Nimit Mai Review
Pacific Institute of Management Science
(Humanities and Social Sciences

Year 3 Issue 2 July-December 2020

HAEMATOLOGICAL PROFILE IN FISH AS AN EFFECTIVE AND SENSITIVE INDEX IN AQUATIC POLLUTION

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Received October 23, 2020 & Revise December 5, 2020 & Accepted December 24, 2020

Abstract

The present investigation assessed toxic effects of aquatic pollution on fish collected from the contaminated station of Chambal River. Both water and fish (Mystus tengara) samples were collected from upstream and downstream of Chambal River at Nagda, Ujjain (M.P. India). Water quality parameters of the water were assessed and fish were used to study the various blood parameters. 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 downstream shown decreased numbers of RBC, Hb and Hct. However the values of WBC count, and Mean Corpuscular Volume (MCV) increased significantly. The Mean Corpuscular Haemoglobin Concentration (MCHC) values were significantly reduced. These outcomes advocate that fish from the polluted station revealed alterations in haematological responses, which possibly point out the health disturbances. Additionally, the results suggest that blood parameters are useful, tools in the monitoring of aquatic pollution. These biomarkers show that fish have macrocytic hypochromic anaemia. Leucocytosis showed general defence response against the pollution-induced toxicity. In conclusion, the results acquired from the current study shown that the fish M.tengara at downstream was exposed to pollution-induced stress that caused a significant reduction in Hb, RBC and Hct values but increased the clotting time, ESR and WBC values. Water from the downstream has strong potential to induce stress making the fish anaemic, weak, and vulnerable to diseases.

Keywords: Biomarkers, River Pollution, Haematological Parameters, MystusTengara.

Introduction

Though industrialization has brought economic affluence but it also resulted obvious stress by discharging huge waste into the soil and water (Reddy, P.B. and Baghel, B.S.,(2012) ;Srivastava, B. and Reddy, P.B. (2017). ;Reddy, P.B. and Singh, R.K. (2011)Xenobiotics and heavy metals present in wastewater could cause negative effects on the flora and fauna. Fishes are extensively used as biomonitoring organisms in ecotoxicological studies as they are sensitive to the probable risks of contaminants introduced in the aquatic environment(Walker, C.H., Sibly, R.M., Hopkin, S.P. and Peakall, D.B., (2005). ;Reddy, P.B. and Rawat, S.S. (2013 ;Reddy, P.B., (2016))Fish are sensitive and very vulnerable to alterations of water quality, which possibly reflected in their blood components.(Reddy, P.B. and Baghel, B.S.,(2012; Reddy, P.B. and Rawat, S.S. (2013; Blaxhall, P.C (1972)

The endurance, distribution, reproduction, and normal metabolism of fish depend on water quality parameters. Blood parameters are most vital markers of the physiological stress that reflect the endogenous or exogenous changes in fish.(Meraj, M., Nizam, M., Wani, S.,

Maqbool, F., Ali, M.N., Ganai, B.A. and Bhat, F.A., (2017)Walia, G.K., Handa, D., Kaur, H. and Kalotra, R.(2013)

Blood indices can offer adequate information about the overall health status and physiological response of fish to environmental changes that affect homeostasis Elahee, K.B. and Bhagwant, S., (2007)Reddy, P.B. and Tiwari, R.K., (2010)

Haematological studies facilitate us in understanding the association of blood indices to the habitat and adaptability of the species to the environment. A huge number of intrinsic and extrinsic factors cause variations in haematological data. (Reddy, P.B. and Tiwari, R.K., (2010);Reddy, P.B. Archana Kushwah, Shehla Ishaque and Baghel, B.S., (2010)Therefore, haematological studies are significant for ecological monitoring of fish and their health status as they are so closely linked with the aquatic environment.

Even though, fish haematology maintains to offer an important tool, but the progress in establishing normal range values has been sparse and information in this area is still incomplete. For that reason, a number of haematological indices such as haemoglobin (Hb), haematocrit (Hct), total erythrocyte (TEC) and 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 is aimed to evaluate haematological parameters of the fresh water fish, Mystus tengarafrom upstream and downstream of the Chambal River at Nagda (India). The informationgenerated from this study may offer a valuabledatabank for upcoming investigations of pollutant effects on haematological parameters in aquatic environment.

Research Methodology

Material and Methods: Study Area: River Chambal at Nagda town (23’27N and 75’25) receives wastewater from various industrial units and sewage from municipality of Nagda town. The surface water samples from upstream and downstream of the River were collected in December 2018 at almost 10 cm below the surface.Acid rinsed glass containerswere used for collection. Collected samples were processed for the analysis of various physico chemical parameters and heavy metals in water according to the protocol given by APHA. (American Public Health Association, A. P. H. A. (1998)

Fish: Live samples of fish Mystus tengara(n = 10), (8.2 ± 0.7cm; 7.1 ± 0.41g) (irrespective of the sex) were collected from upstream (Reference site) and downstream (polluted site) of the River during winter months of 2018 with the help of local anglers. They were immediately transferred to laboratory independently for further haematological studies.

