



## Histopathological alterations in the RBC of *Mystus vittatus* (Bloch) exposed to distillery effluent

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### Abstract

Toxicological effect of distillery effluent in the catfish, *Mystus vittatus* was investigated in the present study. Distillery effluent discharged from distillery unit of Balrampur Chini mill, Balrampur, U.P. was collected and healthy test fish of 7.0-8.0 cm in length and weighting 8.0-9.0 gm were collected from local fresh waterbodies were exposed to a sublethal concentrations (2.5% and 3.0% of LC<sub>50</sub> value i.e., 3.38 %) of the effluent for a period of 15 and 30 days. The histological changes in the RBC morphology in the effluent treated fish and the control group were compared. There was significant reduction in the mean length, breadth and area of erythrocytes in distillery effluent exposed fish. The study revealed marked changes such as membrane disintegration, Anisocytosis, poikilocytosis, double nuclei and rouleaux development, etc. are noticed in the blood tissues of the effluent exposed fish.

**Keywords:** *Mystus vittatus*, histopathology, RBC, distillery effluent, blood smear

### Introduction

Freshwater fish are an important source of animal protein for the human beings. They are adversely affected by aquatic pollutants such as industrial effluents, domestic waste and pesticides. Human activity-induced environmental degradation has made it necessary to develop and improve environmental quality methods. Freshwater sources are polluted with a variety of pollutants as a result of urban, industrial, and agricultural activity, which affects the biota that live there. There is a global tendency to study the chemical and physical parameters as biomarkers of aquatic pollution in order to assess the negative impact of these complex chemical mixes on aquatic organisms (Van der Oost *et al.* 2003; Au 2004) [14, 1].

Histopathological studies can provide information about the health and functionality of organs. Due to the fact that many pollutants require metabolic activation before they may cause cellular change in the affected organism. Such biomarkers could identify recent or long-term exposure to toxins and make it easier to detect fish physiological responses, resulting in a more accurate diagnosis for assessing the health of the ecosystem (Silva *et al.* 2009) [11].

Histopathological biomarkers can be used to predict the impact of various anthropogenic contaminants on aquatic organisms and represent the general health of the entire freshwater ecosystem (Prakash and Verma, 2021) [7]. The amount of the damage must therefore be determined by carefully examining the cellular changes in structure caused by industrial effluent in various fish organs or tissues.

Hematological parameters are important tool in diagnosing the health status of fish exposed to pollutants (Verma and Prakash, 2019a; Prakash and Verma, 2020a) [15, 6]. The blood is one of the connective tissues of the body and entry of any pollutant can be detected by analyzing the blood (Shastri and Gupta, 1994) [10], so histology of blood cells may provide a specific indication of changes within the environment of the test fish. Anisocytosis, poikilocytosis, pyknosis, necrosis, roulex development, etc., are the most frequently noticed abnormalities in blood cells (Rand and Petrocelli, 2003) [8]. A number of researchers including

Prakash and Verma (2019, 2020b) [4, 5] and Verma and Prakash (2019b) [16] worked a lot on various aspects of this fish but quality research on RBCs is still unexplored. Therefore, in the present study an attempt has been made to assess the cellular changes and damages through histopathological studies on RBC of distillery effluent exposed fish, *Mystus vittatus*.

### Materials and methods

Healthy *Mystus vittatus* weighing 8.0–9.0 gm and measuring 7.0–8.0 cm in length were collected from local freshwater bodies of Balrampur and washed with 1% KMnO<sub>4</sub> solution for five minutes to remove dermal infection and then transferred to a plastic jar filled with 50L of dechlorinated tap water for acclimatization. Fish were exposed to laboratory condition for 15 days at room temperature while being fed commercial fish feed every day.

Effluent sample was collected from distillery unit of Balrampur Chini mill Balrampur, U.P. India. The LC<sub>50</sub> values of distillery effluent for *Mystus vittatus* at 96 hours was 3.38 % (v/v) (Tiwari and Prakash, 2021) [12]. Based on 96 LC<sub>50</sub>, fish were exposed to sublethal concentrations (2.5% and 3.0% v/v) for the period of 15 and 30 days. A control group was maintained in an identical environment.

Forty-five healthy fishes were selected from the stock for sublethal exposure study. Two experimental groups and one control group were considered. Each group consisted of 15 fishes. The fish were regularly feed once a day in amounts 2% of their total body with commercial food and the medium was changed once in every two days to maintain the concentration of effluent and also to remove faeces and food remnants.

