Butachlor Induced Alterations in Haematological Parameters of *Schizothorax niger* Heckel

Dissertation Submitted

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CERTIFICATE

Certified that the work presented in this dissertation entitled **"Butachlor Induced Alterations in Haematologcal Parameters of *Schizothorax niger* Heckel"** by **Baby Habiba** has been carried out under my supervision and the same has not been submitted elsewhere for the degree. Certified further that the candidate has fulfilled all the conditions necessary for the M.Phil. degree examination of University of Kashmir. The dissertation is worthy of consideration for the award of M.Phil. degree in Environmental Science.

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Abbreviations

Hb	Haemoglobin
Hct	Haematocrit
PCV	Packed cell volume
RBC	Red Blood Cell
RBCs	Red Blood Cells
WBC	White Blood Cell
WBCs	White Blood Cells
CBC	Complete blood count
TLC	Total Leucocyte Count
TEC	Total erythrocyte count
TRBC	Total Red Blood Cell
ESR	Erythrocyte Sedimentation Rate
MCH	Mean Cell Haemoglobin
MCV	Mean Cell Volume
MCHC	Mean Cell Haemoglobin concentration
Fig	Figure
Figs	Figures
SD	Standard Deviation
%	Percentage
ppm	parts per million
ppb	parts per billion

CHAPTER – 1

Introduction

Aquatic systems are the ultimate sinks of both natural and anthropogenic inputs of contaminants into the environment. Releases of toxic substances have been responsible for dramatic fish kills. Even sub-lethal concentrations of toxicants may prove to be equally devastating to fish populations. The use of biocides is indispensable in modern agricultural technology to control weeds or pests for the production of more food and management of public health and currently about 4,500 pesticides are in general use all over the world, out of which 25 have high toxicity potential to a wide range of flora and fauna (Adhikary and Sahu, 2001).

A number of studies have been carried out regarding the haematological alterations induced by pesticides in general (e.g. Patnaik and Patra, 2006; Ramesh and Saravanan, 2008; Akinrotimi *et al.*, 2012; Parma *et al.*, 2007; Chandrasekar and Jayabalan ,1993; Ali and Rani ,2009; Velisek *et al* .,2009; Kumar *et al.*, 1999 and Ahmed, 2011) and herbicides in particular (e.g. Ramesh *et al*., 2009; Sivakumar *et al*.,2010; Dobsikova *et al.,* 2011;Modesto and Martinez 2010). All the pesticides were observed to cause noticeable changes in the haematological parameters of the fishes.

Butachlor, a pre emergent herbicide i.e., a chemical weed killer applied prior to the emergence of the weed from the soil, was developed by Monsanto Company (USA). It prevents weed seeds from germinating. Butachlor was introduced in 1968 for the control of undesirable grasses and broadleaf weeds in transplanted, direct seeded rice and barley fields. The herbicide is used primarily in rice paddies of Asia, South America, Europe and Africa (Chiang *et al*, 1997; Ishizuka *et al*.1998).

In Kashmir, the most popular herbicide used in rice fields is butachlor. Applied to the paddies, the herbicide dissipates into paddy water and a minor quantity of the

herbicide could be detected in paddy drainage water even several weeks after application (Chiang *et al*., 1987) causing contamination to rivers and lakes (Ohyama *et al*., 1987). Butachlor has a half-life of 1.65−2.48 d in field water and 2.67−5.33 d in soil (Yu *et al*., 1993). Butachlor is moderately to highly toxic to fish and aquatic organisms (Wang *et al*., 1992; Sivakumar *et al*., 2010) and its toxicity persists in the aquatic system even for longer period of time (Chattopadhyay *et al*., 2006). Since, large quantities of this weedicide are used in paddy fields of the valley, most of which lie in the close vicinity of flatland lakes including Dal, there is high possibility that a significant quantity of the weedicide finds its way into these water bodies. Butachlor haematotoxicity has been studied by Sivakumar *et al*, (2010) in *Oreochromis mossambicus* who reported that butachlor caused a significant decrease in white blood cells, haemoglobin, red blood cells, haematocrit, mean corpuscular haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin concentration.

Schizothorax niger Heckel is commercially important fish species inhabiting the flatland lakes of Kashmir including the world famous Dal lake. *S. niger* is the only truly lacustrine Schizothoracine fish in the valley. The deteriorating condition of the lakes in the last two-three decades has resulted in a decline in its population.

The present study was carried out to assess the effect of exposure of *Schizothorax niger* Heckel to butachlor on its haematological parameters under laboratory conditions. The fish for the experimentation was collected from the Dal lake, Kashmir. The observations made are presented in the present dissertation.

CHAPTER – 2

REVIEW OF LITERATURE

The use of haematological parameters in assessment of fish physiology was proposed by Hesser (1960). Since then haematology has been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful conditions such as handling, pollutants, pesticides, metals, hypoxia, anaestehtics and acclimation. The literature pertaining to fish hematotoxicology is quite extensive and a listing of all the studies is beyond the scope of the dissertation. Therefore in the following pages only important contributions in the field have been reviewed.

Bouck and Ball (1966) stated that hematology may be useful tool in monitoring stress levels of aquatic pollution on fish. Eddy and Morgan (1969) observed a mean increase in the Hb concentration from 5.3 to 7.6 g/100ml between control rainbow trout and a group acclimatized to high levels of free carbon dioxide. Enomato (1969) observed a decrease in lymphocyte number during oxygen deficient conditions and Gardener and Yerich (1969) stated that there is probability for eosinophil concentration to increase as a result of environmental stress.

Cameron (1970) studied the impact of temperature on the blood parameters of pinfish *Lagodon rhomboids.* The results revealed increased Hb and erythrocyte count, with decreased haematocrit and mean erythrocyte volume. Smith & Piper (1972) reported increase in Hb, Hct and immature erythrocytes in rainbow trout due to formalin exposure. Larmoyeux and Piper (1973) reported that increased level of ammonia caused increase in haematocrit values in trout due to haematological compensation stimulated by low dissolved oxygen. Soivio *et al.* (1974) studied changes in haematocrit values under lowered oxygen tension and aerobically treated blood samples of rainbow trout, landlocked Baltic salmon, brown trout and lake trout in vitro. The mean haematocrit value increased during 2h incubation under lowered oxygen tension. During corresponding incubation with oxygen, the mean haematocrit value decreased.

Buckley *et al.* (1976) exposed *Onchorhynchus kisutch* to chlorinated municipal sewage and observed reductions in haemoglobin concentration and haematocrit. Lone and Javaid (1976) observed alteration in the blood of *Channa punctatus* due to the effect of dieldrin and DDT. Tandon and Joshi (1976) evaluated total red and white blood cell count of 33 species of fresh water teleosts. The fishes showed a wide range of variations in their total red and white blood cell counts, both of which were reported to be affected by size of the fish, their general habits, feeding habits and metabolic needs. Buckley (1977) recorded haemolytic anaemia with pathological changes in erythrocytes, reduction in packed cell volume and Hb concentration in *Onchorhynchus kisutch* after exposure to chlorinated waste water. McLeay and Gordon (1977) measured the acute stress in salmonid fish, using stressful concentrations of pulp mill effluent. Leucocrit and leucocyte-thrombocyte counts for both coho salmon (*Onchorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) were depressed from stock values after exposure to stressful (high-temperature crowded) conditions. The study revealed that the number of leucocytes and thrombocytes in the circulating blood of fishes reflected more accurately than the number of erythrocytes the fish's reaction to acute stress.

Anees (1978) reported a decrease in erythrocyte count and haemoglobin content in freshwater fish *Channa punctatus* after acute exposure to diazinon. Johansson-Sjobeck *et al.* (1978) reported increased percentage of neutrophils and monocytes and decreased percentage of lymphocytes in the circulating blood of *Anguilla anguilla* exposed to handling stress. Clark *et al*. (1979) studied physiological stress resulting from environmental influences in largemouth bass, *Micropterus salmonids*, and reported that Haematocrit and hemoglobin were positively correlated with fish length and Hb, Hct were positively correlated with fish age, while mean corpuscular hemoglobin negatively correlated with fish age. Both hemoglobin and packed cell volume were related to erythrocyte counts. Koundinya and Ramamurthi (1979) observed a decline in RBC count in *T. mossambica* after exposure to sumithion and sevin. Mishra and Srivastava (1979) observed a decrease

in the number of lymphocytes and an increase of neutrophils and monocytes in a Freshwater Teleost as a result of exposure to sublethal concentrations of zinc. Srivastava and Mishra (1979) studied blood dyscrasia in a teleost fish, *Colisa fasciatus*, associated with cadmium poisoning. Sub-lethal concentration of cadmium as cadmium sulphate induced lymphocytosis and anemia, with simultaneous regeneration of red blood cells. The treatment also evoked thrombocytosis with concomitant hypercoagulability of whole-blood.

Aggarwal and Srivastava (1980) studied blood dyscrasia in a fresh water teleost exposed to experimental manganese poisoning. The results revealed that acute exposure of the fish to manganese causes anaemia and leucocytosis. Hanke (1980) found a decline in the number of erythrocytes after exposure of *C. carpio* to 0.1 mg/l atrazine. Larrsone *et al.* (1980) studied the physiological effects of titanium dioxide industrial effluent on the flounder (*Platichthys flesus*) a marine benthic fish species and reported that the exposure of flounders to the effluent caused increased values for haematocrit, haemoglobin content and number of erythroblasts. Matkovics *et al*. (1981) observed a quick decrease in haemoglobin content of *Cyprinus carpio* in response to paraquat toxicity. Verma *et al*. (1981) worked on the role of ascorbic acid in the toxicity of pesticides, thiotox and malathion in a fresh water teleost. It was found that thiotox and malathion induced significant alteration in blood parameters while ascorbic acid played a protective role. Nieminen *et al.* (1982) studied the effects of pH on the gill ATPase activity and blood composition of whitefish (*Coregonus peled*) and trout (*Salmo trutta fario*) and documented higher value of Hb and PCV in the fishes at low pH due to hypoxia. Wedemyer *et al.* (1983) studied physiological stress response in *Oncorhynchus kisutch* and found that leucocrit was a sensitive indicator of the physiological stress resulting from crowding population densities and to stress of handling and to temperature changes.

Goel (1984) carried out a study on alachlor toxicity to a freshwater teleost *Clarias batrach*us. Exposure of the fish to alachlor resulted in a significant increase in the total leukocyte count, MCV, MCH, and a significant decrease in erythrocyte

count, Hb%, MCHC and PCV. Kumar *et al*. (1984) studied the haematological changes in cold water fish, *Schizothorax plagiostomus* (Heckel). They reported that *O. plagiostomus* naturally infected with metacercarae of *Diplostomium tertare* had decreased total erythrocyte count, PCV, Hb, TLC relative to uninfected controls indicative of pollution.

Dick and Dixon (1985) reported that acute exposure of copper caused leucopenia in rainbow trout. However chronic exposure had no impact on either erythrocyte or total leucocyte concentration, but caused a significant increase in the number of Neutrophils. Mainwaring and Rowley (1985) studied the effects of ethylenediamine-tetraacetic acid (EDTA), heparin and tri-sodium citrate (TSC) on various haematological parameters in the blenny, *Blennius pholis*. Muragesen and Haniffa (1985) studied the impact of textile mill effluent on haematology of *Anabas testudineus*. Significant decrease was observed in RBC, haemoglobin and haematocrit content with increase in effluent concentration. Ralio and Nikinmaa (1985) reported that fish blood parameters like erythrocyte and leucocyte count, hemoglobin, haematocrit and leukocyte differential counts readily respond to incidental factors such as physical stress and environmental stress due to water contaminants.

Dabrowska and Wlasow (1986) exposed *Cyprinus carpio* to a sublethal concentration of ammonia for 3 weeks. The exposure resulted in unfavourable changes in the blood i.e., leucopenia and eosinophilly. Dheer *et al.* (1986) studied the impact of sodium chloride in *Channa punctatus*. Statistically significant changes were observed in all the haematological parameters resulting in microcytic hypochromic anaemia. Vuren (1986) carried out a study on the haematological parameters of *Labeo umbratus* exposed to four different toxicants. Detergent and fertilizer caused decrease in number of erythrocytes, haematocrit, mean corpuscular volume, average corpuscular volume. Metasystox caused increase in MCV and haematocrit.

Bielinska (1987) carried out dielectric, haematological and biochemical studies of detergent toxicity in fish blood and reported that in *Cyprinus carpio* exposed to a sublethal concentration of sodium alkyl benzene sulphonate, a decline was noted in erythrocyte count, haematocrit, blood haemoglobin concentration, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin. Ellsaesser and Clem (1987) studied Cortisol induced haematological and immunological changes in channel catfish (*Ictalurus punctatus*) and reported a reduction in lymphocyte counts. Flos *et al.* (1987) studied the effects of zinc sulphate on haematological parameters in the dogfish *Scyliorhinus canicula*. After treatment of the fish with acute and subacute zinc concentrations, differences were found only in leucocyte number. Rani *et al.* (1987) observed marked decrease in RBCs count in catfish intoxicated with the organophosphate pesticide dichlorvos.

Dheer (1988) studied haematological, haematopoitic and biochemical characteristics of *Channa punctatus* in response to thermal stress. All the parameters tested indicated deviation from the normal healthy conditions. Erythrocytic polycythemias accompanied by an increase in haemoglobin content and haematocrit values were indicative of thermal stress. Bhaskar and Rao **(**1989) evaluated the influence of temperature, salinity, dissolved oxygen, and pH of water on nineteen blood characteristics of juvenile milkfish, *Chanos chanos* from three different brackish water fish farms. No significant differences were reported between fish grown under different farm conditions with regard to most blood values. Cyriac *et al.* (1989) carried out studies on the haemoglobin and haematocrit values of *Oreochromis mossambicus* exposed to copper and mercury. Narain and Srivastava (1989) reported that sewage pollution induce anemic condition in *Heteropneustes fossilis*. Significant decrease in haemoglobin, RBC count, MCHC and increase in PCV, ESR was reported. Reddy and Bashamohideen (1989) studied the fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio* and reported a decrease in hematocrit, hemoglobin and red blood cells values of the fish following exposure to the insecticides. Tucker *et al.* (1989) studied nitrite

induced anemia in channel catfish *Iclaturus punctatus*. It was observed that mean haematocrit and total haemoglobin concentration in fish exposed to the two highest nitrite concentrations indicate only moderate anemia, however there was considerable variation among fish within these groups and some fish were anemic.

Kumar and Benerjee (1990) reported an increase in ESR in *Clarias batrachus* after exposure to sevin. Raja *et al.* (1990) worked on haematological parameters of *Oreochromis mossambicus* living in polluted water tank. Decrease in haemoglobin and RBCs was observed, whereas leucocyte count was high. Ruparelia *et al.* (1990) studied the effect of cadmium on the blood of *Oreochromis mossambicus* in hard and alkaline water. Significant decrease in haemoglobin, haematocrit and red blood cell count was observed. Singh and Reddy (1990) studied the effect of copper sulphate on haematology, blood chemistry and hepatosomatic index of an Indian catfish *Heteropneustes fossilis* (Bloch), and its recovery. Trivedi *et al.* (1990) evaluated hematotoxic effects of two commonly used fertilizers, diammonium phosphate and urea, on *Clarias batrachus*. The toxic effect of diammonium phosphate was reported to result in a sudden fall of Hb, RBC count and Hct at higher concentrations. In urea intoxication, significant decreases in the three parameters were seen only at higher concentrations. Total leucocyte count (TLC) increased during toxicity with both fertilizers, but higher elevations in TLC were produced by diammonium phosphate than by urea. Their study revealed that the toxic effect of diammonium phosphate was more pronounced than that of urea.

Shakoori *et al.* (1991) observed increases in Hb and MCH values in C. idella exposed to sublethal doses of mercuric chloride. Yamamoto (1991) reported increase in Hb, Hct and immature erythrocytes in carp (*Cyprinus carpio*) as a result of formalin exposure. Garcia *et al.* (1992) studied the effect of the volume of blood extracted, number of extractions and weight upon the haemocrit value of fresh water teleosts, rainbow trout and a marine European sea bass and found that from trout with the same weight, the increase in the volume of blood extracted resulted in a significant increase in the haemocrit value. Also for the same volume of blood

extracted in trout of the same weight the second extraction resulted in the significant increase in the haemocrit value. In sea bass, haemocrit values in relation to the aforementioned parameters were not produced. Ghazaly (1992) studied haematological and physiological responses in a fresh water teleost *Tilapia zilli* to sublethal concentration of cadmium. Haematological and physiological changes attributable to cadmium poisoning were observed. Hoglund *et al.* (1992) studied the hematological variations in population of *Anguilla anguilla* naturally infected with *Anguillicola carassus.* Most variables showed no or only minor reactions to the infection. However a marked increase in the gamma fraction of serum protein reduced lymphocyte numbers and increased granulocyte numbers were considered indicative of a humoral and cellular immune response. Sexena and Chauhan, (1992) studied the effect of acid stress on the blood of *Clarius batrachus* (Linn.) and reported that RBC count increased and Hb concentration decreased during acid stress. Shaheen *et al.* (1992) exposed fresh water fish *Clarias batrachus* to 50 -100 ppm urea in aquatic medium. Hb count, erythrocyte count and haematocrit values decreased below controls while the total leucocyte count increased above controls.