Haematological study: Blood sample was collected by cardiac puncture using disposable syringes and kept in separate vials. Haematological parameters like Hb, RBC, WBC, ESR and PCV were estimated following the procedures of Wintrobe, (Wintrobe, M.M.,(2008).and Sood, (1996; Sood, R.(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 observed in the experiment were statistically analyzed for the calculation of standard error (S.E.) and students’’ test was administered for testing the hypothesis with the help of computer software excel program. The data shown are the averages of three replicates ± S.E.

Research Results

Water Quality Parameters: Major fluctuations in the water quality of Chambal River at Nagda were detected between the study stations. The site located at downstream of the River

was characterized by poor water quality conditions. The results of water quality parameters of Chambal River at Nagda are presented in Table.1.

Table 1: Water quality data from Upstream and Downstream (polluted area) of Chambal River at Nagda

Parameter	Upstream	Downstream	% Change over reference
Temperature ^{0}C	22.4±0.33	22.9±0.74	2.232
DO mg/L	7.4±0.42	4.6±0.5	-37.83
pH	7.1±0.09	8.8±0.89	23.94
TDS mg/L	110.10±5.3	392.5 ±5.3	256.49
Total hardness mg/L	200.5 ±9.67	1296.7±9.67	546.73
TSS mg/L	26.1±2.2	136.7±4.6	423.75
BOD mg/L	7.6±0.45	52.4±3.2	589.47
COD mg/L	11.6±1.01	32.3±2.4	178.44

Results point out that virtually all water parameters calculated were higher than the accepted limits laid by the Central Pollution Control Board (CPCB)(Kumar Maurya, P., Malik, D.S., Kumar Yadav, K., Gupta, N. and Kumar, S.,(2019)Most of the assessed parameters shown poorest values at station 2, displaying the worsening of water quality at this station. The discharge of both municipal sewage and industrial pollution contribute to this fall in water quality. Moreover, the low levels of dissolved oxygen observed at station 2 are the result of the increase in BOD, bacterial activity as well as to the rise in the ammonia nitrogen, leading to oxygen consumption downstream from the city sewage discharge.

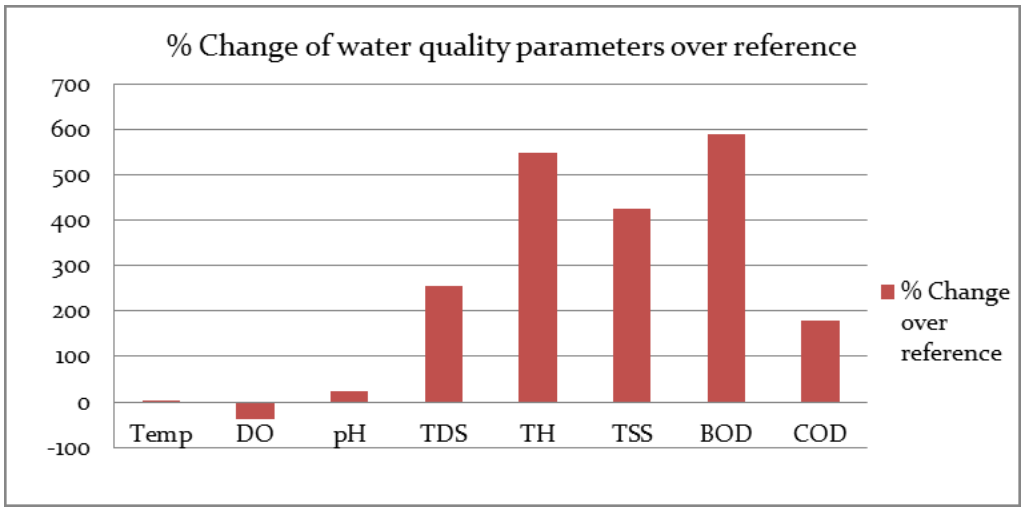

Figure 1 The results of blood analyses

Haematological Parameters: The results of blood analyses (n=10) are given in Table 2. The Hb percentage is significantly decreased (-11.326%) in the fish exposed to pollution.

