Alteration in Hematology of *Triplophysa marmorata* Under the Stress of Pollution from Water Bodies of Kashmir Valley

Maryum Meraj[1], A. R. Yousuf[1], F. A. Bhat[2], Md. Niamat Ali*[1], Bashir A. Ganai[1], M. Rahman[1], N. Shahi[1], M. I. Rather[1] and Hilal Ahmad Ganaie[1]

[1]Centre of Research for Development, University of Kashmir, Srinagar - 190006, J & K, India
[2]Faculty of Fisheries, SKUAST-K, Srinagar - 190006, J & K, India
***Corresponding author: mdniamat@hotmail.com**

Abstract
Blood is an indicator of physiological condition of an animal. Therefore, a field study was conducted to investigate the hematological parameters of Kashmir loach, *Triplophysa marmorata* Heckel collected from Sind and Telbal streams. Blood parameters such as hemoglobin, erythrocyte count, total leucocyte count, hematocrit, differential leucocyte count (DLC) of fish were determined. Statistical analysis revealed noticeable differences in RBC and WBC count and PCV values. A significant decrease was marked in RBC count and PCV values ($p<0.001$), whereas an increase in leucocyte count was observed in fish from Telbal stream. The variation in values of different parameters from these water bodies can be attributed to the presence of various toxicants of pollutants in the water body (Telbal stream).
Keywords: *Triplophysa marmorata*, hemoglobin, hematology, RBC, WBC, PCV, pollution.

Introduction

Blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity (Sampath *et al.,* 1998). Since changes in various environmental parameters result in a new steady state of physiological reactions, patterns of these reactions are expected to provide some indication that the organism is faced with. Fishes can be used as a measure of environmental health, as they are in direct contact with their environment and are as such susceptible to any change that may occur in it. It is expected that such changes would be reflected in the physiology of the fish and particularly in the values of hematological parameters (Blaxhall, 1972). Blood is therefore recognized as a potential index of fish response to water quality (Hickey, 1982), as it can be used to ascertain the effect of pollutants in the environment. Blood parameters have been commonly used to observe and follow fish health, since variations in blood tissue of fish are caused by environmental stress (Shah and Altindag, 2005; Bhaskar and Rao, 1985). Blood parameters in fish have been studied to elucidate physiological adaptation and to assess the health of fishes (Vazquez and Guerrero, 2007). Bouck and Ball (1966) stated that hematology may be a useful 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 (Adams, 1990; Santos and Pacheco, 1996; Cataldi *et al.*, 1998).

Hematological abnormalities have also been studied in various toxicants-exposed fish: *Channa punctatus* to lead (Hymavathi and Rao 2000); *Cyprinus carpio* to carbofuran (Chandra *et al.*, 2001); *C. punctatus* to cadmium (Karuppasamy *et al*., 2005) and *Labeo rohita* to synthetic detergents and sublethal levels of nitrite (Chellan *et al.*, 1999; Acharya *et al.*, 2005). Changes in hemoglobin content under toxic stress are reflected on the oxygen consumption and metabolism. Since oxygen transport in blood depends upon the hemoglobin contents of erythrocytes in blood of fish, the erythrocyte count (TEC) and hemoglobin (Hb) content are taken as reflection of the pollution stress. Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the hematological parameters (Van Vuren, 1986). McLeavy and Brown (1974) reported leukocytosis in zinc-treated fish, *Oncorhynchus kisutch*, due to tissue damage and subsequent removal of debris.

Interestingly, the hematology of fish continues to offer valuable diagnostic tool and progress in establishing normal range values for blood parameters of different fish species, but the information regarding Triplophysa species is limited.

Kashmir loach (Genus *Triplophysa* of sub family Nemachilinae and Family Balitoridae), locally known as 'Ara gurun', is a small fish having elongated and scale-less body, with eyes high on head, and inferior mouth having two rostral, and one maxillary pair of barbells. The Kashmir loaches normally live among pebble and shingle at the bottom of clear rocky streams but some drift into lakes among the hills and become secondarily modified for life in deeper waters (Hora, 1937). The fish are also considered to be very tasty and are a source of cheap nutrition for the poor. Therefore, considering the importance of hematological parameters as indicators of fish health, the present work dealing with synergetic effect of various pollutants on fish from two water bodies was investigated.

