraqi rivers, is highly valued as a source of subsistence and commercial fisheries and is particularly useful as a biomonitor due to its relatively sessile, adaptively plastic behaviour and tissue accumulation of contaminants [4, 5]. Reported outcomes of sewage and heavy metal pollution on catfish and other fish species encompass decreased survivability and development, gill and liver necrosis, impaired reproduction, immunosuppression, and activation of oxidative stress [11, 12]. Increased reactive oxygen species formation, documented by elevated lipid

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peroxidation (malondialdehyde, MDA) and depleted antioxidant defenses (glutathione, SOD, catalase), reflects chronic chemical stress at the cellular level [13, 14].

Although the Tigris River has been identified as having significant importance to the water security in Iraq and the adverse health effects of sewage contamination of sewage on human and aquatic life are known, there were gaps in the literature regarding how fish respond systematically to the effects of sewage discharge on their health using an integrated approach of water quality, morphometric, histopathological, and biochemical measure.

2. MATERIALS AND METHODS

2.1 Study Area and Sampling Design

The study was conducted along a 15-km section of the Tigris River in Baghdad, Iraq, focusing on the area surrounding the Medical City Complex (33.3150° N, 44.3627° E), one of the largest point sources of municipal and medical wastewater in the city [18]. Four sampling sites were selected:

- Reference Site (RS): Located approximately 2 km upstream of the Medical City sewage outfall, representing relatively unpolluted baseline conditions (33.3050° N, 44.3520° E).
- Impacted Site 1 (IS1): Immediately at the sewage outfall point (300 m downstream of Medical City; 33.3145° N, 44.3630° E).
- Impacted Site 2 (IS2): 1 km downstream of the outfall (33.3200° N, 44.3750° E).
- Impacted Site 3 (IS3): 5 km downstream of the outfall, where effluent is progressively diluted with river water (33.3350° N, 44.3900° E).

Sampling was done once a month in March 2023 to February 2024 and included both dry (June to September) and wet (December to February) seasons thus compensating seasonal flow and water quality changes. The whole field work and animal procedures were carried out according to local ethical standards related to research of fish and use of animals.

2.2 Water Quality Analysis

Surface water samples (n=3 replicas per site per month) were collected at a depth of 0.5m in pre-cleaned, acid-washed polyethylene bottles at each site and kept on ice and carried to the laboratory within two hours. The samples were studied using the conventional procedures.(American Public Health Association, APHA):

- Field parameters: Temperature (°C), pH, electrical conductivity (µS/cm), dissolved oxygen (mg/L), and turbidity (NTU) were measured in situ using calibrated handheld probes (Hanna Instruments HI9829).
- Organic load: BOD5 (5-day biochemical oxygen demand) and COD (chemical oxygen demand) were determined by standard procedures (APHA 5210B and 5220D, respectively).
- Nutrients: Ammonium (NH4+-N), nitrate (NO3--N), and phosphate (PO43--P) were analyzed by colorimetric methods using a UV-Vis spectrophotometer (Shimadzu UV-1700).
- Total suspended solids (TSS): Determined by gravimetric analysis after filtration through pre-weighed 0.45-µm membrane filters.
- Heavy metals: Lead (Pb), cadmium (Cd), zinc (Zn), and iron (Fe) were extracted by acid digestion (HNO3) and analyzed by flame atomic absorption spectroscopy (FAAS, Perkin-Elmer AAnalyst 400). Quality control included analytical blanks, duplicate analyses, and reference materials. Recovery rates ranged from 92–108%.

All results were compared with Iraqi national water quality standards (Iraqi Ministry of Environment) and WHO guideline values for aquatic ecosystem protection.

2.3 Fish Collection and Morphometric Analysis

The sites were sampled with African catfish (Clarias gariepinus) to reduce the impact of handling stress by trapping the fish in the dawn with a seine net (6 × 2 m, 5-mm mesh). Fish were also put into aerated containers immediately with site water and taken to the laboratory in the span of 1 hour. Healthy-looking adult fish (fork length 1828 cm, n 1012 per site/month, n 120 total) were only picked in order to reduce the chances of confounding factors in pre-existing disease. The fish were put to death by being immersed in ice-cold water and cervical dislocation in line with the accepted animal care methods. For each fish, the following biometric measurements were recorded:

- Total length (L, cm) measured from snout to caudal fin tip.
- Body weight (W, g) using an analytical balance (±0.1 g).
- Liver weight (LW, g) after careful excision.

From these measurements, the following indices were calculated:

- Condition factor: $K = 100 \times \frac{W}{L^3}$ (Fulton's condition factor), where K represents body mass relative to length. Lower K values indicate poor nutritional status or stress.

- Liver somatic index (LSI): $LSI = 100 \times \frac{LW}{W}$, used as an indicator of hepatic enlargement in response to contaminant exposure and detoxification demands.

Histopathological analysis 2.4.

Samples of the gill arches and liver tissues were promptly cut and then fixed in 10% neutral buffered formalin after 24 hours. The tissues were then subjected to the routine histology procedure: dehydration in graded ethanol, clearing in xylene, embedding in paraffin wax, sectioning with a rotary microtome to 5-m thick sections, hematoxylin and eosin (H&E) staining. The sections were studied using the light microscope (Olympus BX51) under 40x, 100x and 400x magnification.

Histopathological abnormalities were identified and semi-quantitatively scored by a single trained observer (blinded to site designation) using the following scale:

- Score 0: Absent (normal histology).
- Score 1: Slight (minimal deviation from normal structure).
- Score 2: Moderate (distinct but not extensive pathological change).
- Score 3: Severe (extensive tissue damage, substantial loss of normal architecture).

