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Haematological and biochemical and immune responses in commercially valuable brackish water catfish (*Mystus gulio*, hamilton 1822) induced to cadmium chloride

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Abstract

The current exploration pointed towards the impacts of non-essential heavy metals in estuarine catfish *Mystus gulio*. 30 days of exposure in the sublethal concentration of cadmium chloride Cd: 6.2 mg/L shows the effects in haematological, biochemical alterations and immune responses in fish. Fish exposed to 96hrs LC50 in 620 ppm of 1/10th sublethal concentration of CdCl2 in the periods of 30 days were reported. The results showed the cadmium concentration in *M.gulio* was subjected to the exposure of time duration. The analyzed boundaries are hemoglobin (Hb), red blood cells (RBC), white blood cell count (WBC), haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT) and monocytes (Mon), lymphocytes (Lym). WBC and MCV were significantly increased whereas Hct, Hb, MCH, MCHC, RBC, PLT, Lym, and Mon ($P<0.05$) were drastically decreased when compared to the control fish. In biochemically trace differences in albumin (Alb), total protein (TP), total glucose (TG) and immunoglobulin M (IgM). IgM esteems were raised in experimental fish ($P<0.05$). It is clear abundantly that toxic heavy metals induce an early immunity response in the long whiskers' catfish. Overall impressions can be auxiliary to low focuses on sublethal concentrations stimulating the stress responses in infected fish. In this study haematological parameters, biochemical and immune responses showed the alterations in functionally as well as structurally affecting innate immune responses of the brackish water fish *Mystus gulio* has been analyzed to the impact of cadmium chloride toxicity.

Keywords: heavy metal, *M. gulio*, biochemical, haematology, immune response, cadmium

Introduction

The impact of water pollution increased due to the invasion of industrialization in recent days. The Industrial wastes, urban excretes, sewage water treatment, domestic garbage's and anthropogenic pollutants were drained into the freshwater resources whereas it contains non-essential heavy metals, which develops the contaminated aquatic ecosystem. Some of them were most hazardous. Internationally in many coastal area's kinds of seafoods, fish and shellfish were the most economic and main sources of food [1, 2].

In modern techniques agriculture and industries used millions of heavy metals, fertilizers and pesticides. Industrial effluents mixed into the aquatic ecosystem with the help of surface runoff or otherwise, these wastes are discharged into the rivers and streams. The non-targeted organisms, fishes and insects were tending to pose a constant threatening to inorganic industrial wastes [3, 4]. In recent days continuous increase of heavy metals remains in the air and aquatic environment have become a serious concern. [5, 6]. For the most part, marine resources and estuaries contain an assortment of fish, shellfish, seaweed which captivate fishing activities [7]. Cadmium (Cd) is a naturally occurring non-essential HM among metals, and normally tends to accumulate as a superfluous minor component in living organisms which is considered the most dangerous pollutant in the environment [8]. The impacts of cadmium harming people are intense. They incorporate serious stomach illnesses related to queasiness, running stool and vomiting, cerebral pain and dizziness. The persistence of side effects incorporates platelets damage, obliteration of testicular tissue, hypertension, destruction of the kidney [9]. Even at low concentrations cadmium and lead are harmful generally, over a long-time duration [10].

The analysis of the metabolic index in fishes were proved by haematological and biochemical profiles. The study points the haematological and biochemical changes in C. carpio freshwater fish affected by selected HM [11]. Cadmium is broadly utilized in electroplating, binds, batteries, TVs, ceramics, photography, insect toxins, hardware, metal plating, and metallurgical exercises. The toxins can be drained out into the environment by metal-ore refineries, cadmium containing pigmentary, cadmium containing fertilizers, alloys and electrical compounds, phosphate fertilizers, detergents, and refined petroleum products and rechargeable batteries with nickel-cadmium compounds are also a source of cadmium [12].