Materials and Methods

Live and Healthy fishes were collected from Telbal stream (Fig. 1) (34° 08' 41" N – 74° 50' 58" E) carrying the wastes from the adjacent agricultural lands in addition to domestic municipal effluents and control Sind stream (34° 16' 59.5" N - 74° 49' 20.2" E) (fig.2). Fish samples were collected during the year 2012.

Fig. 1. A view of Sind stream near study site

Fig. 2. A view of Telbal stream near study site

Physico-chemical features

The physico-chemical characteristics of water were analysed as per the methods described by the CSIR (1974), Mackereth *et al.,* (1978) and APHA (1998). Water temperature and pH were recorded on the spot, whereas for the estimation of dissolved oxygen water samples were fixed at the sampling site in accordance with the azide modification of Winkler method (APHA, 1998). Measurements were made using the following equipment/method(s): water temperature – Celsius mercury thermometer calibrated up to 0.1°C; hydrogen ion concentration - digital pH meter (Microprocessor pH System-1011E); Total alkalinity – Mackereth *et al*., (1978); Ammonical nitrogen-phenate method (APHA, 1998) and Nitrate-nitrogen - Salicylate method (CSIR, 1974).

Fish collection

Live specimens of the Kashmiri Loach *T. marmorata* (Heckel,1838) were captured with the help of local fisherman and transported to the laboratory within 2 hr after capture into tanks containing 20 L of water. Both sexes were used without discrimination. Hematological parameters were investigated in healthy specimens collected from the sampling area. In the laboratory blood was collected within 24 h after the capture by dissection of the caudal peduncle (Roberts., 1981), into a vial containing dried or powdered potassium salt of ethylene diamine tetra acetic acid (EDTA) as anticoagulant to give a concentration of 5mg/ml of blood samples. The blood sample was rocked gently in the vial to allow thorough mixing of its contents. A further 0.5 ml was taken without EDTA and used to prepare blood films. The blood samples were taken in the morning hours. Fishes were physically examined for any sign of infection or diseased condition (Noga, 1993), and only data from fishes with no sign of infection were used.

Hematological analysis

The Haemoglobin (Hb) content of the blood samples was estimated by the cyanomethaemoglobin method (Brown, 1980). The number of red and white blood cells was determined using a Neubauer haemocytometer after the blood has been diluted with Dacie's fluid (Dacie and lewis, 2001).The Haematocrit (PCV) was determined using wintrobes tube method according to Ramnik (1994). Differential counts, Neutrophils, lymphocytes and monocytes were done on blood film stained with Grumwald Giemsa stain as described by Dacie and lewis method (2001).

Results and Discussion

The physico-chemical parameters of water collected from Sind and Telbal streams are presented in Table 1. The water temperature ranged from 15°C to 26°C, pH from 8.0 to 8.3, dissolved oxygen from 7.2 to 8.8 mg/l, free CO_2 from 7 to14 mg/l, Alkalinity from 50 to 175 mg/l, Ammonia from 17 to 97 mg/l, nitrate nitrogen from 119 to 220 µg/l, while as Orthophosphorus from 15 to 123 µg/l and total Phosphorus ranged from 30 to 194 µg/l. A comparison of the two water bodies showed the least oxygen concentration in the telbal water body. Due to continuous fast current, especially in case of Sind Nalla, the running waters contain relatively higher concentration of oxygen than the water of telbal (Vass *et al.*, 1977 and Qadri *et al.*, 1981). The higher value of the free carbon dioxide content has been related to the eutrophication/pollution of water (Todda, 1970 and Coole, 1979). This is also confirmed by our data from Telbal Nalla, which show much higher concentration than the Sind Nalla. Same was true with respect to pH value as well as total alkalinity. Level of phosphorus and inorganic nitrogen in Telbal was much higher than the Sind Nalla. On the basis of physico-chemical limnology it can be said that Sind stream, which is comparatively free from pollution, as compared to the Telbal is best suited for the growth of *Triplophysa marmorata*. Hematological values of the *T .marmorata* from the two habitats are given in Table 2. Definite variations between the study areas were detected in all hematological values. The hemoglobin value of fish decreased at Telbal compared to Sind stream. A similar trend was observed in RBC value. The mean haematocrit values of *T. marmorata* from the two water bodies were within the range of 21 – 26%. PCV decreased significantly ($P < 0.001$) in Telbal stream in comparison to Sind. Study shows an increase in WBC quantity and leukocyte cell proportions (neutrophil, monocyte) in the fish specimens from Telbal stream.

