Fish Hematopathology Profile is Influenced by the Pollutants in a Lake Water

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Received: 24th August, 2022; Accepted: 15th October, 2022; Published online: 1st November, 2022

https://doi.org/10.33745/ijzi.2022.v08i02.074

Abstract: The present investigation assessed the toxic effects of aquatic pollution on fish collected from the contaminated station of Chakkamkandam lake. Both, water and fish (Mugil cephalus) samples were collected from lake water of Chakkamkandam lake at Guruvayur (Kerala, India). Fish are the most affected organisms by toxicants present in water bodies among all aquatic creatures because they are the dominant species in this ecosystem. When fish consume contaminated food, pollutants/toxicants enter the fish’s digestive system. It can also enter through the fish’s gills and skin. After absorption, they are finally transported by the bloodstream to the organs and tissues where they accumulate.

Water quality parameters were assessed and various blood parameters of the fish were analyzed. The changes in haemoglobin concentration, red blood cells, and white blood cell count were studied. The secondary blood indices were also calculated. Compared to the reference site, the fish from the lake water showed decreased numbers of RBC, Haemoglobin (Hb), and hematocrit (Hct). However, the values of WBC count, and Mean Corpuscular Haemoglobin Concentration (MCHC) increased significantly. The changes in hematological parameters of fish from the polluted station revealed that the health of fish is disturbed. Additionally, the results suggested that blood parameters are useful tools in monitoring of aquatic pollution. These biomarkers show that fish have macrocytic hypochromic anemia. Leucocytosis showed a general defence response against pollution-induced toxicity. In conclusion, the results of the current study showed that the water quality impacted hematological profile of the fish Mugil cephalus collected from lake water.

Keywords: Biomarkers, Lake Pollution, Hematological parameters, Mugil cephalus, RBC, WBC, Haemoglobin

Citation: Nimmy M.V. and Pawlin Vasanthi Joseph: Fish hematopathology profile is influenced by the pollutants in a lake water. Intern. J. Zool. Invest. 8(2): 605-612, 2022.
https://doi.org/10.33745/ijzi.2022.v08i02.074

Introduction

Environments near freshwater are regularly used as landfills for industrial and urban garbage. The aquatic ecology and its biota are badly impacted by such anthropogenic activities. The primary cause of aquatic contamination is anthropogenic activity. Many organic and inorganic substances, including plastics, medications, pesticides, and heavy metals have catastrophic effects on freshwater ecosystems (Reddy, 2016; Srivastava and Reddy, 2020).
Around 1.8 billion people utilize water sources that are contaminated with faeces, putting their health in considerable danger. Around 80 per cent of wastewater worldwide flows back into the ecosystem without being cleaned or reused. A recent study found that India produced roughly 61,948 million litres of sewage from metropolitan areas every day. Although only 38% of this sewage can be handled, the remaining 80% will be released into the environment without adequate treatment (Sengupta, 2018). Fish are susceptible to chemical and physical changes in the water reservoirs since they are surrounded by them constantly. These changes may be reflected in the components of their blood (Sheikh and Ahmed, 2016; Witeska, 2022). In ecotoxicological research, fish are frequently utilized as biomonitoring species because they are sensitive to the potential dangers of toxicants introduced to the aquatic environment (Srivastava and Reddy, 2020).

It is important to consider the physiological and biochemical consequences of changes in harmful environmental elements or pollution to properly interpret the Hematological results in fish. Animal and plant life alike are negatively impacted by water pollution. Physicochemical characteristics of water, such as temperature, pH, dissolved oxygen, nitrites, nitrates, carbon dioxide, pesticides, phosphates, etc., also affect fish health (Sahiti et al., 2018).

Hematological parameters in fish are affected by gender, sexual maturity, temperature, dissolved oxygen, pH, some water quality indicators including calcium, magnesium, and nitrate, illnesses, and other environmental factors in this context (Küçükgül et al., 2019). Monitoring environmental quality and the health of fish living in polluted settings has become more appealing and practical with the use of specific biomarkers. (Fazio et al., 2012).

Fish are largely used for the assessment of aquatic environment quality and are accepted as bioindicators for environmental pollution. The hematological profile represents a good indicator of physiological dysfunctions (Elahee and Bhagwant, 2007) and it provides information not only about the health status of fish and the physical and chemical parameters of the water in which they live, but even to evaluate the relationship among these factors and correlate them with the status of health of organism in respect to environmental conditions (Debala Devi and Usha Anandhi, 2010).