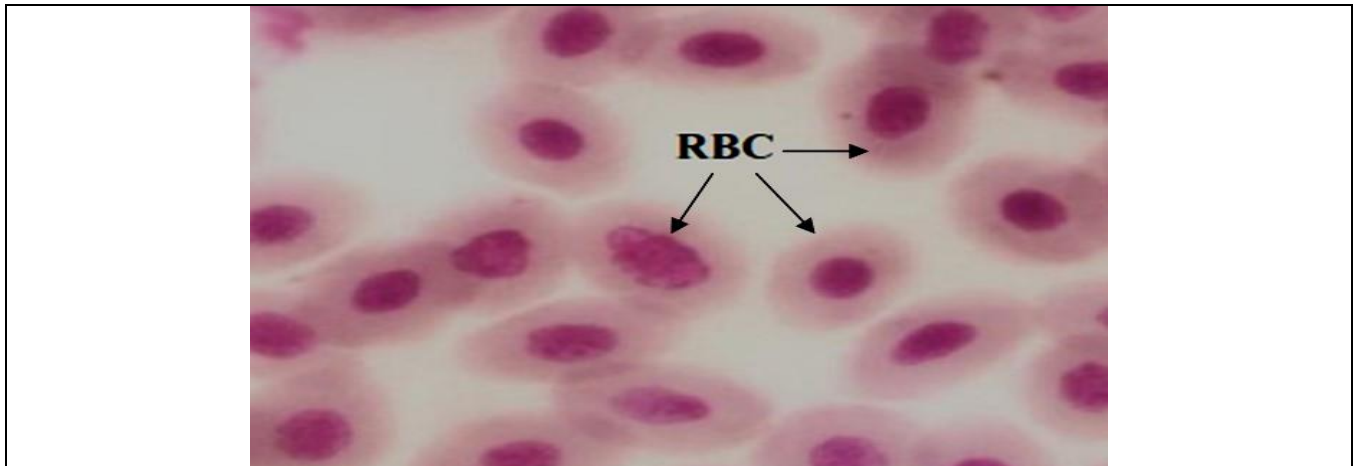
After 15 and 30 days of exposure periods, five fishes were undertaken for each concentration and control group. The blood from control and experimental test fishes were taken from caudal vein and prepare the blood smear then stained with Leishman stain to be evaluated under light microscope later. Once stained, all slides were mounted with DPX.

**Result and discussion**

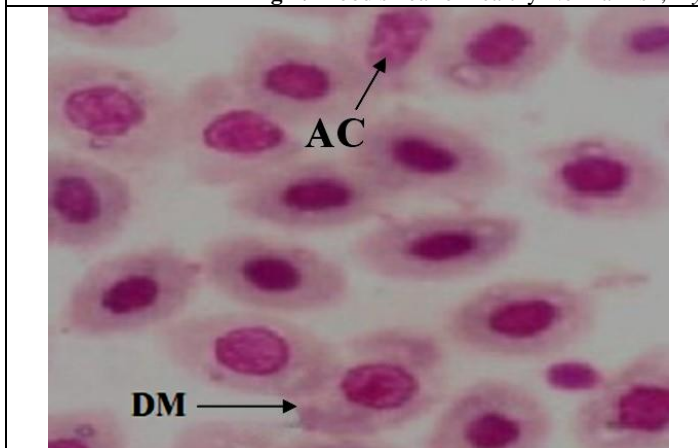
Blood is a connective tissue, and histological studies of this tissue are crucial for understanding how pollution affects it. Blood is a connective tissue, and histological studies of this tissue are crucial for understanding how pollution affects it. The control fish considered showed normal histological structure of different blood corpuscles and their components. The blood smear of fish under control showed

mature normal structure and number of red blood corpuscles (RBC) (Figure.1).

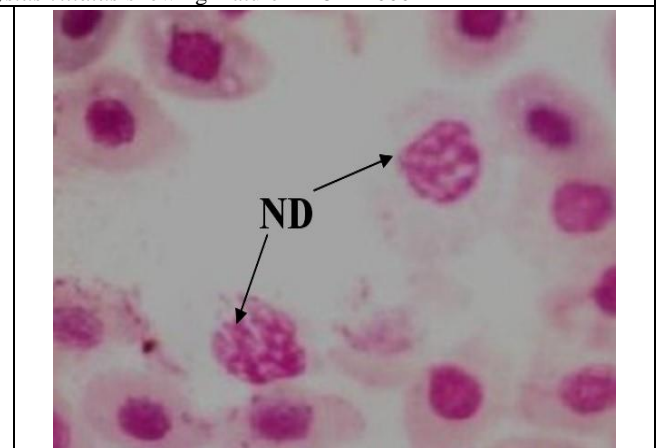
It was noticed that the morphology of RBC of fish exposed to distillery effluent showed deformation. In experimental fishes the histological slide of blood smear showed abnormalities in the shape, size and nucleus of RBC (Figure. 2-5). Due to effluent toxicity, the shape of RBC changed. Similar alterations were observed in paper mill effluent exposed roach fishes (Jeney *et al.*, 1996) [3].