Water parameters are one of the major factors responsible for individual variation in fish hematology. Blood is the most important fluid in the body and its composition often reflects the total physiological condition of an organism. In natural habitat, fish species are pact with different factors such as varied water qualities, pollution, malnutrition, infection and disease, and can adapt themselves such environmental conditions by changing their physiological activities. Although all the above factors are linked to fish health, it is essential to establish and identify the causes of disease in fish which presents as a challenge for the researchers and farmers (Pradhan *et al.*, 2011). Water quality is an important factor, which is responsible for variations in fish hematology, since fishes live in close association with their environment (Casillas and Smith, 1977). The physico-chemical parameters of the different sites showed that telbal is more polluted than Sind. This different eutrophic status of the water bodies has their bearing on the population and density of the *T. marmorata*. Hemoglobin serves to transport oxygen from gills to different tissues of the fish in the form of oxyhemoglobin and carbon dioxide from tissue to the gills in the form of carboxyhemoglobin. The mean hemoglobin value of *T. marmorata* decreased at Telbal compared to Sind stream. The low Hb value in a fish exposed to pollutant may be related to the inhibitory effect of those substances on the enzyme system responsible for synthesis of hemoglobin (Pamila *et al*., 1991). The pollutants entering into fish system is slowly eliminated (Newman and Mitz, 1988; James and Sampath, 1996 and James *et al*., 1996) and hence the blood parameters get affected on account of pollutant toxicity. The low Hb value in Telbal water body may also be associated with less active fishes. Similar results were reported by Engel and Davis (1964) and Rambhaskar and Srinivasa (1986). Eisler (1965) suggested that there was a correlation between hemoglobin concentration and activity of fish. The more active fishes tend to have higher hemoglobin values than the more sedentary ones (Pradhan *et al*., 2011). Consequently, *Pleuronectes annectens* being a relatively quiet and sedentary species (Okafor, 2006) has a slightly lower hemoglobin concentration than more active African teleost such as *Clarias buthupogon* whose mean hemoglobin concentration is as high as 9.88g/dl (Kori-Siakpero and Egor, 1997). The comparatively high hemoglobin content in *T. marmorata* from Sind stream may be related to its preferred environmental conditions. The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Any alteration in the number (quantitative) or morphology (qualitative) of RBCs from normal values can cause various pathological disorders in fish under stressful conditions**.** The high erythrocyte number was associated with fast movement, predaceous nature, and high activity with streamlined body (Rambhaskar and Srinivasa, 1986). The RBC value of *T. marmorata* decreased significantly ($P<0.001$) at Telbal compared to Sind stream. A fall in RBCs count, Hb% and PCV%, in the fishes, due to water pollution, has been reported along with acute anaemia (Singh, 1995). According to Singh *et al*., (2002) the discharge of waste may cause serious problems as they impart odour and can be toxic to aquatic animals. The organic wastes present in Telbal stream seem to cause stress in the fish and as such seem to be responsible for the changes in the hematological parameters. Lowering of TEC count coupled with low Hb content may be due to destructive action of pollutants on erythrocytes as a result of which the viability of the cells may be getting affected (Karuppasamy, 2000 and Zutshi *et al*., 2010). PCV or Haematocrit is an important tool for determining the amount of plasma and corpuscles in the blood (measurement of packed erythrocytes) and is used to determine the oxygen carrying capacity of blood (Larsson *et al*., 1985). Hematocrit or