Allen (1993) exposed *Oreochomis aureus* to two concentrations of lead and cadmium for 24h and 1 week to assess the effect of these pollutants on haematological parameters. Cadmium appeared to be more toxic to *O. aureus* than lead, however lead depresses erythrocyte count. Aziz *et al.* (1993) observed increases in Hb and MCH values in *T. mossambica* exposed to cadmium chloride. Bagarinao and Vetter (1993) found an increase in hematocrit and haemoglobin concentration after exposing *Fundulus parvipinnis* to sulfide for 2 h. Chandrasekar and Jayabalan (1993) studied haematological responses of the common carp, *Cyprinus carpio* L. exposed to the pesticide endosulfan and found that the fish responded with decrease in the levels of haemoglobin and haematocrit. Chaturvedi and Agarwal (1993) reported an increase in ESR in *Heteropneustes fossilis* after exposure to alachlor and rogor. Ghosh and Banerjee (1993) reported lymphopenia and both neutrophil and eosinophil granulocytosis in *Heteropneustes fossilis* after an effect of dimethoate in

96h LC50 concentration. Mukherjee and Sinha (1993) recorded an increase in MCV and MCH with a decrease in RBC, Hb and PCV values for major carp exposed to cadmium chloride for 2 weeks. Salonius & Iwama (1993) reported increase in lymphocyte percentage in Atlantic salmon subjected to handling stress. Sampath *et al.* (1993) studied the haematological changes and their recovery in *Oreochromis mossambicus* as a function of exposure period and sublethal levels of Ekalus.

Allen (1994) evaluated the use of haematological parameters as indicators of mercury pollution. Annune *et al*. (1994) reported a significant increase in RBC count of *C. gariepinus* when subjected to Zn treatment. Chauhan *et al.* (1994) studied rogor induced haematological alterations in *Cyprinus carpio* and reported a decrease in hematocrit , hemoglobin and red blood cell values of the fish after exposure to the insecticide. Martinez *et al*. (1994) studied the effect of the simultaneous influence of weight, temperature, density and O_2 concentration in the water on various blood parameters of *Oncorhynchus mykiss*. Multiple correlation and regression analyses showed a strong dependence of Ht, Hb and RBC on the factors considered, the most influential of which was temperature. Omoregie *et al.* (1994) studied the chronic effects of formalin on erythrocyte counts of Nile tilapia (*Oreochromis niloticus).* Radhakrishnan and Prasad (1994) reported that exposure of tilapia, *Oreochromis mossambicus* to a sublethal concentration of an organophosphorus insecticide Ekalauk (EC-25), elevated the erythrocyte count, haemoglobin content and haematocrit. Rajyasree and Perviz (1994) studied the effect of urea on hematology of *Labeo rohita* fingerlings. Significant decrease in blood parameters was observed at higher concentrations. Rauthan and Grover (1994) reported that blood parameters (total erythrocyte count, hemoglobin, packed cell volume) of *Barilius bendelisis* raised during summer months, whereas lowest values of all parameters were observed in winter months, when ambient temperature was quite low. Sastry and Sachdeva (1994) found that RBC, Hb and PCV decrease in *Channa punctatus* exposed to heavy metals.

Agarwal and Chaturvedi (1995) observed a reduction in hematocrit, hemoglobin and red blood cell values of *Heteropneustes fossilis* following exposure to alachlor and rogor. Al-kahem (1995) studied behavioural responses and changes in some haematological parameters of the cichlid fish, *Oreochromis niloticus*, exposed to trivalent chromium and recorded changes in haematological parameters such as total leucocyte and erythrocyte counts, haemoglobin and haematocrit. Nussey *et al.* (1995) studied the effect of copper on the differential white blood cell counts of *Oreochromis mossambicus* and observed significant increases in the number of lymphocytes (lymphocytosis) and eosinophils (eosinophilia) combined with significant decreases in monocytes (monocytopenia) and neutrophils (neutropenia), after short-term (96 hr) exposure to copper. Singh (1995) studied the effect of copper sulphate and potassium dichromate poisoning on hematological parameters of *Channa punctatus*. RBC, Hb, PCV and MCHC decreased significantly while WBC, MCV and MCH increased. However, independently chromium proved to be more toxic than copper. Woo and Chiu (1995) found significant increase in blood nitrite and methemoglobin of sea bass in response to nitrite exposure to 10 mg/l for 8 days. Total haemoglobin was reduced resulting in an overall decline in functional haemoglobin.

Hussein *et al*. (1996) reported decreased RBC number, haemoglobin concentration and haematocrit percentage of *Oreochromis nitoticus* and *Chrysichthyes auratus* when exposed to 3 and 6 mg/l ATR. Klinger *et al.* (1996) studied the effects of dietary lipid on the haematology of channel catfish, *Ictalurus punctatus*, using soybean oil, menhaden oil, beef tallow, or a combination of these three lipid sources for 90 days. It was observed that the fish fed with menhaden oil diet had significantly lower ($P < 0.05$) hematocrits and higher thrombocyte counts. Their erythrocytes were the least susceptible to osmotic lysis.The erythrocytes of catfish fed the beef tallow diet were the most susceptible to osmotic lysis. Lopez (1996) studied the changes in the haematological characteristics of *Oreochromis niloticus* exposed to sublethal levels of cadmium and reported leucopenia and a

tendency towards neutrophilia in the treated fish. Nath (1996) reported a decrease in haematocrit, hemoglobin and red blood cells values of *Heteropneustes fossils* following exposure to fenvalerate. Nath and Banerjee (1996) reported lymphopenia in *Heteropneustes fossilis* when exposed to methylparathion and cypermethrin. Roche and Boge (1996) made an investigation on classical stress indicators (hemoglobin, and hematocrit) in sea bass (*Dicentrarchus labrax*). Shakoori *et al* (1996) exposed *Ctenopharyngodon idella* to danitol and fenvalarate that caused a significant reduction in the hematocrit value of the fish.

Hrubec *et al*. (1997) investigated the effect of water temperature on haematological and serum biochemical analysis in hybrid stripped brass. Mikryakov and Lapirova (1997) after studying the influence of salts of some heavy metals on the differential blood count in juvenile *Acipenser baeri* reported a decrease in the number of lymphocytes and an increase of neutrophils and monocytes. Nounou *et al*. (1997) found that RBC, Hb and PCV decrease in *Clarias lazera* when exposed to heavy metals. Verdegem *et al.* (1997) studied influence of salinity and dietary composition on blood parameter values of hybrid red tilapia, *O. niloticus* and *O. mossambicus* under similar environmental conditions. Results showed that salinity influence all cellular blood parameters except haematocrit. Dietary composition influenced haematocrit.

Bukowska *et al.* (1998) studied the influence of phenoxy-herbicides and their metabolites on the form of oxy- and de-oxyhemoglobin of vertebrates. Amongst the investigated hemoglobins, the most sensitive one was found to be carp oxyhemoglobin incubated with 2,4-D (2,4-dichlorophenoxyacetic acid) and the least sensitive was human hemoglobin. Manavalaramanujam *et al.* (1998) exposed *Labeo rohita* to sublethal concentration of acid red dye for 30 days. The Hb content of the fish increased throughout the period of experiment. Kumar *et al.* (1999) attempted to explore the impact of deltamethrin on the physiological consequences including certain haematological parameters in fresh water cat fish, *H. fossilis*. Musa and Omoregie (1999) reported reduction in haematological indices following exposure of

C. gariepinus to sublethal concentrations of the therapeutant: malachite green. Santhakumar *et al.* (1999) observed a decline in RBC count in *A. testudineus* after exposure to azodrin.

Bhagwant and Bhikajee (2000) observed hypochromic macrocytic anemia in *Oreochromis* hybrid after exposure to 100mg/l of aluminium. These changes have been attributed to the swelling of the red blood cells, haemodilution and impaired Hb concentration recorded. Kotsanis *et al*. (2000) reported a decrease in the number of lymphocytes and an increase in neutrophils and monocytes in rainbow trout, *Oncorhynchus mykiss*, subjected to metal toxicants. Roche and Bogé (2000) studied in vivo effects of phenolic compounds on blood parameters of a marine fish (*Dicentrarchus labrax*) and reported positive correlations with hemoglobin. Sancho *et al.* (2000) studied the cholinesterase activity and hematological parameters as biomarkers of sublethal molinate exposure in *Anguilla Anguilla*. Molinate exposure produced a decrease in Haematocrit, haemoglobin, erythrocytes and leucocytes which was significant only during the recovery period. Thakur and Bais (2000) studied the impact of sublethal concentration of aldrin and fenvalerate on certain hematological parameters of *Heteropneustes fossilis*. Differential changes in ESR were observed whereas ESR decreased in aldrin treated group. However MCHC, MCH, Hb content, PCV, erythrocyte counts decreased due to pesticide intoxication.

Dorucu and Girgin (2001) reported a decrease in the levels of PVC, Hb, Leucocytes and RBC in carp after poisoning with Cypermethrin. Ezeri (2001) observed no significant differences in the Hb, ESR, WBC and PCV of healthy *C. gariepinus*, those infected with *Pseudonomas florescens* and the infected treated with chloramphenicol. Shalaby (2001) studied the *Protective effect of ascorbic acid against mercury intoxication in Nile tilapia (Oreochromis niloticus) and* reported a significant decrease in RBCs, hemoglobin and packed cell volume of *Nile tilapia* exposed to the heavy metal. Svoboda (2001) assessed an effect of diazinon on common carp (*Cyprinus carpio* L.). The experimental group of one- to two-year-old common carp were found to have lower values of erythrocyte count, haemoglobin

content and haematocrit. It was concluded that diazinon is moderately toxic to *C. carpio* and its use in the fields may be a threat to both aquatic fauna and flora as well as humans. Toussaint *et al.* (2001) assessed the chronic toxicity of chloroform to Japanese medaka fish and reported that hematocrit, leukocrit, cell viability and cell counts of treated fish were not significantly different from those of control fish.

Affonso *et al.* (2002) studied blood parameters and metabolites in the teleost fish *Colossoma macropomum* exposed to sulfide or hypoxia and observed that the physiological responses induced by sulfide resembled those of stress induced by environmental hypoxia. Agbon (2002) carried out a study on acute toxicity of tobacco (*Nicotiana tobaccum*) leaf dust on *Oreochromis niloticus.* Sublethal concentrations of the extract were found to have an inverse relationship with haematological indices assessed. It was concluded that the chronic hematotoxicity of tobacco leaves extract in *O. niloticus* may be useful in pond management. Atamanalp *et al.* (2002) observed a significant increase in the erythrocyte count in *O. mykiss* exposed to cypermethrin (a synthetic pyrethroid). Ateeq *et al.* (2002) studied induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2,4-dichlorophenoxyacetic acid and butachlor. Both the herbicides were found to be genotoxic as well as cytotoxic in this fish. Martinez and Souza (2002) showed that after nitrite exposure, blood samples of *P. lineatus* presented shrunken red cells. Rios *et al.* (2002) studied the effects of long-term food deprivation on respiration and haematology of the neotropical fish *Hoplias malabaricus*. The haematocrit and the number of red blood cells decreased after 150 and 240 days of starvation, respectively. Saxena and Seth (2002) studied the toxic effects of cypermethrin on certain hematological aspects of fresh water fish *Channa punctatus* and observed a significant change in the hematology of the fish.

Atamanalp and Yanik (2003) studied alterations in hematological parameters of Rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. Significant decreases in Hb content and in MCH were observed during exposure to the pesticide. No significant differences were observed in the levels of RBC, MCHC, PCV, MCV and

WBC (P > 0.05). The RBC/WBC level was reported to increase due to the decrease in WBC. Jung. *et al.* (2003) studied effects of formalin on haematological and blood chemistry in olive flounder, *Paralichthys olivaceus* (Temminck et Schlegel). Red blood cell count, haemoglobin, haematocrit, mean corpuscular haemoglobin concentration and percentage of immature erythrocytes were markedly elevated in all formalin-exposed groups, indicating that formalin might have inhibited oxygen transfer by blood of the fish. Mgbenka *et al.* (2003) studied effect of lindane on differential white blood cell counts of *Clarias albenpunctatus.* Compared with control, total white blood cell counts in the treatment groups increased significantly ($p< 0.05$) with increasing lindane concentration. Leucocytosis and lymphocytosis were observed. Osuigwe *et al.* (2003) carried out a study to evaluate the haematological effect of using jackbean (*Canavalia ensiformis*) seed meal as an alternative protein source for *Clarias gariepinus.* Results obtained showed that the haematocrit, red blood cell count, white blood cell count and haemoglobin concentration decreased significantly with increasing dietary levels of JBSM. Poleo & Hytterod (2003) observed a reduction in PCV values recorded for Atlantic Salmon, *salmon salar* exposed to heavy metals.

Barcellos *et al*. (2004) reported a reduction in lymphocyte counts in case of *Rhamdia quelen* after acute and chronic stress caused by usual aquacultural management. Biswas *et al*. (2004) exposed Nile tilapias to different photoperiods for 3 months and observed that lymphocytes increased in shorter light periods (6L: 6D) than in 12L:12D while other variables such as neutrophils and haematocrit remained unaffected. Ezeri *et al.* (2004) studied the haematological response of cultured and wild *Clarias gariepinus* to acclimation and observed a reduction in the values of Packed Cell Volume after acclimatizing the fish for seven days. Gabriel *et al.* (2004) studied the influence of sex, source (pond and wild) acclimation and health status on some blood parameters of *C. gariepinus*. The data collected showed that sex, source of fish, and period of acclimation have some degrees of influence on the blood parameters of *C. gariepinus.* Lermen *et al.* (2004) studied effect of different water

temperatures (15, 23 and 31^0 C) on hematological and metabolic parameters in blood, liver and white muscle of silver catfish, *Rhamdia quelen*, following chronic and acute exposures. In both experiments , haematocrit and hemoglobin remained unchanged. Satyanarayan *et al.* (2004) studied impact of some chlorinated pesticides on the haematology of the fish *Cyprinus carpio* and *Puntius ticto*. The fishes were exposed to sub lethal concentrations of different chlorinated pesticides namely aldrin, dieldrin, DDT, BHC and chlordane. Irrespective of the species and pesticide, the RBC counts uniformly showed decreasing trend with the increase in exposure period, while packed cell volume, PCV (%) showed increasing trend with respect to increase in exposure period in case of aldrin and dieldrin in both the fishes. But DDT, BHC and chlordane showed decreasing trend in PCV (%) values with increasing periods of exposure. Tierney *et al*. (2004) studied the differential leucocytes landscape (monocytes, thrombocytes, lymphocytes and diverse forms of granulocytes) encountered in the blood of four teleost species, coho salmon (*Oncorhynchus kisutch*), pacific herring (*Clupea pallasi*), brook stickle back (*Clupae inconstans*) and feathered minnow (*Pimephales promelas*), and reported that relative leucocyte number responds significantly to changes in water quality. Yousafzai (2004) studied the toxicological effects of industrial effluents dumped in River Kabul on mahaseer, *Tor putitiora* at Aman Garh Industrial area, Nowshera, Pehsawar, Pakistan. The fish collected from polluted sites when compared with the control had lower hemoglobin. RBC counts, PCV and MCHC and higher WBC counts and MCV. Wahbi *et al.* (2004) studied the effect of Pulp and Paper industrial effluent on the striped seabream (*Lithognathus mormyrus*). A significant reduction in both haemoglobin content and packed cell volume were detected at concentration of 20 ml/L of effluent. Blood smears and kidney prints revealed that increasing waste concentration caused increase in small lymphocytes and neutrophils, that emphasize the compensatory and defensive reaction of fish to effluent.

Adeyemo (2005) worked on the haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus.* It was concluded that since cyanide is

a potent respiratory poison, un-detoxified or insufficiently detoxified cyanide-containing liquid wastes could easily contaminate fish and ultimately extinguish aquatic life if discharged into aquatic environments. Blanar *et al.* (2005) investigated the combined effects of toxaphene (an organochlorine pesticide) exposure and infection by the larval stage of the cestode *Diphyllobothrium dendriticum* on hematology of Arctic charr (*Salvelinus alpinus*).It was found that the parasitized charr had decreased hematocrits and increased lymphocyte: erythrocyte ratios. Kopp *et al.* (2005) studied the influence of toxic cyanobacterial water blooms on the hematological indicators of silver carp (*Hypophthalmichthys molitrix* Val.) and recorded changes in fish exposed to the cyanobacterial population in comparison with the control group. Valenzuela *et al.* (2005) studied the effect of acute hypoxia in trout (*Oncorhynchus mykiss*) on immature erythrocyte release and production of oxidative radicals. The main conclusions of this study were that the reduction in ORs production in trout began at 4.8 mgO_2/l, suggesting that acute hypoxia could severely affect disease resistance in fish. Vutkuru (2005) reported a significant decrease in RBCs, hemoglobin and packed cell volume of *Labeo rohita* exposed to heavy metals

Carvalho and Fernandes (2006) evaluated the susceptibility of the neotropical freshwater fish *Prochilodus scrofa* to copper at two temperatures with low and high water pH. On the basis of the results obtained it was suggested that the use of copper sulfate to control algae and fish parasites should take into account the sensitivity of the species to copper and mainly the water pH of aquaculture ponds. Das *et al.* (2006) studied the haematological changes in the three Indian major carps, *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhinus mrigala* (Hamilton) exposed to acidic and alkaline water pH. A change in water pH either to acidic or alkaline conditions exerted stress in fish characterized by swelling of erythrocytes, production of immature erythrocytes, and reductions in the total erythrocyte counts and haemoglobin. Rohu was found to be least affected followed by mrigal to the stress of altered water pH, while catla was the most vulnerable to pH changes. Gbore *et al*. (2006) studied the effects of stress due to handling and transportation on

haematology and plasma biochemistry in the fingerlings of two species of fish. The results indicated reduced values for PCV, Hb and RBC except for the leukocyte and Hb for *Tilapia zilli,* while the blood constants, increased for *C. gariepinus*. The changes in the Hb, leukocyte and MCHC were more significant ($p<0.05$) for fingerlings of *T. zilli* compared to those of *C. gariepinus*. It was concluded that fingerlings generally are susceptible to stress but those of *T. zilli* are more susceptible to physical stresses than those of *C. garipinus*. Giron-Perez *et al*. (2006) noticed a non-significant alteration in TEC, Hb content and Hct in Nile Tilapia (*Oreochromis niloticus*) acutely exposed to 0.422, 0.845, 1.69 and 3.38 ppm of chlorpyrifos. Joseph (2006) studied alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to metasystox and sevin and reported that, WBCs, MCH and MCHC, increased whereas ESR, Hb%, RBCs and PCV decreased in both cases. Patnaik and Patra (2006) studied the haemoatopoietic alterations induced by carbarylin in *Clarias batrachus* (LINN) and recorded the reduction in the number of red blood cells, erythrocytes, packed cell volume, mean corpuscular haemoglobin and mean corpuscular volume thereby indicating anaemia in the fish. Ribeiro *et al.* (2006) studied hematological characteristics in neotropical fish *Hoplias malabaricus* exposed to subchronic and dietary doses of methylmercury, inorganic lead, and tributyltin chloride and reported that changes in hematological and blood indices could highlight some barely detectable metal effects in fish after laboratory exposure to contaminated food. Sweilum (2006) reported that Nile Tilapia (*Oreochromis niloticus*) suffered from erythropenia, marked decline in Hb and Hct values after long-term post exposure to dimethoate and malathion. Trumble *et al*. (2006) studied dietary and seasonal influences on blood chemistry and hematology in seals.