Gills were examined for the following lesions:

- Epithelial hyperplasia and hypertrophy.
- Lamellar fusion and clubbing.
- Edema (swelling) of primary and secondary lamellae.
- Aneurysms (dilated, blood-filled vessels).
- Lamellar atrophy (thinning of respiratory surface).

Liver tissues were examined for:

- Hepatocellular vacuolation (lipid and/or aqueous vacuoles).
- Focal and diffuse necrosis.
- Inflammatory cell infiltration (lymphocytes, macrophages).
- Melanomacrophage aggregates (MMA, brown cells accumulating cellular debris).
- Bile duct hyperplasia.
- Sinusoidal dilation and congestion.

A composite gill lesion index (GLI) and liver lesion index (LLI) were calculated for each fish as the sum of severity scores for all lesions in each organ, allowing semi-quantitative comparison across sites.
The oxidative stress biomarkers identified include those listed below:

2.5 Oxidative Stress Biomarkers

Samples of liver tissues (ca. 200 mg) were homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) with a Teflon-glass homogenizer and centrifuged at 10,000 × g with 15 min at 4 o C. The obtained supernatant (cytosolic fraction) was frozen at -80 o C until analysis of the subsequent parameters of oxidative stress:

- Lipid peroxidation: Determined as the concentration of malondialdehyde (MDA) by thiobarbituric acid (TBA) reaction (colorimetric, 532 nm). Measures were in the form of nmol MDA /mg protein.
- Low glutathione (GSH): Measured through the Ellman method (DTNB reaction) and the absorbance of 412 nm. Outputs are in the form of use of moles of GSH per mg of protein.
- Total protein concentration: It is measured with Bradford assay using the bovine serum albumin in the case of normalization of the biomarker values.

Each analysis was done thrice. Each batch of assays had standards and quality control samples.

2.6 Statistical Analysis

The Shapiro -Wilk test was used to determine whether the data were normally distributed and the Levene test was used to determine whether the data had the same variance. All the morphometric, water quality and lesion index and biomarker data were normally distributed and examined using a one-way analysis of variance (ANOVA) followed by Tukey post hoc test to determine the pair wise differences among the four sites. The associations between variables of water quality (BOD, COD, heavy metals), lesion indices, and oxidative stress indicators were analyzed with the help of linear regression and Pearson correlation coefficient (r). All the statistical tests were two-tailed and the significance was determined at 0.05. The SPSS version 26.0 (IBM Corp., Armonk, NY) was used to conduct statistical analyses.

3. RESULTS

3.1 Water Quality Parameters

Water quality results are presented in Table 1. The reference site (RS) upstream of the Medical City sewage outfall showed water quality within Iraqi national standards and suitable for aquatic life: BOD5 = 6.2 ± 1.8 mg/L, COD = 15.3 ± 3.2 mg/L, TSS = 28.4 ± 8.1 mg/L, Pb = 0.08 ± 0.02 mg/L, and Cd = 0.03 ± 0.01 mg/L.

In stark contrast, all three impacted sites (IS1, IS2, IS3) downstream of the Medical City sewage outfall showed grossly elevated concentrations of organic pollutants and heavy metals:

- IS1 (immediately at the outfall): BOD5 = 125.3 ± 28.5 mg/L (20× reference), COD = 215.8 ± 45.3 mg/L (14× reference), TSS = 412.1 ± 76.2 mg/L, Pb = 0.78 ± 0.15 mg/L (10× reference), Cd = 0.31 ± 0.08 mg/L (10× reference), Zn = 3.8 ± 0.9 mg/L.
- IS2 (1 km downstream): BOD5 = 85.6 ± 19.3 mg/L, COD = 156.2 ± 38.1 mg/L, TSS = 289.5 ± 52.3 mg/L, Pb = 0.52 ± 0.11 mg/L, Cd = 0.18 ± 0.05 mg/L, Zn = 2.1 ± 0.5 mg/L.
- IS3 (5 km downstream): BOD5 = 48.3 ± 12.1 mg/L, COD = 85.4 ± 21.2 mg/L, TSS = 156.2 ± 28.4 mg/L, Pb = 0.35 ± 0.08 mg/L, Cd = 0.12 ± 0.03 mg/L, Zn = 1.2 ± 0.3 mg/L.

One-way ANOVA revealed highly significant differences among sites for all water quality parameters ($p < 0.001$). Tukey's *post hoc* test confirmed that all impacted sites differed significantly from the reference site and from each other ($p < 0.01$). Dissolved oxygen at impacted sites was severely depressed: RS = 7.8 ± 0.6 mg/L (adequate), IS1 = 1.2 ± 0.4 mg/L, IS2 = 2.8 ± 0.5 mg/L, IS3 = 4.2 ± 0.7 mg/L (all critically low). Total coliform and fecal coliform densities at IS1 reached 2–3 million cells per 100 mL and 500,000 cells per 100 mL, respectively, far exceeding safety standards.

3.2 Fish Morphometric Indices

Results for condition factor and liver somatic index are presented in Table 2. Fish collected from the reference site showed normal morphometric values:

- Reference site: K = 1.24 ± 0.14, LSI = 1.6 ± 0.38%.

Fish from sewage-impacted sites exhibited significantly reduced condition factor and markedly elevated liver somatic index:

- IS1: K = 0.68 ± 0.10, LSI = 3.2 ± 0.62%.
- IS2: K = 0.82 ± 0.12, LSI = 2.8 ± 0.51%.
- IS3: K = 0.91 ± 0.11, LSI = 2.1 ± 0.45%.