PCV in the present study decreased significantly ($P < 0.001$) in Telbal stream in comparison to Sind stream. Baxhall and Daisely (1973) have reported the possibility of using haematocrit as a tool in aquaculture and fishery management for checking anaemic condition. Reported values for fish haematocrit are usually between 20% and 35% and scarcely attain values greater than 50% (Clark *et al*., 1979). Joshi *et al*., (2002) and Banerjee & Banerjee (1988) have suggested that pollutant exposure decreases the TEC count, Hb content, and PCV value due to impaired intestinal absorption of iron. PCV values always decrease when a fish loses appetite or becomes diseased or stressed (Zutshi *et al.*, 2010). Similar results were obtained by Larsson *et al*., (1985) after exposure of fish to $KMnO_4$, indicative of anaemia and haemodilution, possibly due to gill damage or/and impaired osmoregulation. In fish, as in mammals, blood cells including WBC are frequently used as indicators of health status in fish because WBC are key components of innate immune defense and leukocytes are involved in the regulation of immunological function in the organism (Duthie and Tort 1985; Gallardo *et al*., 2003; Ballarin *et al*., 2004). There was variation in WBC quantity and leukocyte cell proportions (neutrophil, monocyte) in the fish specimens from Telbal stream. The implication of this result is that the fish has been able to defend itself from invading pathogens both by cell-and antibody-mediated responses (Kumar *et al*., 1992). Similar results were obtained by Sahan and Cengizler (2002) on carp caught from different regions of Seyhan River. Increased levels of TLC have been reported in *Channa punctatus* exposed to lead (Hymavathi and Rao, 2000) and *Clarias batrachus* exposed to mercuric chloride (Joshi *et al*., 2002). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of pollutants in water bodies. This is in conformity with the report by Saravanan and Harikrishnan (1999) in freshwater fish, *Sarotherodon mossambicus*, when exposed to sublethal concentration of copper and endosulfan and by Nanda (1997) in respect of *Heteropneustes fossilis* during nickel intoxication. This may be attributed to alteration in blood parameters and direct effects of various pollutants. These observations are also in good agreement with those of Karuppasamy *et al*., (2005) and Hardikar and Gokhale (2000). In fish, any infestation with any organism activates the cellular and humoral immune system. This is followed by changes in circulating antibodies and percentages and absolute number of the different WBC (Boon *et al*., 1990). Weinreb (1958) used leukocyte count changes as a means of assessing the systematic response of the rainbow trout, *Salmo gairdneri richardsoni* to various injections. Mishra and Srivastava (1980) also reported an increase in leucocytes count of fish due to pollution. Some of the most common causes of pollutant toxicity are inflammatory lesions associated with tissue damage; anemia and neoplasia. The lymphocytes are reported to be responsible for immune response, while neutrophils are reported to show the greatest sensitivity to change in the environment. Their characterization and identification is, therefore, of significance for assessing the changes in the physiological state of fishes. The percentage of these cell types generally decreases during acute exposure to copper (Nussey *et al*., 1995 and Svobodova *et al*., 1994), and in situations of chronic copper exposure, the neutrophil percentage has been reported to increase (Dick and Dixon, 1985). In the present study, the increases in WBC and neutrophil quantities in the samples collected from Telbal seem to be a response of cellular immune system to pollution (Palikova and Navratil, 2001; Şahan and Cengizler, 2002 and Saravanan *et al.*, 2003). The TEC, Hb and PCV and similar other indices based on blood parameters provide vital clues about the overall health of the fish vis-à-vis the condition of the fish environment.