In various species, during stressful conditions, haematological parameters were utilized as a record to identify physiological changes to evaluate the primary and secondary functional health of the fish [13, 14]. Toxins like heavy metals, pollutants can be monitored in fish blood sensitive to pollution delicate to contamination, prompted to pressure. for example, haemoglobin content, haematocrit and the quantity of erythrocytes changes observed in the haematological parameters [15, 16]. The cadmium toxicity in

freshwater fishes has been all around revealed, for instance, an anaemic condition was noted in *Oreochromis mossambicus* and *Channa punctatus* exposed to various concentrations of Cd [17, 18]. Fishes were bioindicators of ecological integrity and aquatic environment [19, 20]. In common carp *C.carpio, O.aureus, O.niloticus* and *Ictalurus melas* Cd acts as an immunosuppressant [21, 22]. It is realized that heavy metals can change physiological and biochemical alterations in fish blood as well as in tissues. Heavy metal toxicity indicates physiological, biochemical and histological modification in fish blood and tissues. Haematological parameters are vital for the assessment of physiological status in the fish. The current investigation embraces the impacts of cadmium chloride in haematological, biochemical and immune responses of the brackish water catfish *Mystus gulio*.

Materials and Methods

The collection site and selection of experimental fish

The brackish water commercial fish Mystus gulio were collected with the help of local fishermen from Vellar estuary (Longitude 790 46'E and Latitude 110 29'N) Parangipettai, Southeast coast of Tamil Nadu, India. The estuary is semi-diurnal, flows eastwards and collect the wastes from adjacent area mixed with the Bay of Bengal on the southeast coast. Prior to the toxicity test average weight of 52g ± 5g and length 12cm ± 5cm of the fishes were measured and properly washed in tap water and after treated with 0.02% KMnO4. To remove any external infection fish were treated with 0.005% solution. In 15 d of acclimatization period, the fishes were fed with worms, small pieces of tissues and soy meal alternatively. Uninfected healthy fishes were selected for the experiment process.

Experimental design and water quality parameters

The fishes were acclimated in the plastic trough (90×40×50 cm) in chlorine-free tap water with adequate aeration. Water quality parameters were estimated and maintained throughout the experiment period. Water quality conditions (Table 1) such as temperature, dissolved oxygen, salinity, alkalinity and water quality were checked regularly. The photoperiod of the laboratory was maintained. In the experimental tank water was cleaned 100%, removed food remains and faeces once a day.

Heavy metal composition and exposure

Cadmium as Cadmium chloride ($CdCl_2$) was purchased from Isochem Laboratories, Angamaly, Kochi, Kerala, India (C 1040) without further purification used for this experiment. Finney method [23] was used to calculate the LC50 value of $CdCl_2$. In *M.gulio* 620 ppm 96hrs LC50 value was found for $CdCl_2$. The experiments were followed in a triplicate manner. After determination of LC50 values, 10 fishes were introduced into a 40 L plastic trough containing 20 L of water. The set of 10 fishes in group I served as a control and the set of 10 fishes in group II as an experimental trough exposed to sublethal concentration of $CdCl_2$ 6.2 mg/l in $1/10^{th}$ of 96hrs LC50. Every day the concentration of toxins in the water was renewed. The fish were fed every alternative day at the rate of 3% body weight throughout the experiment period. Continuous observation of control and experiment fishes for 30 days with enough aeration.

Collection of blood

Towards the end of the 30^{th} days, 5 samples were collected from the control and treated fish were anaesthetized using clove oil and 1ml of blood samples were taken from the caudal vein of fish with disposable heparinized syringes and the blood was stored in Eppendorf vials coated with EDTA to analyze haematological and biochemical changes.

Haematological analysis

The standard procedure [24] was used for the estimation of haematological profiles. Neubauer Haemocytometer special kit (Improved Neubauer Weber Scientific Ltd) was used to enumerate the WBC and RBC count [25]. The blood was diluted with 3 g of sodium citrate, 1 ml of formalin and 99 ml of distilled water counting the corners and centers of the slide.

Haemaglobin was measured by adding 0.1 N HCL and the blood was converted into a brown colour acid fluid called haematin compared with standard reference using haemoglobinometer. The lower meniscus shows the gm% reading on the scale. Dacie & Lewis [26] method was followed to MCHC, MCH, MCV's were calculated by erythrocyte indices. Earlier protocol [25, 26] was followed by the standard micro-haemotocrit method used to determine Haematocrit (Hct) in percentages. Differential counting was performed to calculate leukocytes per millilitre of the sample blood. In this method, a blood smear was taken from heparinized blood decolourized using Wright-Giemsa [27] and the smear was examined by light microscope under oil immersion at 100× magnification. Snieszko method [28] was used to count platelets (PLT).