Table 2: Changes in Haematological Parameters in Freshwater Fish, M.Tengara

Parameter	Reference fish	Exposed fish	% Change over reference
Hb%	10.33±0.06	9.16*±0.08	-11.326
RBC (x10 6/mm3)	3.95±0.04	3.11*± 0.12	-21.26
WBC (x10 3/mm	62.88±0.27	89.13*± 1.33	41.74
Hct%	43.42±1.20	28.31*±1.01	-34.79
ESR (mm/hr)	8.23± 0.57	16.94*±0.88	105.83
Ct/Sec	49.54± 0.03	58.88*± 0.15	18.85
MCV	144.36± 6.05	126.42*± 7.59	-12.42
MCHC	24.11± 0.82	27.16*± 1.37	12.65
MCH	34.87± 0.61	25.72± 2.10	-26.24

MCV= mean corpuscular volume, MCHC= mean corpuscular haemoglobin concentration, MCH= mean corpuscular haemoglobin. Values are mean of three replicate SE and (*) significance of $P<0.05$

The RBC number (-21.26%) and Hct percentage were decreased significantly in the exposed fish when compared to the fish from reference site. However,a significant increase in total white blood cells (WBC) count over the controls was observed (41.74%). The erythrocyte sedimentary rate (ESR) and clotting time (CT) registered an increased trend significantly (105.83% and 18.85%)in the fish of downstream in compare with control.The mean corpuscular volume (MCV) value and mean corpuscular haemoglobin (MCH) values decreased (-12.42% and -26.24%) in compare with control fish while mean corpuscular haemoglobin concentration (MCHC) values increased (12.65%) in comparison with control fish.

Discussion of Research Results

Blood is a pathophysiological reflector of the whole body. Aquatic animals are often exposed to various stressors like excess crowding, transport, and pollution, can directly reflect in various biological and haematological responses. Therefore, the study on the changes of haematological parameters of fish can provide a valuable information in the identification of stress, environmental contamination and pathology. (Elahee, K.B. and Bhagwant, S., (2007; Kumar Maurya, P., Malik, D.S., Kumar Yadav, K., Gupta, N. and Kumar, S.,(2019)) Changes in these indices from reference give an indication of disease. Low TEC usually leads to low PCV and Hb levels, which has also been observed in the current study. In the present study, Hb percentage, RBC content and Hct values significantly decreased in the fish of downstream. In contrast, WBC counts, ESR, CT and MCHC values were found notably increased compared to control fish.Reduction in total erythrocyte count, haemoglobin percentage, and Hct values indicates the occurrence of anaemia. Hb seems to be the best blood indicator of environmental stress.[20] Besides, behavioral and morphological adjustments, fish has to adjust to low oxygen levels by altering several physiological and biochemical parameters.(De Almeida-Val, V.M.F., Gomes, A.R.C. and Lopes, N.P., (2005))

Earlier investigations have also reported low DO levels at this station. (Reddy, P.B. and Singh, R.K. (2011;Reddy, P.B., (2017), Nevertheless, we cannot reject the effects of other pollutants (xenobiotics, pesticides, ammonia, heavy metals, etc.) present in the downstream. The reduction in haemoglobin value perhaps due to distraction of iron synthesis in fish exposed to polluted water. The decrease in total RBC, haemoglobin percentage and Hct values in the present study perhaps due to the damage of mature RBCs or inhibition of erythropoiesis by cocktails of pollutants in River. 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 in Heteropneustes fossilis. (Bujjamma, P, and Padmavathi, 2018 ;Kumar Maurya, P., Malik, D.S., Kumar Yadav, K., Gupta, N. and Kumar, S (2019) Decrease in haemoglobin concentration may perhaps due to pollutants induced production of reactive oxygen species (ROS) which might be caused the destruction of the cell membrane of erythrocyte and its function(Kumar Maurya, P., Malik, D.S., Kumar Yadav, K., Gupta, N. and Kumar, S (2019)Pandey, K.B. and Rizvi, S.I.,(2011))Another possible explanation for the decrease in blood indices may be due to haemolysis and haemodilution, a manner of diluting and reducing the effect of the toxicant/pollutants. (Lavanya, S., Ramesh, M., Kavitha, C. and Malarvizhi, A., (2011)

The higher number of WBC in the exposed probablymight be due to the stimulation of the animal's defense mechanism and the immune system by pollutants. Most of the 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 ina low oxygen environment (hypoxia).The decreased levels of RBC, Hct and Hb concentration in the present study maybe demand a well-coordinated comeback to obtain more oxygen from the depleted environment. Conversely, leucocytes are mainly involved in phagocytic and immune responses. The increase in the number of leucocytes (leucocytosis) in the present study is a common response against the entry of contaminants. Accordingly, higher number of WBC count was observed in fish at downstream could be caused by heavy metals exposure or probably a complex mixture of pollutants could be occurring due to mixing ofboth urban and industrial waste.(Reddy, P.B. and Baghel, B.S.,2012 ;Reddy, P.B. and Singh, R.K. ,2011)

The changes in the number of white blood cells are the natural response on the exposure to toxicant (Narra, M.R., Rajender, K., Reddy, R.R., 2017; Lavanya, S., Ramesh, M., Kavitha, C. and Malarvizhi, A.,2011) In the present study, WBC count (Table 1) is significantly increased in the fish exposed to pollution at downstream, 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 haematological parameters in Labeo rohita and Cyprinus carpio. (Saravanan, M., Kumar, K.P. and Ramesh, M.,2011)

