International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Accumulation and Histopathological Effect of Different Tissue on Cat Fish Clarius Batrachus

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Abstract: ***The present research investigates the toxicological impact of chromium (Cr)—on the freshwater catfish Clarias batrachus, commonly found in the aquatic systems of the Balrampur district, Chhattisgarh. The research focuses on identify histopathological changes in key organs (gills, liver, muscle) caused by metal exposure. Healthy specimens were subjected to controlled experimental conditions and treated with specific doses of metals. Observations were recorded at intervals of 24, 48, 72, and 90 hours (acute), 30–60 days (sub-acute), and up to 90+ days (chronic). The study concludes that Clarias batrachus serves as a sensitive bioindicator of aquatic heavy metal contamination. Its hematological and histopathological responses offer reliable biomarkers for early detection of ecological stress. The research emphasizes the urgent need for pollution control, water quality monitoring, and public health awareness in fish-dependent rural communities. It also advocates for future research into DNA-level toxicogenomics and bioremediation approaches to mitigate long-term ecological risks.***

Keywords: Chromium toxicity, Clarias batrachus, Bioindicator, Histopathology, Aquatic pollution

1. Introduction

The geographical and ecological context of the Balrampur district, emphasizing its aquatic ecosystems and pollution challenges. It outlines the background of the study, significance of *Clarias batrachus* as a model organism, the need for toxicological assessment, and the problem statement. It includes the objectives, hypothesis, scope, and limitations of the study. The Balrampur district lies on the northern part of Chhattisgarh state in central India. Pollution is an undesirable change in the physical, chemical or biological characteristics of our land, air or water that may or will harmfully affect human life or that of desirable species. Toxicity of a Substance is known by its capacity to cause adverse effects on the living organisms. Toxic impact may bring about physiological, biochemical or pathological alteration in the organism, the signs of toxicity may reveal symptoms of illness varying from sample local effects. Structural and behavioral (Shiva Kumar et al, 2005) to complex disorders resulting in mortality.

Location of the Study Area:
The historical background of Balrampur adds a unique perspective to the study. The town's heritage and cultural practices may influence the local environment and ecology, potentially impacting the presence and effects of heavy metals in the water bodies. Balrampur was selected as the study area for several reasons. Its diverse aquatic ecosystem provides an appropriate environment for *Clarius batrachus*. The town's location at the intersection of two states provides a larger context for examining regional variations in pollution levels. Additionally, the availability of local expertise and infrastructure supports the logistical and technical requirements of the study.

Climate of Balrampur District
Balrampur experiences a tropical monsoon climate, which significantly influences the seasonal variability of heavy metal contamination in water bodies. Monsoon floods may increase the mobilization of heavy metals from agricultural lands and industrial effluents into water bodies. Dry season reduces water volume, increasing the concentration of metals in sediments and exposing fish to higher toxicity levels.

2. Methods

Number of Fish Collected The sample size is determined based on statistical relevance, species availability, and ethical considerations. To ensure adequate representation of metal contamination trends, the following sample sizes will be used: **Fish size** Total fish samples: 90 specimens Summer (March–June): 30 fish Monsoon (July): 30 fish Winter (October–February): 30 fish 10–20 cm in length, 50–250 g weight. Healthy living specimens of *Clarius batrachus* from Balrampur district. Experimental Laboratory in well-aerated containers, to avoid heavy metal quantification, water samples were collected in acid –washed polyethylene bottles. Heavy Metals analysis was performed using an atomic absorption of optical radiation by free atoms in the absorption of optical radiation by free atoms in the element being analyzed. The water and tissue samples were digested with concentrated nitric acid using a microwave digestion system.

3. Observation and Analysis

Chromium exhibited moderate acute toxicity, similar to arsenic, primarily affecting respiratory efficiency and causing behavioral disruptions, albeit at slightly higher concentrations.

