World-wide running waters (streams and rivers) drain an area of approximately 150 million km\(^2\) of land. Apart from hosting important fish stocks and enriching us with their scenic beauty, they are also characterized by conspicuously rich and complex environments due to their close connectivity between terrestrial and aquatic habitats. Running waters therefore accommodate a broad spectrum of different life forms which additionally provide a series of consumer services such as water purification and the organic matter processing. These processes eventually play an important role in the supply of drinking water for humans and human related activities. Moreover, they are conduits for effluents from industrial, domestic and agricultural sources. The water quality requirements for these processes and the additional requirements for conservation of biodiversity have led to conflicts in the management and conservation of streams. The problem of globalization increases the competition on the international market for food production and industry. The quantity of xenobiotics compounds that are deliberately or accidentally introduced to the natural environment is a serious threat to all forms of life and the direct, indirect and combined effects of these compounds in natural environments is of great concern in terms of biodiversity, ecosystem and environmental sustainability in general (Liess et al., 2005; MEA, 2005). In total, more than 14 million chemicals exist, and at least 100,000 of them are industrially produced. Many of these compounds are not characterized with respect to their ecotoxicity or characterized in terms of occurrence in the natural environment perhaps due to insufficient analytical methods (Von der Ohe et al., 2009) one of these chemicals are agricultural at pesticides. Pesticides that are applied to agricultural fields are transported to surface water recipients via wind drift, surface runoff, drains, groundwater and leakage from point sources (Schulz, 2004). The transport pathways
are strongly governed by local climatic and geological conditions and by the physico-chemical properties of the pesticide compounds. Surface runoff and transport through tile drains are widely acknowledged as the most important transport routes for pesticides from fields to receiving streams (Kreuger, 1998; Kronvang et al., 2004; Liess et al., 1999; Schulz et al., 1998; Wauchope, 1978). Consequently, peak pesticide concentrations are mostly short and occur during heavy precipitation events. Considering peak concentrations is essential as the maximum exposure concentration is proposed to be more important for estimating ecological effects than i.e. exposure duration (Schulz and Liess, 2000). Various chemicals have been used as pesticides in public health programs, veterinary and agriculture. Use of pesticides having acute toxicity is prohibited. However, pyrethroids are extensively used in the world (Aslam et al., 2010; Ahmad et al., 2011).

Pyrethroids are preferred above organophosphates, carbamates and organochlorines as these have high efficiency, low toxicity and easy biodegradability (Sharaf et al., 2010). For more than 30 years, pyrethroids are in use for home formulations and agricultural purposes and these insecticides cover nearly one-fourth of the worldwide market (Ahmad et al., 2012). In the last decade, their use has been increased (Bhushan et al., 2010). Cypermethrin, synthetic pyrethroid, lipophilic in nature, is considered to be less toxic due to its speedy insect killing properties and having low toxicity to mammalian tissues (Aslam et al., 2010). However, it is abstemiously toxic when applied dermally or administered orally (Luty et al., 1998; Aslam et al., 2010).

In the assessment and evaluation of the toxic characteristics of a substance, determination of toxicity is usually an initial step. It provides information on health hazards likely to arise from short term exposure. It is traditionally a step in establishing a dosage regimen in other studies by providing initial information on the mode of toxic
action of a substance. The acute test for a long time has been a major component in toxicity testing. In which acute chemical toxicity is determined as 96 hrs LC$_{50}$ value. The environmental significance of death of individuals after short term exposure to high concentration is questionable. Behavioral characteristics are obviously sensitive indicators of toxicant’s effect. It is necessary, however, to select behavioural indices of monitoring that relate to the organisms behaviour in the field in order to derive a more accurate assessment of the hazards that a contaminant may pose in natural system.