Number of cells = Number of cells counted × dilution/Area counted × depth fluid

Biochemical analysis

A Blood sample was left to coagulate at 4^0 C for 15 min and then the coagulated blood was centrifuged at 3000rpm in 20 min. after centrifugation, the fresh serum was collected from the top of the centrifuge tube and was used for biochemical analyses. The separated serum was used to determine the albumin, serum glucose, serum protein and Immunoglobulin (IgM). Albumin by Bromocresol green method, total glucose (TG) by Phosphomolybdate method, total protein (TP) by Lowry method, and the determination of Immunoglobulin M by assay procedure using ELISA kit followed by the previous researcher [29].

Statistical analysis

Duplicate analysis of all parameters was done. To evaluate and compare the exposure effects of Cadmium and its impact on haematological and biochemical parameters were calculated by using Analysis of Variance (ANOVA) followed by Student t-test. Data were represented as mean ± standard error and the data has been analyzed statistically with the help of IBM SPSS software and differences were considered statistically significant ($P< 0.05$).

Results

Behavioural responses of the fish

In the present examination, no mortality was noted in the control trough and all remain active and healthy. In experimental trough number of behavioural changes and clinical ailments like high secretion of mucous, air gulping,

fin tremor, equilibrium loss, erratic and increased swimming, increased sur-face breathing and faster opercular movement were noted.

Maintenance of water quality variables

Throughout the experimental period, water quality was maintained in the experimental tank. Water quality conditions (Table 1) and mean value of temperature 29.88 ± 1.71^0 C, pH 8.1 ± 0.26, Dissolved Oxygen 5.81 ± 0.53 mg/l, Salinity 12.28 ± 5.87 ppt, Alkalinity 162.6± 4.28, and the water quality was checked regularly. The photoperiod of the laboratory was 12hr D and 12hr L. The change in temperature increases by hot water and decreases by cold water whereas in the case of salinity, the salt content decreases by freshwater and increases by marine water likewise in pH, it increases by the HCl and decreases in the NaOH. In the experimental tank water was cleaned 100% once a day.

Table 1: shows physiochemical parameters

S No	Physiochemical parameters	mean ±SD	Range
1	Temperature (^{0}C)	29.88± 1.71	25-36
2	pH	8.1±0.26	7.5-8.5
3	DO (mg/l)	5.81±0.53	6.5-7.5
4	Salinity	12.28±5.87	5.12-24.89
5	Alkalinity	162.6±4.28	4.02-169.83
6	Photoperiod	12hr D:12hr L	12hr D:12hr L

Haematological variables

In 30 days of exposure to sublethal concentration of CdCl2 in brackish water fish *M.gulio* cause significant alterations in haematological as well as biochemical profiles as shown in Table 2 & 3.

Estimation of Haemoglobin-Hb

Table 2 shows the contents of haemoglobin in the control group after 30 days were 10.82±0.42. Significance decreases in the treated group Hb content 9.95±0.39 compared to control. The percentage changes were shown in figure 1. To reveal the significant difference ($P < 0.05$) between the control and exposure group using one-way analysis of variance (ANOVA).

Estimation of total RBC

In control fish, the average number of RBCs were estimated as 6.35±0.17 whereas in treated fish the RBCs were 6.21±0.16. in treated fish RBCs were showed a significant decrease ($P<0.05$). The percentage changes between control and treated were shows in figure 1.

Estimation of total WBC

The estimation of WBC in control 29.43±0.86 and treated group at the end of 30 days of exposure shows an average of 31.65±0.82 of blood, whereas to compare control group shows a drastic increase of WBC ($P < 0.05$) in the treated group. The percentages of variation for the control and treated groups figure 2.

Haematocrit- HT

The haematocrit exhibits 24.89±1.24 in control, whereas the treated exhibits 22.65±1.19. when compared between control and the treated fish exhibit significantly decreased condition. The percentage of control and treated figure 1 shows accordingly.

Mean corpuscular volume-MCV

In control 68.33±1.26 of MCV level at the end of 30 days. The significant increase 69.84±1.29 in MCV levels ($P < 0.05$) of exposed after 30 days and the percentage of control fish and treated fish shows in figure 1 respectively.

