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Alteration in hematology of *Cyprinus carpio* under the stress of pollution of water bodies of Kashmir valley

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Abstract
Change in the hematological profile of fish captured from stressed environments may represent a reliable tool in revealing the sublethal effects of the pollutants found in the aquatic ecosystems. Therefore a field study was conducted to investigate the blood profile of *C. carpio* collected from two water bodies of Kashmir valley, Dal and Mansbal lake as fish blood shows signs of pathological changes even before the onset of external symptoms of toxicity and it truly reflect the physical and chemical changes occurring due to pollutant accumulation in the fish body. Significant increase (p<0.05) was found in erythropoietic tissues vis-à-vis Hb, PCV and RBC count in *C. carpio* from Mansbal Lake compared to Dal lake. However, a significant decrease (p<0.05) was found in WBC count in *C. carpio* from Mansbal Lake compared to Dal Lake. More pronounced changes (p<0.05) were found in the values from Dal Lake compared to Mansbal Lake. Results showed in all the parameters studied, it was found that fish from Dal Lake was more vulnerable than Mansbal Lake, also suggested the suitability of the test species used as tools in environmental monitoring programs for risk assessment in fresh water environments.

Keywords: *Cyprinus carpio*, Hemoglobin, RBC, WBC, PCV, Pollution, pesticide

Introduction
The aquatic environment plays a fundamental role in the functioning of ecosystems as they are the major recipients of pollutants, which, over time, can have serious consequences for biota that may not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take effective counter measures. A substantial amount of most of the chemicals employed by human beings in agricultural practices such as fertilizers, pesticides, organic manure finds its way into rivers, lakes and ponds [1]. Majority of them has been found to be extremely toxic not only to fish but also to the organisms which contribute to the food chain of fishes [2]. Fishes are generally exposed to pesticides through dermal uptake, direct absorption through the skin, or direct uptake through the gills during respiration. Once taken up through any of the routes, these xenobiotics are potent to cause physiological dysfunctions like haematological changes in fishes [3]. A major part of the world's food is supplied from fish sources since time immemorial and it is very indispensable to make safe the health of fishes [4]. Being at the clemency of their surroundings, any change in the quality of their environment is bound to affect their health vis-à-vis the fishery resource of a water body. This being so, it is pertinent to have an insight in to the health of the fish and the factors responsible for it.

Fish species have been utilized as an efficient bio indicator of aquatic pollution as they are able to accumulate pollutants and show differential physiological and cellular responses against them [5]. As marker species, *Cyprinus carpio* was chosen because of their ecology, wide distribution in a fresh water environment of Kashmir, availability throughout the seasons, and commercial importance make this species as an excellent test specimen for biological indicator of water quality. Blood is the most vital and abundant body fluid and is documented as a potential index of fish response to water quality as it can be used to determine the effect of pollutants in the environment [6]. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations [7]. A thin epithelial membrane separates fish blood from the water and any unfavorable changes in the water body are reflected in the blood [8].

Blood parameters in fish have been studied to reveal physiological adaptation and to assess the health of fish [9]. Sadeghi *et al*. [10] stated that hematology may be a helpful tool in monitoring stress levels of aquatic pollution on fish. Hematological parameters are increasingly used as indicators of the physiological stress response to endogenous and exogenous changes in fish exposed to a complex mixture of available pesticides/pollutants in water bodies [11]. A numeral hematological index such as RBC, WBC, Hb, HCT is used to assess the functional status of the bloodstream and have been used as an indicator of toxicity/pollution in aquatic environments [12].

As the existence of environmental pollutants in ground and surface water reservoirs increasingly grows due to human activities, public concern has likewise grown over the unfavorable effects of environmental pollution to both the environment and human health. Therefore, the present study was undertaken to assess the hematological profile of *Cyprinus carpio* from two fresh water lakes Dal and Mansbal characterized by different pollution levels.