According to Finney's, (1971) Probit Analysis Method in the present work 96 hrs LC$_{50}$ value of Cypermethrin in Rohu (Labeo rohita) was found to be 0.06 ppm. This shows that Cypermethrin is highly toxic to fish. Yılmaz et al. (2004) reported that behavioural changes of male guppies manifested themselves starting at alpha Cypermethrin concentration of 0.015 ppm. The LC$_{50}$ value of alpha-cypermethrin for guppies was 1.794 ppm. Behavioural changes due to Cypermethrin exposure in the present work are similar to those reported by Polat et al., (2002) for Cypermethrin. The authors reported 48 hrs LC$_{50}$ value of Cypermethrin in male guppies as 0.214 ppm. Edwards et al., (1986) reported acute Cypermethrin toxicity in rainbow trout such as gill flailing, hyperactivity, loss of buoyancy and inability to remain upright. However, published experimental work on Cypermethrin fish toxicity is quite limited. Smith and Stratton, (1986) reported the toxic effects (LC$_{50}$) of cis-cypermethrin on various fish species. They found 0.002 (96 hrs) for Atlantic salmon (Salmo salar), 0.006 (96 hrs) for rainbow trout (Salmo gairdneri), 0.009 ppm (24 hrs) and 0.008 ppm (48 hrs) for mosquito fish (Gambusia affinis) and 0.01 ppm (24 hrs) and 0.006 ppm (48 hrs) for desert pupfish (Cyprinodon macularius). The four species of fish Catla catla (Ham.), Anabas testudineus (Bloch), Mystus cavasius (Ham.) and Mystus vittatus (Bloch) were whose susceptibility to carbaryl and 1-naphthol was tested, belonged to three different families. It is surprising to note that the carp, C. catla which is supposed to be a very
sensitive fish, incapable of withstanding environmental stress, was the least sensitive of the four, to both carbaryl and 1-naphthol. It may also be noted that in the case of the carp, only fingerlings were used, whereas in the case of the other three, adults used. *Catla* of the same size as that of the other three species would have a higher LC$_{50}$ value (as it is well assumed that larger size groups have a higher tolerance to toxicants), in which case the disparity in the tolerance of the carp and the other species was very striking. Similar instance of greater sensitivity of air-breathing fish to Endosulfan was reported by Rao, (1979). Toxicity of Lindane to *C. gariepinus* was relatively lower when compared with other species of fishes. The 96 hrs LC$_{50}$ value (0.038 ppm) obtained in the present study was lower than the values reported in literature for other species of fish. Vittozzi and De angelis, (1991) summarized the 96 hrs LC$_{50}$ values of other organophosphate pesticides like Parathion (0.056-0.199 ppm), Methyl Parathion (0.195-0.891 ppm) and Malathion (0.091-0.229) for different species of fishes. These values indicated that these compounds were more toxic to fish than Lindane. The differential toxicity of Lindane to *C. gariepinus* could be attributed to the differences in susceptibility and tolerance related to its accumulation, biotransformation and excretion. Differences in metabolic pathways among species may result in different patterns of biotransformation, leading to more or less toxic metabolites (Johnson and Toledo, 1993; Alkahem *et al.*, 1998). Cypermethrin exposure to *L. rohita* in present study showed the TLm-96 hrs as 0.06 ppm. Examining Cypermethrin toxicity to other aquatic organisms, the work of Clark *et al.*, (1987) reported the Cypermethrin 96 hrs LC$_{50}$ for grass shrimp (*Palaemonetes pugio*) as 0.016 ppm. The 24 hrs aqueous LC$_{50}$ values for selected terrestrial and aquatic insects, when exposed to technical grade Cypermethrin (99.4% purity), were in the range 0.30-49 ng/mg body weight and 0.0013-0.0098 ppm, respectively (Siegfried, 1993). The author concluded that exposure
of aqueous organisms to pyrethroids might have secondarily induced an osmotic imbalance that contributed to their toxicity.

Recent studies on acute toxicity and behavioural responses of common carp, *Cyprinus carpio* (Linn.) to an organophosphate, Dimethoate brought erratic swimming of the test fish, increased surfacing, decreased rate of opercular movement, copious mucus secretion, reduced agility and inability to maintain normal posture and balance with increasing exposure time (Pandey et al., 2009). Mushigeri and David, (2005) reported that fishes slowly became lethargic, hyper excited, restless and secreted excess mucus all over their bodies. Mucus secretion in fish forms a barrier between the body and toxic media thereby probably reduces contact with the toxicant so as to minimize its irritating effect, or to eliminate it through epidermal mucus. Similar observations were made by Rao et al., (2003) and Parma De Croux et al., (2002) in *Prochilodus lineatus* under monocrotophos stress. Opercular movements increased initially in all exposure periods but decreased later, steadily in lethal compared to sub lethal exposure periods. The increased opercular gill movements observed initially may possibly compensate for increased physiological activity under stressful conditions (Shivakumar and David, 2004). The moving of the fish to the bottom of the tank following the addition of Cypermethrin clearly indicated the avoidance behaviour of the fish, which was reported by Murthy, (1987) in trout. The opercular movement of the fish ceased immediately following exposure to cypermethrin. The decrease in opercular movement and corresponding increase in frequency of surfacing of fish clearly indicated that fish adaptively shifted towards aerial respiration and the fish tries to avoid contact with the pesticide through gill chamber Santhakumar et al., (2000). The increased ventilation rate by rapid, repeated opening and closing of mouth and opercular coverings accompanied by partially extended fins was observed in the present study. Increased physical activity, excess secretions of mucus, erratic swimming, respiratory distress,
sudden quick movement, increase in opercula ventilation and prior to death were associated with Cypermethrin toxicity in this study. Findings of the present work the fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium, leading to hyperstimulation behaviour were observed. This agreed with the findings of Alkahem et al., (1998) on Oreochromis niloticus exposed to trichloroform. Omitoyin et al., (1999) reported similar observation in Sarotherodon galilaeus (Tilapia) fingerlings exposed to piscicidal plant extracts of Tetrapleura tetraptera. Fafioye, (2001) also reported similar changes in fish exposed to Parkia bioglobosa and Raffia vinifera.