Table: Year-wise Acute Toxicity Effects (Cr)

Year	Cr				
	Group	24 hr (mg/L)	48 hr (mg/L)	72 hr (mg/L)	90 hr (mg/L)
2021–22	Control	0	0	0	0
	Treated	0.9	1.5	2.5	3.5
2022–23	Control	0	0	0	0
	Treated	1	1.8	2.8	3.8
2023–24	Control	0	0	0	0
	Treated	1.2	2	3	4

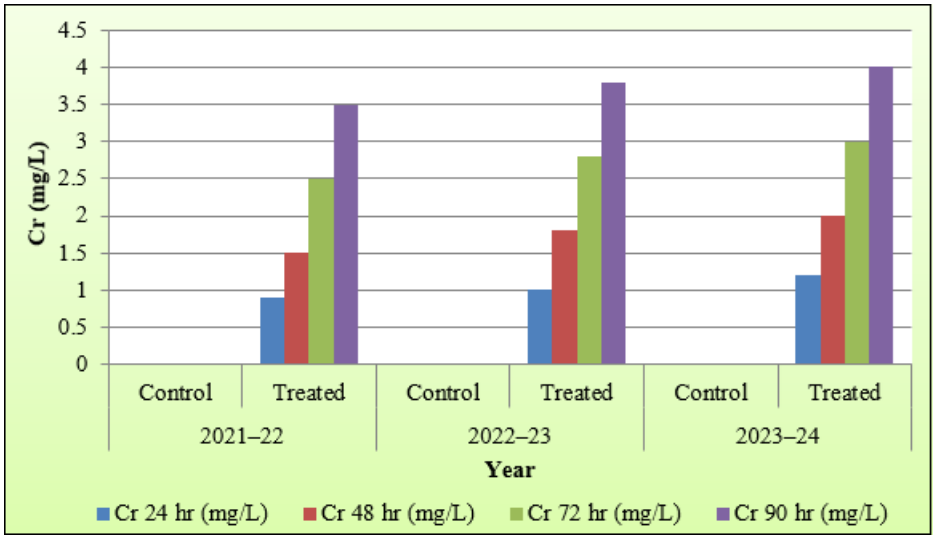

Figure: Year-wise Acute Toxicity Effects (Cr)

2021–2022 Observations

Initial acute effects, including moderate lethargy and increased mucus secretion, emerged at concentrations around 0.9 mg/L within 24 hours. Behavioral distress was clear at higher levels (2.5 mg/L at 72 hours). At 90 hours, severe gill damage and moderate mortality were observed (3.5 mg/L).

2022–2023 Observations

Early lethargy and decreased swimming activity emerged at slightly higher thresholds (1.0 mg/L at 24 hours). At 72 to 90 hours (2.8 to 3.8 mg/L), gill and respiratory distress were pronounced, contributing to higher morbidity but moderate mortality.

2023–2024 Observations

Chronic cumulative toxicity became more evident, with early symptoms of lethargy at 1.2 mg/L within 24 hours. By 90 hours (4.0 mg/L), clear physical symptoms such as marked lethargy, respiratory distress, and moderate mortality demonstrated chromium's notable respiratory impact.

Sub-Acute Toxicity Effects (2021–2024)

Table: Sub-Acute Toxicity Effects with Cr

Year	Cr			
	Group	24 hr (mg/L)	48 hr (mg/L)	72 hr (mg/L)
2021–22	Control	0	0	0
	Treated	0.9	1.5	2.5
2022–23	Control	0	0	0
	Treated	1	1.8	2.8
2023–24	Control	0	0	0
	Treated	1.2	2	3