Acknowledgement

The authors are grateful to the Director of Centre of Research for Development, Kashmir University for providing all the necessary facilities.

Table 1. Some important physico-chemical parameters (Mean ± S.D) of the water bodies

Parameters	**Sind**	**Telbal**
Water Temp. ^{0}C	20.66±7.57	17±2.64
pH	8.1±0.1	8.0±0.36
Dissolved Oxygen(mg/l)	8.13±0.83	7.43±0.35
Free Co_2 (mg/l)	8.33±1.52	12.66±1.52
Alkalinity(mg/l)	66±14.42	161±18.52
Ammonical-Nitrogen (µg/l)	22±4.58	60.33±32.51
Nitrate-Nitrogen (µg/l)	153±49.66	167±48.13
Orthophosphorus (µg/l)	26.33±11.50	87±31.32
Total phosphorus (µg/l)	44±14	166.33±28.00

Table 2: Mean values of the Hematological parameters of *T. marmorata* from Sind and Telbal stream

Parameters	Sind	Telbal
Hb(g/dl)	10.43±0.10	9.65±0.91
RBC(10^6/mm^3)	1.37±0.01***	1.19±0.01***
PCV (%)	26.61±0.55***	21.54±0.79***
WBC(10^4/mm^3)	40.34±1.07***	40.79±0.56***
DLC Lymphocyte (%)	71±5.86	68±6.54
Monocyte (%)	1.66±0.81	2.5±1.04
Neutrophil (%)	25.33±3.88	26.5±6.59
Basophil (%)	1.83±1.16	2.83±0.75
Eosinophil (%)	1.00±0.89	1.16±0.75

Data is presented as Mean ± S.D. Data was analyzed using one way ANOVA. The values were considered significant for* p < 0.05, **p<0.01 and ***p<0.001

References

Acharya, S., Dutta, T and Das, M. K. 2005. Effect of sublethal levels of nitrite on some blood parameters of juvenile *L. rohita* (Ham.). *Indian Journal of Experimental Biology*. **43**: 450–454.

Adams, S. M. 1990. Biological indicators of stress in fish. *American Fisheries Society Symposium Series 8, Bethesda*, Maryland.

APHA, 1998. ***Standard Methods for the Examination of Water and Wastewater.*** (20th edn.) American Public Health Association. Washington, D. C., 874 pp.

Ballarin, L., Dall'Oro, M., Bertotto, D., Libertini, A., Francescon, A., Barbaro, A.2004.Haematological parameters in Umbrinacirrosa (Teleostei, Sciaenidae): a comparison between diploid andtriploid specimens. *Comp Biochem Physiol*. **138**A: 45–51.

Banerjee, V and Banerjee, M .1988. Effect of heavy metal poisoning on peripheral hemogram in *H. fossilis* (Bloch) mercury, chromium and zinc chlorides (LC_{50}). *Comparative Physiology and Ecology*. **13**: 128–134.

Bhaskar, B. R and Rao, K. S. 1985. Some haematological parameters of tarpon, Megalops cyprinoids (Broussonet) from Visakhapathamharbour. *Matsy*. **11**: 63-69.

Blaxhall ,P.C.1972. The hematological assessment of the health of freshwater fish. A review of selected literature. *J Fish Biol*. **4(4):** 593-603.

Blaxhall, P.C and Daisley, K.W. 1973. Routine haematological methods for use with fish blood. *J. Fish Biol***.** **5**: 771 – 781.

Boon, J. H., Cannaerts, V.M. H., Augustijn, H., Machiels, M. A. M., Charleroy, D. De and Ollevier, F. 1990. The effect of different infection levels with infective larvae of *Anguillicolacrassus*on haematological parameters of European eel (*Anguilla anguilla*). *Aquaculture.* **87**: 243-253.

Bouck, G. R and Ball, R. C. 1966. Influence of capture method on blood characteristics and mortality in the rainbow trout (*Salmogairdnari). Trans. Am. Fish. Soc*. **95**: 170-176.

Brown, B. A. 1980. ***Haematology, Principle and Procedure (3rd) Lea and Fabinger***, Philadelphia.

Casillas, E and Smith, L. S. 1977. Effect of stress on blood coagulation and hematology in rainbow trout (*Salmo grindneri*). *J Fish Biol*. **10**: 481–491.

Cataldi, E., DiMarco P., Mandich, A. and Cataudella. S. 1998. Serum parameters of Adriatic sturgeon *Acipenserraccarii* (Pisces: Acipenseriformes); effect of temperature and stress. *Comp. Biochem. Physiol.* **121**: 351-354.

