2. REVIEW OF LITERATURE

Pesticides are well recognized as an economic approach in controlling agricultural pests but at the same time, these are responsible for pollution of our aquatic resources. These are carried into water bodies through surface runoff and alter the physico-chemical properties of water. Worldwide concern is growing over indiscriminate use of chemical pesticides that results in environmental pollution and also affects non-target species. Pesticides even in low concentration interfere with the metabolism of the organisms (Jindal and Kaur, 2015). When large quantities of pollutants are released there may be sudden effect as measured by large-scale immediate mortality of aquatic organisms. Lower levels of discharge may result in an accumulation of the toxicants in aquatic organisms. The end results, which may occur after the pollutants have passed through the environment and include reduced metabolism and damage to various organs. Prolonged exposure may cause a number of pathological and altered biochemical processes and changes in energy demands. The organisms can have energy expenditure to tolerate the toxicity and eventually they show pathological effects. Responses of aquatic organisms to pesticides are broad ranged depending upon the compound, exposure period duration and the exposed species. Literature has been reviewed on pesticide toxicity to fishes with special emphasis on morphological, behavioural, histopathological, ultrastructural, biochemical alterations and bioaccumulation in fish.

2.1. MORPHOLOGICAL AND BEHAVIOURAL STUDIES

Behavior is a sensitive measure of an organism’s response to stress including environmental contaminants. Noticeable changes in morphology and behavior can be found even at sublethal concentrations of chemicals. Since behavior is a link between physiological and ecological processes, it is a particularly important type of response. While much early research focused on avoidance, tremors, complex behaviors such as predator/prey interactions, burrowing, reproductive and social behaviors are much more relevant to ecological impacts. Behavioral toxicity techniques are suggested as useful in setting water quality standards for the aquatic organisms.
Mount (1962), Anderson (1968), Chebbi and David (2010) and Tilton \textit{et al.} (2010) opined that exposure to the toxicant caused the disorder of the central nervous system, the peripheral nervous system and especially the lateral line complex.

Van Duijun (1967) suggested that bulging of eyeball was due to the disturbance in hormonal equilibrium of hypophysis.

Anderson and Peterson (1969) reported that both the central and peripheral nervous systems were affected in the brook trout \textit{Salvelinus fontialis} exposed to sublethal concentrations of DDT. Various behavioural alterations observed in the fishes were rapid and jerky movements of the body and fins, an increased ventilation rate, movement towards the surface, and slow and backward swimming followed by convulsions.

The release of excessive mucous secretion has been observed in \textit{Cyprinus carpio communis} Linn. on exposure to carbaryl by Toor and Kaur (1974) and in \textit{Clarius batrachus} in response to endosulfan has been reported by Gopal \textit{et al.} (1981). As suggested by Pandey \textit{et al.} (1990) it may be due to the dysfunction of pituitary gland under the stress of toxicants.

Pimental and Goodman (1974) observed the loss of locomotor activity in goldfish, \textit{Carassius auratus} on exposure to DDT. Verma \textit{et al.} (1975) observed abnormal behaviour such as erratic swimming, excitation, jerky movements and loss of equilibrium. In \textit{Colisa fasciatus} exposed to lethal and sublethal concentrations of DDT and lindane. With the addition of some toxic chemical into the aquatic environment certain changes in the behaviour of aquatic organisms appeared (Marcucella and Abramson, 1978; Rath and Mishra, 1981; Brewer \textit{et al.}, 2001). They attributed these changes due to the stress conditions caused by the toxic substances.

Murty \textit{et al.} (1983) opined that the rate of opercular movements paralleled to that of oxygen uptake in \textit{Labeo rohita} and \textit{Mystus cavasius} got decline with increasing concentrations of fenitrothion.

Ravikumar and Gupta (1988) opined that both chlordane and malathion caused excitement in silver carp and common carp through the central nervous system, resulting in increased muscle tone which lead to increased oxygen consumption in the exposed fishes.
Ram et al. (1990) and Ram and Gopal (1991) studied various behavioral alterations in fishes upon exposure to the various toxicants.

Behavioural changes induced by the acute exposure of endosulfan to zebrafish (*Brachydanio rerio*) and yellow tetra (*Hyphessobrycon bifasciatus*) included hyperactivity, erratic swimming and convulsions (Jonsson and Toledo, 1993).

