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Haematological and metabolic responses of *Oreochromis mossambicus* to sublethal exposure of methyl parathion and endosulfan

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Abstract
Agricultural pesticides are major contaminants of freshwater ecosystems and pose significant risks to non-target aquatic organisms, particularly fish. The present study investigated the haematological and metabolic effects of sublethal exposure to two widely used pesticides, methyl parathion (organophosphorus) and endosulfan (organochlorine), in the freshwater fish *Oreochromis mossambicus*. Adult fish were exposed to 1/10th of the 96 h LC_{50} concentrations of each pesticide for 10, 20, and 30 days, followed by a 30-day recovery period in pesticide-free water. Haematological parameters including red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and erythrocyte sedimentation rate (ESR) were analyzed, along with metabolic performance assessed through oxygen consumption rate.

Both pesticides induced significant, time-dependent haematological alterations characterized by marked reductions in RBC, Hb, PCV, and MCV, accompanied by increased MCH, MCHC, and ESR, indicating the development of microcytic and hypochromic anaemia and inflammatory stress. Oxygen consumption decreased progressively with exposure duration, reflecting metabolic depression and impaired respiratory efficiency. Endosulfan exerted more pronounced haematological and metabolic toxicity than methyl parathion, consistent with its higher persistence and bioaccumulative potential. Recovery experiments demonstrated partial restoration of altered parameters, suggesting physiological resilience in *O. mossambicus*, although complete recovery was not achieved, particularly after endosulfan exposure.

The findings confirm that haematological indices and oxygen consumption are sensitive and reliable biomarkers for assessing sublethal pesticide toxicity in fish. The study highlights the ecological risks associated with continued use of organophosphorus and organochlorine pesticides and underscores the need for improved pesticide management and monitoring strategies to protect freshwater ecosystems.

Keywords: Pesticides, *Oreochromis mossambicus*, haematology, oxygen consumption, methyl parathion, endosulfan, ecotoxicology

Introduction

Aquatic ecosystems are increasingly threatened by the continuous input of agricultural pesticides, which reach water bodies through surface runoff, leaching, spray drift, and improper disposal practices (FAO, 2022; Sharma *et al*., 2021) [16]. These contaminants pose serious risks to non-target organisms, particularly fish, which are highly sensitive to changes in water quality and chemical stressors. Among the various approaches used to assess pesticide toxicity in fish, haematological and metabolic parameters are considered reliable and early warning biomarkers of physiological stress (Witeska *et al*., 2023) [20].

Haematological indices such as red blood cell count (RBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and erythrocyte sedimentation rate (ESR) provide valuable information on oxygen transport efficiency, erythropoietic activity, and immune status of fish (Kondera *et al*., 2020; Rohani *et al*., 2023) [7, 20]. Alterations in these parameters are often associated with anaemia, impaired respiration, oxidative stress, and metabolic depression caused by toxicant exposure (Nigam *et al*., 2022; Alarape *et al*., 2024) [1, 10].

Organophosphorus and organochlorine pesticides remain among the most commonly detected agrochemicals in freshwater environments. Methyl parathion, an organophosphate, interferes with acetylcholinesterase activity and induces oxidative damage, whereas endosulfan, an organochlorine pesticide, is highly persistent and bioaccumulative, exerting prolonged toxic effects on aquatic organisms (Sharma *et al*., 2019; Witeska *et al*., 2023) [15, 20]. Several studies have reported significant reductions in RBC, Hb, and PCV in fish exposed to these pesticides, suggesting the development of anaemia due to hemolysis, inhibited erythropoiesis, or impaired iron metabolism (Sarkar *et al*., 2018; Chauhan *et al*., 2024) [4, 14].Changes in erythrocyte indices such as MCV, MCH, and MCHC further reflect the nature of pesticide-induced anaemia, often described as microcytic and hypochromic under prolonged exposure conditions (Nigam *et al*., 2022; Rohani *et al*., 2023) [10, 12]. Elevated ESR values reported in pesticide-exposed fish are indicative of inflammatory responses and altered plasma protein composition, serving
as additional evidence of physiological stress (Kumari *et al*., 2020; Alarape *et al*., 2024) [1, 8].

