Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Hematological, Immunohematological, and Biochemical Responses of Fish (Tor putitora) to Marble Factory Effluents

Usama Zahoor
Government Shaheed Abdul Majid Degree College BTK, affiliated with University Of Malakand

Ibad ur Rahman
Government Shaheed Abdul Majid Degree College BTK, affiliated with University Of Malakand

Ajmal Hussain
Research Supervisor and Assistant Professor at Government Shaheed Abdul Majid Degree College BTK, affiliated with University Of Malakand

Syed Hassan Ali Shah
Government Shaheed Abdul Majid Degree College BTK, affiliated with University Of Malakand

Muhammad Ilyas
Director Fisheries, Mahseer Fish Hatchery Thana, Malakand, KPK, Pakistan

Sajjad
Deputy Director Fisheries, Mahseer Fish Hatchery thana malankand,KPK, Pakistan

Talha
Department Of Biological Sciences,University Of Veterinary And Animal Sciences, Lahore. Email: ta357026@gmail.com

Abstract
Marble factory effluents are wastewater containing various toxic pollutants such as heavy metals (Pb, Cu, Cr, Ni, Zn), calcium carbonate ($CaCO_3$), Silica (SiO_2), Phosphates (PO_4^{3-}), Sulfates (SO_4^{2-}), lubricants, oils, and organic compounds along with suspended solids and fine particles.
In this study, the toxicity potential of soluble marble industry effluents was evaluated using fish, particularly *Tor putitora* (commonly known as Mahsheer), as the bioindicator. For 90 days, the effects were elevated on the chronic toxicity of environmentally relevant dosages of soluble marble effluents at 20, 30, and 50% concentrations. Hematological and biochemical analyses of cytotoxicity revealed a dose-response relationship. Compared to the control group, numerous changes were recorded in blood profiles and biochemical indicators in the effluent-exposed groups. RBCs, MCV, MCH, MCHC, Hemoglobin, platelets, and Hct values were substantially declined. Conversely, the Leukocyte counts were considerably enhanced, ranging from 100 to 220 **(**$\times 10^3/\mu L$). Alanine transaminase secretion was markedly elevated from 24 to 150 U/L at a greater concentration than the control, indicating liver injury. At the same time, changes

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

in biochemical markers, such as glucose, total protein, and triglycerides, displayed a biphasic trend. Spectrophotometric nitroblue tetrazolium reduction assay highlighted that respiratory burst activity elevated due to the dose of toxic marble chemicals, ultimately reducing immunity. This study is of utmost importance as it sheds light on the *Tor putitora*, which was neglected for many years in environmental assessments. It also highlights the adverse effects of marble factory chemicals on fish biochemistry and immunity, providing valuable biomarkers for ecological risk assessment of heavy metals in aquatic environments.

Keywords: Marble Factory Effluents, *Tor putitora*, Biochemical Parameters, Immunohematology, Hematology, Chronic Toxicity, RBCs, Leukocytes, Nitroblue tetrazolium assay, Heavy Metals, Biomakers, Environmental Toxicity.

Introduction

Water is a crucial resource for life on Earth, playing a vital role in ecological balance, biodiversity, climate regulation, and human survival. However, human actions such as industrialization, urbanization, and population growth have significantly contributed to environmental pollution. (Kutty & Al-Mahaqeri, 2016).

Among these, marble factory effluents pose a notable threat due to their complex composition, which includes heavy metals (Pb, Cd, Cr, Cu, Ni, Zn, Mn), suspended solids, and other toxic substances like arsenic, uranium, and barium. When discharged into aquatic ecosystems, these pollutants deteriorate water quality and cause ecological imbalances, harming the delicate balance of these ecosystems (Iqbal *et al.,* 2022; Khan *et al.,* 2023).

Liney et al. (2005) observed that Industrial effluents might change key parameters such as dissolved oxygen (D, change the pH to a level where fish cannot survive, and increase turbidity, creating an unsuitable environment for aquatic organisms. Furthermore, bioaccumulation and biomagnification of the heavy metals within the fish tissues are injurious to the fish and dangerous to predators and consumers such as human beings (Danovaro *et al.,* 2023).

Effluents from the marble industry introduce toxic pollutants that can cause oxidative stress in fish, forming reactive oxygen species (ROS). This leads to damage at the cellular level, including lipid peroxidation and DNA harm (Emon et al., 2023; Natalija et al., 2023). Furthermore, heavy metal exposure interferes with metabolic functions and disrupts important biochemical parameters such as glucose, cholesterol, and total protein levels, ultimately threatening the health and survival of fish (Singanan & Narayanan, 2008).

Hematological parameters serve as critical biomarkers of environmental pollution, indicating the physiological condition of fish subjected to contaminants (Fazio, 2019). Changes in hemoglobin levels, hematocrit, red and white blood cell counts, and other blood metrics reveal the degree of stress and toxicity from pollutant exposure. These changes can impair the immune system, resulting in heightened vulnerability to diseases and general physiological dysfunction within fish populations.

The marble industry in Pakistan is expanding, holding around 297 billion tons of granite and marble resources (SBI, 2010). It creates jobs and generates foreign exchange but also leads to significant environmental pollution (Mulk et al.,

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

2015). Untreated factory wastewater contaminates rivers in Khyber Pakhtunkhwa, especially in Thana (Khan et al., 2012). Poor wastewater treatment, weak regulations, and limited funding worsen the problem.

Tor putitora, the golden mahseer, is a significant game fish from the Cyprinidae family, valued ecologically and economically (Yousafzai et al., 2012; Mulk et al., 2016). It has been classified as endangered by the IUCN due to declining populations from pollution, habitat loss, and human activities (IUCN, 2011; Mulk et al., 2016; Khajuria & Langer, 2016). Its most significant threat comes from waste pollution in the marble industry, which risks the bioaccumulation of harmful metals, threatening both its survival and human health.

Although earlier studies have emphasized the harmful effects of industrial pollution on fish populations in various rivers of Pakistan (Mulk et al., 2015, 2016), but studies on the impact of marble factory effluents on *Tor putitora* are inadequate. Information about chronic exposure at environmentally relevant concentrations is still scarce, and additional investigations are required. This study aims to evaluate the hematological, immunohematological, and biochemical changes in *Tor putitora* exposed to marble factory effluents at Thana Fish Hatchery in Khyber Pakhtunkhwa. The findings will offer valuable insights into the extent of pollution-induced physiological stress in fish and will contribute to future efforts in environmental conservation and management.

Material and Methods

Samples of Experimental Fish (*Tor putitora*)

The Government Thana Fish Hatchery provided healthy 36 *Tor putitora*, averaging 20 grams in weight. The specimens were kept in experimental tanks measuring 4 × 2 × 1.5 ft.

Acclimatization of Fish

For 14 days, the fish were acclimated to the lab environment before experimenting and fed high-quality commercial food pellets specially designed for juvenile mnivorous fish. The pellets contained a substantial percentage of animal-based proteins such as fish, shrimp, and krill meal daily, as shown in Fig X. The pellet also included plant-based ingredients like soybeans and wheat but in smaller proportions. *Tor putitora* fingerlings were also used in a study that showed that a 45% protein diet was the optimum for growth and production (Ullah *et al.,* 2021). After relocating to the laboratory, the experimental fish' morphometric parameters were established instantly.

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

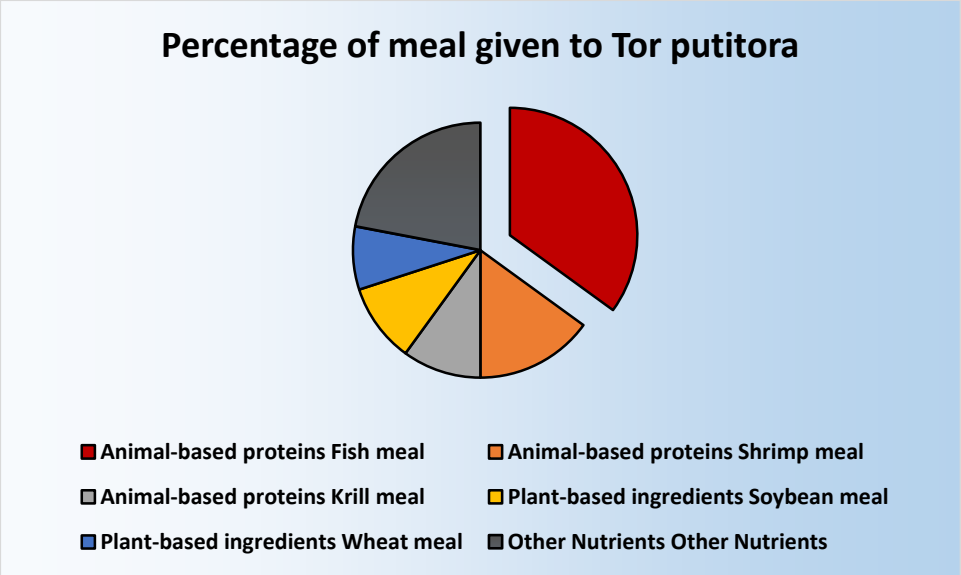

Fig X shows the percentage of various sorts of meal given to *Tor putitora* during experimental period.

Water Chemistry of Fish Tanks

Following the standard OECD guideline 203 (OECD, 1992), the physicochemical properties of experimental tanks and the water at the Thana fish hatchery were examined. pH and temperature were analyzed using a multiparameter analyzer, Consort C1020. Dissolved oxygen was calculated via the Winkler procedure; however, titration practice was employed for measuring total hardness. Fish were provided with tap water to prevent tissue or organ damage.

Experimental Design

There were four experimental tanks. Each had a capacity of 180 Liters of water. The first tank had 20, the second had 30, and the third had 50% soluble marble factory effluents. At the same time, the fourth remained entirely 180 L (100%) of tap water as a control tank. Air stones were distributed throughout all experimental setup tanks, and both tap water and soluble marble factory effluents were renewed daily throughout the 90-day exposure period. The random selection technique divided 36 healthy *Tor putitora* exhibiting the specified morphometric traits (Table 01) and was assigned to experimental and control groups. 9 fish in each tank were exposed to soluble marble factory effluents for 3 months at 20, 30, and 50% of the following environmentally relevant concentrations. Fish toxicity was assessed by analysis according to OECD guidelines 204 (OECD, 1984). During the 90-day experiment, no fish mortality was observed.