Histopathological studies have been conducted to help, establish causal relationships between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical compounds within target organs of fish in laboratory experiments. Histopathology provides a rapid method to detect effects of irritants in various organs. The exposure of fish to chemical contaminants is likely to induce a number of lesions in different organs. Gills, liver, kidney and brain etc. are suitable organs for histological examination in order to determine the effect of pollution (Halis, et. al., 2010). Several authors have reported histopathological changes in the gill tissue of fish exposed to miscellaneous pesticides {Lowe, (1964); Eller, (1971); Jauch, (1979); Nowak, (1992); Rijijohan and Jayabalan, (1993)}. In most cases histological changes were characterized by damage in the epithelial cells and hyperplasia, lamellar fusion and aneurysm which were also reported in present study. The concomitant appearance of inflammatory cells was indicative of a secondary defense mechanism of the body. In the present study the observed changes was the excessive mucus secretion, Lamellar fusion, damages like discontinuity and dilation in central cord, degeneration of secondary gill lamellae, necrosis of gill epithelium, clubbing of ends of the secondary
gill lamellae and necrosis in the primary lamellae were well marked. Beside, these changes vacuolization, lifting of the epithelial layer from the secondary lamellae and aneurysm were also observed significantly. Structure of gill was observed totally damaged at end of experiment. Histopathological changes in the gill of fishes due to pesticides and other contaminants have also been reported earlier (Dutta et al., 1993). According to Leino et al., (1987) the gill of pearl dace and fathead minnows from environmentally polluted Canadian lakes exhibited various cellular, histological and histopathological changes which contributed to problems related to respiration and acid-base balances. The severe damage and rupture of the gill epithelium resulted in hypoxia and respiratory failure. Roy and Datta (1991), reported slight hyperplasia of gill epithelium in *Pineaus monodon* exposed to Gusathion, a commonly used organophosphate. They also reported that provocative alterations of lamellar epithelium and hyperplasia in the gills of freshwater major carp *Cirrhinus mrigala* (Hamilton) during 48 hrs exposure to sublethal dose of Malathion, these finding support the results of present study. Additionally, similar changes were observed in *L. rohita* exposed to Monocrotophos and Fenvalerate (Tilak et al., 2001) and to Cypermethrin (Veeraiah, 2001). All these lesions may damage respiratory function. Filament cell propagation reduce the inter lamellar space and may cause a complete lamellar fusion reducing the total surface area for gas exchange reported by Nowak, (1992). Increase in the distance of the water-blood barrier which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake which can lead to the damages in central cord, discontinuity in central cord, dilation in central cord. This is correctly implicit in present study.