Mean corpuscular haemoglobin- MCH

The MCH level of control fish after 30 days were 17.32±0.05 whereas in treated fish the MCH levels were 16.28±0.02 shows a significant diminish in MCH level compared to treated fish with control fish. The percentage changes over the control and metal treated figure 1. A significant decline in MCH of blood in the $CdCl_2$ treated group ($P < 0.05$) was calculated by one-way ANOVA followed by a t-test.

Mean corpuscular haemoglobin content- MCHC

The MCHC level of the control and the treated were 38.23±0.01 and 37.67±0.22g/dl respectively. In this result, the MCHC level in the treated group diminish significantly compared with the control group. The percentage of variance was in figure 1.

Platelets

In control, the platelets (PLT) count was 37.38±1.12 whereas in treated fish the platelet count was 35.43±1.04 after 30 days respectively. Platelets count of the treated group diminished significantly compared with a control group. The percentage between control and $CdCl_2$ treated the difference between the groups ($P < 0.05$) shows in figure 2.

Lymphocytes

The lymphocytes count in control were 91.72±1.27 while in $CdCl_2$ treated the lymphocyte counts were 89.42±1.16 at the end of 30 days. A significant decrease in treated fish compared to the controlled one. Accordingly, the percentage variance of control and treated were in figure 2.

Monocytes

In the control fishes, the monocyte counts were 10.07±1.04 after 30 days whereas in treated the lymphocytes were 9.61±1.02. The result shows the treated group were significantly decreased than the control. The percentage of variance were in figure 2 accordingly.

Biochemical alterations

Albumin

In control, the albumin (Table 3) contents were 2.10±0.08 after 30 days and in the treated one the albumin contents were 1.90±0.06 ($P < 0.001$) been observed. This was showed a significant (Figure 3) decrease in the treated group compared to the control after 30 days period.

Serum glucose

The control group in the serum glucose contents after 30 days were 58.42±3.56. In treated the glucose contents were 62.81±4.23 and it shows (Figure 3) in the treated group glucose level decreased significantly.

Serum protein

In the control group, the serum protein contents were 4.93±0.47 after 30 days, and in CdCl2 exposed group (Table 3) the protein contents were 3.45±0.32 after 30 days

(P<0.001). Here treated group were significantly diminished than the control group (Figure 3) protein contents.

Immunoglobulin M- IgM

Table 3 reveals the IgM concentration after 30 days period. In the control, IgM values were 8.20±0.08 whereas in the treated the IgM concentrations were 11.16±0.26 at the end of 30 days. In metal treated Immunoglobulin contents (Figure 3) were elevated rather than the control group.

Table 2: Haematological profiles of control *M.gulio* and exposed to sublethal concentration of $CdCl_2$ at the end of 30 days.

Haematological variables	Reference M. gulio	Exposed M. gulio
Haemoglobin (g/dl)	10.82±0.47	9.95±0.39
RBC ×106 (mc/mm3)	6.35±0.17	6.21±0.16
WBC ×103(m/mm3)	29.43±0.86	31.65±0.82
Haematocrit (%)	24.89±1.24	22.65±1.19
MCV (fl)	68.33±1.26	69.84±1.29
MCH (pg)	17.32±0.05	16.28±0.02
MCHC (g/dl)	38.23±0.01	37.67±0.22
Platelet ×103(m/mm3)	37.38±1.12	35.43±1.04
Lymphocytes (%)	91.72±1.27	89.42±1.16
Monocytes (%)	10.07±1.04	9.61±1.02

A row indicates statistically significant differences (P<0.05) with different subtitles.

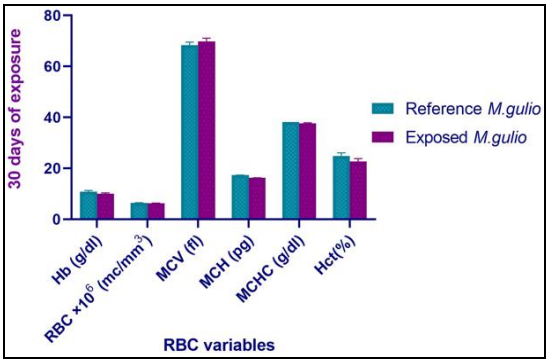

Fig 1: RBC variables of haematological parameters.