The hematocrit (Hct) values are significant to determine the effects of a stressor on the health status of fish and are used to determine the oxygen carrying capacity of blood. Variations in Hct happened in the fish of downstream under hypoxic conditions. The significant increases in Hb concentration in were complemented by increases in the number of erythrocytes perhaps to raise the blood oxygen capacity in order to supply more oxygen to the tissues under hypoxic conditions. We also noticed that the changes in haematological 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 condition in fish of downstream. The decrease of RBC is mostly due to development of hypoxic condition, which successively leads to increase in the destruction of RBC or decrease in the rate of formation of RBC due to non-availability of Hb content in the cellular medium.[31] The anaemic condition in fish results from an abnormal decrease of erythrocytes or with little amount of haemoglobin.(Singh, D., Nath, K., Trivedi, S.P. and Sharma, Y.K., ,2009)

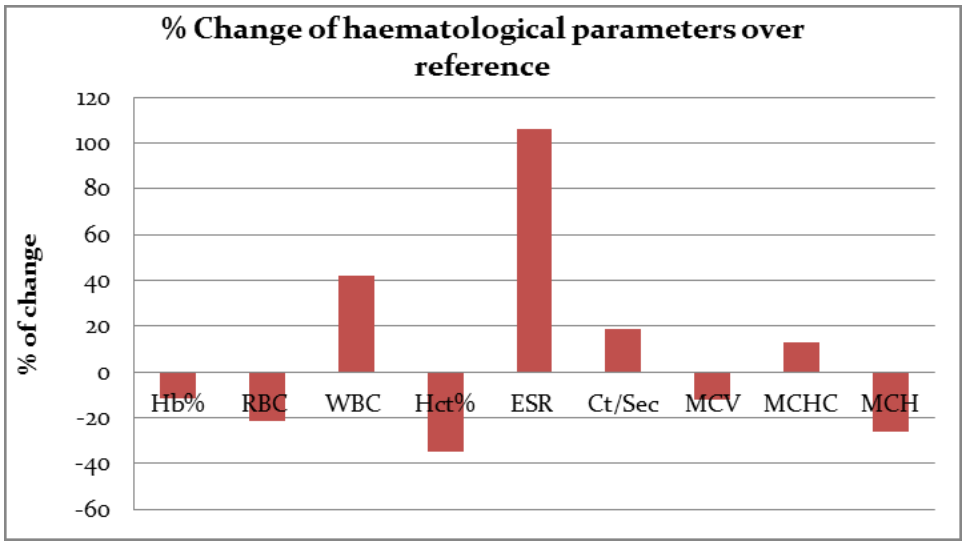

Figure 2 Change of haemalogical parameters over reference

The increase in ESR levels in the fish of downstream possibly due to Pollutants (heavy metals and xenobiotics) induced oxidative stress and caused tissue damage, anaemia, neoplasia an increase in fibrinogen.(Srivastava, B. and Reddy, P.B. ,2017 ;. Reddy, P.B. and Singh, R.K. ,2011) A clot is formed as the product of blood coagulation. The clot under normal conditions undergoes contraction when serum is expressed from the clot, and finally, the clot becomes denser. The blood clotting substance in fish blood is prothrombin, which is present in a high percentage. However, it is less than that of mammalian blood level. A substance released by the platelet (thrombos then in) is responsible for clot retraction. Water pollution caused a significant decrease in RBCs count, Hb and PCV values, in the in Channa punctatus along with acute anaemia. (Singh, D., Nath, K., Trivedi, S.P. and Sharma, Y.K., (2009)) Similar results were also reported in Labeo rohita Zutshi, B., Prasad, S.R. and Nagaraja, R.,2010) and in Channa punctatus exposed to tannery effluent. Parveen, S., Singh, D., Bharose, R., Rout, S., Khan, M.A. and Ansari, E.F., (2017)

Suggestions

The results acquired from the current study clearly displays that the fish M.tengara at downstream was exposed to pollution-induced stress that caused a significant reduction in Hb, RBC, and Hct values but increased the clotting time, ESR and WBC values. In conclusion, the results acquired from the current study shown that the fish M.tengara at downstream was exposed to pollution-induced stress that caused a significant reduction in Hb, RBC, and Hct values but increased the clotting time, ESR and WBC values. Water from the downstream has strong potential to induce stress making the fish anaemic, weak, and vulnerable to diseases. This study will be beneficial for upcoming research in explaining the detailed effects of river pollution in other fish species. The information generated from this study may offer as a valuable databank for upcoming investigations of pollutant effects on haema to logical parameters in an aquatic environment.

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