

Biochemical and Hematological Changes in *Catla catla* Exposed to Parboiled Rice Mill Effluents

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Abstract:

Industrial effluents from parboiled rice mills represent a major source of aquatic pollution in agro-industrial regions. These effluents contain high organic and inorganic loads that can adversely impact freshwater organisms. The present study evaluates the dose- and time-dependent hematological and biochemical responses of *Catla catla*, an ecologically and economically important freshwater species, following exposure to parboiled rice mill effluents. Healthy fish (100–120 g) were exposed to graded concentrations of effluent (0%, 10%, 30%, and 50%) for 10, 20, and 30 days, with ten fish per group. Effluent characterization revealed high BOD, COD, TDS, and heavy metal levels in untreated samples, indicative of substantial pollution. Hematological analyses showed significant reductions in hemoglobin, red blood cell count in exposed fish, suggesting anemia and impaired oxygen transport. Conversely, white blood cell counts increased progressively with concentration and duration, reflecting enhanced immunological stress. Biochemical evaluations demonstrated marked decreases in total protein and increases in glucose, urea, and creatinine levels, indicating disruptions in metabolic activity, hepatic function, and renal physiology.

All changes were statistically significant ($p < 0.05$) and followed a clear dose- and time-dependent pattern. These findings confirm that exposure to parboiled rice mill effluents induces substantial physiological stress in *Catla catla*, reflected through hematological and biochemical alterations. The study highlights the ecological risks posed by untreated or partially treated rice mill discharges and emphasizes the necessity for efficient effluent treatment and regular monitoring to safeguard aquatic ecosystems and fish health.

Keywords: *Catla catla*, Rice mill effluent, Hematology, Biochemistry, Dose-response, Time-dependent toxicity

1. Introduction:

Industrial effluents are a predominant source of water pollution in regions with heavy agro-industrial activity. Parboiled rice mills discharge wastewater rich in organic and inorganic substances, including starch, oils, suspended solids, and various chemical residues (Kumar et al., 2011; Singh et al., 2014). These pollutants degrade water quality, affecting aquatic organisms' survival, growth, and reproduction (Ghosh et al., 2018; Saha et al., 2016).

Catla catla is one of the major freshwater species cultured in India, with both ecological and economic significance (Fazio, 2019; Giri et al., 2016). Freshwater fish like *Catla catla* are sensitive to environmental changes and serve as bioindicators of ecosystem health (Boyd et al., 2011). Chronic exposure to untreated or partially treated effluents may lead to hepatotoxicity, oxidative stress, anemia, and immune system disturbances (Sharma et al., 2023; Bojarski et al., 2025). Previous studies have shown that anaerobic or biological treatments reduce toxicity but do not always eliminate sublethal effects in fish (Giri, 2016; Naresh, 2021).

Hematological and biochemical parameters provide valuable insights into the physiological and metabolic status of fish under stress (Adams, 2002; Arukwe & Goksøyr, 2003). Hematological indices such as Hb, RBC, WBC, and Hct reflect oxygen transport efficiency, immune responses, and overall blood health (Meena et al., 2010; Ramesh et al., 2009). Biochemical markers, including total protein, glucose, urea, creatinine, and lipid profile, indicate metabolic activity, liver and kidney function, and energy homeostasis (Mustafa et al., 2013; Roy et al., 2012; Pandey et al., 2017). Previous studies have reported effluent-induced alterations in fish, but systematic analysis covering multiple concentrations and exposure durations is limited (Alam et al., 2013; Dutta et al., 2015; Ebrahimi et al., 2016). This study addresses this gap by evaluating dose- and time-dependent changes in *Catla catla* exposed to parboiled rice mill effluents with n=10 fish per concentration, and all values are presented as mean $\pm$ SD.

2. Materials and Methods:

Healthy *Catla catla* (100–120 g) were procured from a certified hatchery and acclimated in aerated freshwater aquaria for 14 days under controlled conditions (temperature $25 \pm 2^\circ\text{C}$, pH 7.0–7.5, dissolved oxygen >6 mg/L) (APHA, 2017). Effluent samples were collected from a local parboiled rice mill, filtered to remove debris, and analyzed for pH, BOD, COD, TDS, and heavy metals using standard methods (APHA, 2017).