Some hematological indices such as hemoglobin (Hb), Hematocrit (Hct), total erythrocyte (TEC), total leukocyte counts (TLC) and mean corpuscular haemoglobin (MCH) are considered to measure the health status of the fish and ecological pollution. Therefore, the present study was aimed to evaluate the hematological parameters of the freshwater fish, *Mugil cephalus* collected from the Chakkamkandam lake in Kerala (India). Through analysis of hematological parameters in the blood of *Mugil cephalus* collected from the lake of Chakkamkandam, the goal of this study was to evaluate the effects of pollution and the influence of water quality on fish health.

**Materials and Methods**

*Study Area:*

In the Thrissur district of Kerala, on the southwest coast of India, Chakkamkandam is a hamlet area close to Chavakkad and Guruvayur. The location was well-known for its beauty, and a few years ago, it was an excellent choice for outdoor enjoyment because it is located on the banks of a water body. The lake has become a garbage dump instead of a resource that may generate cash. The sewage from the renowned pilgrimage site of Guruvayoor town merges as a surface river with the water of Lake Chakkamkandam. Then it flows forward to lake Chettuva and then meets the Lakshadweep Sea. Many hectares of rice fields are enriched and the water table is recharged during the course of the current. A portion of the lake is currently cordoned off for aquaculture. The geographical position of the lake is 10° 31′ 4″ N latitude and 76° 2′ 28″ E longitude.
The geographical location and sampling locations of the Chakkamkandam lake are presented in Figure 1.

The collected water samples were processed for physicochemical parameters in water (APHA, 1998).

Fish:
Live samples of both sexes of fish *Mugil cephalus* (n = 10; 8.2 ± 0.7 cm; 7.1 ± 0.41 g) were collected from a fish farm and polluted lake (polluted site) of the Chakkamkandam lake during pre-monsoon season in 2021 with the help of local fisherman. The fish were immediately transported to the laboratory.

Hematological study:
Blood sample was collected by cardiac puncture using disposable syringes and kept in separate vials. Hematological parameters like Hb, RBC, WBC, ESR and PCV were estimated following the procedures of Wintrobe (2008) and Sood (1996). Mean cell haemoglobin concentration (MCHC), Mean cell haemoglobin (MCH), and Mean cell volume (MCV) were calculated using the formulae mentioned by Dacie and Lewis (2001).

Statistical analysis:
The data were statistically analyzed for the calculation of standard error (S.E.) and the students t test was performed by computer software excel program. The data shown are the means of three replicates ± S.E.

Results and Discussion
Water Quality Parameters:
Significant differences in the water quality of Kerala’s Chakkamkandam Lake were found between the survey sites. The area surrounding the municipal sewage flow channel of the lake has poor water quality. The results of the water quality parameters of Chakkamkandam lake in Kerala are presented in Table1.

The present study revealed that water parameters exceeded the permitted limits established by the Central Pollution Control Board (CPCB). Most of the parameters showed the poorest values at the polluted station, displaying the worsening water quality at this station (Fig. 1). The discharge of both municipal sewage and anthropogenic activities contributed to this fall in water quality. Moreover, the low levels of dissolved
Table 1: Water quality data from Upstream and Downstream (polluted area) of Chakkamkandam lake in Kerala

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Upstream</th>
<th>Downstream</th>
<th>% change over reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>31.5±0.10</td>
<td>31.8±0.10</td>
<td>0.95</td>
</tr>
<tr>
<td>DO (mg/l)</td>
<td>5.35±0.19</td>
<td>3.57±0.16</td>
<td>-33.27</td>
</tr>
<tr>
<td>pH</td>
<td>7.19±0.05</td>
<td>7.84±0.10</td>
<td>9.04</td>
</tr>
<tr>
<td>TDS (mg/l)</td>
<td>942.00±201.14</td>
<td>29730±6302.83</td>
<td>3056.05</td>
</tr>
<tr>
<td>Total hardness (mg/l)</td>
<td>174±29.40</td>
<td>12412±1015.54</td>
<td>7033.33</td>
</tr>
<tr>
<td>TSS (mg/l)</td>
<td>296.50±47.52</td>
<td>526.2±49.96</td>
<td>77.47</td>
</tr>
<tr>
<td>BOD (mg/l)</td>
<td>2.59±0.25</td>
<td>8.14±0.29</td>
<td>214.28</td>
</tr>
<tr>
<td>COD (mg/l)</td>
<td>18.50±1.70</td>
<td>53.50±9.70</td>
<td>189.18</td>
</tr>
</tbody>
</table>

Values are mean ± SE

Fig. 1: Analysis of water parameters.