Table 01

Morphometric Characteristics of Experimental Fish

Fish Species	Total Length (cm)	Total Weight	Age (Months)
Mahsheer (*Tor putitora*)	7 ± 0.3	20 ± 0.2	2

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Chemical Used
All the chemical pollutants (soluble marble factory effluents) were brought daily from a nearby marble factory near River Swat in Malakand.

Assessment of effluents
The marble factory effluents were assessed using an ICP-OES spectrometer (Inductively Coupled Plasma Optical Emission Spectroscopy), an extremely sensitive and precise method for detecting and quantifying heavy metals in various samples.

Biochemical Analysis
Biochemical analysis of fish blood samples assessed the toxic effects of marble factory effluents, following Iftikhar and Hashmi (2021). The indices studied were glucose, triglycerides, total protein, and ALT. Blood samples were collected using syringes with gel activators for serum preparation. Serum extraction involved centrifugation at 4,000 rpm for 10-20 minutes, followed by AMP Piccos II Chemistry analyzer analysis.

Respiratory Burst Activity (NBT assay)
An NBT reduction assay, with minor adjustments, was assessed to measure immunohematological alternations described by Zanuzzo et al. (2015). A 0.2% NBT solution and 0.1 mL of heparinized blood were incubated for 40 mins at room temperature. After adding 50 µL of the suspension to 1 mL of N, N-dimethylformamide, the mixture was centrifuged at 2,000 rpm for 10 min. The supernatant's optical density (OD) was measured at 540 nm using a UV-visible spectrophotometer. The blank involved the same steps and components, substituting distilled water for blood.

Hematological Parameters and Blood Samples
A blood sample test was conducted three times within 90 days of exposure to ascertain the impact of applied harmful chemical dosages on the hematological profile of exposed fish, following similar methodologies reported in previous studies (Haider *et al.,* 2014). Blood was collected in EDTA vials via the caudal vein with a heparinized syringe. Vials were shaken to mix anticoagulants. To prevent clots, samples were spun on the LABCON-Spo-MP3 shaker for 10-15 minutes at 300 rpm before hematological analysis. The Sysmex XP-100 analyzer tested for red blood cells (RBCs), white blood cells (WBCs), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelets.

Statistical analysis
The current study's results underwent a two-way analysis of variance (ANOVA) to assess significant differences between the control group and those exposed to marble effluents. A significance level of $p < 0.05$ was established to define critical differences.

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Results and Discussion

Physiochemical parameters of the experimental tank and lake water

To ascertain the water quality of the experimental tanks, specific parameters specified by OECD standards 203 for toxicity studies were examined (OECD, 1992). At the beginning of the experiment, the water quality was assessed and contrasted with the physicochemical values of the water from the Thana Fish Hatchery, where fish samples were taken. Table 2 illustrates the physicochemical analysis of water parameters. The results revealed that mean values of temperature and hardness (26.5 °C and 220 mg/L) were higher in the Thana Fish Hatchery water compared to the experimental tanks (21.5 °C and 210 mg/L). The Thana Fish Hatchery water's notable ($p < 0.05$) rise in temperature and hardness could be caused by a more significant local pollutant load from nearby communities, including household trash and agricultural runoff.

The significant ($p < 0.05$) rise in temperature and hardness in the Thana Fish Hatchery water may be attributed to the increased local pollutant load from surrounding areas, such as agricultural runoff and domestic waste. Local mineral deposits and agricultural activities in the region may also influence the increase in water hardness. Similar findings were reported by Malik et al. (2011), who noted that lake water quality deteriorated near populated areas, while water in less impacted sites remained relatively cleaner and free from organic contaminants.

Table 2: Physiochemical parameters of the experimental tanks and lake water (Thana Fish Hatchery)

	Parameters			
	Mean Values (minimum-maximum)			
	Temperature (°C)	**pH**	**Dissolved Oxygen (mg/L)**	**Hardness (mg/L)**
Experimental Tank	21.5 ± 2.5 (19.0–24.0)	7.2 ± 0.3 (6.8–7.7)	6.5 ± 1.1 (3.8–6.5)	210.0 ± 60.0 (150–270)
Thana Fish Hatchery	26.5 ± 3.0 (23.0–30.0)	7.7 ± 0.3 (7.3–8.1)	6.5 ± 1.2 (5.5–7.5)	220.0 ± 50.0 (190–270)
OECD Guidelines	20–24	6–8.5	80% of air saturation	10–250

Effect of Marble Factory Effluents on Hematological Profile

Alternation in hematological values has been frequently utilized to determine fish's health and physiological status (Blaxhall, 2006). It allows quick and efficient assessment of sub-acute toxicity of xenobiotics on target organs, evaluation of fish's pathophysiological status, and parameters to assist in identifying morphological and physiological or behavioral alternations in fish brought on by chemical contamination.

Figures 1-8 and Table X show variations in RBCs, Hb, WBCs, Platelets, MCV, MCH, MCHC, and HCT after various concentrations of soluble marble factory chemicals in specific tanks. The levels of RBCs, Hb, HCT, MCV, MCH, MCHC, and Platelet counts were reduced over time in the entire exposed groups;

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

however, the values of WBCs were observed to be substantially elevated.

Figure 1 showcases the RBC count of the control and treated groups when exposed to various concentrations of marble factory effluents for 90 days. The control group detected an RBC count of 2.51, 2.48, 2.44 × 106 /µL on the 30th, 60th, and 90th day.

Primarily, on the 30th day, the RBC count changed slightly for all the exposure groups, but as the timeline progressed, a significant ($p < 0.05$) decline in the RBC values was detected by day 60th and day 90th. A massive drop (0.7 × 10^6/µL) was noted for the 50% effluent concentration group after the 90th day.

Measurement of red blood cells is critical because they are an essential part of blood and aid in oxygen circulation, which is necessary for regular body processes. Even a tiny deviation from this might provide significant details about an organism's health. The chemical constituents of marble factory effluents, including heavy metals and suspended particles, can affect the structural integrity of RBC membranes, making them fragile and prone to disruption. Additionally, osmotic imbalances and altered ion and gas exchange may compromise RBC membrane stability during excessive energy demands. Witeska et al. (2023) concluded that exposure to industrial effluents containing metal mixtures could cause macrocytic hypochromic anemia in fish, reducing RBC.

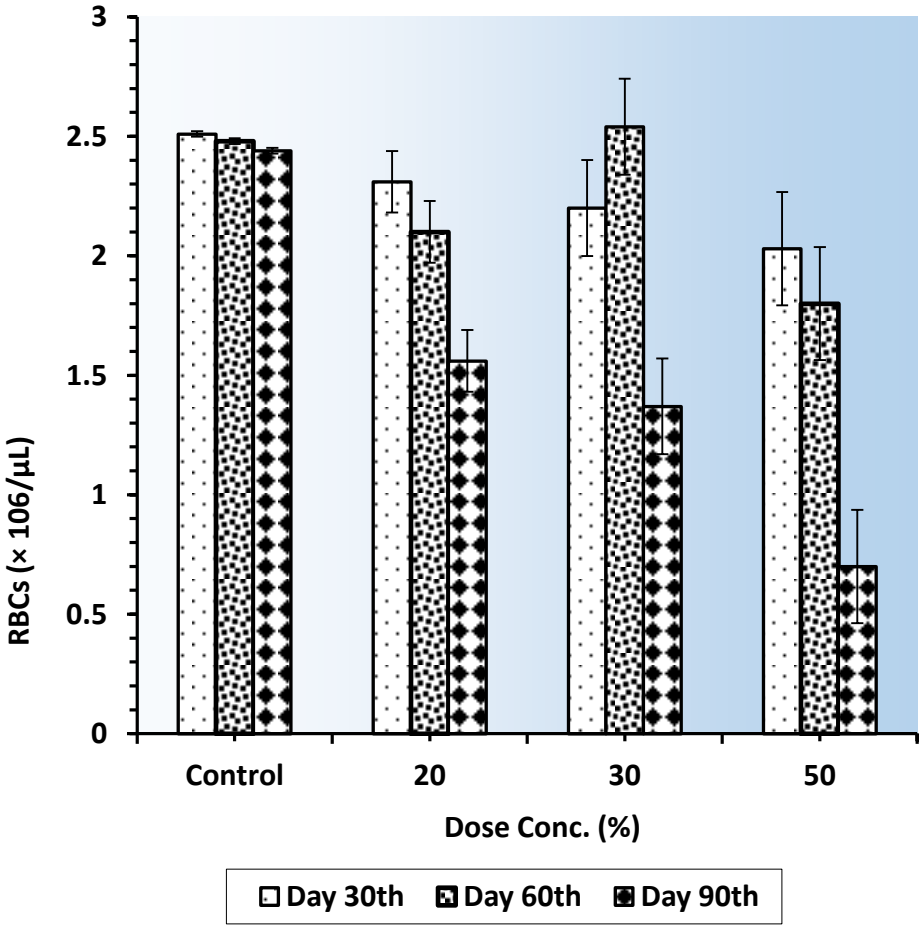

Figure 1 | RBCs value of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 10).

As shown in Figure 2, the Hb showed a similar pattern to RBCs. It decreased in value over time. By the experiment's conclusion, the Hb content has been

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

identified to have decreased compared to the control group, showing values of 5.8 and 5.7 g/dl for the 20% and 30% effluent groups, respectively, on day 90th. A significant decline in Hb content was noted on the 90th day, displaying a value of 5.1 g/dL for the massive dosage group (50%). Lower Hb concentration in this study is a sign of hypochromic microcytic anemia. A modest rise in Hb content detected on the 30th day for all the exposed groups may reflect an initial adaptive response to stress conditions. Hb levels indicate oxygen transport capacity, and during internal or external stress, anoxic conditions may disrupt energy synthesis processes in the body. A similar response to lead was also noticed in *Tor putitora* (Mahseer) fish and *Ctenopharyngodon Idella* (grass fish) (Latif & Zahoor, 2024).

Occult blood loss could cause a drop in the Hb concentration (Goldstein et al., 2011). Stress causes an increase in oxygen transport, which results in a high Hb concentration during the exposure time. The rise in Hb levels in the current investigation suggests either erythrocytosis or the replacement of denatured Hb by heavy metals (Nussey et al., 1995). By the end of the exposure period, the highest concentration exposure group (50%) had reduced hemoglobin levels due to the effects of marble waste on the target fish's erythropoiesis.