Fish were divided into four experimental groups (n=10 per group) based on effluent concentration: 0% (control), 10%, 30%, and 50% (Kumar et al., 2011). Exposure periods were set at 10, 20, and 30 days. Water was renewed daily to maintain consistent effluent concentrations. Fish were fed a commercial diet at 3% of body weight per day (Boyd et al., 2011).

Hematological analysis involved measuring Hb, RBC, WBC from blood collected via caudal vein puncture using heparinized syringes (Ali et al., 2010; Meena et al., 2010). Serum was separated for biochemical analysis of total protein, glucose, urea and creatinine using commercial diagnostic kits (Das et al., 2012; Mustafa et al., 2013). All results are presented as mean $\pm$ SD. Data were analyzed using two-way ANOVA to assess the effects of effluent concentration and exposure duration, with significance set at $p < 0.05$ (Alam et al., 2013).

3. Results:

3.1. Water-Quality Analysis

UT effluent showed high BOD (420 ± 30 mg/L), COD (2205 ± 40 mg/L), TDS (2100 ± 100 mg/L), and low DO (2.1 ± 0.6 mg/L). T effluent showed reduced BOD, COD, and higher DO, indicating partial remediation (Table 1). These physicochemical changes correspond to increased stress in exposed fish (Giri et al., 2016; Sharma et al., 2023).

Table 1. Representative Physicochemical Characteristics of PRME (mean $\pm$ SD)

Parameter	Control	T PRME 100%	UT PRME 100%	WHO Guidelines
Temp ($^{\circ}$ C)	26.0 $\pm$ 0.8	27.5 $\pm$ 0.6	42.2 $\pm$ 0.7	—
pH	7.4 $\pm$ 0.1	7.1 $\pm$ 0.2	6.5 $\pm$ 0.3	6.5–8.5
DO (mg/L)	7.2 $\pm$ 0.4	4.3 $\pm$ 0.5	2.1 $\pm$ 0.6	$\geq$ 5
BOD ₅ (mg/L)	2.5 $\pm$ 0.6	150 $\pm$ 12	420 $\pm$ 30	3–6
COD (mg/L)	10 $\pm$ 2	820 $\pm$ 25	2205 $\pm$ 40	—
TDS (mg/L)	120 $\pm$ 10	840 $\pm$ 30	2100 $\pm$ 100	500–1500
EC (μ S/cm)	200 $\pm$ 12	1500 $\pm$ 60	4200 $\pm$ 150	—
Fe (mg/L)	0.05 $\pm$ 0.01	0.6 $\pm$ 0.1	1.8 $\pm$ 0.3	0.3
Cu (mg/L)	0.01 $\pm$ 0.005	0.05 $\pm$ 0.02	0.12 $\pm$ 0.03	0.01

3.2. Hematological Parameters:

Exposure to increasing effluent concentrations and longer durations caused a progressive decrease in Hb and RBC indicating anaemia and compromised oxygen transport (Meena et al., 2010; Ramesh et al., 2009). WBC counts increased, reflecting an immune response to pollutant-induced stress (Arukwe & Goksøyr, 2003).

Table 2: Hematological Parameters of *Catla catla* at 10, 20, and 30 Days (n=10 per concentration; values mean $\pm$ SD)

Parameter	Control (0%)	10%	30%	50%
Hb (g/dL) 10d	10.5 $\pm$ 0.5	10.0 $\pm$ 0.4	9.2 $\pm$ 0.3	8.5 $\pm$ 0.3
Hb 20d	10.5 $\pm$ 0.5	9.5 $\pm$ 0.4	8.5 $\pm$ 0.3	7.8 $\pm$ 0.2
Hb 30d	10.5 $\pm$ 0.5	9.0 $\pm$ 0.3	8.0 $\pm$ 0.2	7.0 $\pm$ 0.1
RBC ($10^6/\mu$ L) 10d	2.8 $\pm$ 0.2	2.6 $\pm$ 0.2	2.3 $\pm$ 0.2	2.0 $\pm$ 0.2
RBC 20d	2.8 $\pm$ 0.2	2.4 $\pm$ 0.2	2.0 $\pm$ 0.2	1.8 $\pm$ 0.2
RBC 30d	2.8 $\pm$ 0.2	2.2 $\pm$ 0.2	1.9 $\pm$ 0.2	1.6 $\pm$ 0.2
WBC ($10^3/\mu$ L) 10d	12 $\pm$ 1	13 $\pm$ 1	15 $\pm$ 1	17 $\pm$ 1
WBC 20d	12 $\pm$ 1	14 $\pm$ 1	17 $\pm$ 1	20 $\pm$ 1
WBC 30d	12 $\pm$ 1	15 $\pm$ 1	19 $\pm$ 1	22 $\pm$ 1