Materials and methods

Study area

Two hundred live *C. carpio* L. (Family: Cyprinidae and Order: Cypriniformes), were procured with the help of local fisherman using hand nets from the Dal Lake (34° 07′ N, 74° 52′ E) and Mansbal Lake (34° 15′ N, 74° 40′ E). Various morphometric parameters like average length and wet weight (±SD) of *C. carpio* were recorded as 181.2 ± 0.62 mm and 77 ± 6.782 g for Dal Lake and 176.2 ± 1.70 mm and 66.25 ± 17.97 g for Mansbal Lake respectively. An increase in the area of floating gardens, along with the anthropogenic activities has combined with natural processes to reduce the area of Dal Lake, and deteriorate its water quality and ecosystem. Due to the anthropogenic stress, the ecology of lakes has greatly changed over the past 50 years. Ecological stress from human activities not only causes shrinking of the surface area of the lake, but its water quality has also deteriorated badly affecting the aquatic life. Therefore, the lake conditions as a whole continue to deteriorate at an alarming rate. Intensive farming practiced in the surrounding area of Dal Lake and its floating gardens lead to an enhanced vulnerability of crops to pests and indiscriminate use of pesticides. The possible transfer of these hazardous molecules from vegetable fields in the aquatic environment of the Lake poses a potential threat to the aquatic species and human health as well. Recent studies by Zargar *et al*. [13] have attested that the Dal Lake has reached to the level of eutrophic condition, but the level of trophic state varies, with Dal Lake being the most eutrophic and Mansbal Lake being the least nutrient enriched. Also the presence of Organophosphate pesticides was previously evidenced by Meraj *et al*. [14] in the water of Dal lake.

Fish collection

The fish captured from both the sites were transported live in containers to Cytogenetics and Molecular laboratory, Centre of Research for Development (CORD), University of Kashmir and subjected to a prophylactic treatment by bathing in a 0.05% aqueous solution of potassium permanganate for two min to avoid any fungal or dermal infection. The fish were then maintained in well aerated 60L glass aquariums and properly acclimatized for 24 hrs under controlled and constant environmental conditions at 19.7 ± 2.6^{0}C, continuous airing with 24h aged decholrinated tap water (pH 7.6-8.4) and fed ad libitum with commercially available fish food (Feed Royal^R, Maa Agro foods, Visakhapatnam, Andhra Pradesh, India). Every effort as suggested by Bennett and Dooley [15] was made to maintain optimal conditions during acclimatization. The acclimatized fish were then used for the blood collection. Both sexes were used without discrimination.

Collection of blood samples

Prior to blood collection, fish were anesthetized with 0.12 mgL^{-1} benzocaine (Marques *et al*., 2003). The length and weight of fish were recorded before the collection of the blood. Blood samples were withdrawn by heart puncture with a syringe from the heart by stabbing the body wall exactly in midline from the posterior margin of opercular cover and directed dorso-caudally at an angle of 45^o [16]. Blood was collected in glass vials containing anticoagulant ethylene diamine tetra acetic acid at an approximate concentration of 5mg/ml blood [17]. Care was taken to prevent the blood from coming in contact with water.

Analysis of Hematologicalparameters

The hemoglobin was estimated by cyanomethemoglobin method recommended by the International committe for standardization in hematology [18]. The number of red blood cells and white blood cells were counted by Neubauer hemocytometer. Haematocrit (PCV) was determined using the wintrobes tube method.

Data Analysis

Statistical assessment was carried out using the statistical package for the social sciences (SPSS version 16.0) computer program (SPSS Inc. Chicago, IL, USA). All data were first tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests to meet statistical demands. For the comparison, data were subjected to Mann-Whitney U test. Significance was accepted at $p<0.05$. All data are reported as mean ± standard deviation (S.D).

Results and Discussion

The results gave basic information on the effect of pesticides or pollutants present in the Dal Lake on the haematological parameters of *Cyprinus carpio*. In all the parameters studied, it was found that fish from Dal Lake was more vulnerable than Mansbal Lake. Hematological values and cell structures of *Cyprinus carpio* from the two habitats are given in Table 1. Significant variations between the study areas were detected in all the hematological values.