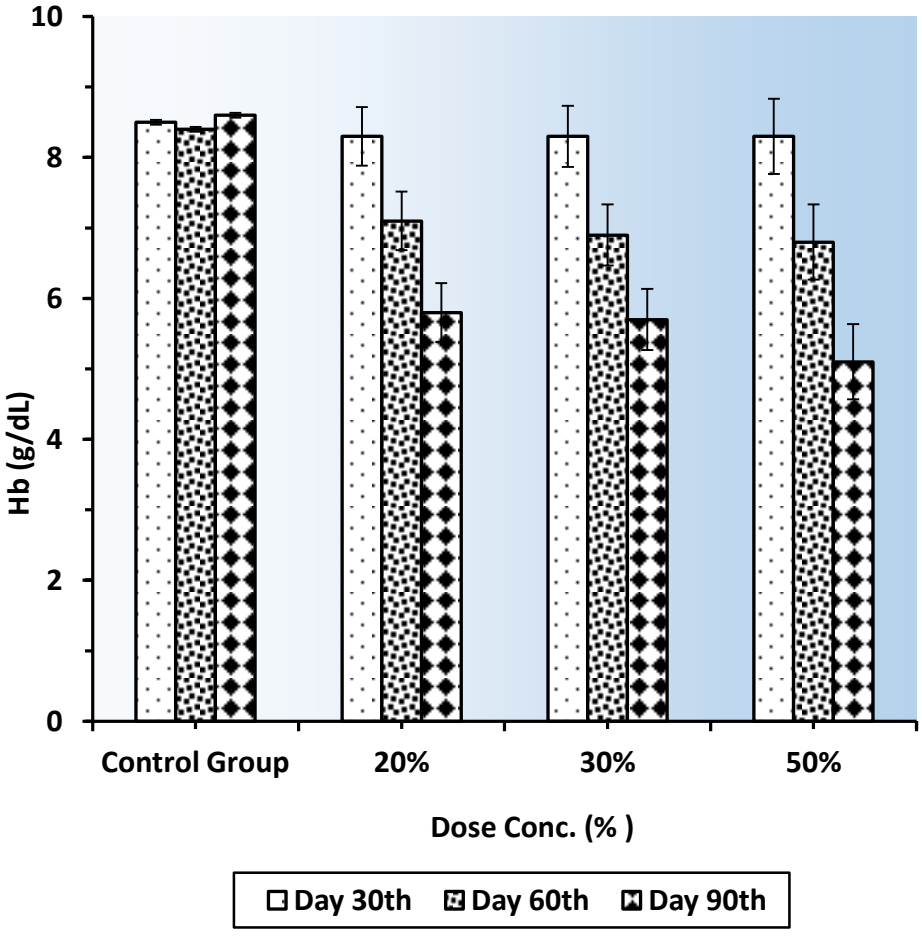

Figure 2 | Hb count of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

White blood cells are essential for the body's defense, and any change in the WBC count could indicate an infection caused by stress or tissue damage. Figure 3 shows the number of WBCs in control and treated fish exposed to soluble marble factory effluents for 90 days. The exposed group's WBCs increased sharply over time and continued until day 90, with values ranging from 115 to 220 ($\times 10^3/\mu L$) for all exposure groups, with a substantial rise being observed for the highest-concentration exposure group (50%) in day 90, with a value of 220 ($\times 10^3/\mu L$). WBCs and mononuclear phagocytes perform phagocytosis in fish, which generate copious amounts of superoxide anion (O_2^-) when stimulated by different xenobiotic substances. It contributes to the greater utilization of oxygen to form hydrogen peroxide (H_2O_2) by dismutation of O_2^-. Heavy metals' likely elevation of H_2O_2 levels may have caused this immunostimulatory impact (Khan et al., 2022). Since the body's immunological response is linked to the WBC count, as confirmed by Lunden and Bylund (2002), the spleen creates new WBCs when there is external stress. WBC count proliferation is a sign that the body is producing antibodies. The immune system plays a vital role in maintaining healthy biological functions and preventing illness. The observed leucocytosis illustrates how fish respond to heavy metals.

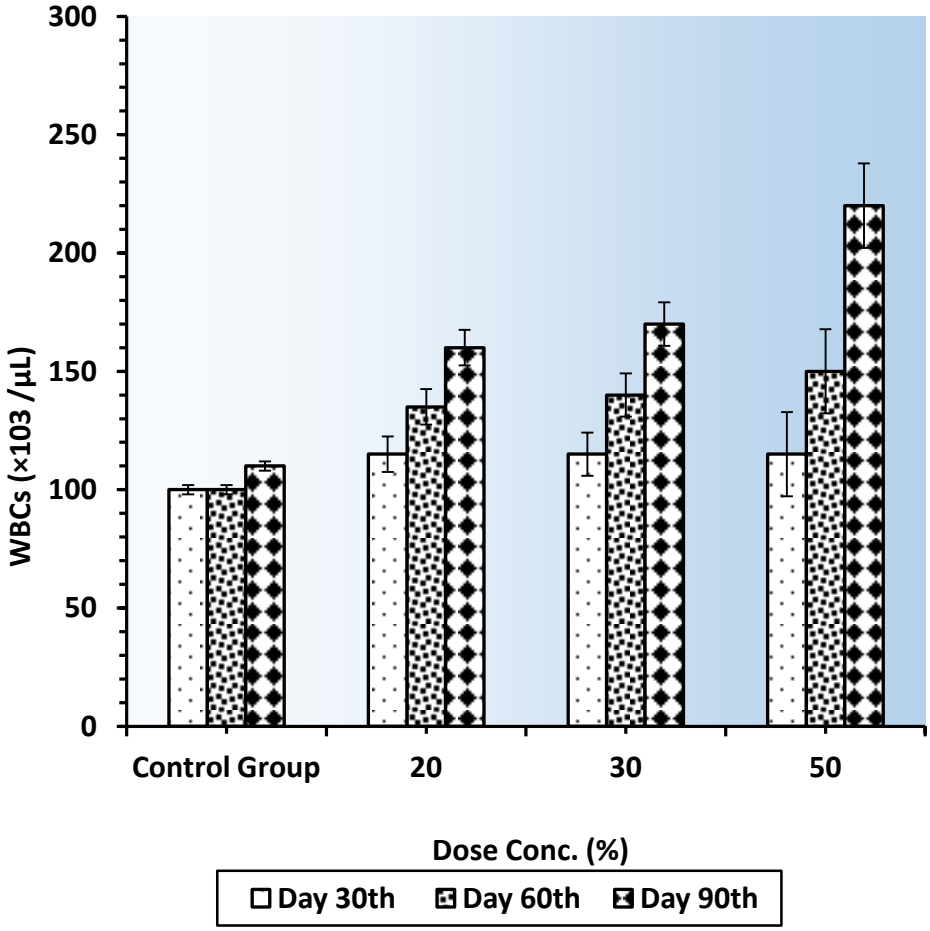

Figure 3 | WBCs numbers of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

For 30, 60, and 90 days, the control group's platelet (PLT) count was 152, 148.34, and 153 × (×10³/µL), respectively, as shown in Figure 4. The exposed group's PLT count gradually decreased compared to the control group, ranging from 153 to 102 × (×10³/µL), signifying the initial appearance of thrombocytopenia—a condition characterized by the reduced number of platelets in the blood. Thrombocytopenia can occur when toxic compounds interfere with platelet production or induce their destruction.

According to Van den Bemt et al. (2004), some pollutants cause the body to produce antibodies that destroy PLT, particularly those affected by lead (Pb) and cadmium (Cd). This condition is called pollutant-induced immune thrombocytopenia. Lead (Pb) and Mercury have been proven to be the leading cause of thrombocytopenia (Khan *et al.,* 2017).

Numerous mechanisms have been correlated with pollutant-induced thrombocytopenia, with marble factory effluents possibly playing a role in this process. These effluents may induce immune responses in fish, activating antibodies that target platelets and decreasing platelet count. Pollutants, like those found in marble factory effluents, could affect specific genes, such as GPIIb/IIIa or GPIb/IX, responsible for platelet aggregation, causing thrombocytopenia in *Tor putitora* (Michelson *et al.,* 1987).

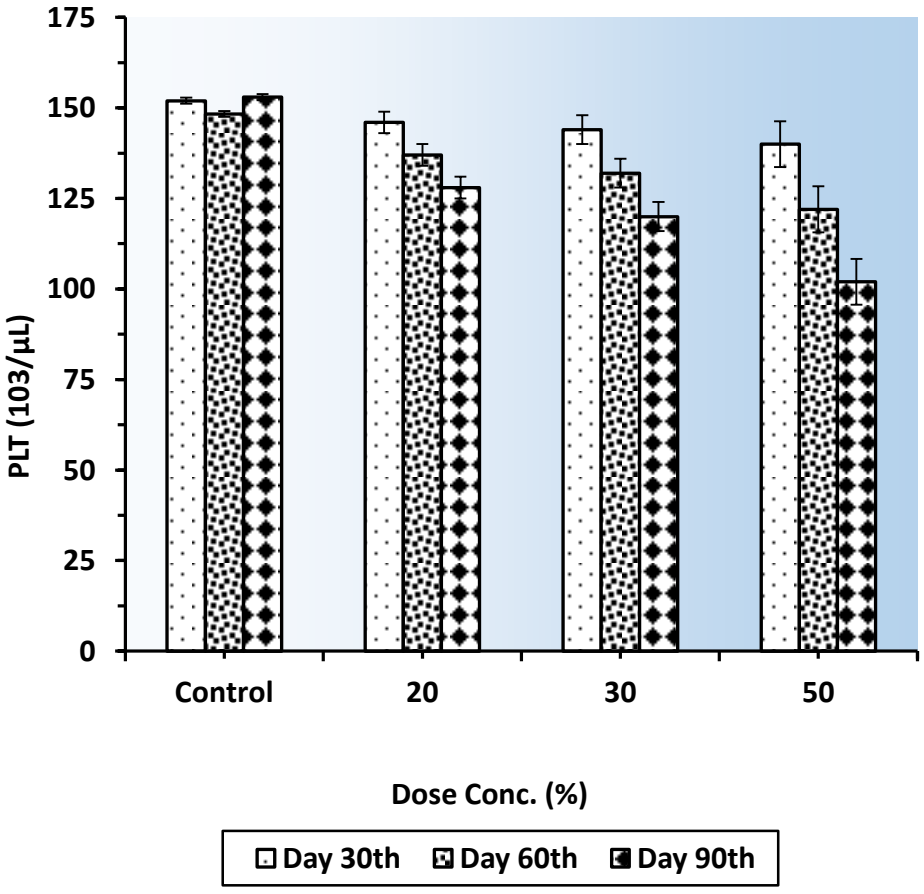

Figure 4 | PLT numbers of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Figure 5 highlights the mean corpuscular volume (MCV) of the control and treated groups when exposed to various concentrations of marble factory effluents for 90 days. The MCV values in the control group were relatively stable throughout the experiment, measuring 110 ± 10, 112 ± 9, and 111 ± 4 fL on the 30th, 60th, and 90th days, respectively.