The findings of Anbu and Ramaswamy (1991) while working on *Channa striatus* on exposure to sublethal concentration of carbaryl revealed direct effect of the pesticide on the behaviour of the fish. The fish exhibited erratic and irregular movements with impaired swimming and also showed increased air gulping and opercular movements.

Alterations in behaviour due to carbofuran in *Carassius auratus* have been observed by Saglio et al. (1996). They reported that short-term exposure to low concentration (5 μg/l) of carbofuran can affect behavioral responses of fish both directly and indirectly by altering the chemical perception of natural substances of ecological importance.

Saglio and Trijasse (1998) studied the effect of atrazine and diuron on goldfish. A 24 hr exposure to 5 μg/l was found to induce significant behavioural alterations like decreased grouping behaviour and sheltering. Some fishes showed an increase in burst swimming reaction. They also inferred that both the pesticides affected the behaviour of fishes by altering their chemical perception for natural substances.

Sarikaya and Yilmaz (2003) observed anxiety, loss of balance, swimming upside down or vertical manner, sudden jerks, respiratory difficulties, lightening in color, excessive mucosal secretion, coming to the surface for breathing and hitting to the side walls of the aquaria in *Cyprinus carpio* on exposure to different concentrations of 2,4-D.

Jindal and Jha (2005) observed the impact of organophosphorous pesticide monocrotophos on the behaviour of *Cyprinus carpio* and noticed alterations including erratic swimming, increased mucous secretion and scale loss.
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Alteration in locomotor behaviour of fishes has been observed by various workers (Kane et al., 2004, Rao et al., 2005 exposed Gambusia affinis to MCP; Yildirim et al., 2006 exposed Oreochromis niloticus to deltamethrin; Ismail et al., 2009 exposed Cyprinus carpio to profenophos; Halappa and David, 2009 exposed Cyprinus carpio to CPF; Cyprinus carpio exposed to dimethoate, Singh et al., 2010).

Siang et al. (2007) determined the effect of acute toxicity of endosulfan on the behaviour of Monopterus albus and reported that after 96 h, the fish exhibited imbalanced position, restless movements, erratic swimming, flashing, tremor and lethargy.

Patil and David (2008) assessed behaviour dysfunction as an index of malathion induced toxicity in Labeo rohita and reported exposed fishes showed irregular, erratic and darting swimming movements, loss of equilibrium, hyper excitability and sinking to the bottom. They attributed the behavioural dysfunction due to inhibition of AChE activity which resulted in excessive accumulation of acetylcholine at cholinergic synapses leading to hyperstimulation.

Marigoudar et al. (2009) made investigations on cypermethrin induced behavioural alterations in Labeo rohita and reported that the carp in toxic media showed erratic, irregular and darting swimming movements, loss of equilibrium and hyper excitability attributed to inhibition of acetylcholinesterase enzyme activity.

Susan et al. (2010a) observed behavioural changes in Labeo rohita, Catla catla and Cirrhinus mrigala exposed to fenvalerate and reported anomalous behaviour like surfacing phenomenon, erratic, irregular and darting swimming movements, loss of equilibrium, hyperexcitability and hitting to the walls of the tank, rapid opercular movements, increased mucus secretion, higher ventilation volume and gulping of air at the surface.

Impact of different pesticides on the behavior of Labeo rohita have been studied by various workers (Anita et al., 2010; Banaee et al., 2011; Nagaraju et al., 2011). Their findings revealed that behavioral changes are the most sensitive indication of potential toxic effects.
Fishes exhibited a number of behavioral changes on exposure to different pesticides: neural paralysis, imbalance, abnormal swimming, severe impatience and paleness in body colour (Mishra et al., 2011; Far et al., 2012; Kamble et al., 2012).

Bhat et al. (2012 a, b) evaluated behavioural responses in *Labeo rohita* exposed to azadirachtin & biopesticide kethrin. The exposed fish exhibited erratic swimming, decreased rate of opercular movement, increased surfacing, reduced agility and inability to maintain normal posture and balance with increasing exposure period and concentration of the toxicant.

Nwani et al. (2013) worked on toxic effects of chlorpyrifos-based pesticide termifos on behavioural responses of *Clarias gariepinus* and reported abnormalities like erratic swimming movements, increased opercular movements, hyperactivity, surfacing to gulp air, secretion of copious amount of mucus and loss of balance.