One-way ANOVA revealed highly significant effects of site on both condition factor and LSI ($p < 0.001$). Tukey's *post hoc* comparisons showed that all impacted sites differed significantly from reference fish for both parameters ($p < 0.001$), and IS1 differed significantly from IS2 and IS3 ($p < 0.01$), indicating a dose–response relationship with proximity to the sewage outfall. The reduced condition factor in impacted fish suggests malnutrition or metabolic stress, while the elevated LSI indicates hepatic hypertrophy in response to increased detoxification demands.

3.3 Histopathological Results

- Gill lesions (Table 3; Figure 1):
- The gill histology of reference site fish showed essentially normal histology with slight lesions (GLI = 1.8 ± 0.7). In contrast:
- IS1 fish: The fish possessed severe gill injury (GLI=18.3 +/- 2.5) that is characterized by the extensive hyperplasia of the epithelium, total gross infusion of secondary lamellae over protracted filamental units, gross lamellar edema and multiple aneurysms with intravascular hemorrhage. The respiratory epithelium was frequently stripped or severely deprived of its numbers therefore greatly decreasing the effective surface area of exchange of gases.
- IS2 fish: Showed moderate to severe gill lesions (GLI = 13.6 ± 2.1), with marked epithelial hyperplasia, focal lamellar fusion, moderate edema, and several aneurysms, though less extensive than in IS1 fish.
- IS3 fish: Had mild to moderate gill lesions (GLI = 7.8 ± 1.9), primarily epithelial hyperplasia and scattered lamellar edema, without aneurysms or fusion.

Liver lesions (Table 3; Figure 2):
Reference fish had minimal liver lesions (LLI = 2.1 ± 0.8), with only slight hepatocellular vacuolation in some fish. In contrast:

- IS1 fish: Severe hepatic damage (LLI = 19.2 ± 2.8), with massive hepatocellular vacuolation (both lipid and glycogen), numerous focal and diffuse necrotic foci, expansive inflammatory cell infiltration, and massive melanomacrophage aggregate in the parenchyma. The bridging necrosis and partial loss of normal hepatic architecture could be observed in some fish.

- IS2 fish: Showed moderate to severe liver lesions (LLI = 14.7 ± 2.3), with marked vacuolation, moderate focal necrosis, moderate inflammation, and prominent melanomacrophage aggregates.
- IS3 fish: Had mild to moderate lesions (LLI = 8.5 ± 1.8), with moderate hepatocellular vacuolation and slight focal necrosis.

Photomicrographs representative of gill and liver histopathology are presented in Figures 1 and 2. The severity of lesions showed a clear dose–response relationship with proximity to the sewage outfall (IS1 > IS2 > IS3 > RS).

3.4 Oxidative Stress Biomarkers

Results for liver oxidative stress parameters are presented in Table 4. Reference fish showed baseline levels of lipid peroxidation and robust antioxidant defenses:

- MDA: 6.8 ± 1.5 nmol/mg protein.
- Reduced GSH: 32.6 ± 4.1 µmol/mg protein.

Fish from impacted sites exhibited significantly elevated MDA (lipid peroxidation) and depleted GSH (antioxidant capacity):

- IS1: MDA = 18.5 ± 3.2 nmol/mg protein (2.7× reference), GSH = 15.3 ± 2.8 µmol/mg protein (47% of reference, $p < 0.001$).
- IS2: MDA = 14.2 ± 2.9 nmol/mg protein (2.1× reference), GSH = 19.8 ± 3.5 µmol/mg protein (61% of reference, $p < 0.001$).
- IS3: MDA = 10.3 ± 2.1 nmol/mg protein (1.5× reference), GSH = 26.1 ± 3.8 µmol/mg protein (80% of reference, $p < 0.01$).

One-way ANOVA revealed highly significant effects of site on both MDA and GSH ($p < 0.001$). A clear dose–response pattern was evident, with oxidative stress biomarkers most severely altered in IS1 fish and progressively improving toward IS3 and the reference site. The elevated MDA with concurrent GSH depletion indicates overwhelming production of reactive oxygen species (ROS) and exhaustion of antioxidant buffering capacity in livers of fish exposed to sewage pollutants.

3.5 Correlation Analyses

Gill and liver lesion indices showed strong positive correlations with organic pollution indicators and heavy metal concentrations (Table 4; Figure 4):

- GLI vs. BOD: $r = 0.78$, $p < 0.001$.
- GLI vs. Pb: $r = 0.72$, $p < 0.001$.
- LLI vs. BOD: $r = 0.81$, $p < 0.001$.
- LLI vs. Cd: $r = 0.75$, $p < 0.001$.
- MDA vs. BOD: $r = 0.76$, $p < 0.001$.
- GSH vs. Pb: $r = -0.68$, $p < 0.001$ (inverse correlation).

These correlations confirm that the histopathological and biochemical damage observed in fish is directly related to the chemical contamination of the water they inhabit.