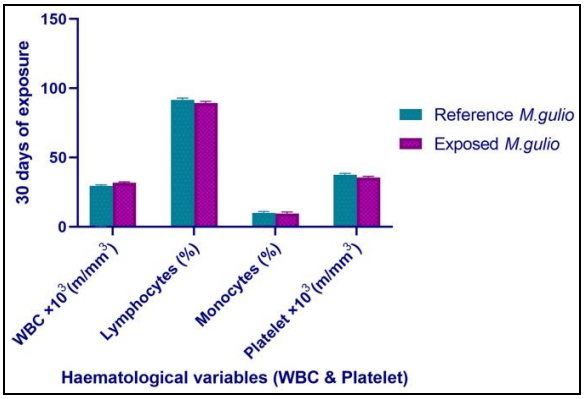

Fig 2: WBC & Platelet variables of haematological parameters.

Table 3: Biochemical profiles of control *M.gulio* and exposed to sublethal concentration of $CdCl_2$ at the end 30 days.

Biochemical variables	Reference M.gulio	Exposed M.gulio
Albumin (Alb)	2.10±0.08	1.90±0.06
Total Glucose (TG)	58.42±3.56	62.81±4.23
Total protein (TP)	4.93±0.47	3.45±0.32
Immunoglobulin (IgM)	8.20±0.08	11.16±0.26

A row indicates statistically significant differences (P<0.05) with different subtitles.

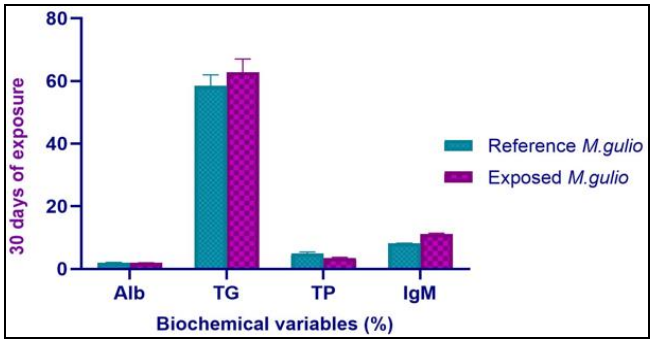

Fig 3: Biochemical variables of *M.gulio*

Discussion

Many researchers found that environmental pollution cause changes in the haematological parameters of the fish [30]. In toxicology to detect the fish health condition under stressful status such as hypoxia, diseases and exposure to heavy metal pollutants, the haematological parameters can be used as a diagnostic tool [31, 32, 33, 34, 35]. The RBC in the blood plays a major role to carry and transporting oxygen to body tissues all over [36]. The time duration and the concentration of HM show depletion in the blood variables [37]. In this present evaluation, the haematological parameters like Hb, RBC, WBC, Hct, MCV, MCH, MCHC, PLT, lymphocytes and monocytes were estimated in the brackish water catfish M.gulio exposed to sublethal concentration of CdCl2. When compared to the control into the exposed haematological indices such as WBC, and MCV contents were significantly ($P < 0.05$) increased whereas Hb, RBC, WBC, PCV, MCV, MCH, MCHC, PLT, lymphocytes and monocytes were significantly ($P < 0.05$) decreased (Table 2). The same evaluations pointed out to various metals exposed results the depletion of RBCs, Hb and Hct contents in the previous studies. Some researchers observed similar findings the significant decrease in the Hb, RBC, WBC, Hct, MCV, MCH, MCHC, PLT, lymphocytes and monocytes whereas the MCV and WBC values were significantly increased in Cyprinus carpio, *C*atla catla exposed to Cd, Cu and some other heavy metals [38, 39, 40, 41]. The result of this present work was also similar to those researchers who reported that exposure to cadmium for 15 and 30 days shows the diminish in the RBC, Hb, Hct, MCH, MCHC in *Oreochromis niloticus*, *Silurus glanis*, and *Clarias gariepinus* [42, 43, 44, 45, 46]. The researcher found in common carp there was a significant decrease in Hct, MCHC, MCV, leukocytes and erythrocytes exposed to sublethal doses of Cd in 60 days period [47]. Same observations were found in *M.gulio* treated to sublethal concentration compared to the control group.