Adeyemo (2007) studied the haematological profile of *Clarias gariepinus* exposed to lead and observed that the packed cell volume and erythrocytes of the treatments decreased significantly while their platelet counts increased. The mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were also reported to increase considerably in all

treatments. Gabriel *et al*. (2007a) studied the haematological characteristics of *Sarotherodon melanontheron* from the brackish water creek of Buguma. The highest range of parameters was recorded in thrombocytes, while the lowest was observed in RBC. Significant differences ($p< 0.05$) between males and females were observed in hemoglobin, haematocrit, red blood cells and thrombocytes. Also, Gabriel *et al.* (2007b) studied the haematology and gill pathology of *Clarias gariepinus* exposed to refined petroleum oil under laboratory conditions.Garcia *et al* (2007) observed that the numbers of total leukocytes, lymphocytes and eosinophils decreased, while the numbers of neutrophils and monocytes increased *in Piaractus Mesopotamicus* when fed with diets supplemented with vitamin C and E, challenged by *Aeromonas hydrophila.* Kapila *et al.* (2007) studied the impact of water pH on haematology and serum enzyme activities in *Schizothorax richardsonii* (Gray) and concluded that the fish thrived well in pH range of 6.0-9.0 but did not tolerate high acidic and alkaline conditions. Parma (2007) studied changes of hematological parameters in *Prochilodus lineatus* exposed to sublethal concentration of Cypermethrin. With the increase of exposure time, total erythrocyte (RBC), hemoglobin (Hb), hematocrit (Ht) and mean corpuscular haemoglobin concentration (MCHC) values were reported to decrease but mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values increased. Puigdoller *et al*. (2007) reported significant increase in hematocrit in Atlantic salmon when exposed to ATR. Sahan *et al*. (2007) carried out a study in agricultural, industrial, domestic, and slaughter house discharging region of Ceyhan river and found that leukocyte values and neutrophil proportion in fish blood were found increased by means of environmental stressors ($p<0.05$).

Bananee *et al*. (2008) reported a decrease in the number of RBC, hemoglobin and hematocrit values of diazinon exposed common carp. Maheswaran *et al.* (2008a) carried out haematological studies of *Clarias batrachus* (L.) exposed to mercuric chloride. Results of their investigation showed that mercuric chloride caused immunological impairment in *C. batrachus*. Maheswaran *et al*. (2008b) observed an increase in hematocrit levels in different fish species after zinc treatments. Mazur *et*

al. (2008) studied the heavy metal content and response of the blood system of Siberian Dace *Leuciscus leuciscus baicalensis* (Cypriniformes, Cyprinidae) under anthropogenic impact. It was established that the most considerable changes in hemopoesis, cellular and humoral links of immunity were in individuals with a high content of heavy metals in the liver from populations inhabiting the Selenga River downstream the Ulan Ude Industrial Center and the Selenga Pulp-and-Cardboard Plant. Mikula *et al*. (2008) observed decreased values of RBC and Hb in common carp exposed to 2.40 mg·l-1 of alachlor in a chloroacetanilide herbicide Lasso MTX. Ramesh and Saravanan (2008) studied the acute toxicity of an insecticide chlorpyrifos on haematological parameters of *Cyprinus carpio* under static conditions and found that the haematological parameters like RBC, haemoglobin level decreased in the insecticide treated fish, whereas WBC level increased. Valenzuela *et al*. (2008) studied the combining effects of different artificial photoperiods and temperatures on the haematological parameters in rainbow trout and observed that the fishes when exposed to a photoperiod of 24L:0D for a period of 14 days demonstrated an increase of haematocrit, number of erythrocytes but lower number of lymphocytes.

Velisek *et al.* (2008) studied the effects of metribuzin on rainbow trout (*Oncorhynchus mykiss*). The experimental group showed significantly lower values of erythrocyte count, haematocrit and significantly higher values of haemoglobin. A significant decrease in both the relative and absolute lymphocyte count and a significant increase in both the relative and absolute count of neutrophile granulocytes was recorded in the fish.

Adedeji *et al.* (2009) studied the acute effects of diazinon on blood parameters in *Clarias Gariepinus*. They reported significantly lower values of erythrocyte count, haemoglobin content and haematocrit and also a significant decrease in leucocyte count as well as in both the relative and absolute lymphocyte count. Adeyeno *et al.* (2009) conducted a study for the induction of acute handling and transport stress that could reproducibly affect haematological changes in African catfish, (*Clarias*

gariepinus. Bruchell, 1822) and found no significant differences ($p< 0.05$) in the haematocrit, white blood cell, hemoglobin and eosinophil of the stressed fish relative to the baseline values. However, significant differences ($p<0.05$) were observed in the values of the neutrophil and lymphocyte of the stressed fish relative to the baseline data. Afaq and Rana (2009) conducted an experiment on toxicological effects of leather dyes on total leukocyte count of *Cirrhinus mrigala* and reported a significant increase in the TLC due to Bismarck brown and acid leather brown. Intoxication of Bismarck brown and acid leather brown induced leukocytosis. Ali and Rani (2009) carried out a study to assess the effect of phosalone on *Oreochromis mossambicus.* It was determined that due to phosalone exposure the total RBCs, WBCs, haemoglobin content, and haematocrit value significantly decreased. The percentages of erythrocyte sedimentation rate, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration showed an increasing trend with respect to the increase in exposure period. Gabriel *et al.* (2009) studied the haematology of *Clarias gariepinus* after intramuscular injection with aqueous leaf extracts of *Lepidagathis alopecuroides.* They concluded that exposure of *C. gariepinus* to *L. alopercuroides* in the open waters may impact negatively on the physiology of the fish as manifested in changes in some of the blood parameters of the fish. Jamalzadeh *et al.* (2009) carried out a study comparing the blood indices in healthy and fungal infected Caspian salmon (*Salmo trutta caspius*) and observed that the white blood cells, neutrophile, and eosinophile recorded higher values ($P < 0.05$) in fungal infected fishes than healthy Caspian salmon. Malla *et al.* (2009) reported a significant increase in ESR (mm/hr) when compared to control group following both acute and sub acute exposure of *Channa punctatus* to chlorpyrifos. Olufayo (2009) studied the haematological characteristics of *Clarias gariepinus* juveniles exposed to *derris elliptica* root powder. His study revealed high mortality rate and deleterious consequences on the health of fish subjected to acute exposure of *Derris.*

Ramesh *et al.* (2009) studied the effect of atrazine (Herbicide) on blood parameters of *Cyprinus carpio* and reported that red blood cells count, hemoglobin

were decreased on exposure of the fish to atrazine, whereas white blood cells count was enhanced. Som *et al.* (2009) conducted an experiment to evaluate hemopoietic responses in *Labeo rohita* following acute copper toxicity. After exposure, percentage of blast cells was found to be increased, whereas percentage of mature erythrocytes decreased. Erythropoietic and leucopoietic efficiencies increased markedly in sublethal-treated fish.

Velisek *et al.* (2009) studied the effects of acute exposure of rainbow trout to bifenthrin on some of its haematological, biochemical and histopathological parameters. Haematologically, fish showed a significant ($P < 0.01$) decrease in mean erythrocyte volume, erythrocyte haemoglobin, and band neutrophil granulocytes compared to controls. Vinodhini and Narayanan (2009) studied the effect of heavy metal pollutants such as, cadmium, chromium, nickel and lead, on *Cyprinus carpio* L. by using a set of biochemical parameters. Concentration of red blood cells was found to be significantly elevated due to the effect of heavy metal pollutants. The study suggested that the presence of toxic heavy metals in aquatic environment had strong influence on the hematological parameters in the fish. Zaki *et al.* (2009) studied the clinicopathological, biochemical and microbiological change on Grey Mullet exposed to cadmium chloride.

Adewoye (2010) studied the haematological and biochemical changes in *Clarias gariepinus* exposed to *Trephosia vogelii* extract and reported an increase in the level of white blood cell (WBC) in treated fish. Akinrotimi *et al.* (2010) studied the effects of acclimation on haematological characters of *Tilapia guineensis* (Bleeker, 1862) and reported significant reduction in the values of haemoglobin; packed cell volume; red blood cell; mean Corpuscular Haemoglobin Concentration and platelets. Gaafar *et al.* (2010) carried out a study on the pathologic and clinicopathologic conditions due to chronic exposure of *Oreochromis niloticus* to the organophosphate fungicide edifenphos. Exposure to the fungicide was reported to lead to adverse effect on some of haematological parameters such as RBCs count, Hb content and blood indices. Kandemir *et al.* (2010) related the high mortality in

Cyprinus carpio, *Leuciscus cephalus*, *Capoeta trutta* ve *Capoeta capoeta umbla* fish to high Copper concentration, organic pollution (Amonium, Suphate) and also low oxygen concentration in Sultansuyu Dam Lake. Kayode and Shamusideen (2010) carried out haematological studies of *Oreochromis niloticus* exposed to diesel and drilling fluid in Lagos, Nigeria. Diesel and drilling fluid evoked significant changes in the haematological parameters of the fish. Modesto and Martinez (2010) evaluated the effects of herbicide roundup transorb (RDT) on the neotropical fish *Prochilodus lineatus* and suggested that the formulation of roundup transorb promotes alterations in hematologic and biochemical parameters of the fish. Ololade and Oginni (2010) studied the toxic stress and hematological effects of nickel on *Clarias gariepinus* fingerlings. All the blood parameters (erythrocyte, leucocytes, hematocrit and haemoglobin count) were reported to decrease with increasing concentration of toxicant.

Safahieh *et al.* (2010) opined that the subacute mercury concentrations cause several changes in the haematological and immunological parameters of *Acanthopagrus latus.* Sivakumar *et al.* (2010) studied the effect of butachlor EC-50 on haematological parameters on *O. mossambicus* and observed that most of the parameters of treated fish showed significant difference when compared to control. Srivastava and Choudhary (2010) studied the influence of artificial photoperiod on the blood cell indices of *C. batrachus* (Linn.) and concluded that exposure to continuous light elicits stress responses in the leukocyte profile of this nocturnal fish. Zarejabad *et al*. (2010) studied the effect of environmental temperature changes on haematological and biochemical parameters of *Hoso hoso* juveniles. The results showed that hematocrit and eosinophil were affected by different temperature. Increasing temperature led to significant increase in haematocrit and eosonophil, but WBC and lymphocytes decreased slightly ($p<0.05$). Zutshi *et al*. (2010) studied alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India and concluded that stress due to various pollutants present in the lakes does create hematological disturbances, erythrocyte destruction

(hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases.

Ahmed (2011) studied acute toxicity and haematological changes in common carp (*Cyprinus carpio)* caused by diazinon exposure. He concluded that the diazinon is moderately toxic to *C. carpio.* Alimohammadi *et al.* (2011) studied the chronic effects of different temperatures in the blood parameters of common carp. They observed that temperature and time of exposure influence blood parameters of *Cyprinus carpio*. Atamanalp *et al.* (2011) studied the alterations in the hematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to cobalt chloride. They reported increase in red blood cells, white blood cells, thrombocyte count, hemoglobin, erythrocyte-sedimentation rate and mean corpuscular hemoglobin concentration. Hematocrit, mean corpuscular volume and mean corpuscular hemoglobin values were found to decrease. Bananee *et al*. (2011) observed a decrease in the number of RBC, hemoglobin and hematocrit values of diazinon exposed rainbow trout (*Onchorhynchus mykiss)*. Dobsikova *et al.* (2011) studied the effect of acute exposure to herbicide Gardoprim Plus Gold 500 SC on haematological and biochemical indicators and histopathological changes in common carp (*Cyprinus carpio* L.). Innocent *et al.* (2011) studied the haematology of *Cirrhinus mrigala* fed with Vitamin C supplemented diet and reported that supplementation of feed with immunostimulant (Vitamin C) improved the total leucocyte counts and granulocyte population, which nonspecifically helped to minimize infection induced stress, improved resistance against infection and faster recovery from stress. Kreutz *et al.* (2011) studied altered hematological and immunological parameters in silver catfish (*Rhamdia quelen*) following short term exposure to sublethal concentration of glyphosate. Silver catfish fingerlings exposed to glyphosate had a significant reduction on blood erythrocytes, thrombocytes, lymphocytes and total leukocytes in contrast to a significant increase in the number of immature circulating cells.

Pamplona *et al.* (2011) evaluated the potential toxic effects of dipyrone on the aquatic environment, using *Rhamdia quelen*. They reported that the constant

dipyrone release to the environment have toxic effects to aquatic organisms. Pereira *et al.* (2011) studied the effects of exposition to polluted environments on blood cells of the fish *Prochilodus lineatus* and reported an increase in the number of leukocytes and white blood cells in the fish. Siakpere and Ikomi (2011) studied the haematological alterations produced on exposure of *Parachanna africans* to the sublethal concentration of cadmium (Cd^{2+}). They found that red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular haemoglobin and mean corpuscular volume levels decreased, but the level of the mean corpuscular haemoglobin concentration increased with an increase in exposure concentration of cadmium. Siakpere and Oboh (2011) concluded that sublethal concentration of tobacco leaf dust have deleterious effects on the haematological parameters of *C. gariepinus* and cautioned that the use of this toxicant in fish ponds needs proper control to avoid reduction in fish production and aquatic fauna.

Ada *et al.* (2012) studied the haematological, biological and behavioural changes in *Oreochromis niloticus* juveniles exposed to Paraquat herbicide. Haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration and erythrocyte sedimentation rate were observed to be negatively related to concentration of Paraquat, whereas packed cell volume, white blood cell count, red blood cell count showed positive relationship. Akinrotimi *et al.* (2012) studied the haematotoxicity of Cypermethrin to *C. gariepinus* and suggested that the exposure to cypermethrin could cause some level of stress in the fish. Al-Ghanim (2012) carried out a study to investigate the toxic effects of malathion in *Oreochromis niloticus* and found that erythrocyte count, haematocrit value and haemoglobin content of Nile tilapia decreased with increased pesticide concentrations, while total production, net returns and profitability of reared fish decreased with increase in concentrations of pesticides. Ergonul *et al.* (2012) found that lead at sub-lethal concentrations had marked effects on red blood cell system, plasma ion concentrations and plasma glucose and lactate levels in *C. carpio*.

STUDIES IN KASHMIR

So far very few reports are available on the fish toxicity in general and fish haematotoxicity in particular. Qadri (2004) studied the impact of aquatic pollution on the haematology of *S. Heckel*. Shafiq-ur-Rehman (2006) studied the effects of endosulfan on hematology of mirror carp, *Cyprinus carpio specularis*. It has been observed that almost all the tested hematological features were found to be significantly declined at 120 hr, with maximum destructions at 240 hr exposure period. Tramboo (2010) carried out a study on antioxidants of fish with special reference to their use as biomarkers of aquatic pollutants. Mir (2011) studied influences of organophosphate pesticides on reproductive activities of female common carp, *Cyprinus carpio Communis.*

From the above review it is quite apparent that although a voluminous literature is available on the impact of biocide residue on the fish haematology worldwide, negligible work has been done on the effect of biocides on the fish occupying different habitats of Kashmir Himalaya. Therefore, the present study was undertaken to fill the gap.

CHAPTER – 3

Methodology

Fish in aquarium for acclimatization

Collection of fish: *Schizothorax niger* Heckel used for the study were collected from Dal lake, Kashmir with the help of a local fisherman who used cast net. Only apparently healthy specimens without any injuries were selected and brought immediately to the laboratory where they were transferred to aquaria for acclimatization. Weight and length of the fish used for the present study ranged from 55-90grams and 17.7-21.7cm respectively. *S. niger* has an elongated and fusiform body; the upper jaw is short, blunt and slightly prognathous and not expanded into wide folds; a series of enlarged scales are present along the anal fin base of the fish. *S. niger* is the only species of Schizothorax in Kashmir valley with almost exclusively 6 dorsal fin rays, the others usually having 6-8. It is endemic to the valley of Kashmir and is the only typical lacustrine schizothoracine species.

Haematology of the fish in field conditions:

The blood samples were taken from live specimens on the spot (Dal lake) and haematological analysis was done within two hours of the blood sample collection.

Pre experimental management:

Aquaria were cleaned and disinfected and filled with 60L of dechlorinated water. Aerators were used to keep the water fully aerated. Water was changed after every forty eight hours. The fish were placed in aquaria for a period of 7 days to get acclimatized to the laboratory conditions, and were normally fed once a day with commercial fish feed of the company Aquasstar. Provision of food was stopped 48 h prior to the start of toxicity experiments.

The toxicity tests were conducted in accordance with the guidelines described for maintenance, care and conducting toxicity tests of fish in Standard Methods for the Examination of Water and Wastewater, American Public Health Association (APHA, 1998) and OECD guidelines on testing for chemicals (OECD 203 “Fish, acute toxicity test” 1992).