In contrast, the exposed groups exhibited a gradual decline in MCV values as the duration of exposure increased. The most substantial decrease was observed in the 50% effluent concentration, where MCV dropped from 102 ± 10 fL on the 30th day to 65 ± 8 fL on the 90th day.

MCV is a critical parameter that reflects the average size of red blood cells (RBCs) and provides insights into the blood's oxygen-carrying capacity. A decline in MCV indicates changes in erythrocyte morphology, potentially caused by exposure to toxicants. The toxic components of marble factory effluents, such as heavy metals and suspended particles, may interfere with erythropoiesis and disrupt cellular metabolism. Additionally, oxidative stress induced by effluents can compromise the structural integrity of erythrocyte membranes, leading to abnormal cell shrinkage.

Mulk et al. (2017) reported that effluents interfere with erythropoiesis, causing the release of more minor, immature RBCs and reducing MCV. They also revealed that effluents alter potassium and sodium levels, causing RBC structural changes that reduce MCV.

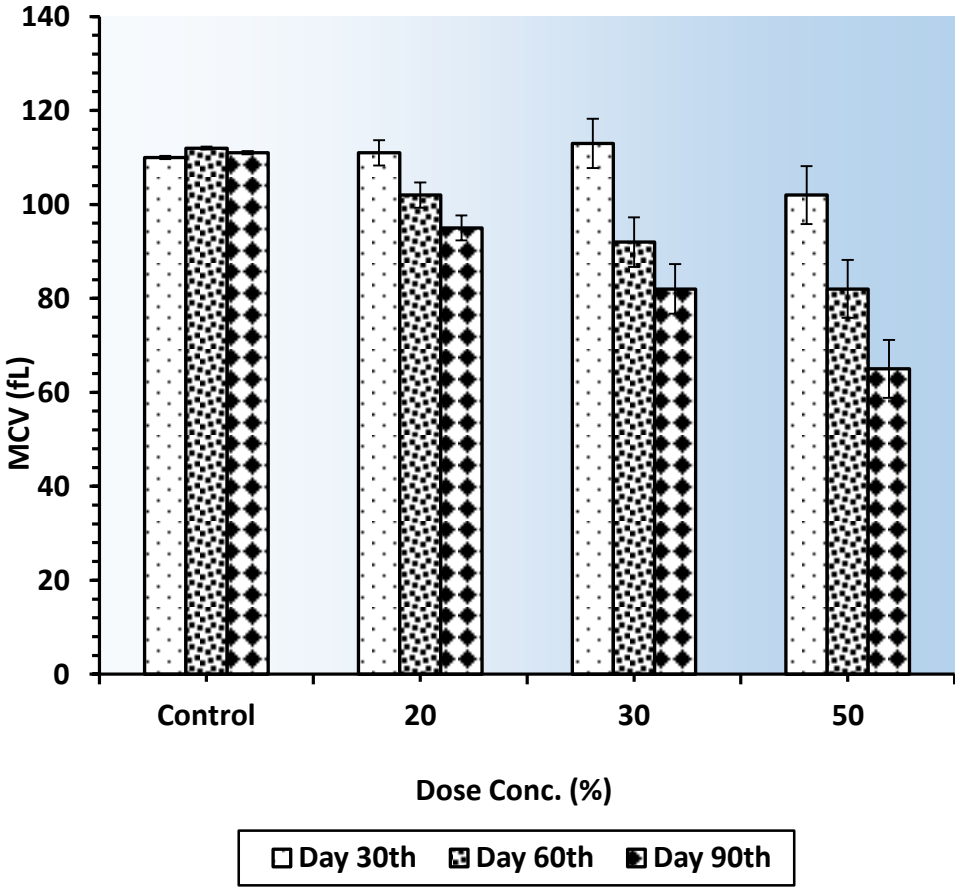

Figure 5 | MCV values of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Mean corpuscular hemoglobin (MCH) is shown in Figure 6 to compare the exposed group with a control group and the different concentrations of the effluent for 90 days. In the control group, the MCH level when minimally changed, and its values on the 30th, 60th, and 90th day amounted to 32±6, 33±3, and 32±7 pg, respectively. However, a significant decrease of MCH level in the normal range was detected in the exposed groups during the time assessment. An effluent concentration of 50% showed the most significant decrease; MCH values decreased from 30 ± 4 pg on day 30 to 15 ± 6 pg on day 90.

MCH is directly related to measuring the average hemoglobin content per red blood cell (RBC) and has particular value in evaluating the blood's ability to transport oxygen in the body. Reduced MCH indicates inadequate hemoglobin production, probably due to the toxic effluents released by the marble factories. These interfere with the production of the hemoglobin substance and distort the normal functioning of the RBCs, thus giving them lower hemoglobin content.

Javed et al. (2016) investigated that marble effluents containing heavy metals damage red blood cell membranes, causing hemolysis and reducing the Mean Corpuscular Hemoglobin Concentration (MCHC).

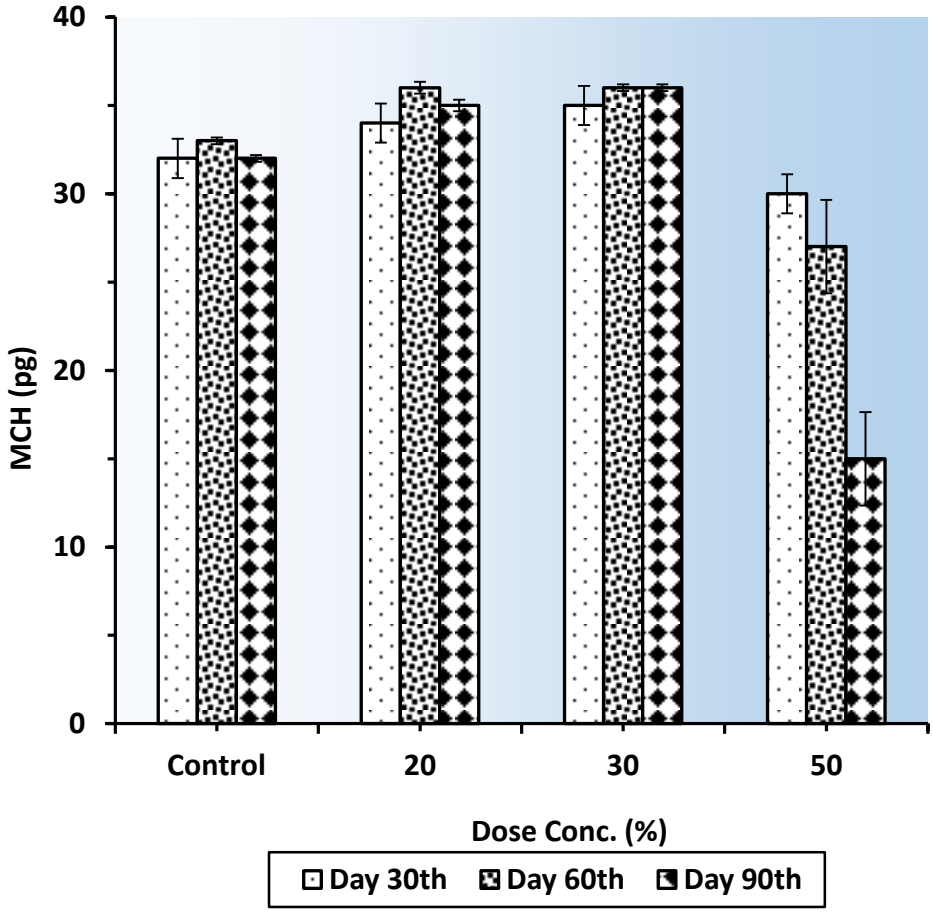

Figure 6 | MCH values of control and treated group for 90 days exposure

(results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Figure 7 showcases the mean corpuscular hemoglobin concentration (MCHC) of the control and exposed groups subjected to varying concentrations of marble wastes over 90 days. In the control group, the value remained constant, measuring 24.3 ± 1.5, 24 ± 5, and 24.6 ± 1.1 (g/dL) on the 30th, 60th, and 90th day, respectively. The exposed group, in contrast, demonstrated a progressive decline in MCHC values over time, with the extent of the decrease being more pronounced at higher effluent concentrations. Notably, the most significant reduction was observed in the 50% exposure group, where the MCHC value dropped from 22.5 ± 2 on the 30th day to 12.5 ± 2.1 g/dL by the 90th day.

MCHC is an important efficiency index of oxygen transport because it shows the density of hemoglobin inside RBCs. The observed decrease in MCHC among the exposed groups may be attributed to the effluent containing heavy metals and other pollutants that affect the synthesis of hemoglobin and erythropoiesis. These pollutants also give rise to oxidative stress, which causes membrane destabilization, hemolysis, and a decrease in Hemoglobin content per cell.

According to Ahmed et al. (2022), the toxic effects of heavy metals can inhibit erythropoiesis, the process of producing new red blood cells. If the rate of RBC destruction exceeds the production rate, this imbalance will lead to a reduction in MCHC and overall anemia.

