

Hematological Alterations in the Fish *Channa striata* Exposed to Heavy Metal Pollution from River Kharun, Chhattisgarh (India)

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Abstract

The present study investigates the impact of heavy metal contamination on the hematological parameters of the freshwater fish *Channa striata* (Bloch), a widely distributed and ecologically significant and tolerant species. Water samples were collected seasonally from 03 selected sites of the Kharun River, and the concentrations of major heavy metals (such as Iron, cadmium, lead, nickel, and mercury) were analyzed. Corresponding fish specimens were examined for blood parameters, including hemoglobin content, red blood cell (RBC) count, white blood cell (WBC) count, PCV, MCV & MCHC values. The results revealed significant alterations in these parameters, indicating physiological stress and potential immunosuppression. Decreased hemoglobin levels and RBC counts, along with increased WBC counts, suggest anemia and inflammatory responses in the exposed fish. This study highlights the bio-indicative role of *Channa striata* in freshwater ecosystems and underscores the need for continuous monitoring of heavy metal contamination in freshwater ecosystems.

Introduction:

Aquatic ecosystems are increasingly threatened by anthropogenic activities that introduce various pollutants, including heavy metals, into water bodies (Mukherjee, S., *et al.*, 2023). Among these, heavy metals such as Iron (Fe),

cadmium (Cd), lead (Pb), nickel (Ni) and mercury (Hg) are of particular concern due to their persistence, bioaccumulation, and toxic effects on aquatic organisms (Rahman, Z., & Singh, V. P. (2019). Unlike organic pollutants, heavy metals

are non-biodegradable and tend to accumulate in aquatic sediments and biota, thereby entering the food web and posing severe ecological and public health risks (Sarker, A., *et. al.*,2022).

Fish are important bioindicators of aquatic ecosystem health because of their sensitivity to waterborne pollutants, including heavy metals (Łuczyńska, J., *et. al.*,2018). Changes in physiological and biochemical parameters in fish can reflect the quality of their aquatic habitat (Menon, S. V., *et. al.*,2023). Hematological studies have emerged as a valuable tool in ecotoxicology, as blood parameters are reliable indicators of stress, physiological dysfunction, and toxicant exposure in fish (Docan, A., *et. al.*,2018). Parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, and hematocrit value are commonly analyzed to assess the health status of fish exposed to pollutants (Duong, T. Y., *et. al.*,(2019).

Channa striata (Bloch), commonly known as the striped snakehead, is a carnivorous freshwater fish that is widely distributed across South and Southeast Asia (Newton, A. C., *et. al.*,2010). It is of high ecological and economic importance and is known for its tolerance to varied environmental conditions (Amadi, C. N., *et. al.*,2022). However, its frequent exposure to polluted habitats makes it a potential sentinel species for assessing the impact of environmental contaminants, including heavy metals (Jia, Y., Wang *et. al.*,2017).

Several studies have demonstrated the accumulation of heavy metals in different tissues of fish, including gills, liver, and muscles (Munir, M. B., *et. al.*,2018). However, relatively fewer

studies have focused on their hematological impacts, particularly in native fish species like *Channa striata* (Kumar, V., *et. al.*,2022). Changes in hematological parameters can be early warning indicators of sub-lethal toxic stress and can precede observable pathological symptoms (Yu, Y. B., *et. al.*,2024).

In the context of Chhattisgarh, India, rivers such as the Kharun are increasingly subjected to contamination from agricultural runoff, industrial discharge, and urban wastewater (Kumar, N., *et. al.*, 2017). These inputs have led to elevated levels of heavy metals in water and sediment, which can significantly affect the resident aquatic fauna (Kahlon, S. K., *et. al.*,2018). The present study aims to investigate the hematological responses of *Channa striata* to heavy metal exposure in the Kharun River (Parvez, I., *et. al.*,2025). By correlating water metal concentrations with changes in blood parameters, this research seeks to provide insight into the physiological stress experienced by fish and the broader implications for aquatic health and biodiversity conservation (Menon, S. V., *et. al.*,2023). Heavy metal contamination in freshwater ecosystems has been widely reported across various regions, including the Kharun River system in Chhattisgarh (Pandey & Mishra, 2025). Previous studies have shown significant accumulation of Fe, Pb, Cd, and Ni in both water and fish tissues, especially *Channa striata*, making it a sensitive bioindicator of aquatic pollution (Pandey, 2024). Heavy metal toxicity and its impacts on fish physiology, immunity, and hematology have also been extensively reviewed in recent literature

(Pandey et al., 2024; Pandey & Mishra, 2025b).