Chandra, S., Ram, R. N and Singh, I. J. 2001. Toxic effects of carbofuran on certain haematological parameters in yearlings of *Cyprinus carpio*. *Aquaculture*. **2**: 137–140.

Chellan, B., Ramesh, M and Ramanujam, R. M. 1999. Lethal and sublethal effects of a synthetic detergents on glucose-6-phosphate-dehydrogenase (G-6-P.D.H) enzyme activity in blood of a freshwater teleost, *Labeo rohita* (Ham, 1822). *Indian Journal of Fisheries.* **46(4):** 397–400

Clarks, S., Whitemore, D. H .Jr and McMahon, R .F . 1979. Consideration of blood parameters of largemouth bass, (*Micropterussalmoides*). *J. Fish Biol.* **14**: 147 – 154.

Coole, G. R. 1979. ***A Text book of Limnology,*** (2nd edn). The Mosley Co. London, New York.

CSIR. 1974. ***Analytical Guide (Laboratory Techniques)*** CSIR, Pretoria, South Africa.

Dacie ,J. V and Lewis, S .M. 2001. ***Practical Haematology*** (9^{th} edn). Churchill Livingstone, London.

Dick, P. T. and Dixon, D. G. 1985. Changes in circulating blood cell levels of rainbow trout, *Salmogairdneri Richardson*, following acute and chronic exposure to copper. *J. Fish Biol*. **26**: 475-480.

Duthie, G .G., Tort, L. 1985. Effects of dorsal aortic cannulation on the respiration and haematology of Mediterranean living *Scyliorhinuscanicula*. *CompBiochem Physiol*. **81**A: 879–885.

Eisler, R. 1965. Erythrocyte counts and hemoglobin contents in nine species of marine Teleosts, *Chesapeake Science*. **6**: 116-120.

Engel, D. M and Davis, E. M. 1964. Relationship between activity and bloodComposition in certain marine teleosts. *Copeia*. **3**: 586–587.

Gallardo, M .A., Sala-Rabanal, M., Ibarz ,A., Padrós, F., Blasco, J., Fernández-Borra, J. and Sánchez, J. 2003. Functional alterationsassociated with "winter syndrome" in gilthead sea bream (Sparusaurata). *Aquaculture* **223**: 15–27.

Hardikar, B. P and Gokhale, K. S. 2000. Study of haematological parameters of sewage fed fish, *Sarotherodon mossambicus* (Peters). *Bulletin of Pure and Applied*. **19**: 7-13.

Heckel, J. J. 1838. FischeausCaschmirgesammelt und herausgegeben von Carl Freiherrn von Hügel, beschrieben von J. J. Heckel. Wien. *Annals and Magazine of Natural History***.** **8:** 1-112.

Hickey, C .R. 1982. Comparative hematology of wild and captive Cunners. *Trans Am Fish Soc*. **111**:242–249.

Hora, S. L. 1937. Comparison of the fish faunas of the northern and the southern faces of the Great Himalayan Range. *I bid.* **39**: 241 - 50.

Hymavathi, V and Rao, L .M. 2000. Effect of sublethal concentration of lead on the haematology and the biochemical constituence of *Channapunctata*. *Bulletin of Pure and Applied Sciences*. **19**: 1–5.

James, R and Sampath, K. 1996. Individual and combind effects of carbaryl and methyl parathion on leucocyte and their recovery in Heteropneustes fossilis. p. 417 – 421. In: Assessment of Water Pollution (S. R. Mishra Ed). A. R. H. Publishing Corporation, Darya Ganj, New Delhi..

James, R., Sampath, K and Alagurathinam, S. 1996. Effects of lead on respiratory on respiratory enzyme activity glycogen and blood sugar levels of the teleost, Oreochromismossambicus (Peters) during accumulation and depuration. *Asian Fish. Sci.* **9**: 86-99.

Joshi, B. D. 1982. Circannual fluctuations in some blood components of the fish Rita rita, in relation to certain eco-physiological conditions. Uttar Pradesh Journal of Zoology. **2**: 62–66.