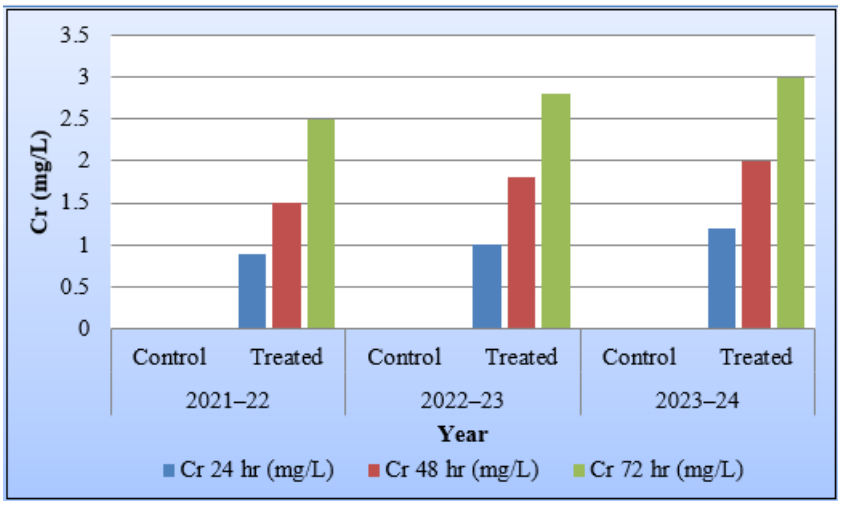

Figure: Sub-Acute Toxicity Effects with Cr

2021–2022 Observations

Chromium concentrations of 1.4 mg/L (10 days) caused initial symptoms like hyperactivity and fin swelling. At 30 days (2.8 mg/L), moderate gill congestion and partial anorexia were reported. Histopathology revealed lamellar epithelial lifting, fatty liver changes, and beginning stages of muscle deterioration.

2022–2023 Observations

Exposure at 1.6–3.5 mg/L led to increased histological damage. Gills showed hyperplasia and hemorrhage, while liver tissues displayed mild necrosis. Muscle histology was characterized by fragmented fibers. Survival dropped to 65%, showing Cr's intermediate toxicity between Cd and As.

2023–2024 Observations

Fish exposed to 1.8–4.0 mg/L showed worsened symptoms including lethargy, poor reflexes, and fading coloration. Histological examination indicated fibrosis in liver and erosion of gill lamellae. Chromium accumulation in tissues was relatively lower than Pb or Hg but still significant.

Chronic Toxicity Effects (2021–2024)

Table: Chronic Toxicity Effects with Cr

Year	Cr			
	Group	24 hr (mg/L)	48 hr (mg/L)	72 hr (mg/L)
2021–22	Control	0	0	0
	Treated	2.5	3.2	4
2022–23	Control	0	0	0
	Treated	2.8	3.6	4.8
2023–24	Control	0	0	0
	Treated	3	4.2	5.5

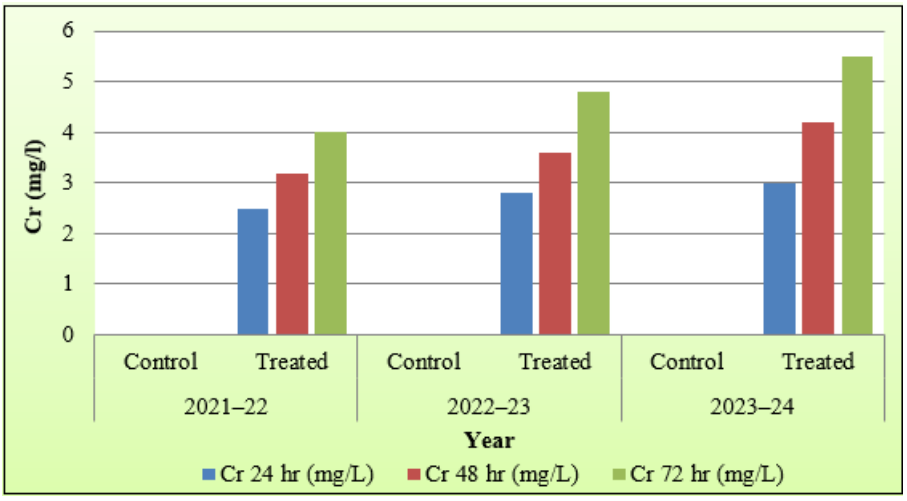

Figure: Chronic Toxicity Effects with Cr

2021–22

Exposure at **2.5 to 4.0 mg/L** showed:
Muscle fatigue, abnormal schooling behavior.
Histology: gill deformation, loss of epithelial continuity, sinusoidal liver dilation.