Fish liver histopathology is an indicator of chemical toxicity and it is a useful way to study the effects of exposure of aquatic animals to toxins present in the aquatic environment (Fernandez et al., 2008). The liver is the primary organ for metabolism,
detoxification of xenobiotics, excretion of harmful substances, etc. Individuals containing higher concentrations of pesticides, such as aldrin, alachlor and dichloroaniline, showed the fibrosis, large necrosis area, leukocyte infiltration and the absence of melanomacrophages in the liver. In the present study, histological lesions were observed in liver of *L. rohita* exposed to sub lethal, lethal and acute lethal concentrations of Cypermethrin; observed similar findings were observed in *Cirrhinus mrigala* on exposure to Cypermethrin, David, (1995) in *Labeo rohita*, Babu et al., (2007) in *Cirrhinus mrigala* on exposure to fenvalerate. Mathur, (1975) showed that dielindrin induced marked degeneration of the liver in *Ophiocephalus punctatus*, *Barbus stigma*, *Trichogaster fascitus* and *Heteropneustes fossilis*. Ray, (1982) worked on the histopathology of *Anabas testudineus* and observed the changes characterized by precipitation of cytoplasm, vacuolation of cells with corresponding increase in cell size, nuclear degeneration, hepatic cord disarray, etc. Similarly, John et al., (2001) described the impact of Endosulfan on the liver of *Cyprinus carpio* and expressed that extensive vacuolation, indistinct cell boundaries, loss of polygonal shape of the cell and degenerative necrosis are the respective histopathological changes. As mentioned here, hepatopathy highly corroborate with our present work on the impact of sub lethal, lethal and acute lethal concentrations of Cypermethrin on the liver of *L. rohita* during exposure period. Wide varieties of insecticides and other toxic by-products tend to accumulate in high concentrations within it and the organ suffers with harmful effects. According to present understanding of the biological structure and function of an organism, reduces the degree of organization and the regularity of functioning. Cirrhosis is a diffuse increase in the fibrous tissue of the liver, usually associated with chronic damage and destruction of hepatocytes. The damage can result from a wide range of stimuli, from longstanding biliary obstruction, heavy metal or pesticide poisoning to chronic parasitism. The mercuric chloride treated *Channa punctatus*
showed vacuolization of hepatocytes, necrosis and rupture of cell membrane (Sastry, 1989). *Labeo rohita* with increasing exposure to the toxicant, King, (1962) found many small vacuoles in hepatic cells of brown trout fry and adult guppies exposed to 0.0032-3.2 ppm DDT. It appeared to be a general feature of the liver of intoxicated fish that the degree of structural heterogeneity is enhanced with increasing concentration of the toxicant (Hawkes, 1980). Kabir and Begum, (1978) and Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis in the liver tissue of *Heteropneustes fossilis* when subjected to acute thiodan toxicity, similar changes were recorded in the present study with Cypermethrin. Shakoori *et al.*, (1988) observed hypertrophy of hepatocytes in rodents after administration of cypermethrin in diet over a period of 6 months. Ahmed *et al.*, (1989) reported extensive degenerative changes such as cloudy swelling, fatty degeneration and necrosis of hepatocytes in rats exposed to sub acute dose of Cypermethrin. Shrunken and pyknotic nuclei indicated that cells became hypo functional and at the end, necrosis was extensive. The stagnation of bile inside the hepatocytes signifies affected metabolism (Fanta *et al.*, 2003). Couch, (1975) reported perivascular lesions in liver of fishes exposed to organic contaminants and pesticides. According to Gingerich, (1982) the vacuolization of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in hepatocytes. In this study, all effects that were observed in the liver reduce the general state of health of *Labeo rohita* at sublethal, lethal and acute lethal concentrations of Cypermethrin. Severe fatty degenerative changes of hepatocytes and in some samples, severe degenerative changes of hepatocytes like necrosis, vacuolation were observed in the test fish. Kabir and Begum, (1978) reported cytoplasmic degeneration, pyknotic nuclei in liver tissues; vacuolation in hepatic cells and ruptured of blood vessels. Shastry and Sharma, (1979) exposed *Channa punctactus* to a sub-lethal concentration
10.01 mg/L) of eldrin and observed hypertrophy of hepatic cells and liver corddisarray, vacuolation of cytoplasm and necrosis, rupture of hepatic cells.

Tissue changes in liver are linked with histological abnormalities of kidney and gill. Once absorbed, toxicant is transported by blood circulation to liver for transformation and storage. If it is transformed in the liver it may be excreted through the bile duct or pass into blood for possible excretion by kidney (Lindstoma-Seppa et al., 1981). Gupta and Dalela, (1987) reported histological changes in kidney of Notopterus notopterus, exhibiting degeneration and dissolution of epithelial cells of renal tubules, hypertrophy and necrosis following subtle exposure to phenolic compounds. Similar observations were made by Csepai, (1978) in Cyprinus carpio chronically exposed to Anthio 40% EC, Satox and Basuden 10G, the organo chlorine and organophosphate compounds. Interestingly, most of the alterations in present study were seen in the tubular cells rather than in the glomeruli which were spared. Moderate to marked cellular infiltrations comprised which might be explained as a defense mechanism in the fish to counter toxic metabolites. Oral administration of Endosulfan at the dose level of 10 mg/day for two and four weeks showed toxic interference with the biochemistry and histology of rat liver and kidney. Similarly pathological alterations were observed in the kidney (Nisha and Joshi, 2003). The toxic sublethal concentration of fenvalerate in vital organs including kidney of Cirrhinus mrigala was evaluated. Renal tubules and haematopoetic tissue were severely affected. The presence of tubule degeneration, coupled with the absence of necrosis in the in the kidney in the present study indicated that the kidney suffered damage after exposure to the Cypermethrin but the short period of confinement might have prevented the establishment of necrosis in this organ. Very interesting changes like occlusion of tubular lumen was observed in the present study. The same had also been reported by Marina and Claudia, (2007).