Joshi, P. K., Bose, M and Harish, D. 2002. Haematological changes in the blood of *Clariasbatrachus*exposed to mercuric chloride. *Ecotoxicol. Environ.Monit*. **12**:119–122.

Karuppasamy, R. 2000. Impact of phenyl mercuric acetate (PMA) on the biomodal respiration in an airbreathing fish *C. punctatus*(Bloch).*Journal of Environmental Pollution*. **7**: 287–293.

Karuppasamy, R., Subathra, S and Puvaneswari, S. 2005. Haematological responses to exposure to sublethal concentration of cadmium in air-breathing fish *C.punctatus*(Bloch).*Journal of Environmental Biology*. **26(1):** 123–128.

Kori-Siakpere, O and Egor, V. E. 1997. Haematological characteristics of the African mudfish: *Clariasbuthupogon*(Clariidae). *Bull. Sci, Ass. Nig*, **21**: 177 – 185.

Kumar, S., Lata, S and Gopal, K. 1999. Deltamethrin Induced Physiological Changes in Freshwater Cat Fish *Heteropneustes fossilis. Bull. Environ. Contam.Toxicol.* **62**: 254-258.

Larsson, A., Haux, C. and Sjobeck ,M .1985. Fish physiology and metal pollution. Results and experiences from laboratory and field studies. *Ecotoxicol. Environ. Saf*. **9**: 250 - 281.

Mackereth, F. J. H., Heron, J and Talling, J. F. 1978. ***Water Analysis: Some Revised Methods for Limnologists.*** Freshwater Biological Association, Scientific Publication. 120 pp

McLeavy, D. J and Brown, D. A. 1974. Growth stimulation and biochemical changes in juvenile Coho salmon (*Oncorhynchus kisutch*) exposed to bleached kraft pulp mill effluent for 200 days. *Journal of the Fisheries Research Board of Canada*. **31**:1043–1049.

Mishra, S and Srivstava, A. K. 1980. The acute toxic effects of copper on the blood of a teleost .Ecotoxicol. *Enivorn.Safety*. **4**:191-194.

Nanda, P. 1997. Haematological changes in the common Indian catfish *Heteropneustes fossilis* under nickel stress. *Journal of Ecobiology*. **9**: 243–246.

Newman, M. C and Mitz, S. V. 1988. Size dependence of zinc elimination and uptake from water by mosquito fish, Gambusiaaffinis (Baird and Girard). *Aquat.Toxicol*, **12**: 17- 32.

Noga, E.J. 1993. Water mold infections of freshwater fish: *Recent advances annual review of fish diseases*. **3**: 291-304.

Nussey, G, Van V. J. H. J, and Du Preez, H. H. 1995. Effect of copper on the differential white blood cell counts of the Mozambique tilapia (*Oreochromis mossambicus*). *Comp. Biochem. Physiol*, **111**: 381-388.

Okafor, A. I. 2006. *Ecophysiological studies on osmotic regulation and aestivation in the African lungfish, protopterusannectens (owen) from Anambara river, Nigeria. Ph. D thesis*, University of Lagos, Nigeria.

Palikova, M and Navratil, S. 2001. Occurrence of *Anguillicolacrassus*in the water reservoir Korycany (Czech Republic) and its influence on the health condition and haematological indices of eels. *Acta Vet. Brno*. **70**: 443-449.

Pamila, D., Subbaiyan, P. A. and Ramaswamy, M. 1991. Toxic effect of chromium and cobalt on *Sartherodon mossambicus*(peters). *Indian J. Environ., Hlth*. **33**: 218 -224.

Pradhan, S. C., Patra, A. K., Sarkar, B and Pal, A. 2012. Seasonal changes in hematological parameters of *Catla catla* (Hamilton 1822). *Comp. Clin. Pathol*. **21 (6):** 1473-1481.

Qadri, M. Y., Naqash, S. A., Shah, G. M and Yousuf, A. R. 1981. Limnology of two streams of Kashmir. *J. Ind. Inst. Sci*. **63**: 137-141.