In addition to haematological disturbances, pesticide exposure significantly affects fish metabolism. Oxygen consumption rate is a widely used indicator of metabolic performance and energy expenditure. Reduced oxygen uptake under pesticide stress reflects impaired respiratory efficiency, mitochondrial dysfunction, and adaptive metabolic suppression aimed at minimizing energy loss (Saha *et al*., 2017; Sharma *et al*., 2021) [13, 16]. Organophosphates and organochlorines have been shown to reduce oxygen consumption by 40–60% in several freshwater fish species, highlighting their severe impact on metabolic health (Rohani *et al*., 2023; Witeska *et al*., 2023) [12].

Oreochromis mossambicus is an ecologically and economically important freshwater fish widely used in ecotoxicological research due to its hardy nature, ease of maintenance, and well-documented physiological responses to pollutants (Sarkar *et al*., 2018; Chauhan *et al*., 2024) [4, 14]. Evaluating the combined haematological and metabolic responses of *O. mossambicus* to sublethal concentrations of methyl parathion and endosulfan provides valuable insights into the comparative toxicity of organophosphorus and organochlorine pesticides.

Therefore, the present study aims to assess time-dependent changes in haematological parameters and oxygen consumption in *Oreochromis mossambicus* exposed to sublethal concentrations of methyl parathion and endosulfan, with emphasis on recovery potential following pesticide withdrawal.

Materials and Methods

1. Experimental Fish

Healthy adult specimens of *Oreochromis mossambicus* (mean length: 12–15 cm; mean weight: 35–45 g) were procured from a local freshwater fish farm and transported to the laboratory in aerated containers. Fish were acclimatized for 15 days in large fiberglass tanks (500 L capacity) containing dechlorinated tap water under laboratory conditions. During acclimation, fish were fed a commercial pelleted feed (30% crude protein) once daily, and uneaten feed was removed to maintain water quality. No mortality was observed during the acclimation period.

2. Water Quality Management

Physico-chemical parameters of water were monitored throughout the experimental period following standard procedures. Temperature was maintained at 26 ± 2 °C, pH at 7.2 ± 0.3, dissolved oxygen at 6.5–7.2 mg/L, total hardness at 120–140 mg/L as $CaCO_3$, and photoperiod at 12 h light: 12 h dark. Partial water renewal (50%) was carried out every alternate day to prevent accumulation of metabolic wastes and pesticide residues.

3. Chemicals and Preparation of Test Solutions

Analytical grade methyl parathion (organophosphorus pesticide) and endosulfan (organochlorine pesticide) were obtained from a certified supplier. Stock solutions were prepared in distilled water and diluted with dechlorinated tap water to obtain the desired sublethal concentrations. Based on preliminary range-finding and reported LC_{50} values, 1/10th of the 96 h LC_{50} concentration was selected as the sublethal dose for both pesticides to avoid mortality while eliciting measurable physiological responses.

4. Experimental Design

Fish were randomly divided into three major groups, each with six individuals (n = 6) per treatment:

- **Group 1 (Control):** Maintained in pesticide-free water
- **Group 2 (Methyl parathion):** Exposed to sublethal concentration of methyl parathion
- **Group 3 (Endosulfan):** Exposed to sublethal concentration of endosulfan

Exposure was carried out for 10, 20, and 30 days. At the end of 30 days, a recovery experiment was conducted by transferring a subset of exposed fish to pesticide-free water and maintaining them for an additional 30 days under the same environmental conditions.

5. Blood Collection

At the end of each exposure period (10, 20, and 30 days) and recovery phase, fish were anaesthetized using MS-222 (tricaine methanesulfonate). Blood samples were collected from the caudal vein using heparinized syringes to prevent coagulation. Samples were processed immediately for haematological analysis.

6. Haematological Analysis

Haematological parameters were analyzed using standard methods:

- **Red blood cell (RBC) count:** Determined using a Neubauer haemocytometer
- **Haemoglobin (Hb):** Estimated by Sahli's acid haematin method
- **Packed cell volume (PCV):** Measured using microhaematocrit centrifugation
- **Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC):** Calculated using standard formulae based on RBC, Hb, and PCV values
- **Erythrocyte sedimentation rate (ESR):** Determined using the Wintrobe method

All measurements were expressed as mean ± standard deviation (SD).