Histopathological changes in optic tectum of brain were observed. Thick covering detached from stratum marginal in optic tectum was noted. Bhattacharya and Mukherjee, (1978) studied the histology of optic tectum of teleost exposed to the industrial pollutants. They observed histopathological changes in five layers of optic tectum in the two fishes exposed to ammonia, sodium sulphide, copper sulphate and phenol. In the present investigation stratum marginal and stratum opticum were observed detached. Coordination of the visual perception with the body movements is performed by the midbrain which also lodges the optic tectum (Sperry, 1950). Stimulation of the tectum results in motar activity (Healey, 1957). Loosening of fibers at these regions was reported by Awasthi, (1982) in Heteropneustes fossilis after the treatment with organophosphorous insecticides. Sheikh, (1985) observed histopathological lesions in optic tectum and telencephalon region in B. dussumieri after the treatment with sublethal concentration of Fluoride. The structural damages may be responsible for the functional inability especially for retarded neurotransmission. Inhibition of the cholinesterase in the brain of Channa punctatus and Channa batrachus by the industrial pollutants and textile effluent (Mukherjee and Bhattacharya, 1974) stood in support of earlier findings.

Haematological parameters are important for the assessment of various systemic functions and health of animals under various environmental conditions importantly, for diagnosis of drugs and chemicals (Atamanalp & Yanik, 2003). Minimum hematological indices include haematocrit (Hct), Hemoglobin (Hb) concentration and Total Erythrocyte Counts (TEC) etc and have been frequently included in toxicological studies (Gad & Chengelis, 1988). However, information regarding hematological alterations following exposure to Cypermethrin is inconsistent. It might be partially due to various non-specific features influencing hematological parameters. These features may include alterations in circulations, rate of food consumption, fluid and salt balance,
food utilization, and feeding pattern. Blood sampling and experimental variables may also influence hematology (Greaves, 2007). According to EPA, statistically significant hematological findings in pyrethroid orally fed animals could be attributed to adaptive reactions rather than persuaded haematotoxicity. However, anemia was reported in mice treated with Fenvalerate (EPA, 1991). Decrease in Hb concentrations and TEC in female rats and decrease in Hct in male rats fed Cypermethrin have also been reported (Anonymous, 1989). The decrease in the haemoglobin concentration has been reported by Rai and Qayyam, (1984) in *Catla catla* due to intoxication of lead; Thakur and Sahai, (1987) in *Channa punctatus* exposed to BHC; Garg et al., (1989) in *Heteropneustes fossilis* due to manganese poisoning, Goswami and Dutta, (1991) in *Heteropneustes fossilis* due to vit. A deficient diet; while Singh and Shrivastava, (1992) in *Heteropneustes fossilis* due to propoxur toxicity; Nath and Banerjee, (1995) in *Heteropneustes fossilis* treated with devithion; Singh, (1995) in *Channa punctatus* due to copper sulphate and potassium dichromate poisoning; Raizada and Rana, (1998) in *Clarias batrachus*, Ananadkumar et al., (2001) in *Heteropneustes fossilis*, Saxena and Seth, (2002) in *Channa punctatus* after Cypermethrin treatment, Das et al., (2004) after nitrate toxicity in *Labeo rohita*. Masud et al., (2005) in *Cyprinus carpio* following mercuric chloride intoxication, Kumar et al., (2006) in *Clarias batrachus* and Singh and Singh, (2007) in *Heteropneustes fossilis*. Haemoglobin is an integral part of RBCs and its decrease is obviously due to decrease in RBCs count after Bismarck brown and acid leather brown administration investigation and also investigated in the present study. Reduction in haemoglobin concentration may also be due to hypohaemoglobinemia. Augmented hemolysis usually lead to reduction in Hb, TEC and Hct and are escorted by elevated reticulocytes counts, amplified an isocytosis, increased red cell dissemination width and volumes. Significantly decreased mean corpuscular Hb concentration (MCHC) after pyrethroid treatment was reported
(Matsushima et al., 2003; Basir et al., 2011). Since hematological parameters are necessary for clinical diagnosis and pathological conditions of a disease. These criteria receive enough attention in assessing the health of the fish with regard to aquatic pollution and have been accepted by many workers. In general, anemia, reduction in the number of red blood cells or of haemoglobin in the blood can reflect impaired synthesis of haemoglobin or impaired production of erythrocytes (Murray et al., 2007). Jung-Hoon Jea et al., (2005) studied the decline in RBC count, hemoglobin concentration and Hct presumably reflects erythrocyte hemolysis and due to either an increase in the rate at which haemoglobin concentration may be destroyed or a decrease in the haemoglobin synthesis. Decrease in haematocrit is attributed to the reduction in RBC count caused either destruction or reduction in size. Rahmen and Siddiqui, (2006) also observed decrease in haematocrit and mean value of hemoglobin. Decreased RBC count, Hb and PCV