Test chemical

Butachlor is a member of the chloroacetanilide class of chemicals. Its chemical name is 2-chloro-2, 6-diethyl-N-(butoxymethyl) acetanilide, with molecular formula

as $C_{17}H_{26}NO_2Cl$ and molecular weight equal to 311.9. Its melting point is 4-5^{0}C and at room temperature it behaves as a clear amber liquid. It is sparingly soluble in water (Water solubility: 20 ppm at 25^{0}C) but is readily miscible with alcohol, ether, acetone and other organic solvents.

Preparation of test solution:

Technical grade (94.8%pure) butachlor, obtained from Fungicides India Limited, Jammu, was used for the experiments. Since butachlor is sparingly soluble in water, therefore its stock solution was prepared in acetone. Necessary dilutions were made from the stock solution. Half of the water in the aquaria was replaced once (at 48 h intervals) during the experimental duration and butachlor solution was added to water to keep the required concentration constant.

Estimation of LC50:

Three types of procedures can be used to determine the LC50 value of any toxicant

- Static test (no flow of test solution)
- Semi static test (batch wise renewal of test solution)
- Flow through test (water is constantly renewed)

For butachlor, the semi static test procedure was preferably adopted because of its being fairly stable compound. Initially few random doses ranging from 0.1mg/l (1/3 LC50 value of butachlor for common carp, Tramboo 2010) to 1.7 mg/l, were selected and mortality response if any, of the fish was recorded against these concentrations. Accordingly seven different concentrations (1.3, 1.5, 1.7, 1.9, 2.1, 2.3 and 2.5mg/l) of butachlor were chosen for determining the LC50 value. All these concentrations were added to 100L aquaria filled with 60L of dechlorinated and continually aerated water. Control group was also maintained during the experiment. The fish were placed in aquaria with a density of six fish per aquarium. The fish were exposed for 96 hours, dead fish were counted at 12 h intervals and removed from aquaria. A Fish was considered dead when it showed no visible movement (e.g., gill movements) and touching its caudal peduncle did not produce any reaction (OECD,

1992). Fish status and behaviour along with water temperature, pH and oxygen saturation were monitored throughout the test. The water was continuously aerated and hence well oxygenated.

Stats Direct statistical software (version 2.5.6) was used for making all calculations.

Experimental design:

Experiments employing different doses of sublethal concentrations of butachlor were carried out in 100L glass aquaria filled with 60 litres of dechlorinated water. Three sublethal concentrations of butachlor employed for the experiment are depicted in Table 1. Since, stock solution of butachlor was prepared in acetone therefore, all the three treatment groups were run with their respective positive and negative controls. The difference in weight and length of the fish between the first two treatment groups (Fish exposed to 0.45mg/l and 0.9mg/l) was insignificant but the weight and length of the fish in the last treatment group (Fish exposed to 1.35mg/l) was significantly more ($p<0.001$) when compared with that of the first two treatment groups. The experiment was carried out in replicates.

Table 3.1: Sublethal concentrations of butachlor employed in the experiment

Name of the group	Concentration employed	Dose administered	Number of fish exposed to each dose	Body weight(g) of the fish in treatment group	Body weight(g) of the fish in control group
Group 1	25% of LC50 value	0.45mg/l	6	64.1±5.5	63.9±6.1
Group 2	50% of LC50 value	0.9mg/l	6	66.2±5.3	64.1±3.9
Group 3	75% of LC50 value	1.35mg/l	6	86.0±3.6	84.1±4.2

Duration of tests:

The fish were exposed to different concentrations of butachlor for 96 hours. Important physico-chemical characteristics of the aquaria water (pH, oxygen content, temperature and conductivity) were determined as per standard methods to make it sure that the water in aquaria was fit for fish survival. Almost same conditions as in acute toxicity test were maintained during the experiment. At the end of exposure time, the blood was collected from the test fish and studied for different haematological parameters viz., haemoglobin, total erythrocyte count, packed cell volume, total leukocyte count, MCV, MCH, MCHC and differential leucocyte count.

Collection of blood samples:

The fish were bled alive. The length & weight of the fish were recorded after the collection of the blood. Blood was drawn out with a syringe from the heart by stabbing body wall exactly in midline from the posterior margin of opercular cover & directed dorso-caudally at an angle of 45^0 (Lucky, 1977). Care was taken to prevent the blood from coming in contact with water. Part of the blood sample was used directly to make smears on clean and dry slides for staining. For determining other

haematological parameters, samples were collected in glass vials containing EDTA as anticoagulant at an approximate concentration of 5mg/ml of blood (Blaxhall & Daisley, 1973).

HAEMATOLOGICAL TECHNIQUES

3.1 Estimation of Hb concentration:

The haemoglobin was estimated by cyanomethaemoglobin method recommended by International Committee for Standardization in Haematology (ICSH 1996 and 1997). In this method, the alkaline solution of ferricyanide converts haemoglobin ferrous (Fe^{2+}) iron to the ferric (Fe^{3+}) state to form Cyanomethaemoglobin. The colour developed was measured spectrophotometrically at 540 nm with the help of Systronics 106 Spectrophotometer.

Drabkins solution used for the quantitative, spectrophotometric determination of haemoglobin concentration in whole blood at 540 nm, was prepared by mixing the following reagents in the proportion :

Sodium bicarbonate	1.0 gm
Potassium cyanide	0.05gm
Potassium ferricyanide	0.2gm
Distilled water	1000cc

Drabkin's Solution reacts with all forms of haemoglobin except sulfhemoglobin, a pigment that normally occurs in only minute concentrations in blood. The broad absorption peak of cyanmethemoglobin permits its measurement using both wide and narrow bandwidth instruments (530-550 nm).

Calculation:

$$\text{Hb (g/100ml)} = \frac{A_{540}\ \text{test sample}\ \text{x } 15.06\ (\text{Std .Conc .as stamped on the vial x}0.25)}{A_{540}\ \text{standard}}$$

3.2 Total erythrocyte count:

Hayems diluting fluid, which had the following composition, was used for RBC count:

Mercuric chloride : 0.5gm

Sodium chloride : 1.0gm

Sodium sulphate : 5.0 gm

Distilled water : 200ml

An improved Neubauer's counting chamber was used for counting RBC (Baker and Silverton, 1982). Using RBC pipette, the blood was drawn upto 0.5 mark and the diluting fluid to the mark 101. Although fluid is drawn to the mark 101 but the real dilution is 0.5:100 or 1:200 because the fluid in the capillary tube is discarded before the count.

3.3 Total leucocyte count:

A white cell count (TLC) estimates the total number of white cells in a cubic millimetre of blood. WBC diluting fluid or Turk fluid contains a weak acid to lyse the red blood cells and Gentian violet stain for staining the nucleus of white blood cells.

The Turks fluid with following composition was used for TLC:

Glacial acetic acid :1.5ml

1% aqueous soln of Gentian violet :1 ml

Distilled water :100ml

Neubauer's hemocytometer (Baker and Silverton, 1982) was used for counting of leucocytes. Using white cell pipette, the blood was drawn upto 0.5 mark and the diluting fluid to 11 mark, thus the dilution was 1:20.

3.4 Haematocrit or Packed cell volume:

This was obtained by centrifuging blood (containing 5mg/ml EDTA) in a graduated tube until corpuscles were packed down to a constant volume. The volume of packed cell was then expressed as a %age of the original volume of blood. With the aid of capillary pipette a Wintrobes haematocrit tube was filled to the 100 mark with the anticoagulated blood and centrifuged for 5-10 min at ~ 700RPM. As the original column of blood in the tube is 100mm long, the volume of packed cell is

read directly as %age. The analysis was done according to England and Walford (1972).

3.5 Erythrocyte Indices

Wintrobe (1974) introduced calculation for determining the size, content and Haemoglobin concentration of red cell. These erythrocyte indices were found very useful in the morphological characterization of anaemia.

3.5.1 Mean cell volume (MCV):

The MCV is the average volume of red cells and was calculated from the haematocrit (Hct ,packed cell volume) and red cell count (TRBC).

$$\text{MCV} = \frac{\text{PCV (\%)X 10 cubic microns}}{\text{RBC [millions /⍰l]}}$$

3.5.2 Mean cell Haemoglobin (MCH):

The MCH is the content (Weight) of the Hb of the average red cell. It was calculated from the Hb concentration and red cell count.

$$\text{MCH} = \frac{\text{Hb (g/dl) x 10 micro grams}}{\text{RBC (millions /⍰l)}}$$

3.5.3 Mean cell Haemoglobin concentration (MCHC):

The Mean cell haemoglobin concentration in g% for 100 ml erythrocytes was calculated by following formula:

$$\text{MCHC} = \frac{\text{Hb (g/dl) x100 ml}}{\text{PCV (\%)}}$$

3.6 Differential leucocyte count:

A thin blood film was made by spreading a blood drop evenly on clean grease free slide using smooth edged spreader. Giemsa's and Leishman's stains were employed for the staining of blood films. Using the 40X objective high-power lens, 100 leucocytes were counted in the blood smear. The percentage of each of the five basic leucocytes (Neutrophils, Eosinophils, Basophils, Lymphocytes, Monocytes) was calculated and DLC reported in percentage.

Staining of Blood Films

Leishman's stain:

Blood smears were stained with Leishmans stain for 2 minutes (undiluted stain) and 5-15 minutes (diluted stain) as the former acts faster than the latter.

Composition of Leishman`s Stain

Leishman powder	1.5gms
Methyl alcohol	1 litre

Leishman's stain is used for staining blood smears. It provides excellent stain quality and is generally used to differentiate and identify leucocytes. Cytoplasmic details and granules are better stained by Leishman's stain.

Giemsa stain:

The stain introduced by Geimsa is a modification of a stain made by Ramanowsky who mixed methylene blue and red stain eosin, so that three colours red, purple and blue were present on the stained slide. Blood films were stained for one hour with Geimsa stain (10% stain in phosphate buffered water, pH 7.2) having the following composition:

Geimsa powder	0.3gms
Glycerine	25.0ml
Acetone free methyl alcohol	25.0 ml

Microscopy and Photomicrography:

Light microscopy was conducted under Olympus microscope with lense combination of 10x eye piece and 40x and 100x objectives. Images were captured by digital camera (Leica DMLS2). Also, photographs were taken under compound microscope Leica with the help of a digital camera.

Statistical Analysis:

A computer program (SPSS 12.0 for windows) was used for data analysis. The descriptive data was given as a mean ± standard deviation (SD).

CHAPTER – 4

Results And Discussion

The aim of the present study was to see the impact of butachlor, a herbicide most extensively used in Kashmir valley on various haematological parameters of *Schizothorax niger* Heckel.

The present chapter is comprised of three parts:

4.1: Haematology of *Schizothorax niger* Heckel under field conditions.

4.2: LC50 of butachlor for *Schizothorax niger* Heckel.

4.3: Impact of butachlor on haematological parameters of the fish.

4.1: Haematology of the fish under field conditions

Blood of *Schizothorax niger* being similar to any other vertebrate, comprises of plasma and cellular components. Plasma consists of 97% water, dissolved salts, electrolytes and hormones. The cellular components include erythrocytes and leucocytes. It is these formed elements whose variations from normal values forms the basis of diagnosis for health status of human beings and in the same way, the fish too. The cellular components of the fish are shown in (Figs. 4.1 and 4.2). To appreciate butachlor induced alterations in haematological parameters of *Schizothorax niger* Heckel, normal range values for all these parameters were initially measured in the fish. The haematological profile of the fish in field conditions is presented in Table 4.1.

The specimens weighing 55-90 grams showed the Hb to be varying in the range of 8.5-11.6g/dl, while the RBC varied in the range of 1.5 million-1.94 million cells/cumm. The PCV values varied in the range of 28.3-38.6% and WBC values were obtained in the range of 23.3thousand-30.8thousand cells/cumm. MCV values varied in the range of 155.6-226 μm^3, MCH in the range of 43.8-75µg and MCHC varying in the range of 25-39.9%. Coming to differential leucocyte count, basophil varied in the range of 2-9%, eosinophils in the range of 1-3% and neutrophils varied in the range of 15-22% while lymphocytes and monocytes varying in the range of 61-69% and 6-11% respectively.

The cellular components of *Schizothorax niger* Heckel

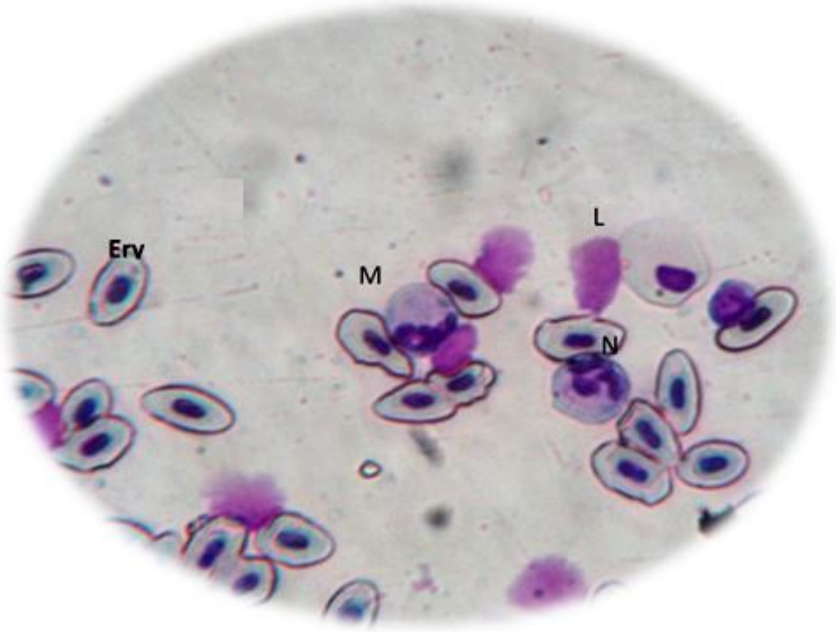


Fig. 4.1: Ery (Erythrocyte); M (monocyte); N (Neutrophil); L (Lymphocyte)

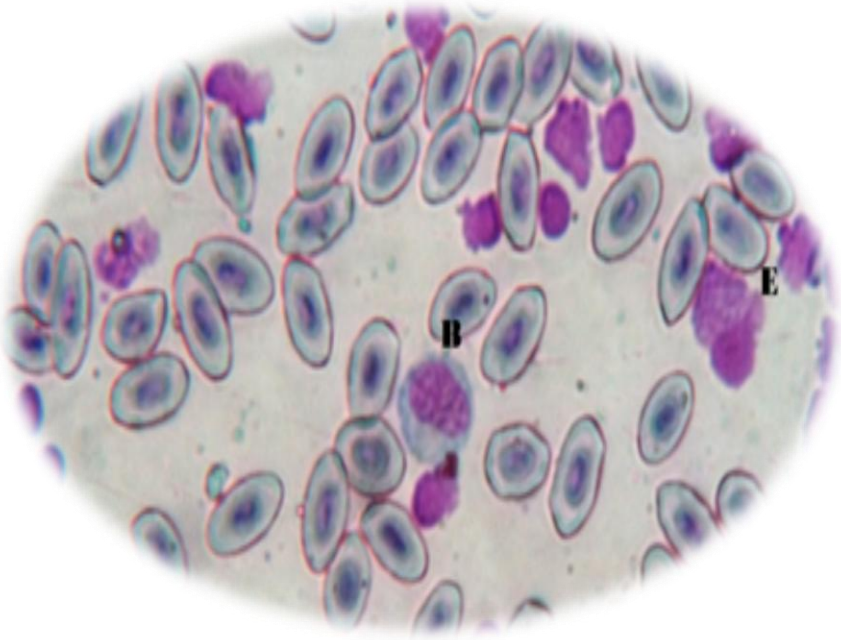


Fig. 4.2: B (Basophil); E (Eosinophil)

Table 4.1: Haematological profile of *Schizothorax niger* Heckel under field conditions.

	Weight (g)	Total length	Hb (g/dl)	RBC (10^6/cumm)	PCV (%)	WBC (10^3/cumm)	MCV (μm^3)	MCH (µg)	MCHC (%)	BAS (%)	EOS (%)	NEU (%)	LYM (%)	MON (%)
	55	18	10.5	1.8	30.7	30.8	170.5	58.3	34.2	2	2	21	65	10
	59	19	8.9	1.87	29.1	29	155.6	47.6	30.6	5	1	17	62	11
	61	18.4	9.8	1.5	28.8	29.7	192	65.3	34	5	1	15	69	10
	63	18.4	8.5	1.94	28.3	23.3	166.5	43.8	30	5	3	21	61	10
	64.4	19.3	8.7	1.7	34.2	27	180	51.2	25.4	7	3	20	65	9
	66	18.5	8.8	1.6	29.6	30.5	185.3	55	29.7	9	3	18	64	6
	67.1	20.5	10.6	1.59	33.4	30.2	210.1	66.7	31.7	4	3	15	68	8
	70	21.2	11.4	1.8	38.6	30	214.4	63.3	29.5	4	2	22	66	6
	73.7	19.4	11.2	1.5	33.9	28.5	226	75	33	7	3	19	65	9
	84	20.3	11	1.9	30.5	26.1	160.5	57.9	36.1	4	1	19	68	7
	86.8	20.8	9.4	1.69	37.6	23.8	222.5	55.6	25	7	1	21	69	7
	90	21.4	11.6	1.7	29.1	27.5	171.2	68.2	39.9	3	1	19	66	11
Range	**55-90**	**18-21.4**	**8.5-11.6**	**1.5-1.94**	**28.3-38.6**	**23.3-30.8**	**155.6-226**	**43.8-75**	**25-39.9**	**2-9**	**1-3**	**15-22**	**61-69**	**6-11**

4.2: General behavioural abnormalities due to butachlor exposure:

Abnormalities in behaviour due to butachlor exposure were observed in the fish which were followed by mortality, if at all it occurred. As the fish came in contact with butachlor, restlessness was observed which was supplemented by sudden rapid movement in circles. The fish tried hard to swim and swam in half circles. Secondly, the fish secreted an increased amount of mucus to coat the body, especially gills, to get relief from the irritating effects of the toxicant. Sometimes the fish showed erratic movements and lost buoyancy with occasional jerking of their bodies. The extent of behavioural abnormality appeared to be directly proportional to the butachlor concentration.