Oxygen observed at the station are the result of the increase in BOD and bacterial activity as well as the rise in ammonia nitrogen, leading to oxygen consumption from the municipality sewage discharge.

Hematological Parameters:

The results of blood analyses (n=10) are given in Table 2. The Hb percentage is significantly decreased (-6.20%) in the fish collected from the pollutants containing lake water.

The RBC and hemoglobin content (-20% and -6.20%) decreased significantly in the fish collected from lake when compared to the fish collected from fish farm (control). However, a significant increase in total white blood cell (WBC) count over the controls was observed (14.63%) (Fig. 2). The mean corpuscular volume (MCV) value and mean corpuscular hemoglobin (MCH) values decreased (-0.92% and -3.14%) in comparison with control fish while mean corpuscular hemoglobin concentration (MCHC) values increased (2.79%) in comparison with control fish. Platelet count values decreased (4.54 %) in comparison with control fish. There were no changes in neutrophil percentage between the lake fish and control fish (Table 2).

Aquatic animals are frequently exposed to a variety of stresses, such as overcrowding, pollution, and transportation, which can directly affect their biological and hematopoietic responses. Changes in fish’s hematological characteristics can offer useful information for identifying stress, environ-
Table 2: Changes in hematological parameters in freshwater fish, *Mugil cephalus*

| Parameter                  | Control fish | Lake Fish     | % Change over control | Equal variance assumed (t Statistics) | DF | Prob>|/t/| |
|---------------------------|--------------|---------------|-----------------------|---------------------------------------|----|--------|
| WBC (x10³ µl⁻¹)           | 820±0.57     | 940±0.33**    | 14.63%                | 179.5                                 | 4  | 0.000  |
| RBC (x10⁶ µl⁻¹)           | 4.5±0.057    | 3.9±0.033**   | -20%                  | 9.5                                   | 4  | 0.001  |
| HB (%)                    | 12.9±0.03    | 12.1±0.033**  | -6.20%                | 15.55                                 | 4  | 0.000  |
| PCV (%)                   | 38.9±0.057   | 38.3±0.057**  | -1.54%                | 7.348                                 | 4  | 0.002  |
| MCV (fl)                  | 86.4±0.057   | 85.6±0.11**   | -0.92%                | 6.19                                  | 4  | 0.003  |
| MCH (pg)                  | 28.6±0.057   | 27.7±0.15**   | -3.14%                | 5.511                                 | 4  | 0.005  |
| MCHC (%)                  | 32.2±0.11    | 33.1±0.18**   | 2.79%                 | 3.96                                  | 4  | 0.017  |
| Platelet count (X 10⁵)    | 22000±1.52   | 21200±1.15**  | -4.54%                | 417.78                                | 4  | 0.000  |
| Neutrophils (x10³. µl⁻¹)  | 3.22±0.88    | 1.02±0.88**   | -68.32%               | 4.427                                 | 4  | 0.011  |
| Lymphocytes (x10³ µl⁻¹)   | 10.3±0.33    | 8.18±0.57**   | -20.58%               | 4.24                                  | 4  | 0.013  |

MCV= mean corpuscular volume, MCHC= mean corpuscular hemoglobin concentration, MCH= mean corpuscular hemoglobin, PCV= Packed cell volume. Values are mean ± SE; ** indicates significance at p<0.01

Fig. 2: Analysis of blood parameters of fish.
mental contamination, and pathology (Srivastava and Reddy, 2020). Changes in these indices indicate disease. Low PCV and Hb levels are typically associated with low TEC, which has also been seen in the current investigation. In the present study, Hb percentage, RBC count and Hct values significantly decreased in the fish collected from downstream. In contrast, WBC counts, and MCHC values were found notably increased compared to control fish. Anaemia is indicated by a decrease in the total erythrocyte count, haemoglobin percentage, and Hct. The best blood indicator of environmental stress appears to be Hb. In addition to behavioural and morphological changes, fish must modify several physiological and biochemical characteristics to adapt to low oxygen levels (Martínez et al., 2011). Previous studies have similarly confirmed low DO levels at this site. We must take into account the effects of additional toxicants existing in the polluted lake, such as sewage contamination and anthropogenic activity.