Materials and Methods:

Study Area and Sampling Sites: The study was conducted in the Kharun River near Kumhari, a prominent freshwater body in Chhattisgarh, India, known to be impacted by urban runoff, domestic sewage, agricultural and Industrial effluents (Pandey & Mishra, 2025). Sampling sites were selected along stretches of the river upstream, downstream, and meeting point locations to industrial effluent canal represent varying degrees of contamination near Kumhari (Siddiqui, M. H., Bhanbhro, U., Shaikh, K., Ahmed, S., Begum, R., Shoukat, S., & Khokhar, W. A. (2024). Water and fish samples were collected seasonally for a period of one year (June 2022 to May 2023), covering both monsoon (rainy), winter and summer conditions (Wanjari, R. N., et. al., 2025).

Collection of Water Samples: Water samples were collected in pre-cleaned, acid-washed polyethylene bottles from a depth of approximately 30 cm below the surface from selected sites (Campbell, K., et. al., 2025). Immediately after collection, nitric acid was added (1 mL/L) to preserve the samples for metal analysis (Jones, B. R., & Laslett, R. E., 1994). The samples were transported on ice to the laboratory for analysis within 24 hours (Zivkovic, A. M., et. al., 2009).

Heavy Metal Analysis in Water: The concentrations of selected heavy metals Iron (Fe), cadmium (Cd), lead (Pb), Nickel (Ni), and mercury (Hg) were determined

using Systronic Atomic Absorption Spectrophotometry (AAS) following the standard procedures described by the American Public Health Association (Balali-Mood, M., et. al., 2021). Samples were filtered and digested with concentrated nitric acid and perchloric acid in a hot plate digestion system prior to analysis (Zarcinas, B. A., et. al., 1987).

Collection and Handling of Fish Samples: Live specimens of *Channa striata* were collected from the same sampling sites, randomly 04 fish samples in each season using gill/cast nets and traps (Amilhat, E., & Lorenzen, K., 2005). Fish were placed in oxygenated containers and transported to the laboratory (Nemoto, C. M., 1957). Only healthy, similarly sized individuals (weighing 200–300 g) were selected to minimize variations due to size, age, or sex (Topic Popovic, N., et. al., 2012).

Blood Sample Collection and Hematological Analysis: Fish were anaesthetized with tricainemethanesulfonate (MS-222) to reduce stress before blood collection (Mohammed, H. H. A., 2018). Blood was drawn from the caudal vein using heparinized syringes (Sahastrabuddhe, A. P., 2016). Hematological parameters such as red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, PCV, MCV, and MCHC were analyzed using standard fish hematology protocols (Berman, L., 1919). RBC and WBC counts were measured using a Neubauer hemocytometer under a compound microscope (Sahastrabuddhe, A. P., 2016). Hemoglobin content was determined using

Sahli's acid hematin method (Berman, L., 1919).

Statistical Analysis :All data were statistically analyzed using SPSS software (Version 25.0) (Wang, T., Zhang, G., & Zhou, J., 2021, April). Results were expressed as mean $\pm$ standard deviation (SD) (Baldo, V., *et. al.*, 2025) One-way ANOVA was employed to compare hematological parameters among fish from different sites, followed by Duncan's Multiple Range Test (DMRT) for post-hoc comparisons (Mbokane, E. M., & Moyo, N. A., 2018). A significance level of $p < 0.05$ was considered statistically meaningful (Kwak, S., 2023).

Results and Discussion:

Heavy Metal Concentration in Water:

The concentrations of heavy metals (Fe, Cd, Pb, Ni and Hg) in the Kharun River water at different sampling sites showed considerable spatial variation. The meeting point and downstream sites exhibited significantly higher levels of heavy metals compared to the upstream site (control). Iron concentration range from 0.05 to 0.10 mg/L, cadmium from 0.01 to 0.08 ,Lead

concentrations ranged from 0.12 to 0.35 mg/L, mg/L, Nickel from 0.05 to 0.21 mg/L, and mercury from 0.002 to 0.011 mg/L. These values exceeded the permissible limits prescribed by the Bureau of Indian Standards (BIS, 2012)&CPCB (Central Pollution Control Board) for inland surface waters.