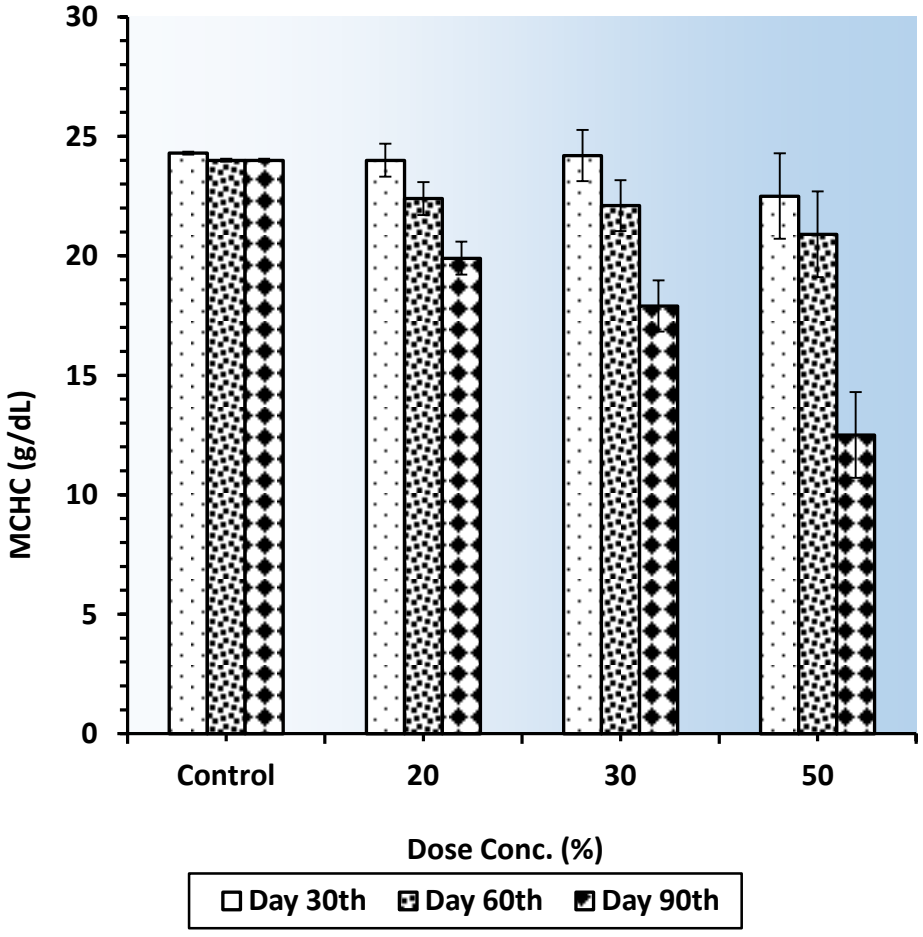

Figure 7 | MCHC values of control and treated group for 90 days exposure

(results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Figure 8 showcases the hematocrit (Hct) levels of the control and treated groups exposed to varying concentrations of marble factory effluents over 90 days. The hematocrit levels in the control group were 35 ± 2, 35.3 ± 2, and 35.8 ± 2% on the Day 30th, 60th, and 90th, respectively.

Initially, all exposure groups showed a slight drop in Hct levels on Day 30th compared to the control. However, as exposure time rose, Hct levels were shown to decrease significantly ($p < 0.05$). The most pronounced decline (19 ± 5%) was recorded in the 50% effluent concentration group by the 90th day.

Hematocrit is an essential indicator of oxygen-carrying capacity and overall blood health in fish. Reduced hematocrit levels can signify anemia, impaired oxygen transport, or physiological stress caused by environmental contaminants. Toxicants, such as heavy metals and other harmful constituents in marble factory effluents, can induce oxidative stress and impair erythropoiesis.

According to Barathinivas et al. (2012), heavy metals cause oxidative stress and hemolysis, contributing to reduced hematocrit (Hct) levels in various fish.

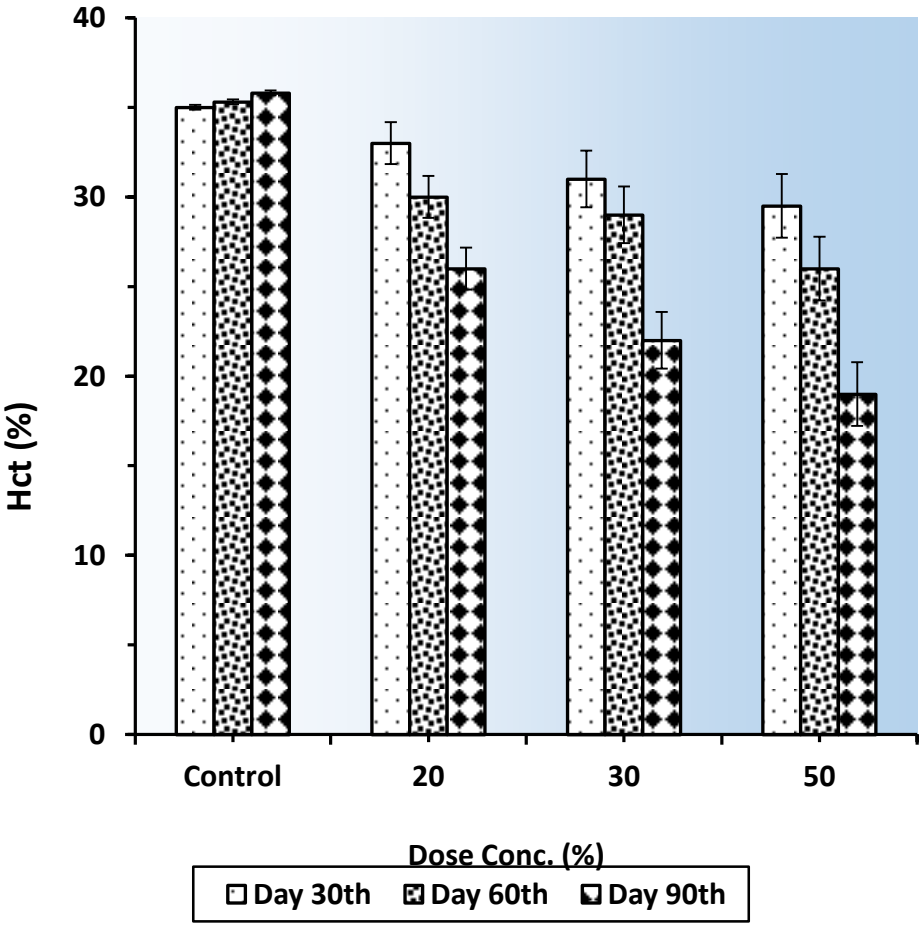

Figure 8 | Hct values of control and treated group for 90 days exposure

(results are highlighted as mean ± SE, n = 9).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Alternation in all hematological profile values in Table form

Fig X shows the values of various hematological parameters

Parameters	Group/Exposure	Day 30TH	Day 60TH	Day 90TH
RBC	**Control**	2.51 ± 0.2	2.48 ± 17	2.44 ± 0.1
	20%	2.31 ± 0.2	2.1 ± 0.3	1.56 ± 0.4
	30%	2.2 ± 0.2	2.54 ± 0.3	1.37 ± 0.5
	50%	2.03 ± 0.3	1.8 ± 0.4	0.7 ± 0.5
Hb	**Control**	8.5 ± 0.5	8.4 ± 0.8	8.6 ± 0.3
	20%	8.3 ± 0.5	7.1 ± 0.5	5.8 ± 0.6
	30%	8.3 ± 0.6	6.9 ± 0.8	5.7 ± 0.12
	50%	8.3 ± 0.8	6.8 ± 0.7	5.1 ± 0.8
WBC	**Control**	100 ± 02	100 ± 10	110 ± 14
	20%	115 ± 10	135 ± 14	160 ± 18
	30%	115 ± 14	140 ± 16	170 ± 22
	50%	115 ± 20	150 ± 18	220 ± 25
PLT	**Control**	152 ± 02	148.34 ± 15	153 ± 0.2
	20%	146 ± 2	137 ± 1	128 ± 2
	30%	144 ± 0.5	132 ± 9	120 ± 2
	50%	140 ± 0.5	122 ± 0.3	102 ± 0.6
MCV	**Control**	110 ± 10	112 ± 9	111 ± 4
	20%	111 ± 9	107 ± 8	104 ± 10
	30%	113 ± 9	106 ± 9	103 ± 12
	50%	108 ± 10	102 ± 01	92 ± 08
MCH	**Control**	32 ± 6	33 ± 3	32 ± 7
	20%	34 ± 2	36 ± 3	35 ± 4
	30%	35 ± 3	36 ± 4	36 ± 5
	50%	30 ± 4	27 ± 4	15 ± 6
MCHC	**Control**	24.3 ± 1.5	24 ± 5	24 ± 1.1
	20%	24 ± 1.5	22.4 ± 1.4	19.9 ± 1.8
	30%	24.2 ± 1.8	22.1 ± 1.7	17.9 ± 2
	50%	22.5 ± 2	20.9 ± 1.9	12.5 ± 2.1
HCT	**Control**	35 ± 2	35.3 ± 2	35.8 ± 2
	20%	33 ± 2	30 ± 2	26 ± 3
	30%	31 ± 2	29 ± 3	22 ± 4
	50%	29.5 ± 3	26 ± 4	19 ± 5

Biochemical parameters

Assessing biochemical parameters is crucial in understanding an organism's physiological systems. In toxicology studies, these parameters, including glucose and total protein levels, are instrumental in determining normal bodily function. Figure 9 illustrates the effect of soluble marble factory effluents on glucose levels in fish. Compared to the control group (55.5, 56.3, 57.1 mg/dL), the glucose levels in the exposed group varied significantly. A general decline in values was noted, though levels rose on the 60th day, reaching 60, 61.5, and 70 mg/dL across all exposure levels. Values ranged from 57 to 40 mg/dL, with the lowest recorded at 40 mg/dL on the Day 90th for the group exposed to 50%.

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Glucose breakdown in the body is crucial for energy production. Stress can alter glucose levels in fish, particularly under high energy demands. This occurs when fish face stressors, like antibiotics, triggering a hormone release (corticosteroids, epinephrine, dopamine) that reactivates glycogenesis. The changes in glucose levels from antibiotics are linked to carbohydrate metabolism and cortisol production, a primary stress response.

Shaffi (1980) observed that following the initial spike, prolonged exposure to marble effluents can lead to the depletion of glycogen reserves due to ongoing stress and sustained energy requirements. As fish attempt to sustain homeostasis in toxic environments, their capability to generate and store glucose declines, resulting in reduced blood glucose levels.