Significant decrease ($p<0.05$) in hemoglobin content was observed in the fish from Dal Lake compared to Mansbal Lake which may be due to the disruptive action of the pesticides/pollutants dumped into the lake, on the erythropoietic tissue as a result of which the viability of the cells might be affected. According to Pamila *et al*. [19] the reduction in hemoglobin content in a fish exposed to toxicant could be due to the inhibitory effect of those substances on the enzyme system responsible for synthesis of hemoglobin. The decline in Hb content of *Cyprinus carpio* L. was also observed by Chauhan *et al.* [20] and Ramesh and Saravanan [21] after exposure to dimethoate and chlorpyrifos respectively. The high hemoglobin content of the blood in the Mansbal fish compared to Dal Lake may further be related to the large anaerobic metabolic capacity of the species and its preferred environmental conditions, suggesting that *C. carpio* could survive environmental extremes. Packed cell volume or Haematocrit values showed a declining trend in *C. carpio* in

Dal Lake compared to Mansbal Lake which may be due to contamination in the Dal Lake fish caused by pesticide pollution because of the intensive agriculture dominant in the catchment and by possibly a complex mixture of contaminants could be occurring because of a confluence of both urban and agricultural pollution. This may also be due to impaired oxygen supply to various tissues, resulting in a slow metabolic rate and low energy production. Recently, Ramesh and Saravanan [22] also opined that the decrease in the PCV of *C. carpio* treated with sublethal concentration of chlorpyrifos was the result of either rapid oxidation of haemoglobin to methaemoglobin or release of oxygen radical brought about by toxic stress of the insecticide. The RBC value of *C. carpio* was also found to be decreased significantly ($P<0.05$) at Dal Lake compared to Mansbal Lake. The lowered RBC values would be attributable to the destructive action of pollutants released into the lake, because decreased RBC values are reported to be indicative of accelerated destruction of the cells and hemolysis which occur in response to toxicity [23]. Such destructions results in alteration of the selective permeability of the cell membrane. The effluents discharged into the Dal Lake contain agricultural products that could be partly responsible for the destructive activity on fish RBC. According to Singh *et al*. [24] the discharge of waste may cause serious problems as they impart odour and can be toxic to aquatic animals. Declined RBC count was also reported by Svobodova *et al*. [25] after exposure of *Cyprinus carpio* to chloropyrifos. Significant increase ($P<0.05$) in the number of leucocytes (leukocytosis) in fish captured in the Dal Lake was directly relative to the severity of stress condition and resulted from the direct stimulation of immunological defense due to the presence of pesticides/pollutants present in their aquatic environment. Okechukwu *et al*. [26] reported significantly higher values of white blood cells in *C. carpio* after exposure to chlorpyrifos-ethyl. This alteration could be the result of the activation of the immune system in the presence of a contaminant, which may in turn be an adaptive response of the organism, resulting in a more effective immune defense response [27].

Conclusion

In conclusion, the current study has assessed the toxic effect of agricultural runoff on the fish from Dal lake compared to those from Mansbal lake, and it is evident that the fish health status is negatively affected by this pollution menace. Moreover, the Dal Lake is impacted by pesticides in concentrations enough to affect the health condition of *C. carpio*. It is important to note that the situation reported in our study may be worse in the future because of the continuous discharges of agriculture and domestic effluents into the Dal Lake, which can pose threat to both inhabiting biota and public health. Regular monitoring and strict law enforcement are needed to develop an approach to deal with the environmental hazards due to these rudiments and improve environmental protection of this area.

Table 1: Hematological parameters of *Cyprinus carpio* collected from Dal and Mansbal Lake (mean ± S.D)

Parameters	Dal Lake	Mansbal Lake
Hb (g/dL)	7.62±0.49[a]	8.81±0.45[a]
Ht (%)	24.95±0.55[a]	27.89±0.95[a]
RBC (10^6 /mm^3)	1.86±0.11[a]	2.96±0.04[a]
WBC (10^4 /mm^3)	31.70±1.48[a]	25.97±1.41[a]

[a] represents a significant difference between Dal and Mansbal Lake *(Mann- Whitney U test*; p value =0.009).

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