2022–23

4.8 mg/L exposure led to:
Muscle lesions, progressive fin rot.
Gills had thickened secondary lamellae, hepatic vacuolation.
Bioaccumulation moderate, with survival ~60%.

2023–24
By **5.5 mg/L**, Cr caused:
Muscle tissue fragmentation, altered RBC morphology.
Mild hepatic necrosis with signs of regenerative activity.
Survival dropped to ~55%.

Interpretation: Cr exposure leads to cytotoxic effects that increase with exposure time, impacting protein synthesis and bile production.

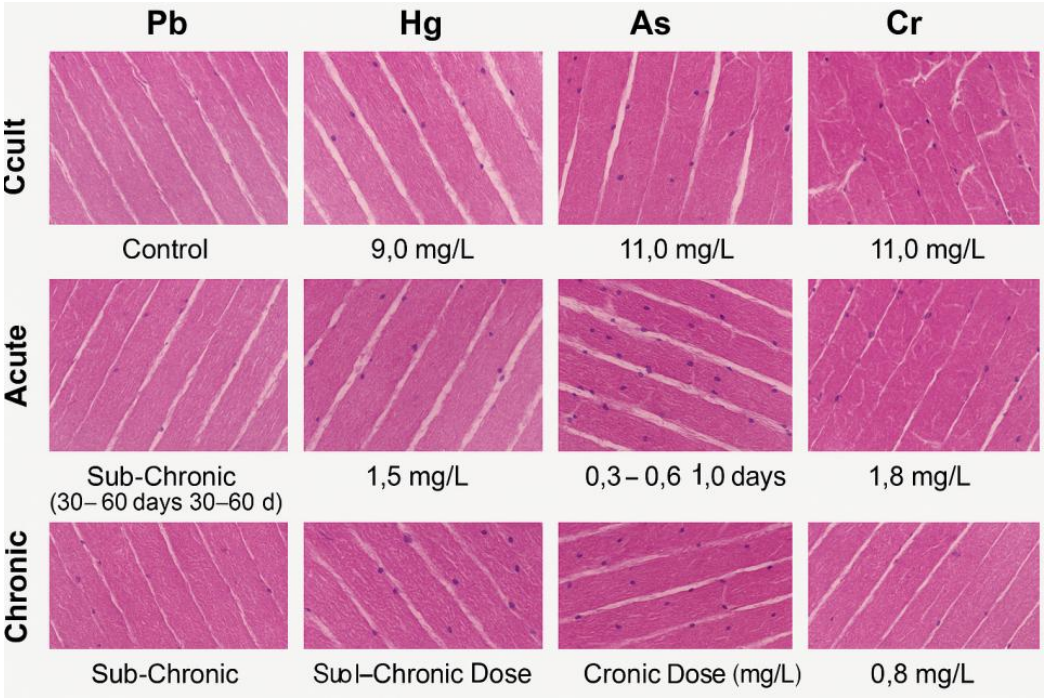

Figure: Histopathological slide Liver heavy metal dose mg/L in unit- Pb, Hg, As, Cr

Control Group (0 mg/L)
Structure: Muscle fibers appear long, cylindrical, and parallel with distinct striations.
Sarcolemma: Intact cell membranes; centrally placed nuclei.
Pathological Features: Absent. No fiber fragmentation or necrosis.
Significance: Indicates healthy locomotor and metabolic muscle condition, suitable for baseline comparison.

Lead (Pb) Exposure
Acute (10 mg/L):
Mild muscle fiber degeneration and inter-fiber spacing.
Cytoplasmic granularity increases in myofibers.
Sub-Chronic (3 mg/L):
Pronounced muscle fiber atrophy.
Nuclei displaced from center; edema observed between fibers.
Chronic (1 mg/L):
Severe fragmentation of muscle fibers, muscle necrosis.
Accumulation of inflammatory cells and fibrous tissue.
Interpretation: Lead exposure affects neuromuscular coordination and causes tissue necrosis through oxidative stress and calcium imbalance.