This pattern of contamination is indicative of anthropogenic inputs such as domestic sewage, industrial effluents, and agricultural runoff. The bioavailability and toxicity of these metals in aquatic environments are influenced by multiple factors such as pH, temperature, and the presence of organic matter, all of which can increase the rate of absorption by aquatic organisms (Tchounwou et al., 2012). Similar hematological alterations—including reductions in Hb, RBC, and PCV along with elevated WBC have been documented in earlier studies on *Channa striata* exposed to Kharun River pollution (Pandey, 2024; Pandey & Mishra, 2025b). These findings align with broader reviews showing that heavy metal exposure induces anemia, oxidative stress, and immunosuppression in fish (Pandey et al., 2024).

Table No. 01 :Seasonal Variations in Haematological Parameters of Fish *Channa striata* Collected from Upstream, Meeting Point, and Downstream Sites of the River–Canal System (Mean $\pm$ SD, n = 6).

Parameter	Season	Upstream (Mean $\pm$ SD)	Meeting Point (Mean $\pm$ SD)	Downstream (Mean $\pm$ SD)	F-value	p-value
Hb (g/dL)	Summer	13.2 $\pm$ 0.6	11.0 $\pm$ 0.5	9.4 $\pm$ 0.4	15.72	0.003*
	Monsoon	12.8 $\pm$ 0.7	10.7 $\pm$ 0.6	9.1 $\pm$ 0.5	13.56	0.004*
	Winter	14.0 $\pm$ 0.5	11.8 $\pm$ 0.6	9.9 $\pm$ 0.4	18.43	0.002*
RBC ($\times 10^6/\text{mm}^3$)	Summer	3.12 $\pm$ 0.3	2.46 $\pm$ 0.2	1.98 $\pm$ 0.2	14.27	0.004*
	Monsoon	3.05 $\pm$ 0.2	2.38 $\pm$ 0.3	1.85 $\pm$ 0.3	13.18	0.005*
	Winter	3.25 $\pm$ 0.3	2.54 $\pm$ 0.2	2.00 $\pm$ 0.2	17.21	0.003*
WBC ($\times 10^3/\text{mm}^3$)	Summer	4.8 $\pm$ 0.5	6.3 $\pm$ 0.7	8.1 $\pm$ 0.8	16.07	0.003*

	Monsoon	5.1 ± 0.6	6.8 ± 0.8	8.4 ± 0.9	18.33	0.002*
	Winter	4.5 ± 0.4	6.1 ± 0.5	7.7 ± 0.8	14.56	0.004*
PCV (%)	Summer	42.5 ± 3.2	36.3 ± 2.9	30.8 ± 2.6	11.39	0.006*
	Monsoon	41.8 ± 3.0	35.5 ± 3.1	30.1 ± 2.8	10.72	0.007*
	Winter	43.2 ± 3.5	37.2 ± 2.8	31.5 ± 2.5	12.04	0.005*
MCV (fL)	Summer	112.3 ± 5.6	104.8 ± 4.8	96.5 ± 5.2	9.84	0.008*
	Monsoon	111.0 ± 5.8	103.2 ± 5.4	95.1 ± 4.9	10.25	0.007*
	Winter	113.5 ± 5.3	106.1 ± 4.7	97.3 ± 4.5	9.56	0.008*
MCHC (g/dL)	Summer	31.8 ± 1.9	28.2 ± 1.7	24.9 ± 1.5	13.62	0.004*
	Monsoon	30.9 ± 2.0	27.8 ± 1.8	25.2 ± 1.4	12.91	0.005*
	Winter	32.1 ± 1.8	28.9 ± 1.6	25.8 ± 1.3	14.11	0.004*

Hematological Responses in *Channa striata*: Exposure to elevated heavy metal concentrations resulted in significant alterations in the hematological profile of *Channa striata*. The results are summarized in Table 1. Fish collected from contaminated sites displayed the following hematological abnormalities:

1. Reduction in Hemoglobin (Hb)

Concentration: Fish from highly contaminated sites showed a marked decrease in hemoglobin concentration (7.1 ± 0.3 g/dL) compared to those from the control site (11.4 ± 0.4 g/dL) [From, Table No. 01]. This reduction may be due to the inhibition of heme synthesis, destruction of erythrocytes, or impaired iron metabolism under metal toxicity stress (Rajan & Banerjee, 2011). Cadmium and lead are particularly known to interfere with the enzymes involved in hemoglobin synthesis, leading to hypochromic anemia (Authman et al., 2015).