levels was observed in rats treated with thiodan 35% E.C (Solanke and Singh, 2000), chloropharm (Fujitani et al., 2001), Endosulfan (Choudhary and Joshi, 2002), and Lindane and Endosulfan (Azhar, 2007), deltamethrin (Yekeen et al., 2007). MCV, MCH and MCHC showed significant decrease in all doses in the present investigation due to destruction of RBC (size and shape) and decrease in Hb synthesis and hemoglobin content. These symptoms imply the microcytic hypochromic anemia. Increase in total leukocytes count has been suggested to be due to stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue. Such lymphocyte response might be due to the presence of toxic substances may be associated with the pollutant induced tissue damage and severe disturbance of the non-specific immune system leading to increased production of leukocytes. A decreased percentage of neutrophils in peripheral blood observed in animals poisoned with chlorpyrifos may suggest, neutrophils involves in phagocytosis during xenobiotics intoxication, during which some of the neutrophils might rupture. In the present
experiments with *Labeo rohita*, significant differences were observed in the levels of RBC, WBC, Hb, PCV, MCV, MCHC and MCH. On the other hand, Atamanalp *et al.*, (2002) and Atamanalp and Yanik (2003) also found a significant decrease in the levels of RBC, Hb, MCH, MCHC, thrombocyte count and erythrocyte sedimentation rate in rainbow trout (*Oncorhynchus mykiss*) following Cypermethrin and Mancozeb acute exposure. The MCV, MCH, MCHC, Values are completely depend upon the factors of PCV, RBC count and hemoglobin concentration. In the present study, the PCV, RBC and hemoglobin concentration was completely altered affective the values of MCV, MCH and MCHC. Altered MCV, MCH and MCHC values were observed in *Channa punctatus* under Cypermethrin toxicity. The decrease in hematological indices could be due to macrocytic hypochromia, iron deficiency and increased haemolysis (Gupta and Rajbanshi, 1979). The qualitative and quantitative haematological evaluations of present work show the similar trend and supportive decrease in RBC and Hb content in the present study are also comparable to those reported by Chen, (2002) for catfish *Clarias leather* exposed to phenol. The decrease in RBC and Hb concentration indicate acute anemia. The anemia could be due to the destruction of RBC (Waluga, 1966, b; Andres and Kurazhovskaia, 1969) triggered by the influx of phenol into the erythrocytes (Swift, 1978). In the present investigation, haemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. The fall in haematological parameters might be due to decreased rate of production and to an increased loss of destruction of RBC (Larsson, 1975). Another reason for RBC suppression could also be the damage to the haemopoietic tissue. PCV appeared to be positively correlated with RBC counts, hence, a decrease in PCV was observed. Similar results have been reported for several freshwater fishes exposed to pesticides (Khalaf Allah, 1999; Balathakur and Bais, 2000; Rehulka, 2000).
Authentic and less time consuming way for monitoring the genotoxic effects of pollutants and mutagens is to perform micronuclei test. There has been an increasing interest in the use of micronucleus test (MNT) as an index of cytogenetic damage in fish and other marine vertebrates and invertebrates (Al-Sabiti, 1994). Rahman et al., (1990) observed incidence of micronuclei test on *Oreochromis mossambicus* exposed to liquid water of Quinidin which produced alarmingly higher number of micronucleated erythrocytes, at lethal concentration as compared to control. Similar results were observed by Bahari and Noor, (1994) in *Clarius gariepinus* with anti cancer drug, Mitomycin-C. Various studies have shown that the peripheral erythrocytes of fish have a high incidence of micronuclei under laboratory conditions. Pesticides have been reported to lead to DNA damage which appears in the form of micronucleus formation, chromosome aberrations and mitotic aberrations (Kocaman and Topaktas, 2010; Sharaf *et al*., 2010; Sankar *et al*., 2010; Hussain *et al*., 2011, 2012). Poongothi *et al*., (1996) reported induction of micronuclei in five species of fishes from polluted sewage water and in fish exposed to heavy metals. Rahman and Khuda Bukhsh, (1992) carried out study on genotoxic potential of industrial effluent and some of their individual polluting components with reference to micronuclei of peripheral erythrocytes of the fish *Oreochromis mossambica*. Loprieno *et al*., (1992) studied micronuclei count on fisherman who ate fishes contaminated with mercury. Micronucleus appearance in the cytoplasm is considered as biomarker of DNA damage (Saleh & Sarhan, 2007). With the treatment of pyrethroids, micronucleus could result when the entire or chromosome fragments were not incorporated in the main nucleus after cell division (Sankar *et al*., 2010). Cypermethrin has mutagenic affect like other numerous pesticides (Sankar *et al*., 2010; Muranli & Guner, 2011). Due to exposure of these pesticides, micronuclei formation, chromosomal aberrations have been documented (Kocaman & Topaktas, 2010). Fastac 10% EC (a pyrethroid) in high