LC50: The semi static test procedure was preferably adopted to determine LC50 of butachlor for *Schizothorax niger*. Seven different concentrations (1.3, 1.5, 1.7, 1.9, 2.1, 2.3 and 2.5mg/l) of butachlor were added to 100L aquaria filled with 60L of dechlorinated and continually aerated water. Six fish were placed in each aquarium. Control group was also maintained during the experiment. The fish were exposed for an experimental duration of 96h, dead fish were counted at 12h intervals and removed from aquaria. During the test water temperature was about 16^0C and pH remained close to 8.0 units. The results obtained regarding the acute toxicity (mortality) test of butachlor for the fish are presented in Table 4.2. The dosage-mortality studies were conducted for 96h period. 16 % mortality was recorded upto 1.5mg/l, 33% in 1.7mg/l, 66% mortality was recorded in both 1.9mg/l and 2.1 mg/l, 83% in 2.3mg/l and 100% mortality was recorded in 2.5mg/l. The data clearly showed the relationship between the concentration of the herbicide and the percentage mortality. Fish exposed to higher concentrations underwent rapid death. The LC50 value of butachlor for *S. niger* Heckel in 96 h time are given in Fig. 4.3. The 96 h LC50 is the basic value in the acute toxicity test and it was 1.8 mg/L for butachlor against the test fish. Calculation of LC50 was based on the cumulative mortality observed at the end of 96 h exposure and was calculated by plotting a graph of percent mortality (probit value) against butachlor

concentrations in accordance with Clesceri et al (1998). StatsDirect statistical software (version 2.5.6) was used for making all calculations.

Table 4.2: Mortality of *S. niger* Heckel as a function of butachlor concentration for an exposure time of 96 hours.

concentration (mg/l) of butachlor	**number of specimens exposed**	**number of specimens dead**
control	6	0
1.3	6	0
1.5	6	1
1.7	6	2
1.9	6	4
2.1	6	4
2.3	6	5
2.5	6	6

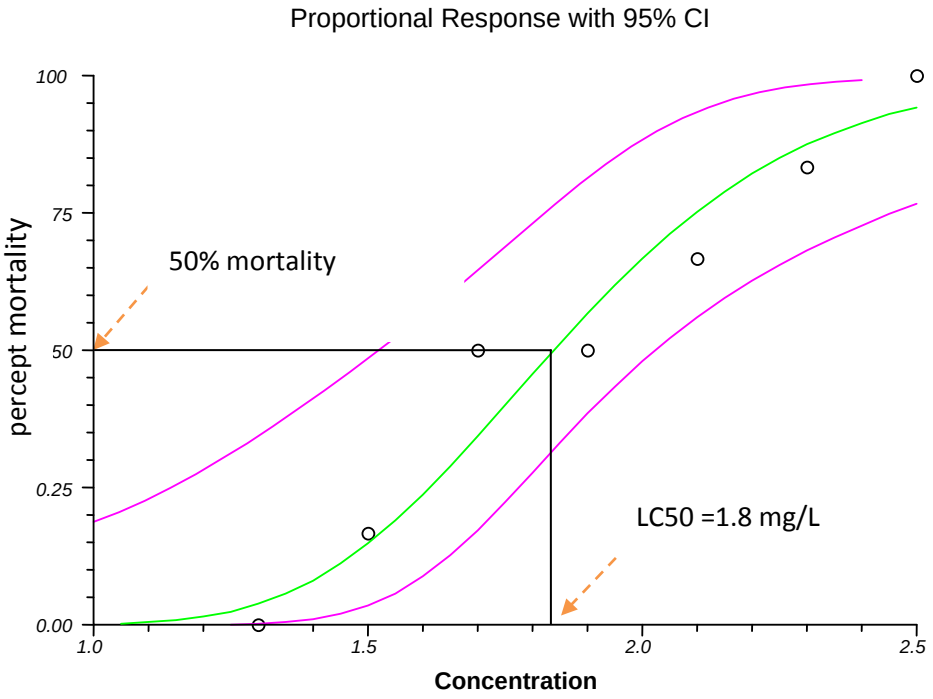


Fig 4.3: Graphic representation of butachlor concentrations verses percent mortality for determining the LC50 of butachlor in *S. niger*

Median sublethal concentration is the statistically derived dose of any chemical that is expected to cause death in 50% of the population of organisms under a defined set of experimental conditions after 96 hours of exposure period. Many authors have worked on acute toxicity of butachlor. Farah et al (2004) reported LC50 values of 2.34 ppm, 3.25 ppm and 2.82 ppm in *Heteropneustes fossilis*, *Clarias batrachus* and *Channa punctatus* respectively. However, the LC50 value (1.8mg/l) recorded in the present study was higher than the values reported (0.52mg/l) for rainbow trout, (0.44mg/l) bluegill sunfish, (0.32mg/l) carp and (0.14mg/l) channel catfish (Tomlin 1994) and (0.33mg/l) common carp (Tramboo 2010), which can possibly be better explained by the fact that the more distant relationship between the two species, the more different is their response to chemical toxicity (LeBlanc, 1984). Further, there is ample data available to prove that a species can only represent itself consistently and not a group. This can be related to their differences in susceptibility and tolerance related to accumulation,

biotransformation and excretion of a toxicant. According to Johnson and Toledo, 1993, differences in metabolic pathways among species may result in varied patterns of bio-transformation, leading to more or less toxic metabolites. The magnitude of toxic effects of pesticides also depends on length and weight, corporal surface/body weight ratio and breathing rate (Singh and Narain, 1982; Murty, 1986).

4.3 Impact of butachlor on haematological parameters of *Schizothorax niger* Heckel.

Many workers have assessed the effect of various pesticides on the haematological responses of various species of fish and have found varying responses after exposing the fish to varying sublethal concentrations using the 96 hour toxicity tests. The purpose of present study was to assess the heamatological changes in *Schizothorax niger*, an endemic fish of Kashmir valley following the exposure of the fish to butachlor. The fish were exposed to different sublethal concentrations of butachlor (0.45mg/l, 0.9mg/l and 1.35mg/l) for 96 hours. All the three treatment groups were run with their respective controls. No mortality (100% survivability) was observed in case of control groups. Also, no mortality was recorded in the fish treated with 0.45mg/l and 0.9mg/l of butachlor, while 16.6% mortality was recorded in fish exposed to 1.35 mg/l concentration in 96 hours. At the end of exposure time, the blood was collected from all the fish and studied for different haematological parameters. The results obtained are presented in Tables 4.3-4.5 and Figs 4.4 - 4.15.

4.3.1 Effect of butachlor on haemoglobin (Hb) (g/dl):

The normal range of Hb for the fish was 8.5-11.6 (Table 4.1). The Hb value in all the three treatment groups of fish was found to be higher than the control groups (Fig.4.4 and Tables 4.3-4.5). The Hb value was 12.5±0.5 in fish treated with 0.45mg/l butachlor while it was 10.34±0.7 in the control group run parallel with the treated group. Hb value of fish treated with 0.9 mg/l butachlor was 13.8±0.85 while it was 9.9± 0.9 in the control group. In the fish exposed to 1.35mg/l butachlor the value was 13.6±0.85 while in the control group it was

10.4 ± 0.8. The difference in Hb value of treated fish was very significant ($p < .001$) when compared with the respective controls. The data clearly indicated that the treated fish had increased Hb values.

4.3.2 Effect of butachlor on RBC count (10^6/cumm):

The normal range of RBC count for the fish was 1.5-1.94 (Table 4.1). The RBC count in all the three treatment groups of fish was found to be higher than the RBC count of the control groups (Fig.4.5 and Tables 4.3-4.5). The RBC count was found to be 2.2±0.2 in fish treated with 0.45mg/l butachlor and 1.63±0.1 in the control group. In case of fish treated with 0.9mg/l butachlor it was found to be 2.4±0.3 and 1.73±0.14 in the control group. The RBC count of the fish treated with 1.35mg/l was found to be 2.46±0.3, while it was 1.83 ± 0.1 in the corresponding control group. The difference observed in the fish treated with 0.45mg/l and 0.9 mg/l was highly significant ($p<.001$) in comparison to control group, while in case of the fish treated with 1.35mg/l it was significant at 1% level when compared with the respective control.

4.3.3 Effect of butachlor on PCV (%) :

The normal range of PCV for the fish was 28.3-38.6 (Table 4.1). The PCV in all the three treated groups of fish was higher than the control groups (Fig. 4.6 and Tables 4.3-4.5). The PCV was 39.0±1.4 in the fish treated with 0.45mg/l butachlor and 31.4±3.2 in the corresponding control group. In case of fish treated with 0.9mg/l butachlor it was 32.68 ± 3.5, while in the corresponding control group it was 31.3± 2.5. The PCV of the fish treated with 1.35mg/l butachlor was 40.6±1.8 against 34.3±2.6 recorded in the corresponding control group. The difference observed in the fish treated with 0.45mg/l and 1.35 mg/l butachlor was significant at 0.1% level ($p= 0.001$) when compared with the respective controls, while that in the fish treated with 0.9mg/l was insignificant when compared with the control.

4.3.4 Effect of butachlor on WBC count (10^3/cumm):

The normal range of WBC count for the fish was recorded as 23.3- 30.8 (Table 4.1) The WBC count in case of treated as well as control groups of fish are presented in Fig 4.7 and Tables 4.3-4.5. The WBC count in all the three treatment groups of fish was found to be higher than the WBC count of the control groups. The WBC count was 115.1±8.3 in fish treated with 0.45mg/l butachlor against 40.6±2.4 recorded in corresponding control group. The WBC count of fish treated with 0.9mg/l butachlor was 197.1±15.87 against 40.95±1.6 recorded in the control group. The WBC count of the fish treated with 1.35mg/l butachlor was 99.4±10.89 against 35.78 ± 4.79 in the control group. The difference observed was found to be highly significant ($p<.001$) when compared with the respective controls.

4.3.5 Erythrocyte Indices:

Mean Corpuscular Haemoglobin (MCH) i.e, average Hb content of single RBC, Mean Corpuscular Haemoglobin Concentration (MCHC) i.e., average Hb concentration in 100 ml of haematocrit and Mean Corpuscular Volume (MCV) i.e, size/state of RBCs constitute red blood indices.

i) Effect of butachlor on MCV (μm^3):

The normal range of MCV for the fish was 155.6-226 (Table 4.1) The MCV values in all the three treatment and control groups of fish are presented in Fig. 4.8 and Tables 4.3-4.5. The MCV value in the three treated groups of fish was found to be lower than the control groups of fish. The MCV value was 177.9±8.7 in the fish treated with 0.45mg/l butachlor and 194±12.4 in the control group. MCV value of the fish treated with 0.9mg/l butachlor was found to be 138.5±15.6, while it was 181.9±18 in the control group. The MCV value of the fish treated with 1.35mg/l butachlor was found to be 167.58±24.6, while it was 187.35 ± 13.25 in the control group. The difference in MCV between the fish treated with butachlor and the control group was significant ($p= 0.001$) only in case of 0.9 mg/l, while the difference observed using 0.45mg/l and 1.35mg/l butachlor was found to be insignificant.

ii) Effect of butachlor on MCH (µg) :

The normal range of MCH for the fish was 43.8-75 (Table 4.1). The MCH values in all the three treatment and control groups of fish are presented in Fig 4.9 and Tables 4.3-4.5. The MCH value was found to be 57.3±5.5 in the fish treated with 0.45mg/l butachlor, while in the corresponding control group it was 63.9±7.0. In case of the fish treated with 0.9mg/l butachlor it was 58.9±8.7 against 57.5± 6.9 in the corresponding control group. The MCV value of the fish treated with 1.35mg/l butachlor was found to be 55.96±6.9, while it was 56.8 ± 4.3 in the control group. The difference in MCH values between the treatment groups and respective control groups was statistically insignificant.

iii) Effect of butachlor on MCHC (%) :

The normal range of MCHC for the fish was 25-39.9 (Table 4.1). The MCHC values in all the three treatment and control groups of fish are presented in Fig 4.10 and Tables 4.3-4.5. The MCHC value was found to be 32.2±2.3 in the fish treated with 0.45mg/l butachlor, while in the corresponding control group it was 33.4±4.9. In case of the fish treated with 0.9mg/l butachlor it was recorded to be 42.5±3.96 against 31.8± 4.7 in the corresponding control group. The MCHC value of the fish treated with 1.35mg/l butachlor was found to be 33.56±3.1, while it was 30.45 ± 3.21 in the corresponding control group. The difference observed in the fish treated with 0.9 mg/l with the corresponding control was significant at 1% level ($p<.01$). However, in case of the other two treatment groups the differences were found to be insignificant.

4.3.6 Differential Leucocyte Count (DLC):

i) Effect of butachlor on basophils:

Basophil %age of presently studied fish have been found to range from 2-9 (Table 4.1). The basophil %age in all the three treatment and corresponding control groups are presented in Fig 4.11and Tables 4.3-4.5. The basophil % age in all the three treatment groups of fish was found to be lower than the control groups of fish. It was found to be 1.8±0.80 in the fish treated with 0.45mg/l butachlor and 2±0.9 in the control group. The basophil %age of the fish treated with 0.9mg/l butachlor was found to be 1.3±0.5 and 1.8± 0.75 in the control

group. In the fish treated with 1.35mg/l butachlor, it was found to be 1.33±0.51 and 1.83 ± 0.75 in the control group. The difference in basophil %age between the treatment and control groups was statistically insignificant.

ii) Effect of butachlor on eosinophils:

Eosinophil contribution in the fish was found to range from 1-3% in natural habitat (Table 4.1). The eosinophil %age in all the three treatment and control groups of fish are presented in Fig 4.12 and Tables 4.3-4.5. The eosinophil %age in all the three treatment groups of fish was found to be lower than the corresponding control groups of fish. It was found to be 0.8±0.4 in fish treated with 0.45mg/l butachlor against 1.3±1.0 recorded in the corresponding control group. The eosinophil %age of fish treated with 0.9mg/l butachlor was found to be 1.16±0.75 while it was 1.3± 0.8 in the corresponding control group. In the fish treated with 1.35mg/l butachlor it was found to be 1.33±0.51 and 1.5 ± 0.5 in the control group. The difference in eosinophil %age between the treatment and control groups was found to be statistically insignificant.

iii) Effect of butachlor on neutrophils:

Neutrophil concentration in the fish was recorded to range from 15-22% (Table 4.1). The neutrophil %age in all the three treatment and control groups of fish are presented in Fig 4.13 and Tables 4.3-4.5. The neutrophil %age in all the three treatment groups of fish was found to be higher than the control groups of fish. It was found to be 15.2±0.8 in fish treated with 0.45mg/l butachlor against 13.3±2.6 in the control group. In case of fish treated with 0.9mg/l butachlor it was recorded to be 16.2±1.9 against 14.3± 1.5 in the control group. In the fish treated with 1.35mg/l butachlor it was found to be 15.66±1.86 against 15±2.4 in the control group. The difference in neutrophil %age between the treatment and control groups was found to be statistically insignificant.

iv) Effect of butachlor on lymphocytes:

Lymphocyte content in the fish from natural habitat was found to range from 61-69% (Table 4.1). The lymphocyte %age in case of fish maintained in laboratory, both treated and control groups of fish, are presented in Fig 4.14 and Tables 4.3-4.5. The lymphocyte %age in all the three treatment groups of fish was found to be higher than the control groups of fish. It was found to be 78.7±1.2 in fish treated with 0.45mg/l butachlor against 76.3±2.7 in the control group. The lymphocyte %age of the fish treated with 0.9mg/l butachlor was found to be 80.3±1.6 against 75.7± 1.75 in the control group. In the fish treated with 1.35mg/l butachlor it was found to be 81.16±1.16, while it was 76 ± 2.8 in the control group. The difference observed in the fish treated with 0.9mg/l was highly significant **(** p= 0.001), while difference was significant at 1% level in the fish treated with 1.35mg/l. No significant difference was observed in the fish treated with 0.45mg/l butachlor when compared with the control.

v) Effect of butachlor on monocytes:

Monocyte %age of the present fish was found to range from 6-11% (Table 4.1). The monocyte %age in all the treated fish and those maintained as control groups are presented in Fig 4.15 and Tables 4.3-4.5. The monocyte %age in all the three treatment groups of fish was found to be lower than the corresponding control groups. It was found to be 3.5±0.8 in fish treated with 0.45mg/l butachlor against 7± 0.9 in the corresponding control group. In case of the fish treated with 0.9mg/l butachlor it was found to be 1±0.6 against 6.8± 1.5 in the control group. In the fish treated with 1.35mg/l butachlor it was found to be 0.5±0.54, while it was 5.66 ±1.0 in the control group. In all the three treatments the difference in the monocyte level was significantly high ($p < .001$) than the corresponding control fish.

Table 4.3: Effect of 96h exposure to 0.45 mg/l butachlor on haematological profile of *Schizothorax niger* Heckel.