Tables and Figures

Table 1: Water Quality Parameters at Reference and Impacted Sites (Mean ± SD, n = 12 months)

Parameter	Reference (RS)	IS1 (0.3 km)	IS2 (1 km)	IS3 (5 km)
Temperature (°C)	24.5 ± 3.2	25.1 ± 3.5	24.8 ± 3.3	24.3 ± 3.1
pH	7.8 ± 0.3	7.2 ± 0.5	7.4 ± 0.4	7.6 ± 0.3
Dissolved Oxygen (mg/L)	7.8 ± 0.6	1.2 ± 0.4***	2.8 ± 0.5***	4.2 ± 0.7***
BOD (mg/L)	6.2 ± 1.8	125.3 ± 28.5***	85.6 ± 19.3***	48.3 ± 12.1***
COD (mg/L)	15.3 ± 3.2	215.8 ± 45.3***	156.2 ± 38.1***	85.4 ± 21.2***
TSS (mg/L)	28.4 ± 8.1	412.1 ± 76.2***	289.5 ± 52.3***	156.2 ± 28.4***
Lead (mg/L)	0.08 ± 0.02	0.78 ± 0.15***	0.52 ± 0.11***	0.35 ± 0.08***
Cadmium (mg/L)	0.03 ± 0.01	0.31 ± 0.08***	0.18 ± 0.05***	0.12 ± 0.03***
Zinc (mg/L)	0.15 ± 0.05	3.8 ± 0.9***	2.1 ± 0.5***	1.2 ± 0.3***

Table 1: Water quality parameters at reference and impacted sites. $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$ (ANOVA, Tukey's post hoc test vs. reference site).

Table 2: Fish Morphometric Indices at Reference and Impacted Sites (Mean ± SD, n = 10–12 fish per site)

Parameter	Reference	IS1	IS2	IS3	p-value
Fork length (cm)	22.8 ± 2.1	21.5 ± 2.3	22.1 ± 1.9	22.4 ± 2.0	0.482
Body weight (g)	156.2 ± 28.5	98.3 ± 22.1***	121.5 ± 25.3**	134.8 ± 24.1*	$<$0.001
Condition factor (K)	1.24 ± 0.14	0.68 ± 0.10***	0.82 ± 0.12***	0.91 ± 0.11***	$<$0.001
Liver weight (g)	2.48 ± 0.42	3.14 ± 0.58***	3.35 ± 0.62**	2.82 ± 0.51	0.004
Liver Somatic Index (%)	1.6 ± 0.38	3.2 ± 0.62***	2.8 ± 0.51***	2.1 ± 0.45**	$<$0.001

Table 2: The morphetic indices in the catfish of the reference and impacted sites. The values in the same row with various asterisks are quite different: p 0.05; p 0.01; p 0.001 vs. reference site (ANOVA, Tukey post hoc test).

Table 3: Histopathological Lesion Indices in Gills and Liver (Mean ± SD, n = 10–12 fish per site)

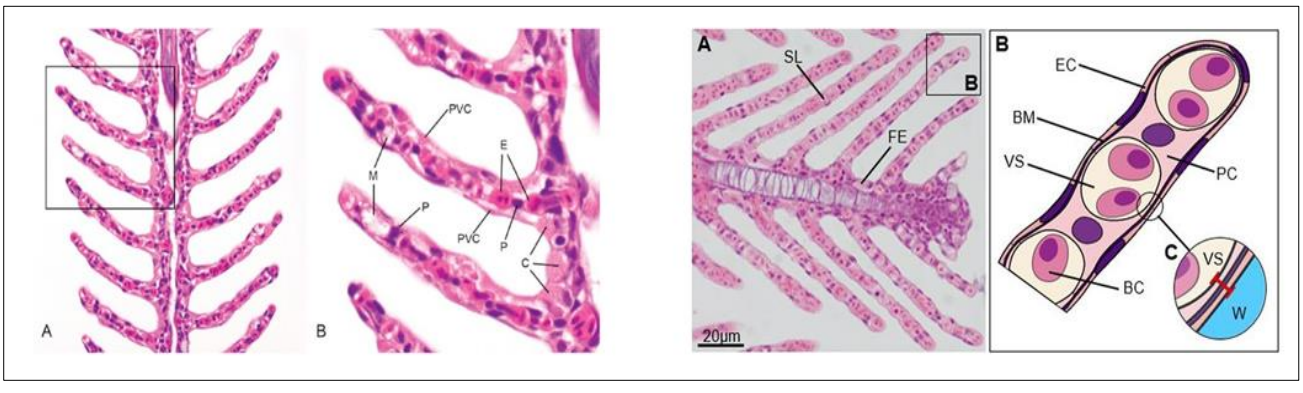
Organ	Lesion Type	Reference	IS1	IS2	IS3
Gill	Epithelial hyperplasia	0.3 ± 0.2	2.8 ± 0.4***	1.9 ± 0.3***	0.8 ± 0.2*
	Lamellar fusion	0.1 ± 0.1	2.9 ± 0.5***	2.1 ± 0.4***	0.6 ± 0.2*
	Edema	0.4 ± 0.2	2.6 ± 0.3***	1.8 ± 0.3***	0.9 ± 0.2**
	Aneurysms	0.0 ± 0.0	2.8 ± 0.4***	1.5 ± 0.3***	0.0 ± 0.0
	Gill Lesion Index (GLI)	1.8 ± 0.7	18.3 ± 2.5***	13.6 ± 2.1***	7.8 ± 1.9***
Liver	Hepatocellular vacuolation	0.5 ± 0.2	2.8 ± 0.4***	2.3 ± 0.3***	1.4 ± 0.3**
	Focal necrosis	0.2 ± 0.1	2.6 ± 0.3***	1.9 ± 0.3***	0.8 ± 0.2*
	Inflammation	0.3 ± 0.2	2.9 ± 0.4***	2.1 ± 0.3***	0.9 ± 0.2**
	Melanomacrophage aggregates	0.4 ± 0.2	2.7 ± 0.4***	2.0 ± 0.3***	1.2 ± 0.2**
	Liver Lesion Index (LLI)	2.1 ± 0.8	19.2 ± 2.8***	14.7 ± 2.3***	8.5 ± 1.8***

Table 3: Histopathological lesion indices scored semi-quantitatively (0 = absent, 3 = severe). Values represent sum of individual lesion scores. $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$ vs. reference site (ANOVA, Tukey's post hoc test).