7. Measurement of Oxygen Consumption

Oxygen consumption was measured to assess metabolic activity using a closed respirometry method. Individual fish were placed in airtight respirometer chambers filled with aerated water. Dissolved oxygen levels were measured at the beginning and end of a fixed incubation period using a Winkler titration method. Oxygen consumption was expressed as ml O_2/g body weight/hour.

8. Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences between control and treatment groups. Post-hoc comparisons were performed using Tukey's multiple comparison test. Differences were considered statistically significant at $p < 0.05$. Statistical results are presented as mean ± SD.

Results

1. Haematological Responses to Pesticide Exposure

Haematological parameters serve as sensitive biomarkers for detecting physiological and metabolic disturbances in fish under chemical stress. In the present study, exposure of *Oreochromis mossambicus* to sublethal concentrations of methyl parathion and endosulfan resulted in significant, time-dependent alterations in haematological indices (Tables 1 and 2).

1.1 Red Blood Cells (RBC), Haemoglobin (Hb), and Packed Cell Volume (PCV)

RBC counts, haemoglobin concentration, and PCV exhibited progressive declines with increasing duration of pesticide exposure. In methyl parathion-treated fish, RBC decreased from 2.85 × 10^6 cells/mm³ in controls to 1.78 × 10^6 cells/mm³ after 30 days. Hb decreased from 9.6 g/dL to 6.8 g/dL, and PCV from 32.4% to 21.5%. Following a 30-day recovery period, these values partially restored to 2.60 × 10^6 cells/mm³ (RBC), 9.0 g/dL (Hb), and 30.2% (PCV).

Endosulfan exposure caused more pronounced reductions, with RBC decreasing to 1.55 × 10^6 cells/mm³, Hb to 6.1 g/dL, and PCV to 18.9% at 30 days. Recovery treatments restored values to 2.38 × 10^6 cells/mm³ (RBC), 8.6 g/dL (Hb), and 28.4% (PCV), indicating partial reversibility of haematological damage upon cessation of pesticide exposure.

1.2 Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC)

Both pesticides induced a decline in MCV, indicating microcytic anaemia. MCV values decreased from 113.7 fL in controls to 96.4 fL (methyl parathion) and 90.8 fL (endosulfan) after 30 days. Conversely, MCH and MCHC values increased progressively, indicating hypochromic changes. In methyl parathion-treated fish, MCH rose from 33.7 pg to 38.2 pg, and MCHC from 29.6% to 33.6%. Endosulfan-treated fish showed MCH and MCHC values of 39.2 pg and 34.8%, respectively, at 30 days. Recovery periods restored these indices closer to control values.

1.3 Erythrocyte Sedimentation Rate (ESR)

ESR increased in a time-dependent manner in both pesticide treatments. Methyl parathion exposure elevated ESR from 3.2 mm/hr to 7.8 mm/hr at 30 days, while endosulfan increased ESR to 9.0 mm/hr. Recovery treatments reduced ESR towards baseline, demonstrating that the physiological stress caused by sublethal pesticide exposure was partially reversible.

2. Oxygen Consumption Under Pesticide Stress

Exposure to sublethal concentrations of methyl parathion and endosulfan significantly reduced the metabolic rate of *O. mossambicus* (**Table 3**). Oxygen consumption decreased progressively with exposure duration. Methyl parathion-treated fish showed reductions of 27.9%, 44.2%, and 51.2% at 10, 20, and 30 days, respectively. Endosulfan caused greater inhibition, with reductions of 32.6%, 48.8%, and 58.1%, indicating that endosulfan is more toxic than methyl parathion. Recovery treatments facilitated partial restoration of oxygen consumption, demonstrating the capacity of fish to regain metabolic function after cessation of chemical stress.