concentrations had reported to damage the mitotic spindle, clastogenic activity and amplified occurrence of micro-nucleated erythrocytes in tadpoles (Bosch et al., 2011). Micronuclei could also take place by the loss of complete or parts of chromosomes at anaphase from daughter nuclei and occur distinctly in the cell from the main nuclei (Sankar et al., 2010). Not only pyrethroids like Cypermethrin caused DNA damage (Muranli & Guner, 2011) but other insecticides like Malathion (organophosphate) also caused the same damage (Sarabia et al., 2009). Other than the above hypothesis of nuclear changes, these could be due to intracellular generation of reactive oxygen and nitrogenous species (Altuntas & Delibas, 2002). These nuclear abnormalities could also be due to over generation of caspase activated DNase which is responsible for the cleavage of cytoskeletal (gelsolin, fodrin & vimentin) and nuclear proteins (Banerjee et al., 2001). It is alluring to speculate that blebbed, notched and lobed nuclei could result from aneuploidy, i.e., a process leading to formation of chromosomal abnormalities (Cavas & Ergene-Gozukara, 2005). The comet assay is another method to observe damaged DNA which appears as a fluorescing material around the nuclei, making a tail of variable length along the electric field. These pesticides not only damage DNA in erythrocytes but also in hepatocytes, lymphocytes, and other cells in the body (Hussain et al., 2011; Cortes-Gutierrez et al., 2011). In present study micronuclei and Comet Assay test affected the alteration in DNA due to Cypermethrin exposure of *L. rohita*.

The karyotypes of *Labeo rohita* have been studied by different workers (Khuda-Bukhsh and Chakrabarty, 1994; Mujumdar and McAndrew, (1986); Zhang and Reddy, 1991 and Jana, 1993). All these workers reported the diploid number (2n) in these two species as 50. In catla, the karyotype according to Zhang and Reddy (1991) consisted of 12 metacentric, 16 submetacentric, and 22 sub-telocentric chromosomes. In Rohu, the karyotype according to these authors consisted of 10 metacentric, 18 submetacentric and 22 sub-telocentric chromosomes. Later study with regards to comparative
karyotype of these carps (Jana, 1993) also more-or-less agree with the observations of Zhang and Reddy (1991). Krishnaja and Rege, (1982) have also shown a significant increase number of chromosomal aberrations in *B. dussumieri* by Mitomycin-C, Hg, Se and Cr compounds. Gadhia *et al.*, (1990) reported effects of Cadmium Nitrate on metaphase chromosome of common carp *Cyprinus carpio*. Hence chromosomal aberrations and micronuclei test provide record of damage in genetic material which is very significant to access the toxicity of water and heredity is poorly understood (Denton, 1973).

Chromosomes are made up of genetic material present in the cell. Hence the damage in chromosomes is very important tool to study the damage in chromatin material at molecular level. Micronuclei are small incomplete nuclei originated from chromatin lags in anaphase thus acts as cytological indicator of damage in genetic material. Dichlorvos concentration of 0.01 ppm caused chromosomal aberrations in the form of centromeric gaps, chromatid gaps, chromatid breaks, sub-chromatid breaks, attenuation, extra fragments, pyknosis, stubbed arms etc in kidney cells of *Channa punctatus* after exposure periods of 24, 48, 72 and 96 h (Rishi and Grewal, 1995). Interestingly, there was an inverse relationship between duration of exposure and aberration frequency. Longer exposures to Dichlorvos were associated with lower frequencies of aberrations. The toxicity of Dichlorvos has also been related to alterations in DNA replication which causes mutations and cellular hyper proliferation as a result of local irritation (Mirsalis *et al.*, 1989; Oshiro *et al.*, 1991; Benford *et al.*, 1994). The Thiocarbamate pesticide malinate and vernolate have been reported to cause changes like SCE (sister-chromatid exchange) and chromosomal aberrations *in vitro* and increased frequency of polychromatic erythrocytes in mouse bone marrow cells (Pinter *et al.*, 1989). Different chromosomal aberrations, such as breaks, rings and dicentric chromosomes, have been detected in kidney cells after the
injection of three fish species (common carp, *Cyprinus carpio*, tench, *Tinca tinca*; grass carp, *Ctenopharyngodon idella*) with aflatoxins B, aroclor 1254, benzidine, benzo[a]pyrene and 20-methylcholanthrene (Al-Sabiti, 1985; Cajaraville et al., 2003). Das & John (1999) evaluated the effects of two organophosphorous pesticides, methyl parathion and phosphamidon on *Etroplus suratensis* using chromosomal aberrations as the genetoxicological test tool. Cypermethrin also significantly decreased the level of nucleic acids in the various tissues of the fish *C. fasciatus*. Several reports are available on the reduction in DNA and RNA level on exposure to different pesticides (Tarig et al., 1977; Nordenskjold et al., 1979). The present work also showed that the Cypermethrin generally possessed the specialized chemical properties which facilitated their interaction with nucleus and effect the DNA molecule and resulted in genotoxic effects in the form of chromosomal aberrations like acentric fragments, rings, chromosomal break, double minutes, endoreduplication, premature separation of chromosome and pulverizations.