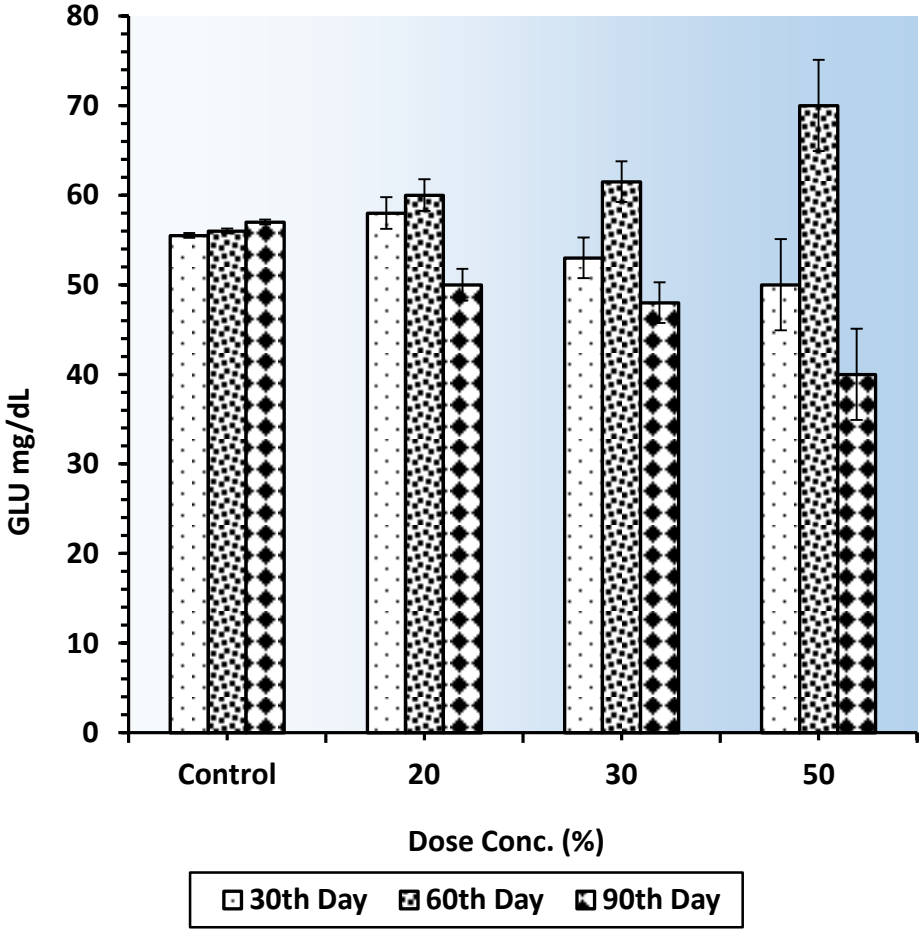

Figure 9 | GLU level of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

Protein levels fluctuated, as highlighted in Figure 10, decreasing from 25 to 30.6 mg/dL compared to control values (8.2, 8.8, 8.3 mg/dL) on Days 30th, 60th, and 90th, respectively. All groups had higher protein levels on day 30th, with a peak value of 30.6 mg/dL for 50% exposure that dropped until the final week. A significant reduction to 3.5 mg/dL occurred on day 90th for the 50% effluent concentration.

Protein is a key component of all body cells, essential for tissue formation, repair, and creating enzymes, hormones, and other chemicals. It is also found in bones,

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

muscles, skin, and blood. In this study, fish exposed to marble effluents showed reduced protein levels compared to controls, indicating a potentially stressful impact from marble factory elements, leading to declined protein formation.
Zhang et al. (2021) reported that fish in polluted environments display altered serum biochemical values. Research indicates that while stress can increase total protein levels, toxic environmental conditions ultimately hinder effective protein synthesis, leading to a decrease in total proteins. Additionally, changes in the globulin-to-albumin ratio reflect shifts in immunologic capacity and overall health.

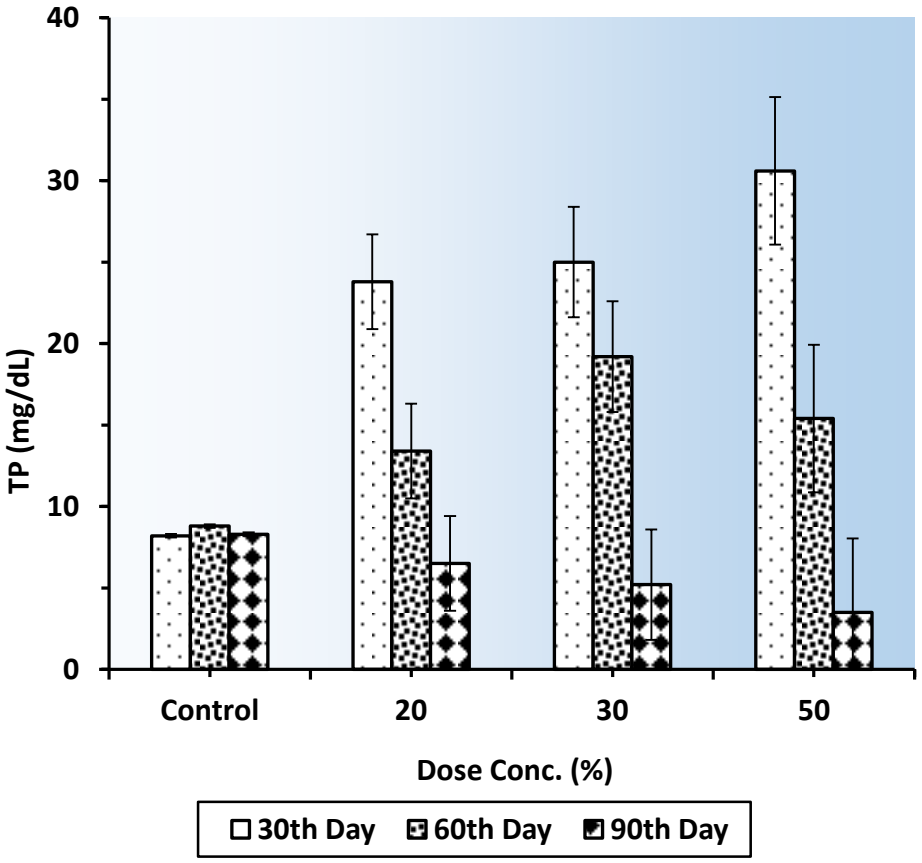

Figure 10 | TP level of control and treated group for 90 days exposure

(results are highlighted as mean ± SE, n = 9).

Triglycerides (TGs) displayed biphasic fluctuations, peaking at 0.25, 0.27, and 0.29 mg/dL for the 20, 30, and 50% effluent exposure groups, respectively, on day 60th, as illustrated in Figure 11. However, TG levels significantly reduced after day 60th compared to the control group, with the highest dosage group (50%) showing a value of 0.1 mg/dL on day 90th.
TGs are vital energy sources during stress, with increased levels possibly stemming from lipid mobilization due to the energy demands from marble factory effluents (Tan et al., 2018). Elevated blood TG may also occur from their transport for oxidation or storage. Toxic pollutants in marble effluents likely disrupt lipid metabolism, damaging cell membranes and initially raising TG levels. Liver dysfunction further exacerbates these issues (Gaber et al., 2013). The

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

oxidative stress from these effluents can damage lipid-containing membranes, resulting in higher TG levels in fish. A decline in TG levels later may result from reduced feed intake, impaired absorption from gut and liver dysfunction, or membrane biogenesis initiation (Van Meer et al., 2008).

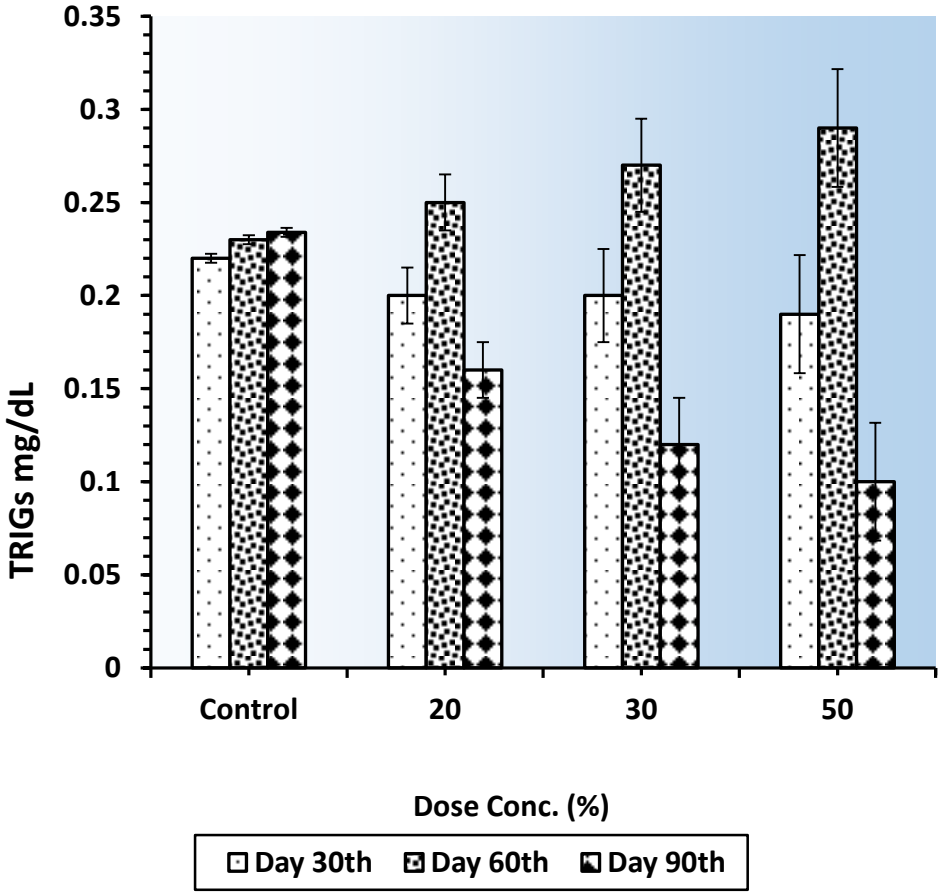

Figure 11 | TRIGs level of control and treated group for 90 days exposure (results are highlighted as mean ± SE, n = 9).