Table 1: Haematological Parameters of *Oreochromis mossambicus* Exposed to Sublethal Concentrations of Methyl Parathion (Mean ± SD, n = 6)

Parameter	Control	10 Days	20 Days	30 Days	Recovery (30 Days)
RBC (×10^6 cells/mm³)	2.85 ± 0.12^{a}	2.42 ± 0.10^{b}	2.05 ± 0.08^{c}	1.78 ± 0.07^{d}	2.60 ± 0.11^{ab}
Haemoglobin (g/dL)	9.6 ± 0.4^{a}	8.4 ± 0.3^{b}	7.6 ± 0.3^{c}	6.8 ± 0.2^{d}	9.0 ± 0.3^{ab}
Packed Cell Volume (%)	32.4 ± 1.2^{a}	28.6 ± 1.1^{b}	24.8 ± 1.0^{c}	21.5 ± 0.9^{d}	30.2 ± 1.1^{ab}
MCV (fL)	113.7 ± 4.2^{a}	108.2 ± 3.8^{b}	102.5 ± 3.5^{c}	96.4 ± 3.1^{d}	110.6 ± 3.9^{ab}
MCH (pg)	33.7 ± 1.1^{d}	34.6 ± 1.2^{c}	36.8 ± 1.3^{b}	38.2 ± 1.4^{a}	34.6 ± 1.2^{c}
MCHC (%)	29.6 ± 1.0^{d}	30.8 ± 1.1^{c}	32.1 ± 1.2^{b}	33.6 ± 1.3^{a}	30.1 ± 1.0^{c}
ESR (mm/hr)	3.2 ± 0.4^{d}	4.6 ± 0.5^{c}	6.2 ± 0.6^{b}	7.8 ± 0.7^{a}	4.0 ± 0.4^{c}

Note: Different superscripts (a–d) within a row indicate significant differences at $p < 0.05$ (Tukey's post-hoc test).

Table 2: Haematological Parameters of *Oreochromis mossambicus* Exposed to Sublethal Concentrations of Endosulfan (Mean ± SD, n = 6)

Parameter	Control	10 Days	20 Days	30 Days	Recovery (30 Days)
RBC (×10^6 cells/mm³)	2.85 ± 0.12^{a}	2.30 ± 0.09^{b}	1.92 ± 0.07^{c}	1.55 ± 0.06^{d}	2.38 ± 0.10^{ab}
Haemoglobin (g/dL)	9.6 ± 0.4^{a}	8.0 ± 0.3^{b}	6.9 ± 0.2^{c}	6.1 ± 0.2^{d}	8.6 ± 0.3^{ab}
Packed Cell Volume (%)	32.4 ± 1.2^{a}	26.8 ± 1.0^{b}	22.5 ± 0.9^{c}	18.9 ± 0.8^{d}	28.4 ± 1.0^{ab}
MCV (fL)	113.7 ± 4.2^{a}	104.5 ± 3.6^{b}	97.6 ± 3.2^{c}	90.8 ± 3.0^{d}	107.3 ± 3.7^{ab}
MCH (pg)	33.7 ± 1.1^{d}	35.1 ± 1.2^{c}	37.4 ± 1.3^{b}	39.2 ± 1.5^{a}	35.3 ± 1.2^{c}
MCHC (%)	29.6 ± 1.0^{d}	31.6 ± 1.1^{c}	33.2 ± 1.2^{b}	34.8 ± 1.4^{a}	30.6 ± 1.1^{c}
ESR (mm/hr)	3.2 ± 0.4^{d}	5.2 ± 0.6^{c}	7.1 ± 0.7^{b}	9.0 ± 0.8^{a}	4.8 ± 0.5^{c}

Table 3: Oxygen Consumption Rate of *Oreochromis mossambicus* Under Pesticide Stress (Mean ± SD, n = 6)

Treatment	Oxygen Consumption (ml O_2/g/hr)	% Change from Control
Control	0.86 ± 0.04	—
Methyl Parathion (10 days)	0.62 ± 0.03	−27.9
Methyl Parathion (20 days)	0.48 ± 0.02	−44.2
Methyl Parathion (30 days)	0.42 ± 0.02	−51.2
Endosulfan (10 days)	0.58 ± 0.03	−32.6
Endosulfan (20 days)	0.44 ± 0.02	−48.8
Endosulfan (30 days)	0.36 ± 0.02	−58.1

Note: Values are mean ± SD (n = 6). Significant differences from control may be determined using ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

3. Summary

Both methyl parathion and endosulfan induced significant haematological and metabolic alterations in *Oreochromis mossambicus*, characterized by:

- Decreased RBC, Hb, PCV, MCV, and oxygen consumption
- Increased MCH, MCHC, and ESR

These changes are indicative of microcytic and hypochromic anaemia. Endosulfan exerted more pronounced effects than methyl parathion. Recovery treatments over 30 days partially restored haematological and metabolic parameters, highlighting the resilience of *O. mossambicus* to sublethal pesticide exposure.