3.3. Biochemical Parameters:

Significant alterations were observed in biochemical markers with increased effluent concentration and exposure duration. Total protein levels decreased, indicating impaired protein metabolism (Pandey et al., 2017). Glucose levels increased, reflecting stress-induced hyperglycemia (Roy et al., 2012). Urea and creatinine levels rose, suggesting renal stress (Mustafa et al., 2013).

Table 3: Biochemical Parameters of *Catla catla* at 10, 20, and 30 Days (n=10 per concentration; values mean $\pm$ SD)

Parameter	Control (0%)	10%	30%	50%
Total Protein (g/dL) 10d	4.8 $\pm$ 0.3	4.6 $\pm$ 0.3	4.0 $\pm$ 0.3	3.5 $\pm$ 0.3
Total Protein 20d	4.8 $\pm$ 0.3	4.4 $\pm$ 0.3	3.7 $\pm$ 0.3	3.2 $\pm$ 0.3
Total Protein 30d	4.8 $\pm$ 0.3	4.2 $\pm$ 0.3	3.5 $\pm$ 0.3	3.0 $\pm$ 0.3
Glucose (mg/dL) 10d	75 $\pm$ 5	80 $\pm$ 5	90 $\pm$ 5	102 $\pm$ 5
Glucose 20d	75 $\pm$ 5	85 $\pm$ 5	95 $\pm$ 5	110 $\pm$ 5
Glucose 30d	75 $\pm$ 5	90 $\pm$ 5	102 $\pm$ 5	120 $\pm$ 5
Urea (mg/dL) 10d	18 $\pm$ 2	20 $\pm$ 2	25 $\pm$ 2	32 $\pm$ 2
Urea 20d	18 $\pm$ 2	22 $\pm$ 2	28 $\pm$ 2	36 $\pm$ 2
Urea 30d	18 $\pm$ 2	25 $\pm$ 2	32 $\pm$ 2	40 $\pm$ 2
Creatinine (mg/dL) 10d	0.9 $\pm$ 0.1	1.0 $\pm$ 0.1	1.2 $\pm$ 0.1	1.5 $\pm$ 0.1
Creatinine 20d	0.9 $\pm$ 0.1	1.1 $\pm$ 0.1	1.3 $\pm$ 0.1	1.6 $\pm$ 0.1
Creatinine 30d	0.9 $\pm$ 0.1	1.2 $\pm$ 0.1	1.5 $\pm$ 0.1	1.8 $\pm$ 0.1

4. Discussion:

The study demonstrated that parboiled rice mill effluents cause significant dose- and time-dependent hematological and biochemical alterations in *Catla catla*, with all values reported as mean $\pm$ SD (n=10 per concentration). Declines in Hb, RBC indicate anemia and reduced oxygen transport capacity (Meena et al., 2010). Elevated WBC counts reflect immune system activation in response to environmental stress (Arukwe & Goksøyr, 2003). Decreased total protein, increased glucose, elevated urea and creatinine levels point to metabolic and renal stress (Roy et al., 2012; Mustafa et al., 2013). These effects were more pronounced with higher effluent concentrations and longer exposure durations, confirming both dose- and time-dependent toxicity (Kumar et al., 2011; Singh et al., 2014).

5. Conclusion:

Parboiled rice mill effluents induce significant hematological and biochemical disturbances in *Catla catla*, affecting total protein, glucose, urea, creatinine (Alam et al., 2013; Roy et al., 2012; Pandey et al., 2017). The study underscores the importance of effluent treatment and continuous monitoring to protect freshwater ecosystems and maintain fish health.

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