Table 2 shows the depletion in Hb contents and the decline of RBC level due to the haemopoietic system impairment leads to anaemia and erythropenia in fishes. This result incorporates with the result of some researchers who worked in Eel and Perch short-term and long-term exposure of Cd [48]. A researcher pointed out that the fish exposed to toxic substances leads to diminishing and prolonged decrease of haemoglobin, blood dyscrasia, impaired O_2 supply, erythrocytes degeneration and hypoxia [49, 50, 51, 52]. The low number of RBCs or Hb leads to the fish into anaemic condition. A similar observation was found by the previous researcher, the increased demolition of erythrocytes even though the spleen size remains unchanged but the reduced synthesis of RBC in the circulation of blood [53]. The researcher suggests the exposure of Cd decrease the

erythroblast in *C.auratus, Salmo gairdneri* [54]. In the present results noticed the decreased erythropoiesis leads to the production of RBCs. This is corporate with the haemolysis of blood cause stimulatory action due to toxicity and altering the action of cell membrane permeability [55]. The counts of Hb and platelets decreased the fish *M.gulio* exposed to heavy metal concentration clearly noticed in gill damage, impaired osmoregulation and haemodilution which was incorporated with the fish *C.carpio koi* exposed to $CdCl_2$ and some other heavy metals were clearly demonstrated by some other researchers [56, 57, 58, 59]. The sudden eruption of WBCs in exposed fish due to the stimulation in lymphomyeloid tissues, then the lymphopoiesis release lymphocytes to fighting against the toxicants in the present study, these findings parallel to some researcher's findings stated the pollutants enters into the tissues combine with biochemical contents of the cells leads to xenobiotics, the production of WBCs increased to eradicate the toxins to avoid the fatal condition of the fish [60, 61, 62].

In this study biochemical analysis bring forth valuable information to detect the fish health condition. The results of the present study, experimental fish exposed to sublethal concentration of cadmium in *M.gulio* showed significant elevations in the blood glucose level. This result shows the significant increase of glucose concentration mainly due to indicate stress response. Glucose level increased in the treatments related to the researcher demonstrate in the combination of Cd and MP or Cadmium alone increase the glucose level exposed to Cd and other metal. Serum protein contains albumin and globulin plays a vital role in the time of stress condition. The researcher marked the same function and uses of albumin in dominant and alter in the level of protein in stress and diseased situation [63]. In catfish the glucose level increased at the end of 30 days cause vulnerable stress conditions due to HM in toxicity somehow a similar result was found by the researcher [11]. Total protein in plasma where deplete called hypoproteinemia leads to reduction of serum protein in 30 days of exposure for this study agreed with the researchers [43, 64]. In our report heavy metal exposure is responsible for the depletion of serum protein and albumin. These results parallel the work carried out in an *Onchorynchus mykiss* and *Oreochromis niloticus M. tengara* and *M. vittatus* [65, 66]. The high liver damage leads to the production of more albumin and the fish meet high energy. Immune resistantance occur in *M.gulio*, fish need globulins in low quantity to meet high energy demands to sustain in water contain heavy metal pollution [66].

Immunoglobulin M (IgM) is the major antibody, plays a vital role in the fish primary immune response. In this study, IgM was significantly higher in concentration fish exposed to toxic metal $CdCl_2$ in sublethal condition. The author noticed IgM decreases in the first week of exposure likewise at the end of 30 days the IgM levels were drastically increased due to hyperglycaemia in the exposed group. The fish immunity is suppressed because of toxic infective agents.

Insignificant increase of serum IgM at the end of 30 days likewise the depletion of albumin in protein, the result was somewhat obtained in some heavy metals like Cd, Pb, Cr and this work was carried in few fishes by some other researchers which more or less corroborate with present observations [10, 67, 68].

Conclusions

The observation shows the harmful impact of heavy metals in fish in the current study. The heavy metals contaminated estuarine water affects the species *Mystus gulio* as an indicator of cadmium exposure act as a stressor, haematological, biochemical and immune responses lead to changes in some blood parameters. It is clear that heavy metals bioaccumulation induced an early innate immune response and alteration in structurally as well as functionally including biochemical and genetic effects in the fish tend to cope with or prevail the conditions. Therefore, this species *M.gulio* is used as a biomarker of organisms and it shows the innate immune system increase susceptibility to diseases of exposed fish. Hence biomarkers are more important for monitoring environmental hazards induce alteration to admit the impacts in xenobiotic level. Hence it is to be considered and recommended that all kinds of sewage, agricultural and wastewater must be treated well before it drains into the aquatic ecosystem.

Conflict of Interest

The authors declare that they have no conflict of interest.

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