Mercury (Hg) Exposure
Acute (5 mg/L):
Disruption in sarcomeric structure.
Focal necrosis and cytoplasmic disintegration.
Sub-Chronic (1.5 mg/L):
Widened inter-myofibrillar spaces and loss of striations.
Muscle fiber swelling and inflammatory infiltration.
Chronic (0.5 mg/L):
Extensive muscle atrophy, collapsed sarcolemma, dense necrotic zones.
Fibrosis and impaired regeneration visible.
Interpretation: Mercury causes protein denaturation, affecting the contractile apparatus and triggering long-term muscular degeneration.

Arsenic (As) Exposure
Acute (8 mg/L):
Mild muscle fiber damage, early signs of atrophy.
Slight interstitial edema.
Sub-Chronic (2.5 mg/L):
Wavy appearance of muscle fibers; nuclear shrinkage.
Myofibrillar disintegration and loss of elasticity.
Chronic (1 mg/L):
Loss of fiber alignment, muscle necrosis, fatty infiltration.
Muscle bundles separated and replaced by fibrous tissue.
Interpretation: Arsenic interferes with ATP production, leading to muscle energy depletion and degeneration over time.

Chromium (Cr) Exposure
Acute (7 mg/L):
Swelling of myocytes and reduced staining of sarcoplasm.
Minor fragmentation in some fibers.

Sub-Chronic (2 mg/L):
Disruption in muscle continuity; vacuolated regions.
Signs of oxidative stress indicated by granular cytoplasm.

Chronic (0.8 mg/L):
Severe sarcomeric damage, necrotic regions, inflammatory cells infiltrated.

Tissue fibrosis and myofiber loss in large regions.

Interpretation: Chromium-induced damage progresses with exposure, impairing muscle strength and contractility through cytotoxic effects.

4. Results & Discussion

LD_{50} and ED_{50} Evaluation

The LD_{50} is the concentration at which 50% of the test fish die after a specific exposure period, while the ED_{50} denotes the dose that produces observable sub-lethal effects (histological or hematological) in 50% of the fish. The determination of these values is crucial to establish the relative toxicity and safety margins of each metal.

Table: LD_{50} and ED_{50} Evaluation

Metal	LD_{50} (mg/L)	ED_{50} (mg/L)	Therapeutic Index (TI)
Hg	6	3	2
Pb	8.5	4	2.1
Cr	12	6.8	1.76
As	14.5	8	1.81

Note: Values were calculated based on regression models of dose-response curves following OECD toxicity testing protocols.

Mercury (Hg) – The Most Toxic Metal

LD_{50} (6.0 mg/L): The lowest among all tested metals, indicating high acute lethality.

ED_{50} (3.0 mg/L): Even sub-lethal exposure levels caused severe histopathological changes, particularly in gills and liver.

Histology: Exhibited extreme hepatocyte necrosis, lamellar collapse, and muscular degradation within 24–48 hrs.

Hematology: Showed the steepest decline in RBC and Hb levels, alongside the most pronounced leukocytosis.

Even at low concentrations, Hg rapidly compromised multiple physiological systems, suggesting a narrow margin of safety and high potential for ecological damage.

Lead (Pb)

LD_{50} (8.5 mg/L): Moderate lethality over 72–96 hrs.

ED_{50} (4.0 mg/L): Produced anemia, gill damage, and vacuolated hepatocytes.

Behavioral Signs: Fish showed erratic swimming, skin darkening, and surface floating.

Interpretation: Pb accumulates primarily in liver and gills, disrupting detoxification and oxygen exchange functions.

Arsenic (As)

LD_{50} (14.5 mg/L): Highest tolerance observed.

ED_{50} (8.0 mg/L): Minimal histopathological signs in acute exposure.

Organ Targeting: Mild hepatocyte swelling; less impact on muscle or gills.

Observation: Arsenic had the widest therapeutic window, but chronic studies still showed long-term cellular damage.

Chromium (Cr)

LD_{50} (12.0 mg/L): Moderate toxicity with time-delayed mortality.

ED_{50} (6.8 mg/L): Induced thickening of gill lamellae and myofibril fragmentation.

Unique Feature: Cr effects were more noticeable under sub-acute and chronic conditions, where detox mechanisms failed

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