Changes in hematological value could be used as a preliminary indicator of the toxicant’s effects on fish health. The improper chelator induces a new equilibrium between cells that require iron and the cells, changing the iron homeostasis in the exposed cells (the pollutant or its catabolite). Fish exposed to contaminated water may experience adverse biological effects due to the disruption of functional iron homeostasis that results from environmental pollutants (Schreinemachers and Ghio, 2016). Hb among the hematological parameters is an indicator for evaluation of stress. The increased breakdown and decreased synthesis of Hb are the main causes of the decrease in blood Hb and Hct (Imtiaz Ahmed et al., 2022). The decrease in haemoglobin concentration may possibly be due to the pollutants-induced production of reactive oxygen species (ROS) which might have destroyed the cell membrane of erythrocyte and its function (Kumar Maurya, 2019). The decrease in blood indices may be due to haemolysis and haemodilution, a manner of diluting and reducing the effect of the toxicant/pollutants.

The lake’s toxic concoction may harm mature RBCs or prevent erythropoiesis, which would explain the decline in total RBC, haemoglobin percentage, and Hct measurements seen in the current study. One more possible explanation for the reduction in RBC and Hb may be due to the cytotoxic effects of pollutants on the hematopoietic tissue as reported in Heteropneustes fossilis (Bujjamma and Padmavathi, 2018; Kumar Maurya et al., 2019).

The higher number of WBC in the fish collected from lake probably might be due to the stimulation of the animal’s defense mechanism and the immune system by pollutants. Most of the oxygen inspired by fish (95%) is utilized for ATP production. Fish react to hypoxia with mixed behavioral, functional, and cellular responses to maintain homeostasis and functional physiology in a low-oxygen environment (hypoxia). The changes in the number of white blood cells are the natural response to exposure to toxicants (Narra et al., 2017). In the present study, WBC count is significantly increased in the fish collected from the lake, which may be due to stimulation of the defense mechanism of the fish to counteract the stress of toxicant. Similar results were recorded on the toxicity and recovery of insecticides on Hematological parameters in Labeo rohita and Cyprinus carpio (Saravanan et al., 2011).

The lower levels of RBC, Hct, and Hb concentration in this study may call for a well-coordinated recovery to replenish the environment’s reduced oxygen supply. On the other hand, leucocytes play a major role in phagocytic and immunological responses. The increase in the number of leucocytes (leucocytosis) in the present study is a common response against the entry of contaminants. Accordingly, a higher WBC count observed in fish collected from lake could be caused by exposure to a complex mixture of pollutants occurring due to the mixing of both urban and industrial waste (Reddy and Baghel, 2012).

Stress may change the values of red blood parameters (Hct, Hb, RBC, MCV) and various
biochemical indices (e.g. glucose, catecholamine and cortisol levels). An increase in the values of red blood parameters often occurs due to stress since stress reaction involves higher energy expenditure ("fight or flight") and an increase in oxygen transport is one of the stress-related adaptive mechanisms. In fish, it may involve erythrocyte swelling (increase in MCV and Hct).

Stress-induced increases in red blood indices were reported by Fazio et al. (2015) and Aguirre-Guzman et al. (2016). On the other hand, stress may affect white blood parameters. Short-term stress sometimes results in an increase in WBC but chronic and/or strong stress usually causes leukopenia (Tort, 2011). Stress may also affect differential leukocyte count causing the shift from lymphocytes to neutrophils and monocytes (Grzelak et al., 2017). Both acute and chronic thermal stress affected all red and white blood parameters in a different way depending on acclimation temperature (Witeska, 2022).

Conclusion

The changes in hematological parameters are associated with low levels of dissolved oxygen (DO). The reduction in blood values might also be due to the disrupting action of water contaminants on erythropoietic tissue, which consecutively induced anaemic conditions in the fish of the lake.

References