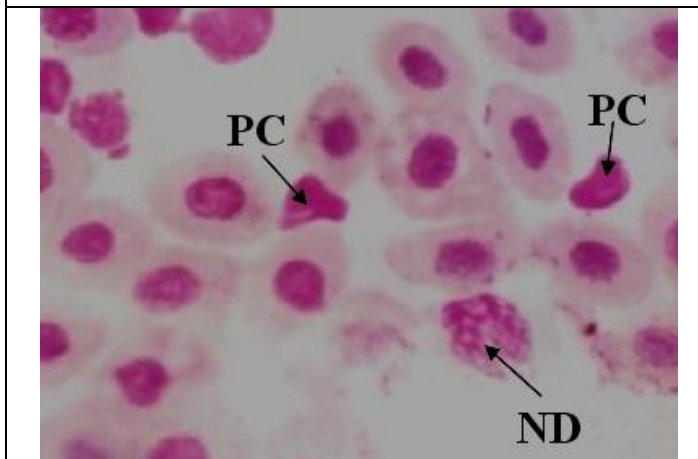
**Fig 1:** Blood smear of healthy Normal fish, *Mystus vittatus* showing mature RBC X 1000



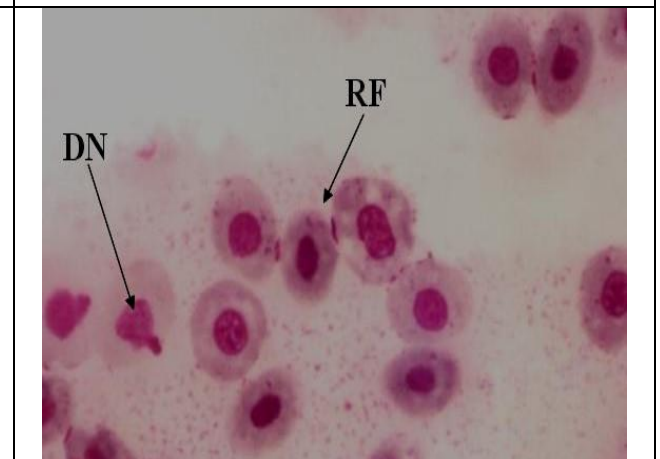
**Fig 2:** Blood smear of 15 days exposed fish at 2.5 mg/l concentration of distillery effluent showing membrane disintegration (DM) and Anisocytosis (AC) i.e., unequal size of RBC. X 1000



**Fig 3:** Blood smear of 15 days exposed fish at 3.0 mg/l concentration of distillery effluent showing Nucleus disintegration (ND) of RBC. X 1000



**Fig 4:** Blood smear of 30 days exposed fish at 2.5 mg/l concentration of distillery effluent showing Nucleus disintegration (ND) and Poikilocytosis (PC) i.e., abnormal shape RBC Nucleus. X 1000



**Fig 5:** Blood smear of 30 days exposed fish at 3.0 mg/l concentration of distillery effluent showing double nuclei (DN) and Rouleaux formation (RF) of RBC. X 1000

The abnormal conditions such as, membrane disintegration (DM), anisocytosis (AC), nucleus disintegration (ND) and poikilocytosis (PC) were noticed in both sublethal concentrations of distillery effluent exposed *Mystus vittatus* for 15 and 30 days. Presence of double nuclei (DN) in which one small and another normal nucleus and rouleaux formation (RF) was observed in 3.0% concentrations of distillery effluent exposed fishes for 30 days (Figure 2-5). Presence of double nuclei in which one small and another normal nucleus is not definitively known what effect these anomalies have on the organism.

Presence of double nuclei (DN) in which one small and another normal nucleus and Rouleaux formation (RF) was observed in 3.0% concentrations of distillery effluent exposed fishes for 30 days. Similar observation was observed in metal exposed catfish, *Clarias batrachus* (Ranu *et al.*, 2006) <sup>[9]</sup>. Presence of double nuclei in which one small and another normal nucleus is not definitively known what effect these anomalies have on the organism.

The decrease in erythrocyte surface area (Poikilocytosis) indicates that fish body tissues are under-oxygenated as a result of damage to the gill tissue. The RBC of exposed fishes were shrunked due to distortions of their cell wall with crenated margins, and their nuclei hypertrophy (Jayaprakash and Shettu, 2013) <sup>[2]</sup>. The erythrocytes' evident distortion may have decreased their ability to carry oxygen, leading to a certain degree of hypoxia. Oxygen consumption was decreased to a significant level in distillery effluent exposed catfish, *Mystus vittatus* (Tiwari and Prakash, 2022) <sup>[13]</sup>. Thus, morphological changes in erythrocytes is a serious threat to freshwater fishes and that can be used as indicators of industrial effluent pollution related stress in fish (Witeska, 2004) <sup>[17]</sup>.

### Conclusion

The most sensitive indicators for assessing the toxicity of distillery effluent at sub-lethal concentrations are fish erythrocyte histological studies. Since fish are the most sensitive aquatic species, even a small change in their living environment may have an immediate impact on their physiology. In experimental fishes the histological assessment of blood smear showed abnormalities in the structural view of different types of blood cells. The abnormal conditions such as, anisocytosis (AC), poikilocytosis (PC), double nuclei (DN) and rouleaux formation (RF) were noticed in distillery effluent exposed fish. Therefore, it is suggested that the discharged of industrial effluent in the fresh waterbodies should be judicious and controlled.

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