Discussion

The present study demonstrates that sublethal exposure to methyl parathion and endosulfan induces marked haematological and metabolic disturbances in *Oreochromis mossambicus*, with effects intensifying over time. These findings support the use of blood indices and oxygen consumption as sensitive biomarkers of pesticide-induced stress in freshwater fish (Witeska *et al*., 2016; Banaee, 2019; Rohani *et al*., 2023) [2, 19, 20].

The observed decline in RBC count, haemoglobin concentration, and packed cell volume indicates the development of anaemia, likely resulting from haemolysis, inhibited erythropoiesis, and oxidative damage to erythrocyte membranes (Velisek *et al*., 2011; Banaee *et al*., 2014; Monteiro *et al*., 2019) [3, 9, 18]. Endosulfan caused more pronounced haematological alterations than methyl parathion, reflecting its higher persistence and bioaccumulative nature and prolonged physiological stress in fish (Silva *et al*., 2015; Sharma *et al*., 2019; Chauhan *et al*., 2024) [4, 15, 17].

Alterations in erythrocyte indices, particularly reduced MCV with increased MCH and MCHC, indicate microcytic and hypochromic anaemia, a common response to chronic pesticide exposure (Kondera *et al*., 2020; Kumari *et al*., 2020; Rohani *et al*., 2023) [7, 8, 12]. Elevated ESR values further suggest inflammatory responses and changes in plasma protein composition, confirming systemic stress, especially in endosulfan-exposed fish (Gabriel *et al*., 2018; Alarape *et al*., 2024) [1, 6].

The progressive reduction in oxygen consumption reflects metabolic depression caused by impaired gill function, mitochondrial dysfunction, and suppression of aerobic metabolism under pesticide stress (Saha *et al*., 2017; Ramesh *et al*., 2018; Witeska *et al*., 2023) [11, 13, 20]. Greater inhibition in endosulfan-treated fish highlights its stronger impact on respiratory physiology. Partial recovery of haematological and metabolic parameters following pesticide withdrawal indicates physiological resilience in *O. mossambicus*, though incomplete restoration—particularly after endosulfan exposure—suggests lingering toxic effects due to pesticide persistence (Banaee *et al*., 2014; Silva *et al*., 2015) [3, 17].

Overall, the results confirm that prolonged sublethal pesticide exposure can significantly impair fish health, even in the absence of mortality, underscoring the ecological risks posed by continued pesticide contamination in freshwater ecosystems.

Summary and Conclusion

The present study evaluated the haematological and metabolic responses of *Oreochromis mossambicus* exposed to sublethal concentrations of methyl parathion and endosulfan. The major findings can be summarized as follows:

- Both pesticides induced significant, time-dependent reductions in RBC count, haemoglobin concentration, packed cell volume, mean corpuscular volume, and oxygen consumption.
- Increases in MCH, MCHC, and erythrocyte sedimentation rate indicate the development of microcytic, hypochromic anaemia and inflammatory stress.
- Endosulfan exerted more pronounced haematological and metabolic toxicity than methyl parathion, highlighting its higher persistence and bioaccumulative potential.
- Recovery experiments demonstrated partial restoration of altered parameters, suggesting some degree of physiological resilience in *O. mossambicus*, though complete recovery was not achieved, especially after endosulfan exposure.

In conclusion, the study confirms that haematological indices and oxygen consumption are sensitive and reliable biomarkers for assessing sublethal pesticide toxicity in fish. The results underscore the ecological risk posed by continued use of organophosphorus and organochlorine pesticides in agricultural practices and emphasize the need for stricter regulation, monitoring, and adoption of eco-friendly pest management strategies to protect freshwater ecosystems.

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