The ALT level rose significantly ($p < 0.05$) with higher dose concentration and exposure time, as shown in Figure 12. Goessling and Sadler (2015) concluded that toxic substances induce stress in fish, altering metabolism and exacerbating liver injury, leading to higher ALT levels. Various studies link stress to elevated ALT, showing that environmental stressors impact liver function in aquatic organisms. High ALT levels indicate hepatotoxicity caused by industrial waste, especially Cadmium (Cd) and Lead (Pb).

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

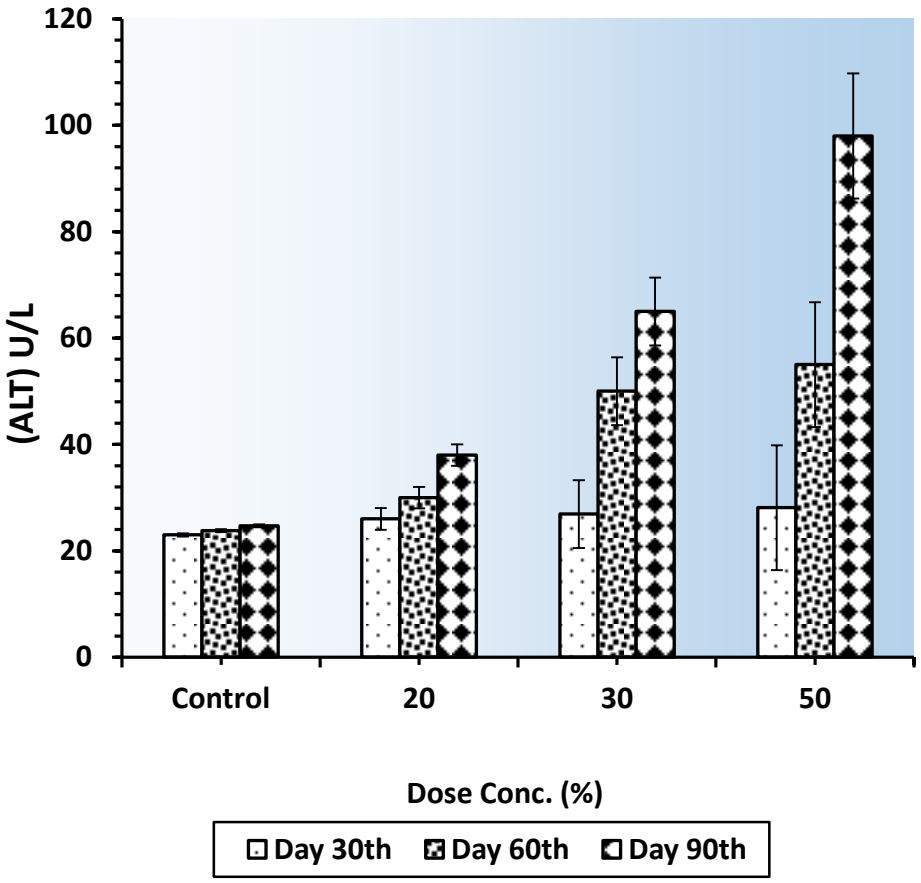

Figure 12 | ALT level of control and treated group for 90 days exposure

(results are highlighted as mean ± SE, n = 9).

Respiratory Burst Activity (NBT)

Figure 13 illustrates a significant rise in respiratory burst activity over time. On day 90th, 1.2, 1.3, and 1.6 values were recorded for the 20, 30, and 50% effluent-exposed groups. Respiratory burst activity reflects the activation of neutrophils and macrophages, producing reactive oxygen species (ROS) like hydrogen peroxide and superoxide anions. Cytokines aid neutrophils and macrophages in phagocytosing bacteria by generating ROS during this activity (Secombes et al., 2001). The study found that marble factory wastes significantly ($p < 0.05$) increased phagocytic activity in Tor putitota. Biller and Takahashi (2018) noted that marble factory effluents can increase oxidative stress in fish, heightening respiratory burst activity as a compensatory response to infections or damage from pollutants.

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

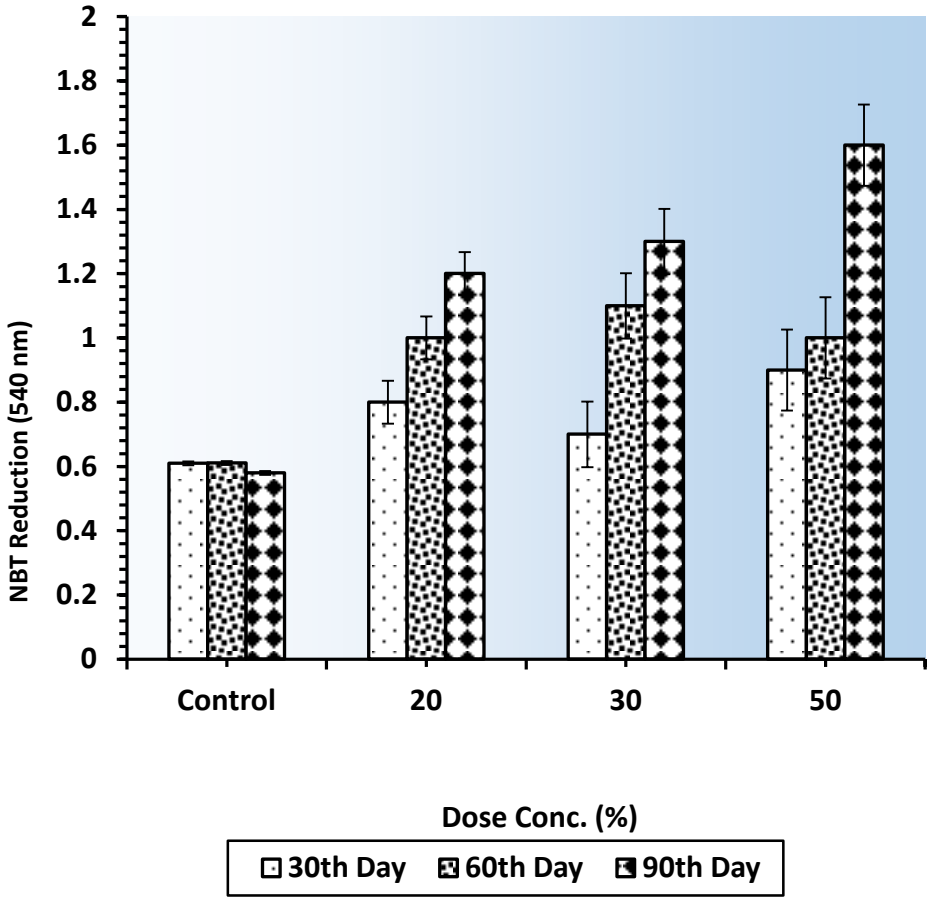

Figure 13 | Respiratory burst activity in control and treated group for 90 days exposure (values are expressed as mean ± SE, n = 9).

Conclusion

The study's result offers critical insights into the adverse effect of marble factory effluents on fish species. It presents significant alternation in the hematological profile, biochemical parameters, and respiratory burst activity of *Tor putitora* due to the toxicity of heavy metals and other pollutants. These results emphasize the potential health risks to aquatic organisms and underscore the necessity for stringent waste management and regulatory measures to mitigate the release of untreated industrial effluents into marine environments.

Furthermore, fish's observed physiological and biochemical changes are reliable bioindicators for assessing environmental pollution. The current study contributes to the comprehensive understanding of ecotoxicology and stresses the significance of sustainable practices in industrial operations to preserve aquatic biodiversity and ecosystem health. Notably, future research could explore advanced remediation techniques to minimize the impact of such pollutants and further investigate long-term effects on marine species and ecosystems.

Acknowledgments

We are incredibly grateful to the Government of Thana Fish Hatchery for providing fish samples and experimental tanks. The authors also acknowledge the unwavering support of the hatchery staff, especially Muhammad Ilyas, the

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Fisheries Director of Malakand Division, and Mr. Sajjad, the Deputy Director of Fisheries at the Government Fish Hatchery Thana Malakand. The current research were conducted under the supervison of Mr. Ajmal Hussain.

References

Kutty, A. A., & Al-Mahaqeri, S. A. (2016). An investigation of the levels and distribution of selected heavy metals in sediments and plant species within the vicinity of ex-iron mine in Bukit Besi. *Journal of Chemistry*, 2016, Article ID 2096147. https://doi.org/10.1155/2016/2096147

Iqbal, Q., Musarat, M. A., Ullah, N., Alaloul, W. S., Rabbani, M. B. A., Al Madhoun, W., & Iqbal, S. (2022). Marble dust effect on the air quality: An environmental assessment approach. *Sustainability, 14*(7), 3831. https://doi.org/10.3390/su14073831

Khan, S. N., Nafees, M., & Imtiaz, M. (2023). Assessment of industrial effluents for heavy metals concentration and evaluation of grass (*Phalaris minor*) as a pollution indicator. *Heliyon, 9*(9), e20299. https://doi.org/10. 1016 /j.heliyon.2023.e20299

Liney, K. E., Hagger, J. A., Tyler, C. R., Depledge, M. H., Galloway, T. S., & Jobling, S. (2005). Health effects in fish of long-term exposure to effluents from wastewater treatment works. *Environmental Health Perspectives*, *114*(Suppl 1), 81–89. https://doi.org/10.1289/ehp.8058

Danovaro, R., Cocozza di Montanara, A., Corinaldesi, C., Dell'Anno, A., Illuminati, S., Willis, T. J., & Gambi, C. (2023). Bioaccumulation and biomagnification of heavy metals in marine micro-predators. *Communications Biology, 6*, 1206. https://www.nature. com/ articles /s42003-023-05539-x

Emon, F. J., Rohani, M. F., Sumaiya, N., Jannat, M. F. T., Akter, Y., Shahjahan, M., Kari, Z. A., Tahiluddin, A. B., & Goh, K. W. (2023). Bioaccumulation and bioremediation of heavy metals in fishes—A review. *Toxics, 11*(6), 510. https://doi.org/10.3390/toxics11060510

Natalija, P., Igor, S., & Milan, D. (2023). Oxidative stress in fish: Biomarkers and environmental relevance. *Fish Physiology and Biochemistry, 49*, 587-603. https://doi.org/10.1007/s10695-022-01122-7

Singanan, V., & Narayanan, M. (2008). Bioaccumulation of heavy metals in organs of freshwater fish *Cyprinus carpio* (Common carp). *International Journal of Environmental Science and Technology, 5*(2). http://dx.doi.org/10.1007/BF03326011