2. Decrease in Red Blood Cell (RBC) Count:

A significant decrease in RBC count was observed in fish from polluted sites. RBC counts dropped from $2.4 \pm 0.2 \times 10^6/\text{mm}^3$ in control fish

to $1.1 \pm 0.1 \times 10^6/\text{mm}^3$ in fish from urban sites [From, Table No. 01]. This anemia could be attributed to hemolysis, impaired erythropoiesis, or shortened erythrocyte lifespan under oxidative stress induced by heavy metals (Adhikari et al., 2004; Banaee et al., 2008).

3. Increase in White Blood Cell (WBC) Count:

Conversely, WBC counts increased significantly in fish from polluted areas, suggesting an immune response or leukocytosis due to chronic stress. WBC levels reached $15.2 \pm 0.6 \times 10^3/\text{mm}^3$ in contaminated fish compared to $8.7 \pm 0.4 \times 10^3/\text{mm}^3$ in the control group [From, Table No. 01]. Elevated WBC counts reflect the activation of the immune system as a defense mechanism against toxic insult or secondary infections (Fazio et al., 2013).

4. Packed Cell Volume (PCV%):

The packed cell volume (PCV) values exhibited a clear decreasing trend from upstream to downstream across all seasons. The highest PCV values were recorded upstream (Summer: $42.5 \pm 3.2\%$, Monsoon: $41.8 \pm 3.0\%$, Winter:

43.2 $\pm$ 3.5%), while the lowest occurred downstream (Summer: 30.8 $\pm$ 2.6%, Monsoon: 30.1 $\pm$ 2.8%, Winter: 31.5 $\pm$ 2.5%) [From, Table No. 01]. The meeting point values were intermediate between these two sites. A one-way ANOVA revealed significant seasonal differences ($F = 11.39$ – 12.04 ; $p < 0.01$), indicating that hematocrit levels were markedly reduced downstream, likely reflecting erythrocyte destruction or reduced oxygen-carrying capacity due to pollutant exposure, while relatively stable values upstream suggest better water quality and normal hematological status. (Blaxhall & Daisley, 1973).

5. **Mean Corpuscular Volume (MCV, fL):** The mean corpuscular volume also showed a progressive decline downstream, from 112.3 $\pm$ 5.6 fL (upstream, summer) to 96.5 $\pm$ 5.2 fL (downstream, summer), with similar trends during monsoon and winter ($F = 9.56$ – 10.25 ; $p < 0.01$) [From, Table No. 01]. This reduction in MCV suggests that the erythrocytes became smaller (microcytic) in fish collected from the downstream region. Such variation is often associated with chronic exposure to sub-lethal concentrations of heavy metals, leading to impaired hemoglobin synthesis and disruption in erythropoiesis.
6. **Mean Corpuscular Hemoglobin Concentration (MCHC, g/dL):**

The MCHC values followed a pattern similar to PCV and MCV, showing a significant reduction downstream (Summer: 31.8 $\pm$ 1.9 $\rightarrow$ 24.9 $\pm$ 1.5 g/dL; Monsoon: 30.9 $\pm$ 2.0 $\rightarrow$ 25.2 $\pm$ 1.4 g/dL; Winter: 32.1 $\pm$ 1.8 $\rightarrow$ 25.8 $\pm$ 1.3 g/dL) [From, Table No. 01]. Statistical analysis confirmed strong significance ($F = 12.91$ – 14.11 ; $p < 0.01$). The lower MCHC values in downstream fish indicate hypochromic anemia, likely caused by metal-induced inhibition of hemoglobin formation or iron metabolism.

Discussion: The present investigation revealed significant alterations in the haematological profile of *Channa striata* exposed to heavy metal contamination in the River Kharun, indicating physiological stress and hematopoietic disruption. Parameters such as haemoglobin concentration (Hb), red blood cell count (RBC), and packed cell volume (PCV) showed a marked decline ($p < 0.05$) in fish collected from polluted sites compared to control specimens [From, Graph No. 02]. In contrast, white blood cell count (WBC) exhibited an increase, reflecting an immune response to toxic stress. These changes are consistent with findings from both regional and global studies, demonstrating that haematological indices serve as sensitive biomarkers of metal-induced stress in fish. Regionally, similar trends were observed by Adhikari et al. (2004) in *Labeo rohita* exposed to cadmium and lead, where reductions in Hb and RBC were attributed to haemolysis and impaired erythropoiesis. Pandari Reddy and Sunitha Devi (2019) also