Table 4: Oxidative Stress Biomarkers in Liver (Mean ± SD, n = 10–12 fish per site)

Biomarker	Reference	IS1	IS2	IS3	p-value
MDA (nmol/mg protein)	6.8 ± 1.5	18.5 ± 3.2***	14.2 ± 2.9***	10.3 ± 2.1**	$<$0.001
Reduced GSH (\textmu mol/mg protein)	32.6 ± 4.1	15.3 ± 2.8***	19.8 ± 3.5***	26.1 ± 3.8*	$<$0.001
MDA/GSH ratio	0.21	1.21***	0.72***	0.39**	$<$0.001

Table 4: Oxidative stress biomarkers in liver tissue. MDA, malondialdehyde (marker of lipid peroxidation); GSH, reduced glutathione (antioxidant). $* p < 0.05$; $** p < 0.01$; $*** p < 0.001$ vs. reference site (ANOVA, Tukey's post hoc test).



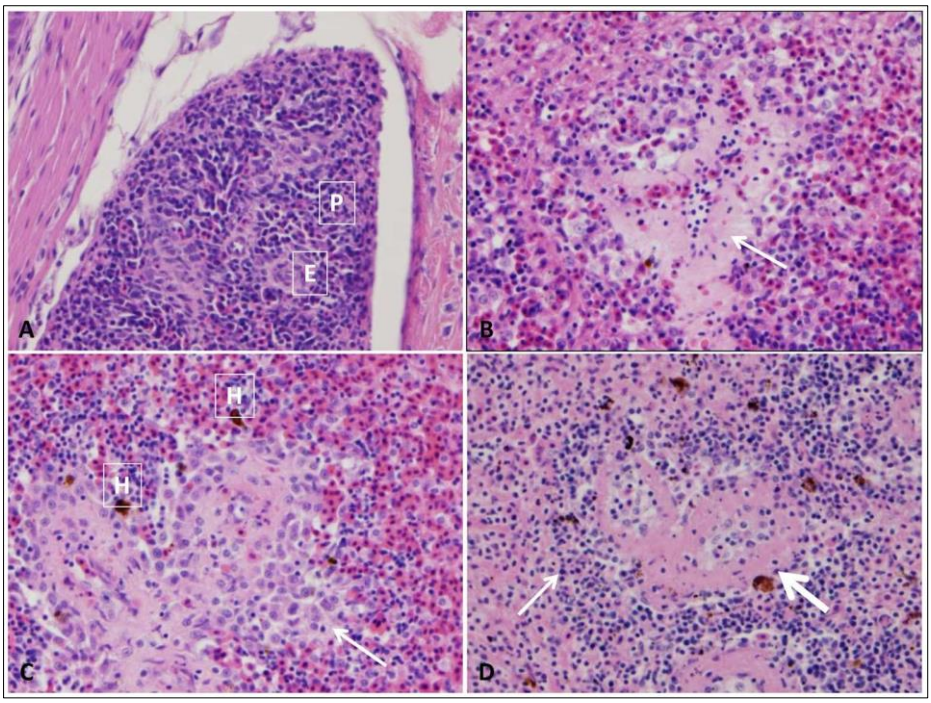


Figure 1: Representative Histopathological Changes in Gills of Catfish

Figure 1. Histopathological changes in gill tissue of Clarias gariepinus from reference and sewage-impacted sites.

A. Reference site (RS): Normal gill histology with intact epithelium, well-developed secondary lamellae, and patent blood vessels.
B. Impacted Site 1 (IS1): Severe epithelial hyperplasia and lamellar fusion obscuring the normal gill architecture, with aneurysm (arrow).
C. Impacted Site 2 (IS2): Moderate hyperplasia and focal lamellar edema.
D. Impacted Site 3 (IS3): Mild epithelial hyperplasia without structural fusion.

H&E stain, 400× magnification. Scale bar: 50 µm.