Fazio, F., Ahmed, I., & Zakiya, A. (2022). Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: A review. *Frontiers in Environmental Science, 10,* Article 919204. https://doi.org/10.3389/fenvs.2022.919204

SBI. (2010). Marble and granite sector in Pakistan: Overview and analysis. *Small and Medium Enterprises Development Authority (SMEDA), Pakistan*. Retrieved from https://www.smeda.org

Mulk, S., Yousafzai, A. M., & Khattak, M. I. (2015). Impact of industrial effluents on fish biodiversity in the River Kabul. *Pakistan Journal of Zoology, 47*(2), 389-398. https://www.pjzoology.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

Yousafzai, A. M., Khan, A. R., & Shakoori, A. R. (2012). Bioaccumulation of heavy metals in *Tor putitora* as an indicator of freshwater pollution. *Pakistan Journal of Zoology, 44*(2), 489-500. https://www.pjzoology.com

Mulk, S., Khattak, M. I., & Yousafzai, A. M. (2016). Heavy metal accumulation in *Tor putitora* from industrial wastewater. *Environmental Monitoring and Assessment, 188*, 486. https://doi.org/10.1007/s10661-016-5475-9

IUCN. (2011). *IUCN Red List of Threatened Species*. Retrieved from https://www.iucnredlist.org

Khajuria, A., & Langer, S. (2016). Threats to freshwater biodiversity in the Indian subcontinent: A review. *International Journal of Aquatic Science, 7*(1), 67-81. https://www.aquaticsciencesjournal.com

Ullah, N., Said, A., Israr, M., Rasool, A., Akbar, F., Ahmad, S., Mehmood, S. A., Jabeen, H., Islam, M., Muhammad, S., Noureenh, S., Habiba, U., Ahmed, D., Shah, M., Khan, M. A. A., & Siraj, M. (2021). Effect of different protein-based feed on the growth of mahseer. *Brazilian Journal of Biology*, *82*, e243670. https://doi.org/10.1590/1519-6984.243670

Organisation for Economic Co-operation and Development 1992 'Fish, Acute Toxicity Test' OECD Guideline for Testing Chemicals 203. OECD, Paris, France. https://www.oecd.org/content/dam/oecd/en/publications/reports/2019/06/test-no-203-fish-acute-toxicity-test_g1gh28f5/9789264069961-en.pdf

Organisation for Economic Co-operation and Development 1984 Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study. OECD Publishing, Paris, France.

Iftikhar, N., & Hashmi, I. (2021). Assessment of immunohematological, hematological and biochemical responses in cultivable fish *Cyprinus carpio* exposed to an antibiotic sulfamethoxazole (SMX). *Journal of Water and Health, 19*(1), 108–119. https://doi.org/10.2166/wh.2020.183

Zanuzzo, F. S., Urbinati, E. C., Nash, G. W., & Gamperl, A. K. (2015). Steelhead trout *Oncorhynchus mykiss* metabolic rate is affected by dietary *Aloe vera* inclusion but not by mounting an immune response against formalin-killed *Aeromonas salmonicida*. *Journal of Fish Biology, 87*(1), 43–53. https://doi.org/10.1111/jfb.12690

Haider, M. J., & Rauf, A. (2014). Sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in Indian carp, *Cirrhinus mrigala* (Hamilton). *Brazilian Archives of Biology and Technology, 57*(6). https://doi.org/10.1590/S1516-8913201402086

Svobodova, Z., Pravda, D., & Palackova, J. (1991). Unified methods of haematological examination of fish. *ScienceOpen*. https://www.scienceopen.com/document?vid=88c9d31c-b8f3-41ea-baad-370c5443154b

Malik, R. N. & Nadeem, M. 2012 Spatial and temporal characterization of trace elements and nutrients in the Rawal Lake Reservoir, Pakistan using multivariate analysis techniques. Environmental Geochemistry and Health 33 (6), 525–541. https://doi.org/10.1007/s10653-010-9369-8

Blaxhall, P. C. (2006). The haematological assessment of the health of freshwater fish. *Journal of Fish Biology, 4*(4), 593–604. https://doi.org/10.1111/j.1095-8649.1972.tb05704.x

ISSN Online: 3007-3154
ISSN Print: 3007-3146

Witeska, M., Kondera, E., & Bojarski, B. (2023). Hematological and hematopoietic analysis in fish toxicology—A review. *Animals (Basel)*, 13(16), 2625. https://doi.org/10.3390/ani13162625

Latif, M., & Zahoor, M. (2024). Bioaccumulation of lead in different organs of *Ctenopharyngodon idella* (grass fish) and *Tor putitora* (Mahseer) fish. *Brazilian Journal of Biology, 84*. https://doi.org/10.1590/1519-6984.260355

Goldstein, J. L., Chan, F. K. L., Lanas, A., Wilcox, C. M., Peura, D., Sands, G. H., Berger, M. F., Nguyen, H. & Scheiman, J. M. 2011 Haemoglobin decreases in NSAID users over time: an analysis of two large outcome trials. Alimentary Pharmacology & Therapeutics 34 (7), 808–816. https://doi.org/10.1111/j.1365-2036.2011.04790.x

Nussey, G., Van Vuren, J. H. J. & Du Preez, H. H. 1995 Effect of copper on blood coagulation of Oreochromis mossambicus (Cichlidae). Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology 111 (3), 359–367. https://doi.org/10.1016/0742-8413(95)00062-3

Khan, M. S., Javed, M., Rehman, M. T., Urooj, M., & Ahmad, M. I. (2020). Heavy metal pollution and risk assessment by the battery of toxicity tests. *Scientific Reports*, 10, 16593. https://doi.org/10.1038/s41598-020-73434-0

Lunden, T. & Bylund, G. 2002. Effect of sulphadiazine and trimethoprim on the immune response of rainbow trout (Oncorhynchus mykiss). Veterinary Immunology and Immunopathology 85 (1–2), 99–108. https://doi.org/10.1016/S0165-2427(01)00422-6

Van den Bemt, P. M., Meyboom, R. H. & Egberts, A. C.2004. Drug-induced immune thrombocytopenia. Drug Safety 27 (15), 1243–1252. https://doi.org/10.2165/00002018-200427150-00007

Khan, S. A., Suleman, M., & Asad, M. (2017). Assessment of pollution load in marble wastewater in Khairabad, District Nowshera, Khyber Pukhtunkhwa, Pakistan. *International Journal of Economic and Environmental Geology, 8*(2), 35–39. https://www.researchgate.net/publication 19005514 Assessment of Pollution Load in Marble Waste Water in Khairabad District Nowshera Khyber Pukhtunkhwa Pakistan

Michelson, A. D., & Barnard, M. R. (1987). Thrombin-induced changes in platelet membrane glycoproteins Ib, IX, and IIb-IIIa complex. *Blood, 70*(5), 1673–1678. https://doi.org/10.1182/blood.V70.5.1673.1673

Javed, M., Ahmad, I., Ahmad, A., Usmani, N., & Ahmad, M. (2016). Studies on the alterations in haematological indices, micronuclei induction and pathological marker enzyme activities in *Channa punctatus* (spotted snakehead) Perciformes, Channidae exposed to thermal power plant effluent. *SpringerPlus, 5*(1), 761. https://doi.org/10.1186/s40064-016-2478-9

Ahmed, I., Zakiya, A., & Fazio, F. (2022). Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: A review. *Frontiers in Environmental Science, 10*. https://doi.org/10.3389/ fenvs. 2022.919204

Dialogue Social Science Review (DSSR)
www.thedssr.com

ISSN Online: 3007-3154
ISSN Print: 3007-3146

DIALOGUE SOCIAL SCIENCE REVIEW

Vol. 3 No. 3 (March) (2025)

Barathinivas, A., Ramya, S., Neethirajan, K., Jayakumararaj, R., Pothiraj, C., Balaji, P., & Faggio, C. (2022). Ecotoxicological effects of pesticides on hematological parameters and oxidative enzymes in freshwater catfish, *Mystus keletius*. *Sustainability, 14*(15), 9529. https://doi.org/10.3390/su14159529

Shaffi, S. A. (1980). The acute industrial effluent toxicity to freshwater fish. *Toxicology Letters, 5*(3-4), 183–190. https://doi.org/10.1016/0378-4274(80)90057-0

Zhang, Y.-L., Shang, Z.-H., Wang, G.-Y., You, K., & Mi, D. (2021). High concentrations of environmental ammonia induced changes in large-scale loach (*Paramisgurnus dabryanus*) immunity. *Ecology and Evolution, 11*(13), 8614–8622. https://doi.org/10.1002/ece3.7675

Tan, C., Sun, D., Tan, H., Liu, W., Luo, G. & Wei, X. 2018 Effects of stocking density on growth, body composition, digestive enzyme levels and blood biochemical parameters of Anguilla marmorata in a recirculating aquaculture system. Turkish Journal of Fisheries and Aquatic Sciences 18 (1), 9–16. https://doi.org/10.4194/1303-2712-v18_1_02

Gaber, H. S., El-Kasheif, M. A., Ibrahim, S. A. & Authman, M. 2013 Effect of water pollution in El-Rahawy drainage canal on hematology and organs of freshwater fish. World Applied Sciences Journal 21, 329–341.

Van Meer, G., Voelker, D. R. & Feigenson, G. W. 2008 Membrane lipids: where they are and how they behave. Nature Reviews Molecular Cell Biology 9 (2), 112–124. https://doi.org/10.1038/nrm2330

Goessling, W., & Sadler, K. C. (2015). Zebrafish: An important tool for liver disease research. *Gastroenterology*, 149(6), 1361–1377. https://doi.org/10.1053/j.gastro.2015.08.034

Secombes, C. J., Wang, T., Hong, S., Peddie, S., Crampe, M., Laing, K. J., Cunningham, C. & Zou, J. 2001 Cytokines and innate immunity of fish. Developmental & Comparative Immunology 25 (8–9), 713–723. https://doi.org/10.1016/S0145-305X(01)00032-5

Biller, J. D., & Takahashi, L. S. (2018). Oxidative stress and fish immune system: Phagocytosis and leukocyte respiratory burst activity. *Anais da Academia Brasileira de Ciências*, 90(7). https://doi.org/10.1590/0001-3765201 820170730