Parameters	**Control**		**Treated**	
	range	**mean±sd**	**range**	**mean±sd**
Body weight (g)	55-69.4	63.9 ± 6.1	57-70.8	64.1± 5.5
Body length (cm)	18.5-19.2	18.8± 0.3	18-19.3	18.7 ± 0.4
Hb (g/dl)	9.4-11.36	10.34 ±0.7	11.9-13.3	12.5 ±0.5***
RBCs (10^6/cumm)	1.5-1.82	1.63± 0.1	1.97-2.41	2.2 ± 0.2***
PCV (%)	28-36.3	31.4± 3.2	37.3-41.11	39.0± 1.4#
WBCs (10^3/cumm)	37.9-43.7	40.6± 2.4	103.1-127.8	115.1± 8.3***
MCV (μm^3)	153.8-234.2	194± 12.4	169.2-189.3	177.9± 8.7
MCH (µg)	57.7-75.7	63.9 ± 7.0	51.7-66.0	57.3± 5.5
MCHC (%)	25.9-37.6	33.4 ± 4.9	30.4-35.5	32.2 ± 2.3
Basophils (%)	1- 3	2± 0.9	1-3	1.8 ± 0.8
Eosinophils (%)	0-3	1.3 ± 1.0	0-1	0.8 ± 0.4
Neutrophils (%)	11-18	13.3 ± 2.6	14-16	15.2 ± 0.8
Lymphocytes (%)	72-79	76.3± 2.7	77-80	78.7 ± 1.2
Monocytes (%)	6-8	7± 0.9	2-4	3.5 ± 0.8***

Values shown represent mean± S.D. Asterisks indicate significant difference with Control. ***$p<0.001$, # $p= 0.001$, when compared with the control.

Table 4.4: Effect of 96h exposure to 0.9 mg/l butachlor on haematological profile of *Schizothorax niger* Heckel.

Parameters	Control		Treated	
	range	mean±sd	range	mean±sd
Body weight (g)	59-69.2	64.1 ± 3.99	59-73	66.2± 5.3
Body length (cm)	17.7-19	18.3 ± 0.5	18.0-20.2	18.8 ± 0.76
Hb (g/dl)	8.7-11.2	9.9 ± 0.9	12.6-14.8	13.8± 0.85***
RBCs (10^6/cumm)	1.59-1.9	1.73 ± 0.14	1.99-2.76	2.4± 0.3***
PCV (%)	28.5-34.8	31.3 ± 2.5	28.9-37.6	32.68± 3.5
WBCs (10^3/cumm)	38.8-42.9	40.95± 1.6	135.2-214.1	197.1± 15.87***
MCV (μm^3)	158.3-211.3	181.9 ± 18.0	118-154.5	138.5± 15.6#
MCH (µg)	47.9-64.8	57.5± 6.9	50.4-74.4	58.9± 8.7
MCHC (%)	25.7-36.1	31.8 ± 4.7	37.8-48.7	42.5± 3.96**
Basophils (%)	1-3	1.8 ± 0.75	1-2	1.3 ± 0.5
Eosinophils (%)	0-2	1.3± 0.8	0-2	1.16± 0.75
Neutrophils (%)	13-16	14.3± 1.5	14-19	16.2± 1.9
Lymphocytes (%)	73-78	75.7± 1.75	78-82	80.3 ± 1.6#
Monocytes (%)	5-8	6.8 ± 1.5	0-2	1± 0.6***

Values shown represent mean± S.D. Asterisks indicate significant difference with control. ** $p < 0.01$, ***$p<0.001$, # $p= 0.001$, when compared with the control.

Table 4.5: Effect of 96h exposure to 1.35 mg/l butachlor on haematological profile of *Schizothorax niger* Heckel.

Parameters	Control		Treated	
	Range	**mean±sd**	**range**	**mean±sd**
Body weight (g)	77-89	84.1± 4.2	80.5-90	86.0± 3.6
Body length (cm)	18.8-21.5	20.47± 0.96	19.3-21.9	20.85 ± 0.95
Hb (g/dl)	9.2-11.4	10.4± 0.8	12.6-14.9	13.6 ± 0.85***
RBCs (10^6/cumm)	1.7-2.0	1.83± 0.1	2.07-2.90	2.46 ± 0.3**
PCV (%)	30.8-37	34.3 ± 2.6	37.5-42.2	40.6± 1.8#
WBCs (10^3/cumm)	27.2-40.1	35.78± 4.79	89.1-118.8	99.4 ± 10.89***
MCV (μm^3)	171.4-205.5	187.35± 13.25	141.0-200.5	167.58 ± 24.6
MCH (µg)	50.3-62.1	56.8 ± 4.3	45.5-65.2	55.96 ± 6.9
MCHC (%)	25.8-35.7	30.45± 3.21	29.9-38.1	33.56± 3.1
Basophils (%)	1-3	1.83 ± 0.75	1-2	1.33± 0.51
Eosinophils (%)	1-2	1.5 ± 0.5	1-2	1.33± 0.51
Neutrophils (%)	12-19	15 ± 2.4	13-18	15.66 ± 1.86
Lymphocytes (%)	73-80	76± 2.8	80-83	81.16 ± 1.16**
Monocytes (%)	4-7	5.66 ± 1.0	0-1	0.5± 0.54***

Values shown represent mean± S.D. Asterisks indicate significant difference with control. ** $p < 0.01$, ***$p<0.001$, # $p= 0.001$, when compared with the control.

Table 4.6: Range of haematological values of *Schizothorax niger* Heckel exposed to different sublethal concentrations of butachlor and that of the fish in the field conditions.

parameters	Control 1	0.45mg/l	Control 2	0.9mg/l	Control 3	1.35mg/l	Field conditions
Body weight (g)	55-69.4	57-70.8	59-69.2	59-73	77-89	80.5-90	55-90
Body length (cm)	18.5-19.2	18-19.3	17.7-19	18.0-20.2	18.8-21.5	19.3-21.9	18-21.4
Hb (g/dl)	9.4-11.36	11.9-13.3	8.7-11.2	12.6-14.8	9.2-11.4	12.6-14.9	8.5-11.6
RBCs (10^6/cumm)	1.5-1.82	1.97-2.41	1.59-1.9	1.99-2.76	1.7-2.0	2.07-2.90	1.5-1.94
PCV (%)	28-36.3	37.3-41.11	28.5-34.8	28.9-37.6	30.8-37	37.5-42.2	28.3-38.6
WBCs (10^3/cumm)	37.9-43.7	103.1-127.8	38.8-42.9	135.2-214.1	27.2-40.1	89.1-118.8	23.3-30.8
MCV (μm^3)	153.8-234.2	169.2-189.3	158.3-211.3	118-154.5	171.4-205.5	141.0-200.5	155.6-226
MCH (µg)	57.7-75.7	51.7-66.0	47.9-64.8	50.4-74.4	50.3-62.1	45.5-65.2	43.8-75
MCHC (%)	25.9-37.6	30.4-35.5	25.7-36.1	37.8-48.7	25.8-35.7	29.9-38.1	25-39.9
Basophils (%)	1- 3	1-3	1-3	1-2	1-3	1-2	2-9
Eosinophils (%)	0-3	0-1	0-2	0-2	1-2	1-2	1-3
Neutrophils (%)	11-18	14-16	13-16	14-19	12-19	13-18	15-22
Lymphocytes (%)	72-79	77-80	73-78	78-82	73-80	80-83	61-69
Monocytes (%)	6-8	2-4	5-8	0-2	4-7	0-1	6-11

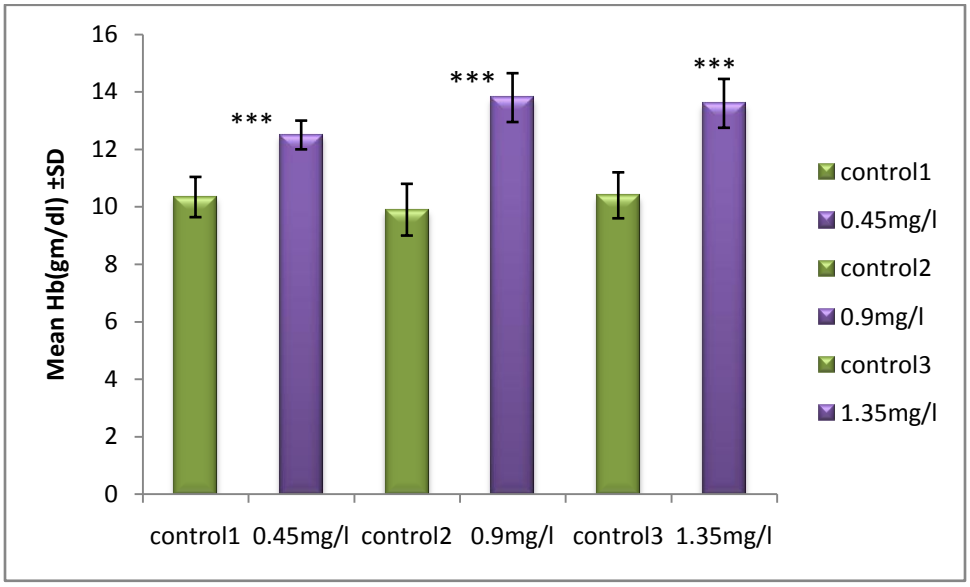


Fig 4.4: Comparison of Hb values of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

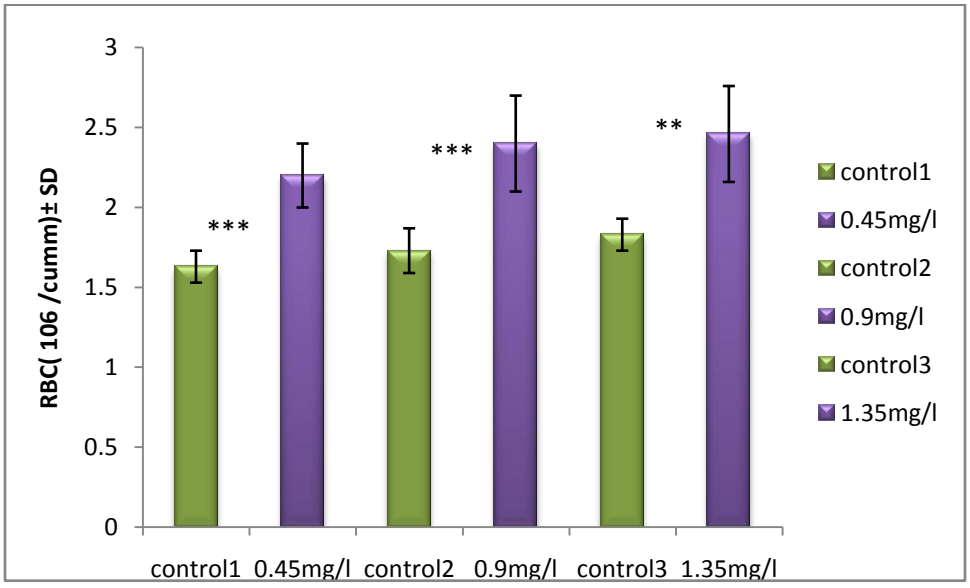


Fig 4.5: Comparison of RBC count of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

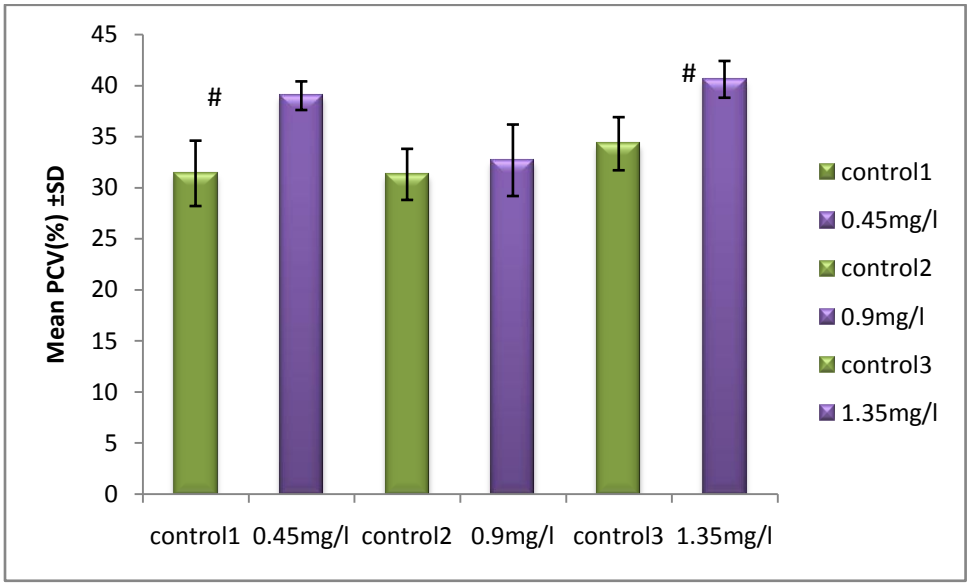


Fig 4.6: Comparison of PCV of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

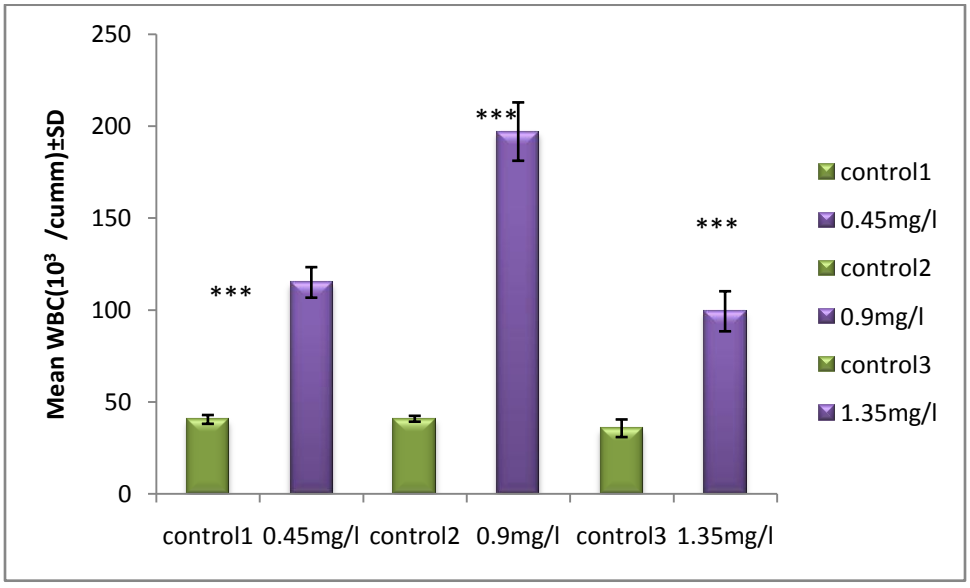


Fig 4.7: Comparison of WBC count of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

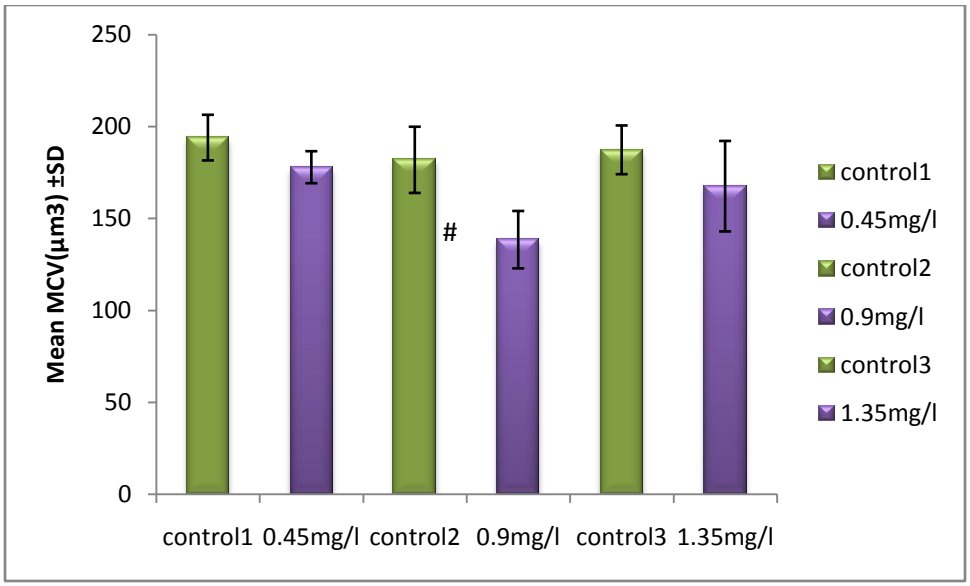


Fig 4.8: Comparison of MCV of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

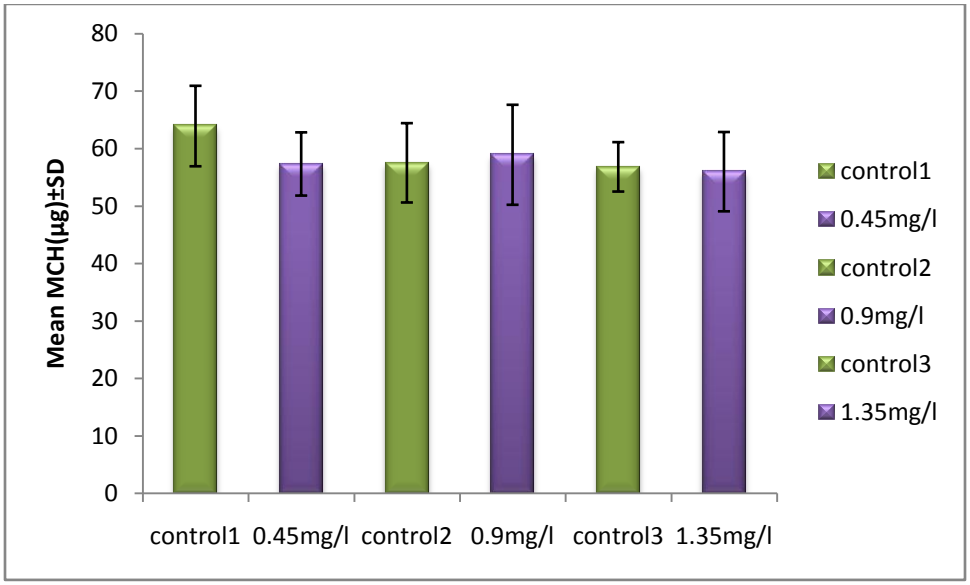


Fig 4.9: Comparison of MCH of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