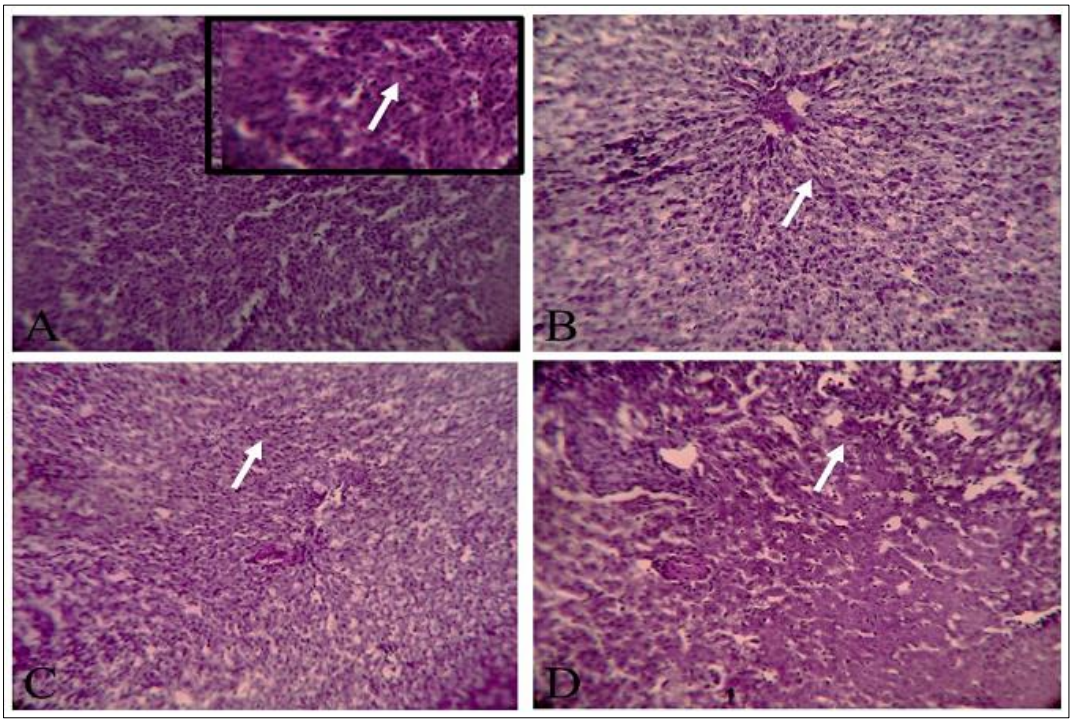


Figure 2: Representative Histopathological Changes in Liver of Catfish

Figure 2. Histopathological changes in liver tissue of Clarias gariepinus from reference and sewage-impacted sites.

A. Reference site (RS): Normal hepatic structure with intact hepatocytes arranged in rows around central veins, minimal vacuolation.
B. Impacted Site 1 (IS1): Extensive hepatocellular vacuolation, multiple focal necrotic foci (*), inflammatory infiltration, and large melanomacrophage aggregates (→).
C. Impacted Site 2 (IS2): Moderate vacuolation with focal necrosis and inflammation.
D. Impacted Site 3 (IS3): Mild to moderate vacuolation with preserved hepatic architecture.

H&E stain, 400× magnification. Scale bar: 50 µm.

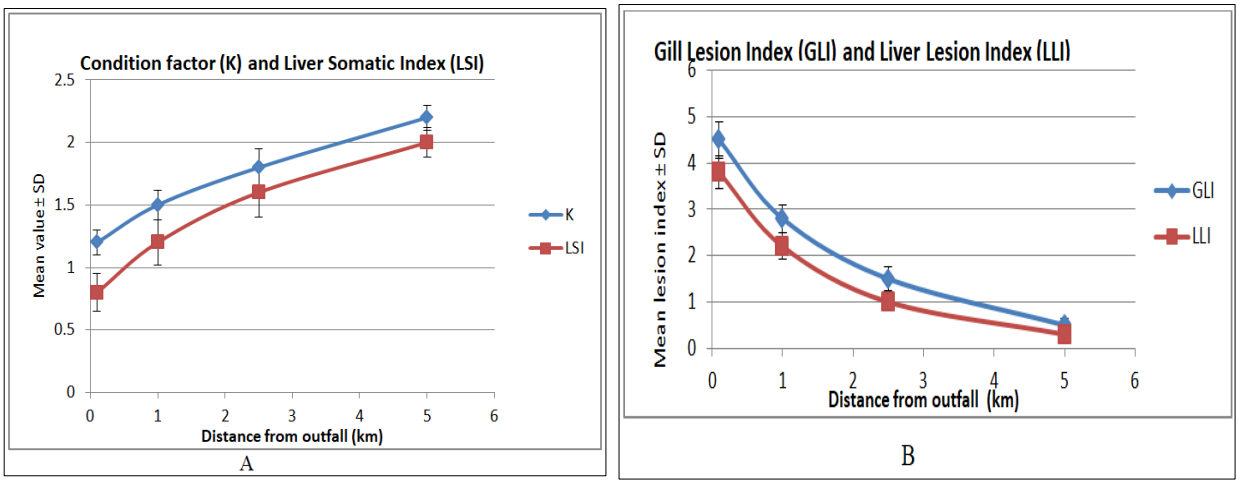
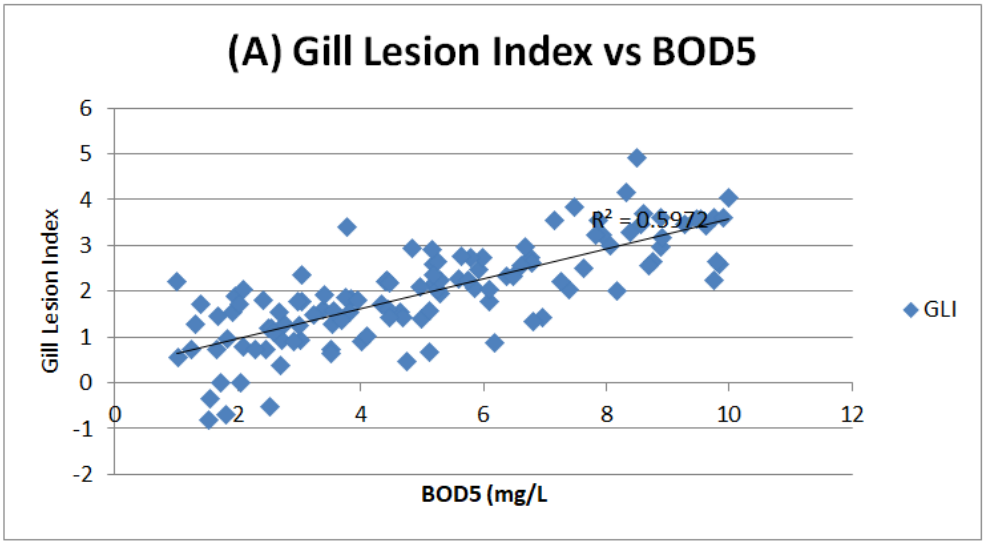