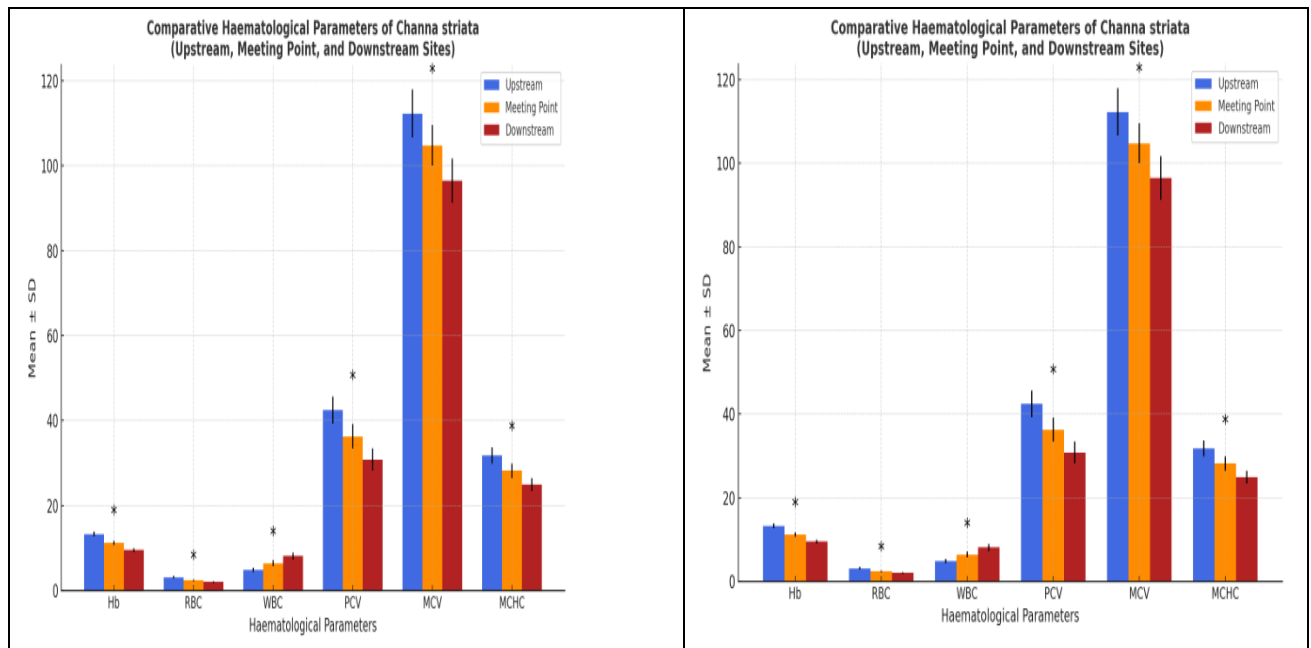
reported comparable declines in *Channapunctatus* exposed to chromium and nickel, suggesting disruption of oxygen transport due to cellular membrane damage. The results of the present study agree with these observations, highlighting that metal bioaccumulation in Indian freshwater systems leads to physiological alterations across species. Globally, Witeska et al. (2010) demonstrated similar haematological suppression in *Cyprinus carpio* exposed to lead, linking it to oxidative injury and inhibition of haemoglobin synthesis. Fazio et al. (2013) also found significant declines in Hb and erythrocyte counts in *Mugil cephalus* subjected to environmental stress, indicating that such responses are not species-specific but represent a general physiological adaptation to pollutant exposure. Moreover, Authman et al. (2015) documented haematological impairment in *Oreochromis niloticus* inhabiting metal-polluted Egyptian waters, confirming that fish from diverse geographic regions exhibit convergent haematological responses under heavy metal exposure.

The elevation in WBC observed in the current study reflects immune activation, a mechanism often reported in fish exposed to sublethal metal concentrations (Adeyemo, 2007; Vinodhini & Narayanan, 2009). The increase in leukocyte levels likely represents an adaptive response to counteract the stress-induced tissue damage and maintain homeostasis. Similarly, reductions in MCV and MCHC may indicate microcytic anaemia resulting from impaired iron metabolism or erythrocyte destruction (Gill & Eppler, 1993). The consistency of these findings with international studies reinforces the utility of haematological parameters as reliable indicators of environmental stress. Collectively, the results suggest that chronic exposure to heavy metals such as cadmium, lead, and zinc disrupts the oxygen-carrying capacity and immune competence of freshwater fish. These alterations may compromise the survival, growth, and reproductive fitness of *Channa striata*, emphasizing the urgent need for biomonitoring and effective regulation of industrial effluents in Indian aquatic ecosystems.