Rambhaskar, B. and Srinivasa, R. K. 1986. Comparative haematology of ten species of marine fish from Visakhapatnam Coast. *J Fish Biol.* **30**: 59–66.

Ramnik, S. 1994. ***Medical Laboratory Technology***. (4th Ed). Jaypee Brothers, New Delhi. 187 pp.

Roberts, R. J. 1981. ***Patologı´a de los peces***. Mundiprensa, Madrid. 366 pp

Sahan, A. and Cengizler, I. 2002. Determination of some heamatological parameters in spotted barb (*Capoetabarroisi*, 1894) and roach (*Rutilusrutilus*, 1758) Living in Seyhan River (Adana City Region), (in Turkish). *Turk.J. Vet. Anim. Sci.* **26**: 849-858.

Sampath, K., James, R and Akbar A. K. M. 1998. Effects of copper and zinc on blood parameters and prediction of their recovery in *Oreochromis mossambicus* (pisces). *Indian Journal of Fisheries.* **45(2):** 129–139.

Santos, M. A. and Pacheco, M. 1996. Stress biomarkers recovery in clean water and secondary treated pul mill effulent. *Ecotoxicol and Environ. Safety*. **35**: 96-100.

Saravanan, J. S and Harikrishnan, R. 1999. Effect of sublethal concentration of copper and endosulphan on haematological parameters of the freshwater fish, *Sarotherodonmossambicus*(Trewaves). *Journal of Ecobiology*. **11**: 13–18.

Saravanan, T. S., Aneez, M., Mohammad, R., Chandrashekhar, M and Sundramoorthy .2003. Freshwater fishes as indicators of Kaveri river pollution. *J. of Environ. Biol*. **24 (4):** 381-389.

Shah, S. L and Altindag, A. 2005.Alteration in the immunological parameters of tench (Tincatinca L) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. *Turk J. Vet. Anim Sci*. **29**: 1163-1168.

Singh, K .D., Srivastava, B and Sahu, A. 2002. Non-conventional absorbents for fresh water treatment containg phenolic compounds, 22nd Annual Session, NBFGR, Lucknow. The Academy of Environ. Biol. Abstracts. 73-74.

Singh, M .1995. Hematological responses in freshwater teleost *Channapunctatus* to experimental copper and chromium poisoning. *J. Env. Biol.* **16(4):** 339-34.

Svobodova ,Z., Kroupova, H., Modra, H., Flajshans, M., Randak, T., Savina, L. V. and Gela, D. 2008. Haematological profile of common carp spawners of various breeds. *J Appl Ichthyol*. **24**: 55–59.

Svobodova, Z., Vykusova, B. and Machova, J. 1994. The effects of pollutants on Selected haematological and biochemical parameters in fish. P. 36 In*: Sublethal and Chronic Effects of Pollutants on Freshwater Fish* (R. Müller and R. Lloyd, eds.). Fishing New Books, London.

Todda, B. K. 1970. ***Water Encyclopedia***. Water Information Centre. Port Washington, New York.

Van Vuren, J. H. J., Van Fer Merwe, M and Du Preez, H. H. 1994. The effect of copper on the blood chemistry of *Clarias gariepinus* (Clariidae). *Ecotoxicology Environment Safety*. **29**: 187–199.

Vass, K. K., Raina, H. S, Zutshi, D. P and Khan, M. A. 1977. Hydrobiological studies on River Jhelum., *Geobios*. **4**: 238-242.

Vazqez, G. R and Guerrero, G. A. 2007. Characterization of blood cells and haematological parameters in Cichlasomadimerus (Teleostei, Perciformes). *Tissue and cell*. **39**: 151 – 160.

Weinreb, E. L.1958. Studies on the histology and histopathology of the rainbow trout, *Salmogairdneri* (Richardson). 1. Haematology. Under normal and experimental conditions of inflammation. *Zoologica Ny*. **46**:145 – 153.

Zutshi, B., Raghu Prasad, S. G. and Nagaraja, R. 2010. Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India. *Environ Monit Assess*. **168**: 11–19.