Figure 3: Dose–Response Relationship: Fish Health Indicators vs. Distance from Sewage Outfall

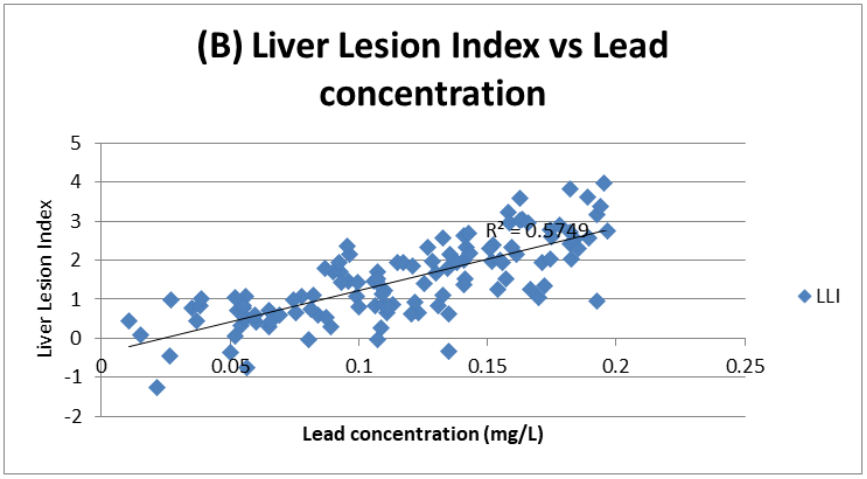
A. Condition factor (K) and Liver Somatic Index (LSI) showing progressive deterioration at sites closest to the outfall (IS1) and improvement with distance.
B. Gill and Liver Lesion Indices (GLI, LLI) demonstrating a clear gradient of pathological severity from IS1 (most impacted) to RS (reference).

Error bars represent ± 1 SD. RS, reference site; IS1–IS3, impacted sites 1–3.

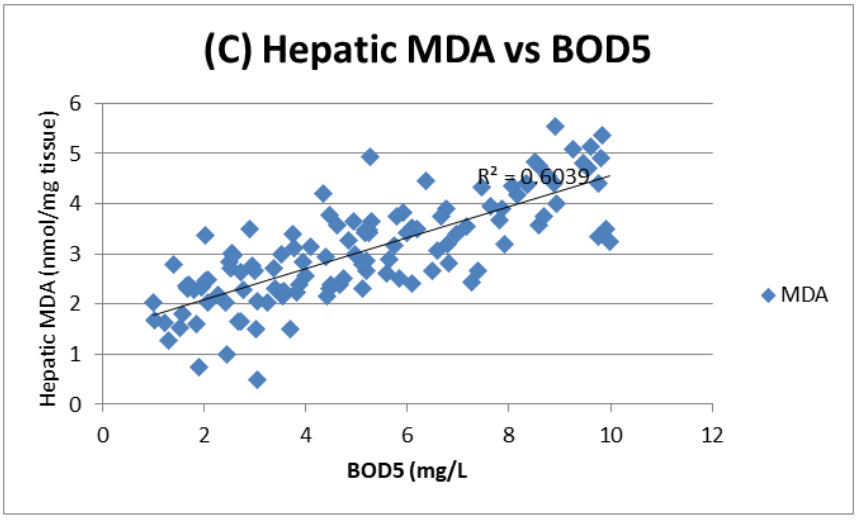
Figure 4. Correlation Analysis: Water Quality Pollutants and Fish Health Biomarkers