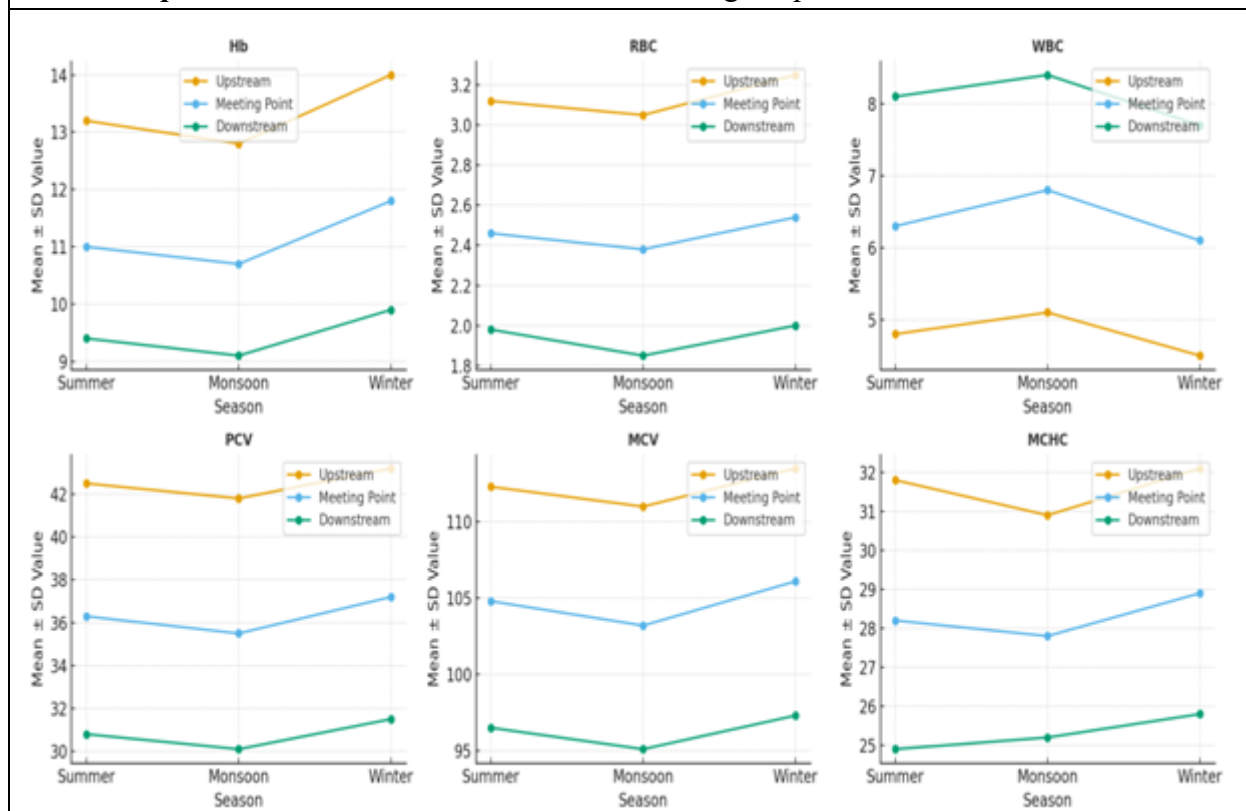
Graph No. 01

(A) A professionally formatted bar graph showing the comparative haematological parameters (Mean $\pm$ SD)

(B) *Channa striata* from Upstream (blue), Meeting Point (orange), and Downstream (red) sites.



Graph No. 02: Seasonal variation of haematological parameter at different sites.



Conclusion: This study provides clear evidence that exposure to heavy metal-contaminated water induces hematological disturbances in *Channa striata*. Declines in Hb, RBC, PCV, MCV, and MCHC,

coupled with elevated WBC counts, indicate anemia and immune stress. These parameters can serve as sensitive biomarkers for early detection of metal-induced toxicity in aquatic ecosystems.

Continuous biomonitoring and stringent regulation of industrial effluents are strongly recommended for the conservation of freshwater biodiversity in Chhattisgarh.

Ethical Considerations: All procedures involving fish handling and sampling were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC), and efforts were made to minimize stress and suffering of experimental animals (CPCSEA, India).

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