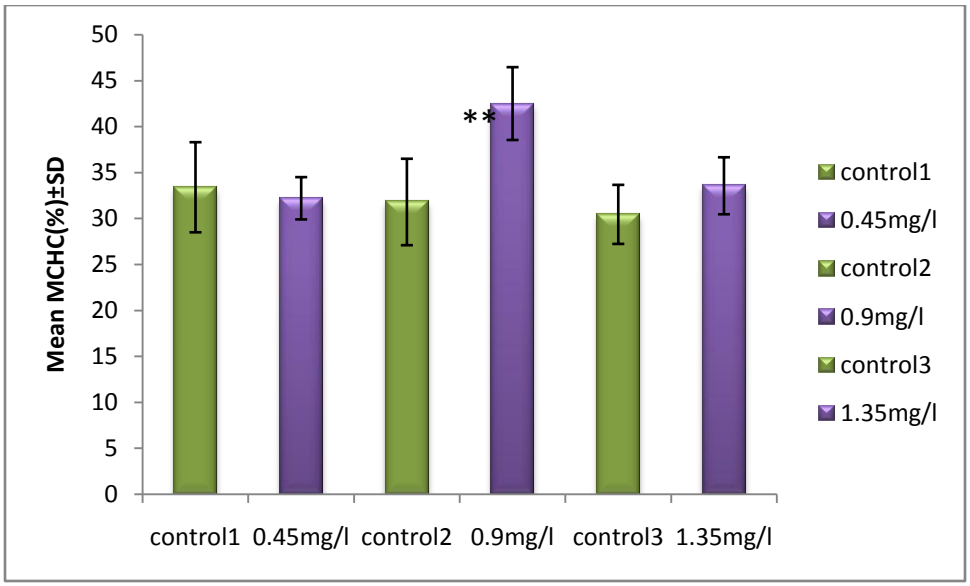


Fig 4.10: Comparison of MCHC of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

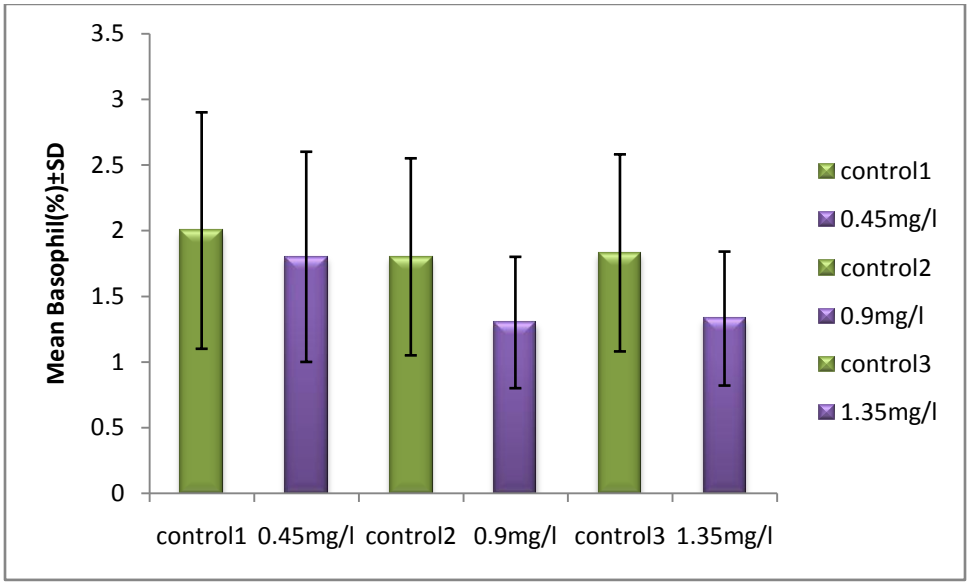


Fig 4.11: Comparison of basophil %age of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

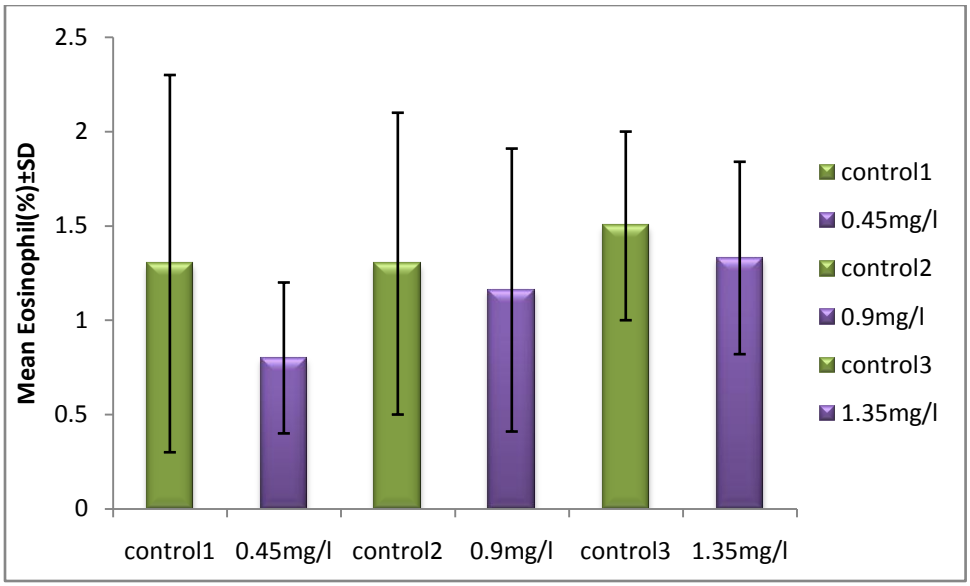


Fig 4.12: Comparison of eosinophil %age of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

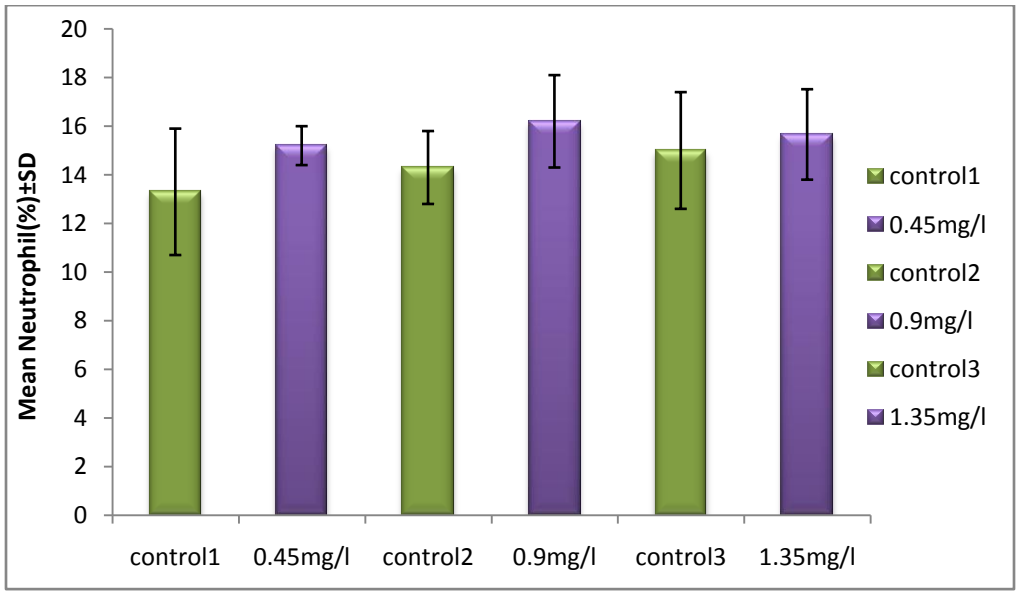


Fig 4.13: Comparison of neutrophil %age of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

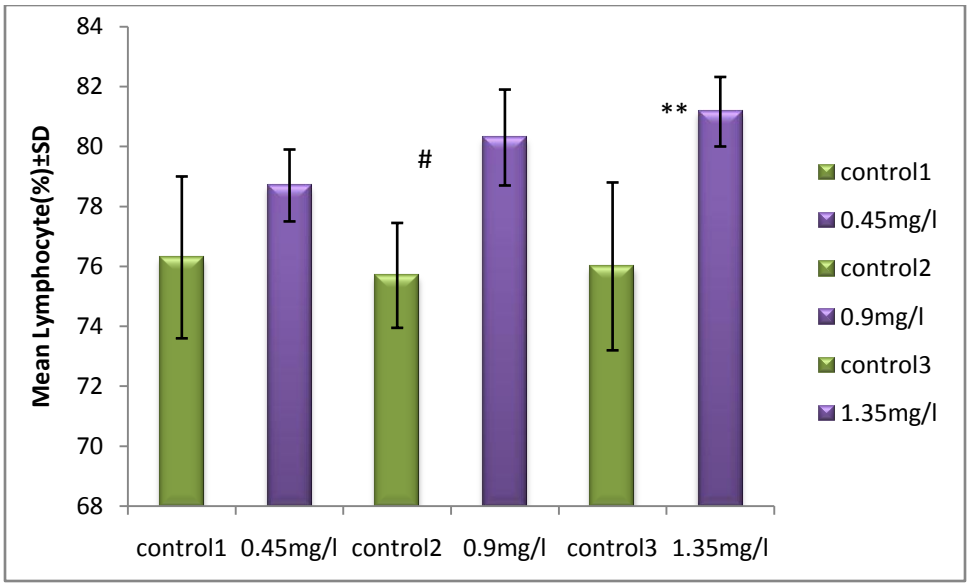


Fig 4.14: Comparison of lymphocyte %age of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

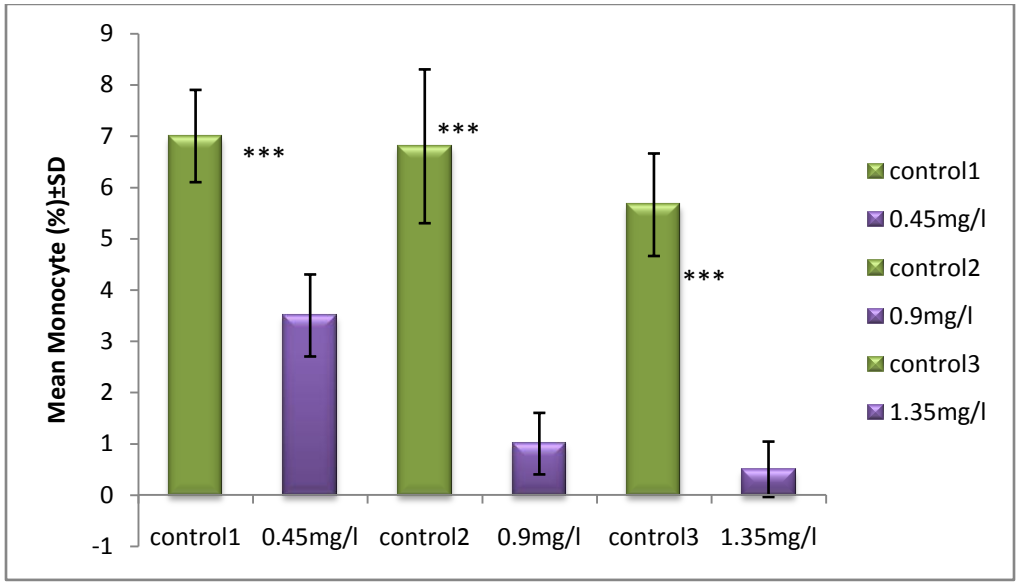


Fig 4.15: Comparison of monocyte %age of *Schizothorax niger* exposed to different sublethal concentrations of butachlor for 96 hours.

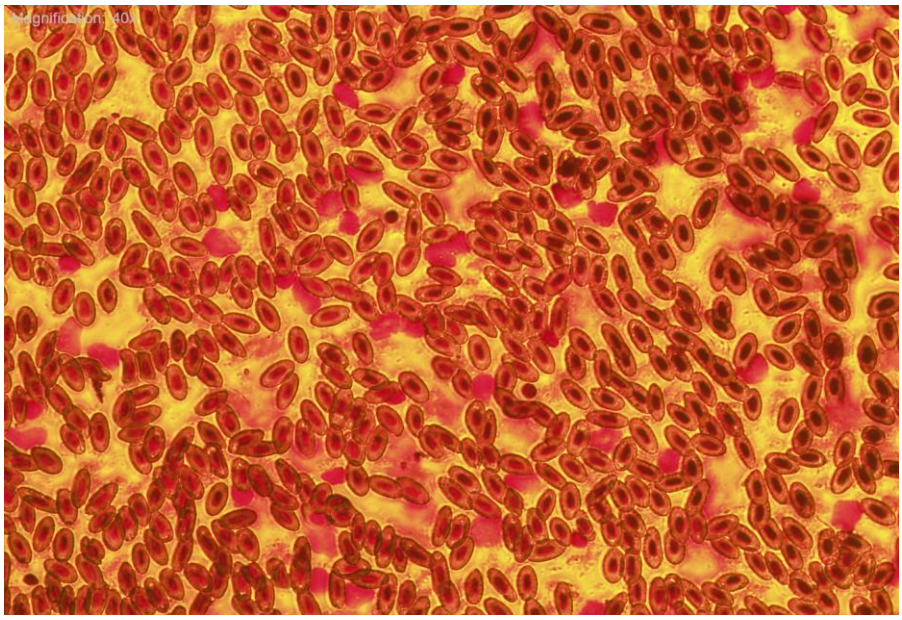


Fig 4.16 (a): RBC count increased in the fish treated with 0.9mg/l of butachlor

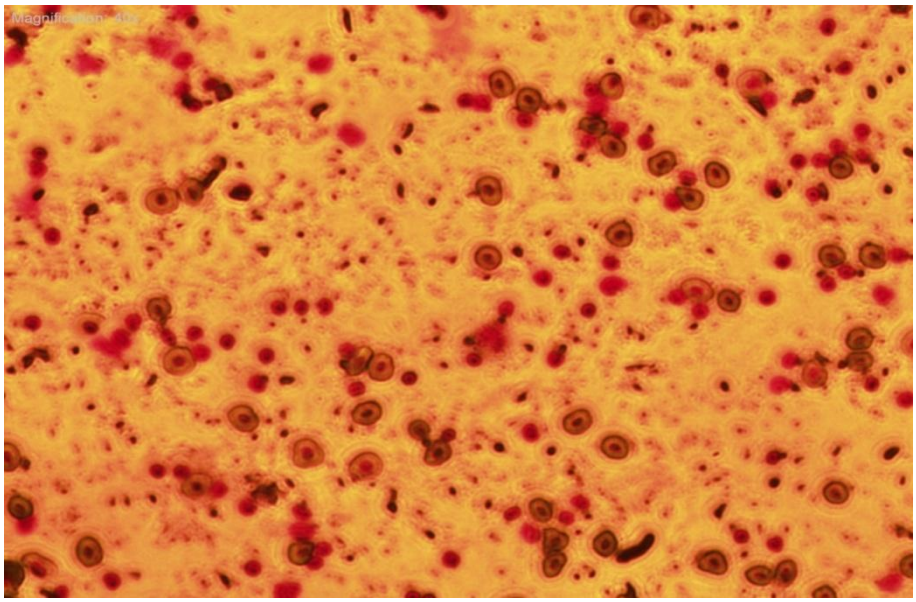


Fig. 4.16 (b) : RBC count in the control fish

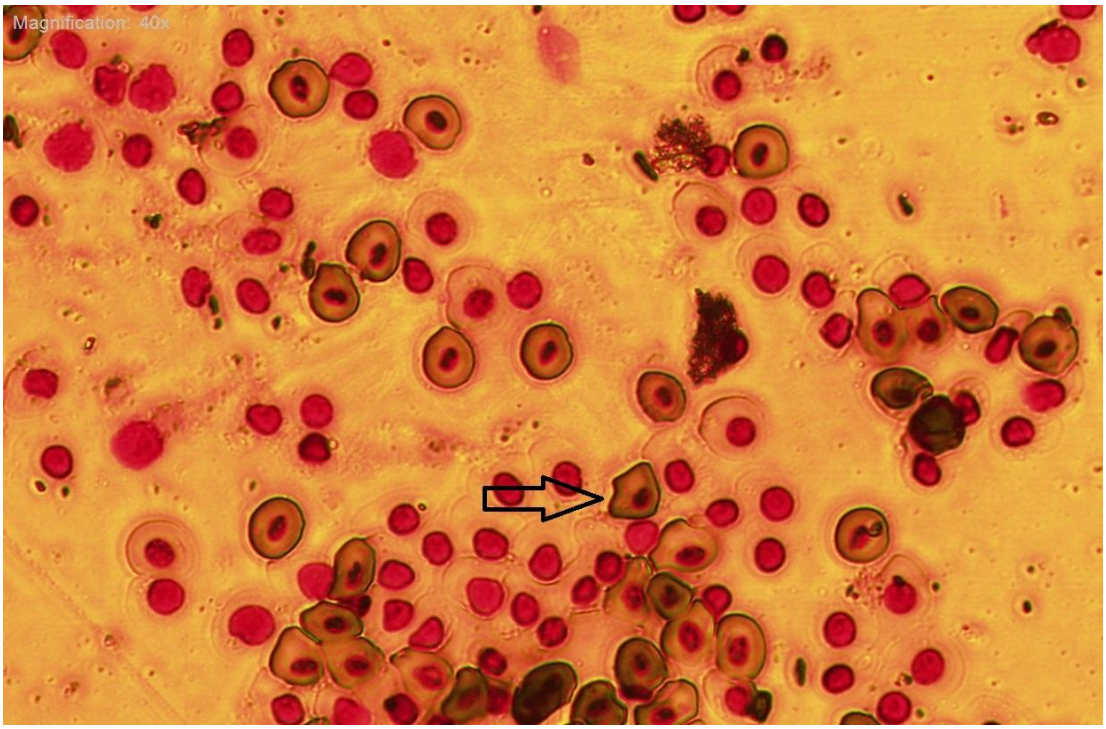


Fig 4.16 (c): Irregularity in the shape of RBCs due to butachlor exposure

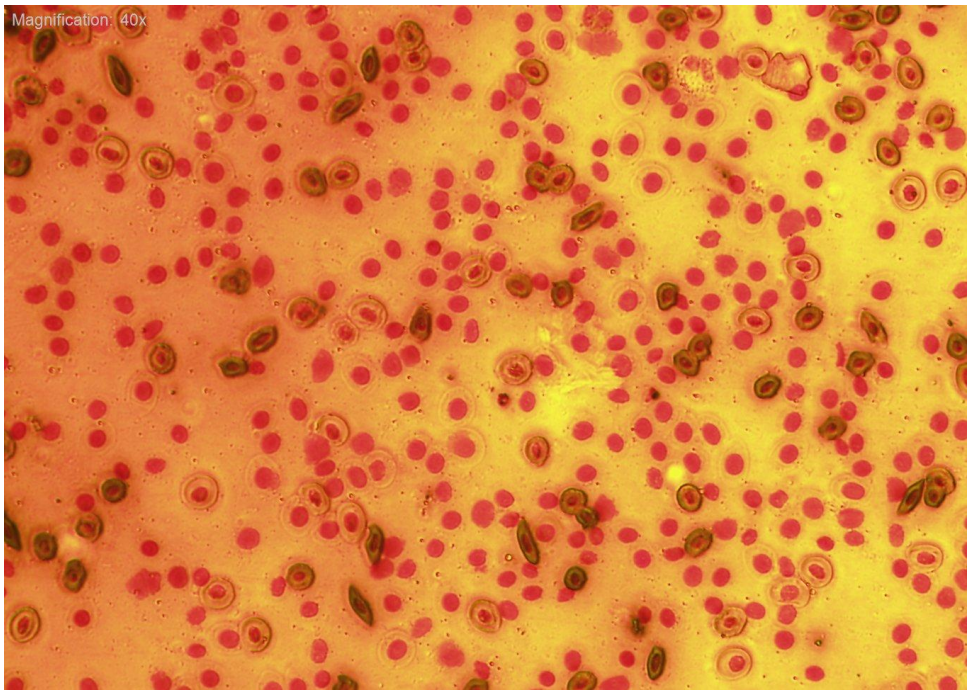


Fig 4.17 (a): Leucocyte count increased in the fish treated with 0.9mg/l of butachlor