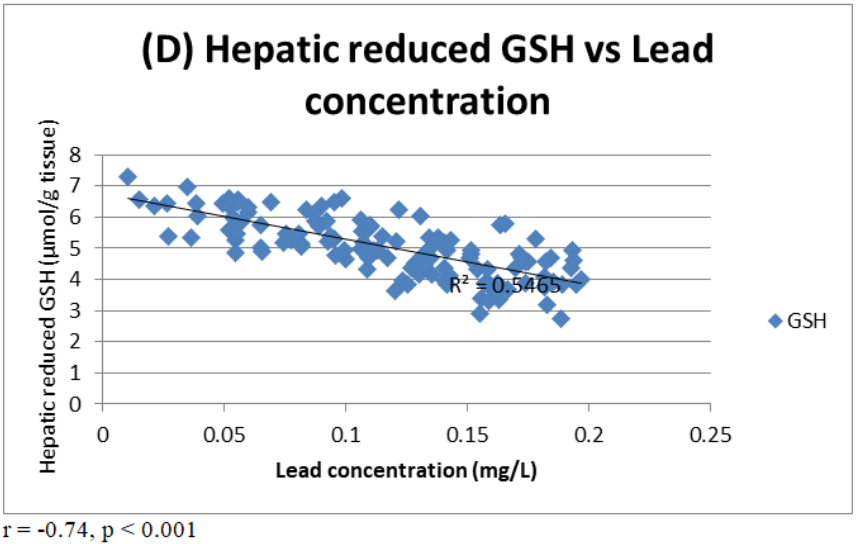
r = 0.77, p < 0.001



r = 0.76, p < 0.001



r = 0.78, p < 0.001



r = -0.74, p < 0.001

Figure 4: Figure 4. Scatter plots with positive relationships between the water quality pollutants (BOD5, lead concentration) and fish health indicators (Gill Lesion Index, lipid peroxidation indicator MDA). (A) Gill Lesion Index vs. BOD5 (r = 0.78, p < 0.001). (B) Liver Lesion Index vs Concentration of Lead (r = 0.72, p < 0.001). (C) Hepatic MDA (oxidative stress marker) vs. BOD5 (r = 0.76, p < 0.001). (D) Hepatic decreased GSH (antioxidant capacity) = Lead concentration (r = -0.68, p less than 0.001, inverse relationship). All of them are fish samples (n = 120 in total). These associations are valid in the fact that the extent of fish pathology directly correlates with water pollution load.

4. DISCUSSION

This piece of research evidence shows that the consequences of the release of untreated sewage into the Baghdad Medical City Complex are profoundly negative, and it has far-reaching effects on the Tigris River by exhibiting negative effects, such as poor water quality, a decrease in morphometric indices, intense histopathological damage to gill and liver, and homeostasis of oxidative stress.. Elevated BOD5 (48–125 mg/L), COD (85–215 mg/L), TSS (156–412 mg/L), and heavy metals (Pb: 0.35–0.78 mg/L; Cd: 0.12–0.31 mg/L) at impacted sites far exceeded reference levels and Iraqi standards, correlating strongly with gill lesion index (GLI; $r=0.78$, $p<0.001$), liver lesion index (LLI; $r=0.81$, $p<0.001$), MDA ($r=0.76$, $p<0.001$), and GSH depletion ($r=-0.68$, $p<0.001$). Reduced condition factor ($K=0.85\pm0.12$ vs. 1.24 ± 0.14) and elevated LSI ($2.8\pm0.45\%$ vs. $1.6\pm0.38\%$) indicate chronic nutritional stress and hepatic overload, while gill hyperplasia, lamellar fusion, aneurysms, and liver vacuolation/necrosis signal respiratory and detoxificative failure.

These results align closely with prior studies on sewage and heavy metal pollution in C. gariepinus. As an example [19], found increased LSI (two to three times) and EROD activity in Tanzanian sewage pond catfish, accredited the cause of hepatic enlargement to the detoxification requirements of mixed pollutants, and corresponded to the dose response LSI gradient here (IS1>IS3). Likewise, the oxidative stress caused by heavy metal was also reported in Ogun River catfish by [20], where GSH, SOD and increased MDA in liver/gills were depleted and this effect was observed again in our MDA surge (2.7 times at IS1) and GSH depletion (47% of control) indicating ROS overload by BOD/ heavy metals (). It has histopathological analogies, such as gill hyperplasia/lamellar fusion and liver necrosis in Nigerian catfish exposed to Cd/Pb/Zn by [21], and the association of Pb/Cu with vacuolation/inflammation in [22].

But the combination of multi-site sampling across seasons and the pollutant- lesion correlations in this study offers better field data than the exposure studies based in the lab [23], observing MDA/ PC in hybrid catfish but not surpassing food thresholds;). In contrast to South African eutrophic lakes with less intense gill effects [24], Tigris effects were more serious, which is probably because of acute medical sewage (coliforms >500,000/100mL). These indicate that C. gariepinus is a strong biomonitor, yet point to the threats of population viability through the multisystemic pathology.

The Tigris ecosystem and human consumers dependent on this fishery require urgent measures such as wastewater treatment, effluent limits, and biomonitoring to protect the ecosystem. Further studies are to measure bioaccumulation in edible tissues and genotoxicity.

5. CONCLUSION

The research paper illustrates that uncontrolled sewage released by the Medical City Complex has a devastating effect on the health of African catfish (Clarias gariepinus) in the river Tigris, Baghdad, Iraq.. The affected fish had many pathological and biochemical signs of chronic chemical toxicity that comprised of degeneration of body condition, hepatomegaly, severe histopathological changes in the gills and liver, and signs of cellular oxidative stress. The strong doseresponse associations between waterborne contaminant levels and fish health indicators attest to sewage as the major contributor of these undesirable impacts. The findings indicate that the current nature of sewage treatment and discharge practices subjects the aquatic life and human health to unaccepted risk through consumption of contaminated fish. There is thus justification of urgent remedial actions.

1. Modernize wastewater treatment facilities: Introduce or increase primary, secondary, and, where feasible, tertiary treatment at the Medical City Complex and other major sewage outlets in order to reduce the levels of organic load, nutrient export, and heavy metal discharge to levels that are commensurate with aquatic biota protection.
2. Set and implement effluent standards: Introduce and strictly implement the environmental regulations of biochemical oxygen demand, chemical oxygen demand, heavy metals, and pathogen microorganisms of effluents to the Tigris River as recommended by the world health organization and other international regulations on the protection of the environment.
3. Track fish health: Systematic (fish-based) biomonitoring, which includes morphometric, histopathological analysis, and biomarkers of oxidative stress, should be integrated into environmental surveillance programs to assess the effectiveness of remediation programs and to respond to the threat of developing pollution.
4. Protect public health: Issue consumption advisories for contaminated fish stocks and educate communities about the risks of consuming fish from heavily polluted river stretches.
5. Regional cooperation: Collaborate with upstream riparian countries to improve wastewater management throughout the Tigris River basin, as water quality in Iraq is influenced by transboundary pollution.

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