Among the recent assays, single cell gel electrophoresis or called comet assay has immense use in the detection and evaluation of genotoxic compounds in several test systems (Singh et al., 1988; Collins, 2004; Pandey et al., 2006). Several reviews have been published on the acceptance of comet assay in monitoring the effects of several potent genotoxic agents on the DNA of different animals (Moller et al., 2000; Bolognesi, 2003; Collins, 2004). This assay has been carried out in fishes, such as bullhead (*Ameriurus nebulosus*) Common carp (*Cyprinus carpio*) (Pandrangi et al., 1995), brown trout (*Salmo trutta*) (Belpaeme et al., 1996), flounder (*Pleuronectes americanus*) (Nacci et al., 1996), rainbow trout (*Oncorhynchus mykiss*) (Devaux et al., 1999), butterfish (*Pholis gunnellus*) (Bombail et al., 2001), zebra fish (*Danio rerio*) (Schnurstein and Braunbeak, 2001) tilapia (*Tilapia mossambica*) (Banu et al. 2001) and *Channa punctatus* (Pandey et al., 2006).
In relation to the application of the comet assay in *P. lineatus* as a bio-indicator, results corroborate the researchers developed by another authors (De Campos Ventura *et al.*, 2008), which showed that the comet assay in fish erythrocytes seems to be efficient to detect the genotoxicity of chemicals. Damage in the present work represents an increase in DNA fragments that have migrated out of the cell nucleus, which form a distinctive comet tail. The tail length and DNA fragment contented in it are directly proportional to the amount of DNA damage. Comet assay has proved to be a useful tool for measuring the relationship between DNA damage and exposure of aquatic organisms to genotoxic pollutants (De Andrade *et al.*, 2004), being considered more sensitive than cytogenetic techniques to detect DNA damage. The evaluation of DNA damage in fish using the comet assay frequently involves the utilization of erythrocytes because of their ready availability and ease of collection. Interaction of genotoxic agents with DNA can form alkaline labile adducts and other modifications which can contribute to an increase level of DNA strand breaks via enzymatic removal of damaged nucleotides. The DNA fragmentation or DNA strand breaks are considered a kind of lesion potentially pre-mutagenic with the production of breaks in DNA strands being related to mutagenic and carcinogenic properties of chemicals (De Campos Ventura *et al.*, 2008). If not repaired, these DNA lesions can initiate a cascade of biological consequences at cellular, organic, individual, and finally at population and community levels. DNA damage in a variety of aquatic animals has been associated with reduced growth, abnormal development and reduced survival of embryos, larvae and adults. Several studies (Nacci *et al.*, 1996; Frenzilli *et al.*, 2009) confirm that environmental contaminants can affect the genetic material of wildlife species and several mechanisms have been proposed to link these exposures with DNA strand breakage and repair. The significance of malignancy incidence in wild species, however, needs to be considered in the light of emerging scientific priorities, where
humans are seen as part of the ecosystem. In this context, it has also emerged that increasing pollution could lead to higher incidence of cancer in the human population and concurrently, contribute to loss of biodiversity, the main aim of ecotoxicological studies (Jha, 2008). In the present study, the comet assay for one of the most common native fish species *L. rohita* was standardized. In view of the results obtained in this work concluded that the comet assay is an effective short-term test for in vivo monitoring of genotoxic effects in aquatic species and promising sentinel organism for the evaluation of substances potentially mutagenic, teratogenic and carcinogenic in aquatic environments.