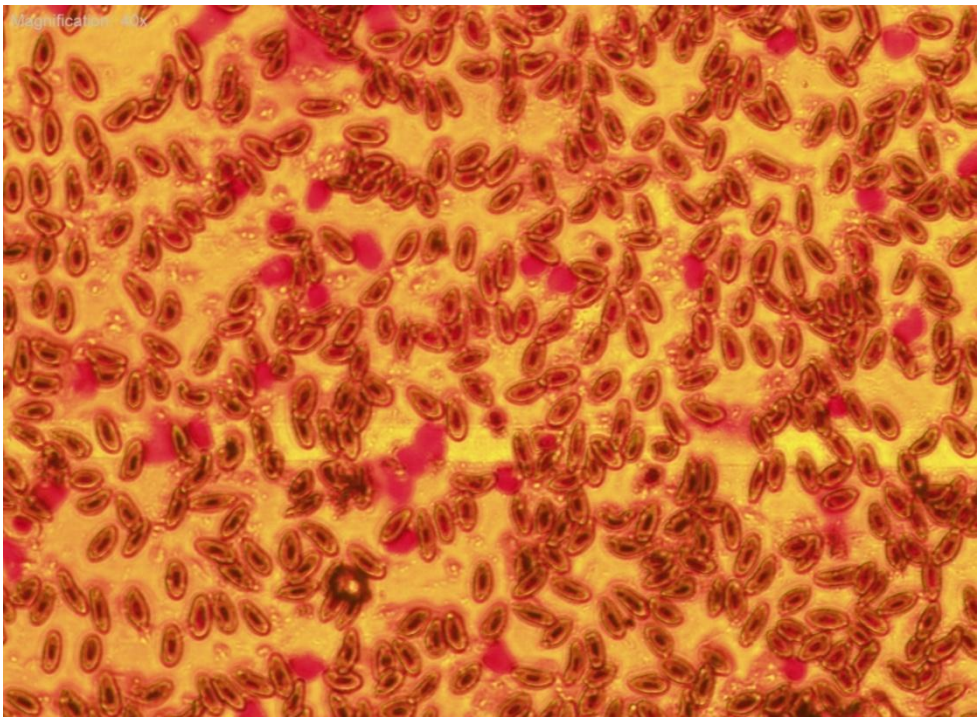


Fig. 4.17 (b): Leucocyte count in the control fish

Aquatic toxicology is the study of the effects of manufactured chemicals and other anthropogenic and natural materials and activities on aquatic organisms at various levels of organization, from subcellular through individual organisms to communities and ecosystems. It is a multidisciplinary field which integrates toxicology, aquatic ecology and aquatic chemistry. While basic research in toxicology began in the 19th century, it was not until around the 1930s that the use of acute toxicity testing, especially on fish, was established. The degree of ecosystem contamination by toxic chemicals can be estimated by the analysis of haematological changes. Blood is the most important fluid in the body and its composition often reflects the total physiological condition of an organism. Haematological parameters are nowadays not only used for clinical diagnosis of physiology but also help in addressing the effects of toxic substances on the fish (Wendlaar, 1997). 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 haematological parameters (Van Vuren, 1986). Blood cell responses are important indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either an increase or decrease in haematological levels depending on fish species, age and cycle of sexual maturity (Golovina, 1996; Luskova, 1997). Butachlor is widely used in paddy culture in the valley of Kashmir and frequently significant residual quantities of this toxicant find their way into natural aquatic ecosystems. Since, *S. niger* is a common food fish present in most of the flatland lakes of the valley, it was decided to have an insight into the impact of sublethal concentrations of butachlor on the said fish by assessing the changes found in the haematology of the fish.

Haemoglobin serves to transport oxygen from gills to different tissues of the fish in the form of oxyhaemoglobin and carbon dioxide from tissue to the gills in the form of carboxyhaemoglobin and its concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. The Hb value in all the three treatment groups of fish was found to be significantly higher ($p<.001$) than the control groups which may be attributed to the fact that the oxygen

carrying capacity of the fish was affected by the herbicide. Similar results were reported in Common carp (*Cyprinus carpio*) exposed to diazinon (Ahmed 2011). Butachlor appears to interfere with the ability of hemoglobin to bind oxygen during respiration. Due to an insufficient supply of oxygen, respiration was not maintained efficiently. As a result, the demand for hemoglobin content increased. Hence, the increase in Hb values can be interpreted as a compensatory response that improves the O_2 carrying capacity to maintain the gas transfer.

Red blood cells are the most numerous formed elements in blood. Cytoplasm of RBC appears light violet or pinkish with darkly stained purple coloured nucleus when stained with Leishman-Geimsa stain. 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 present study clearly indicated that the RBC count increased significantly on exposure to butachlor(Figs. 4.16a and 4.16b). The increase seems to be attributable to the stimulation of haemopoietic machinery of fish as their response to butachlor toxicity. It seems that erythropoiesis has been accelerated to avoid anaemic state leading to higher production of erythrocytes (Svoboda *et al.,* 2001, Modesto and Martinez, 2010, Rao, 2010 and Ahmed, 2011). It is documented that under stress conditions, fish become hyperactive perhaps to get out of the stressful medium and would require an increased amount of oxygen to meet their energy requirement. In order to get relief from the irritating effects of toxicant, the fish increases the secretion of mucous. But this increased mucous reduces the gaseous exchange through the gill. The increased utilization of oxygen and reduced supply of it may cause a hypoxic condition in fish (Pandey *et al.,* 1979 and Alkahem *et al.,* 1998). Under hypoxic condition, erythropoietic organs start releasing new erythrocytes so as to improve the oxygen carrying capacity of blood (Schindler and DeVries, 1986; Murad and Mustafa, 1989; Alkahem, 1993; Al-Ghanim *et al.*, 2008; Al-Akel *et al*., 2010).

The microscopic examination of blood smear of butachlor treated fish revealed marked irregularities in the shape of the RBCs (Fig 4.16c). It clearly showed that the fish not only responded to the toxicant by increasing the quantity of RBCs, but also by changing the quality of these cells (Katalay and Parlak, 2004). The morphological abnormalities may result in reduced and abnormal functioning of RBC and hence lead to a reduction in their functional capability including oxygen carrying capacity.

Haematocrit (Hct) expresses the volume of RBCs in 100 ml of whole blood. Any deviation from its normal values can lead to various pathological conditions. PCV increased on exposure to butachlor (significantly against 0.45 and 1.35mg/l concentrations). Elevated numbers of red blood cells and hemoglobin levels seem to be an indication of the increase in newly formed immature red blood cell population and shortening of the life span of mature red blood cells. Ergonul (2012), also came up with more or less similar results. Hence the increase in PCV points towards the fish suffering from a physiological disorder due to butachlor exposure.

Leucocytes or white blood cells (WBCs) are the defence cells of the body which provide protection to the organism against environmental as well as anthropogenic stress. Total number of leucocytes per cubic millimetre (TLC) is a diagnostic feature of many diseases. The significant increase ($p<0.001$) in WBC count was observed in the fish due to butachlor exposure (Figs 4.17a and 4.17b), which could be the result of the activation of the immune system in the presence of the herbicide (Kumar *et al.*, 1999, Barreto-Medeiros *et al*., 2005, Ramesh *et al.,* 2009 and Modesto and Martinez, 2010). The study of the DLC clearly indicates that increase in TLC upon butachlor exposure of the fish has been due to the increase in lymphocytes. Johansson-Sjobeck and Larsson (1978) has also attributed the increase in total leucocyte count (TLC) to an enhanced release of lymphocytes from lymphoid tissues and Fink and Salibian (2005) has related the WBC increase to an induced proliferation of pluripotential hematopoietic cells due to chemical toxicity. The tremendous increase in WBC count in the present study simply indicates the stress condition of the fish caused by butachlor which might have produced hypoxia and gill damage.

Erythrocyte indices:

MCV gives an indication of the status or size of RBCs (Alwan *et al*, 2009). MCV value was significantly lower ($p=0.001$) in fish treated with 0.9mg/l of butachlor while MCHC significantly increased ($p<0.01$) against the same concentration of butachlor. The increases in MCHC of the blood conform to the erythrocyte count and their production in the disorder. A decrease in MCV indicates that the erythrocytes have shrunk, either due to hypoxia, stress or impaired water balance or a large concentration of immature erythrocytes that have been released from the erythropoeitic tissue (Kumar *et al.,* 1999). Further, since MCV and TEC are inversely related to each other, therefore in the present study, decrease in MCV can also be attributed to elevation in TEC values observed in the fish.

The MCHC is a good indicator of red blood cell swelling or shrinkage (Wepener *et al*., 1992). The increase in the MCHC values in the exposed fish is thus probably an indication of shrinking of the red blood cells and/or an increase in haemoglobin synthesis. The increased Hb content may also be attributed to increased erythropoiesis and haemoglobin synthesis which, in turn, explains an increased MCHC.

Concentrations of circulating blood cell types are important parameters for use in detecting and evaluating the sublethal effects of toxicants on fish (Dick and Dixon, 1985). Many investigators have demonstrated that the leucocytes of teleosts are extremely sensitive to toxicants (Mishra and Srivastava, 1980). The evidence to date strongly suggests that the lymphocytes of fish probably play a role similar to lymphocytes in higher vertebrates because they are immunocompetent cells (Ellis, 1981). Increase in the number of lymphocytes was found in the fish exposed to butachlor which can be the result of activation of the immune system in the presence of contaminant, which in turn may be an adaptive response of the organism resulting in a more effective immune defense (Barreto-Medeiros et al., 2005). Monocytosis (increased number of monocytes) and monocytopenia (decreased number of monocytes) are the pathological condition generally observed as a reflection of stress caused by xenobiotics. Significant reduction in the number of monocytes was observed in fish after exposure to butachlor

which may be the result of elevated phagocytic activity in affected tissues, such as gills, liver and kidneys which were damaged by butachlor (Wepener, 1990; Gey van Pittius, 1991; Vander Merwe, 1992).

Eosinophills are traditionally grouped along with neutrophills and basophills as granulocytes. They also serve a critical function in mitigating allergic response since they have an ability to neutralize histamines as well as inhibit mast cell activation. Reduction in the %age of eosinophils and monocytes observed in the present study is in agreement with the findings of Benariji and Rajendranath (1990) in *Clarias batrachus* exposed to dichlorvos.

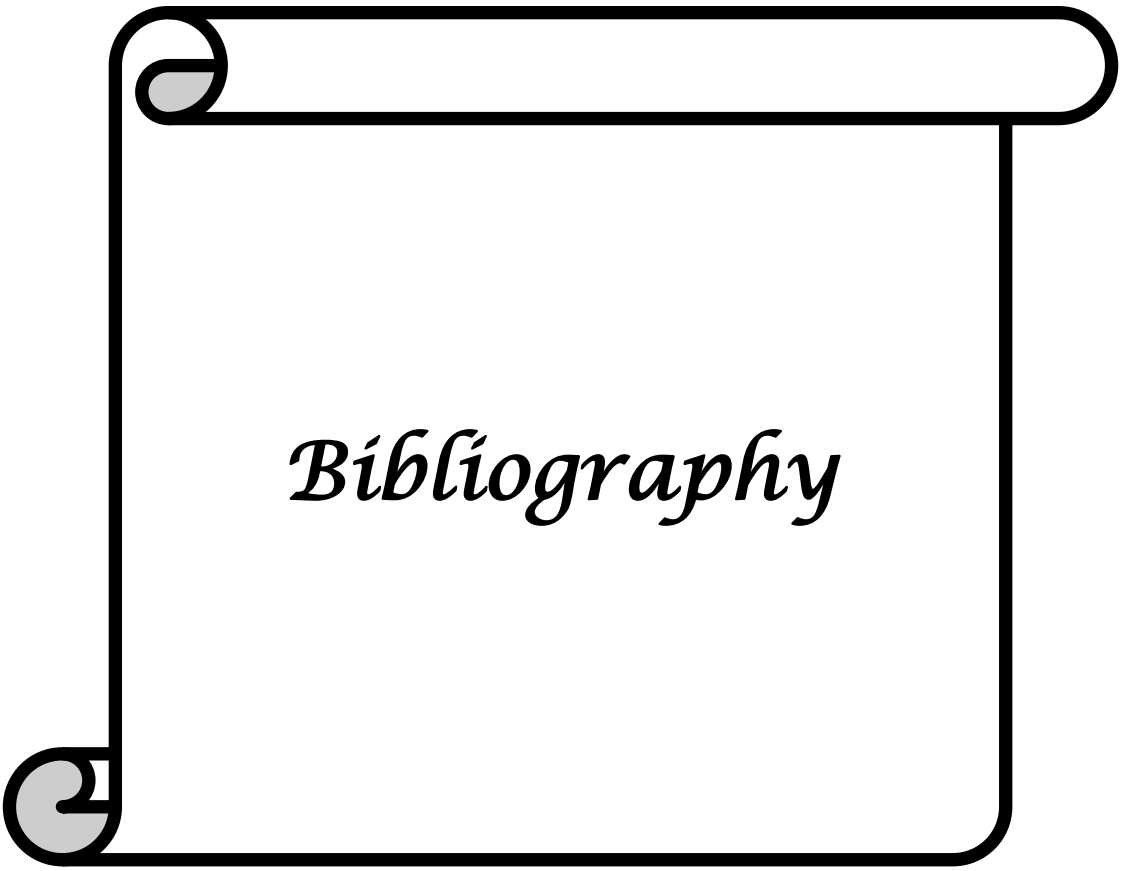
Certain haematological parameters like WBC count and DLC in the control groups of fish slightly deviated from their normal range values which is purely attributable to the stress caused to the fish under laboratory conditions.

On the whole the present study revealed that butachlor toxicity brings about considerable changes in haematological parameters of *Schizothorax niger* Heckel under laboratory conditions. The fish under field conditions may face the stress of butachlor and similar other toxicants which can lead to alterations in the normal physiological characteristics of the fish even if present for a few hours to a few days. Such, changes if unattended, can prove detrimental for the health of the fish and consequently the fish may easily fall prey to secondary infections. Water bodies like Dal lake is not an exception to the case where fishes survive and at the same time exhibit physiological / metabolic crisis due to xenobiotics.

CHAPTER – 5

Summary And Conclusion

- The LC50 value of butachlor for *Schizothorax niger* Heckel was found to be 1.8 mg/l, indicating that butachlor is moderately toxic to the fish.
- The Physiological changes induced by butachlor were apparent in hematology of *S. niger* Heckel.
- The fish of small size were more prone to the effect of toxicant than the larger fish.
- Exposure to sub-lethal concentrations of butachlor resulted in significant haematological alterations in the fish. These changes suggest that the treated fish were faced with a serious metabolic crisis. The elevated values of RBC count, hemoglobin concentration and hematocrit values in the exposed fish are indicative of stress mediated production of RBC and haemoglobin by the fish.
- Exposure to butachlor resulted in tremendous increase in WBC count of the fish due to the activation of the immune system.
- Butachlor exposure caused an elevation in lymphocyte percentage of the fish.
- Exposure to different concentrations of butachlor resulted in significant reduction in the percentage of monocytes due to elevated phagocytic activity in affected tissues, such as gills, liver and kidneys damaged by butachlor.
- The results clearly indicate that the usage of the pesticides in the fields may be a threat to both aquatic fauna and flora as well as humans.
- The use of herbicides in agricultural fields should be controlled to prevent possible contamination by leaching into the aquatic environments. In this way aquatic organisms could be protected from these kinds of toxic chemicals.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