Panigrahi et al. (2014) studied monocrotophos impact on behaviour of *Cyprinus carpio* and observed abnormalities like erratic swimming, loss of equilibrium, dispigmentation, coughing and opercular movement.

Das and Gupta (2014) exposed *Esomus danricus* to malathion and the exposed fish exhibited faded color along with copious mucous secretion, irregular, erratic and jerky movements.

On exposure of *Ctenopharyngodon idellus* to chlorpyrifos, Jindal and Kaur (2014a) reported that the fish showed erratic, speedy and jerky movements, hyperactivity, violent behaviour and jumping out of the tanks violently (escape behaviour). The intensity of altered behaviour was found to be related to toxicant concentration and time dependent.

As reported by Jin et al. (2015), after prolonged exposure of the fish to the toxicant, it becomes sluggish & showed escape tendency with decreased swimming activity. Similar behavioural changes on the impact of different pesticides on other fishes have been studied by various workers (Far et al., 2012; Huang et al., 2014; Walsh-Monterio et al., 2014; Naserabad et al., 2015).

2.2. HISTOPATHOLOGICAL STUDIES

Histopathology of fish tissues is a monitoring tool, which allows the assessment of the environmental stress in fish (Fernandes et al., 2008; Leonardi et al.,
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2009). A number of researchers have documented the histopathological alterations induced by different pesticides in various organs like gills, liver and kidney.

Loeb (1963) worked on the acute oral toxicity of thiodan to carp and reported that it got absorbed more easily across gills and oral membrane. Microscopic alterations of the fish liver induced by pesticides and related chemicals have been studied by Couch (1975). Dalela et al. (1979) made toxicological studies on exposure of Channa gachua to endosulfan and noticed histopathological changes in the gills. On acute exposure, there was separation of respiratory epithelium from basement membrane, pronounced hyperanemia, necrosis, fusion of adjacent secondary lamellae, erosion at the distal end of gill filaments and loss of basement membrane. Mallat (1985) in a review on toxicant induced changes in the gills, suggested that such changes tend to be largely non-specific and reflect physiological adaptation of the fish to stress.

Palarp and Ted (1987) worked on the histopathological effects of paraquat on gills of Puntius gonionotus. The morbid changes found in the gills were marked swelling of the secondary gill lamellae, especially under the basement membrane of endothelial and epithelial cells in which hydropic vacuolization were noticed. Gill et al. (1988) reported lesions in gills, liver and kidney of Puntius conchonius on exposure to varying concentrations of carbaryl and dimethoate. The pesticides induced wilting of pillar cells, occurrence of lamellar thrombosis, curling and ballooning of secondary lamellae and hypertrophy of chloride cells. They also reported degeneration of secondary lamellae due to oedema and lifting of epithelial lining. The curled up secondary lamellae revealed lysed blood cells in sinuses and degeneration of chloride cells in interlamellar crypts.

Rani and Ramamurthi (1989) and Braunbeck et al. (1990) revealed severe hepatic injury with reduction of cell size and glycogen depletion in toxicant treated fishes. Laurent and Perry (1991) considered the morphological changes in fish gills, as a consequence of environmental changes and as adaptive attempts in conserving some physiological functions. Bhatnagar et al. (1992) reported erosion of basement membrane, degeneration of gill lamellae, necrosis, clumping of blood cells and development of lacunae in the secondary gill lamellae of Clarius batrachus exposed to endosulfan.
Padgaonkar and Parab (1993) noted the histopathological changes in the fish, *Etroplus maculatus* (Bloch.) after exposure to acute and subacute concentrations of DDT. From their findings, they inferred that the gills, liver and kidney were the organs which have been most affected by the pesticide. Dutta *et al.* (1993) found the alteration in the size of hepatocytes in malathion exposed *Heteropneustes fossilis* (Bloch).

Bhatnagar *et al.* (1994) worked on the impact of malathion poisoning to *Clarius batrachus* and reported severe damage in the histological architecture of kidney, gills and liver of the fish. In kidney, apart from shrinkage of glomerular tuft, enlargement of nuclei and their migration towards the lumen of uriniferous tubules were observed.

Krishna and Ram (1994) and Banerjee and Bhattacharya (1994) reported the histopathological changes in the toxicant exposed kidney of *Channa punctatus* like hypertrophy, nuclear abnormalities, formation of vacuoles, necrosis, shrinkage of glomeruli and swollen capsules.

Oulmi *et al.* (1995) assessed the effects of linuron on kidney and liver of rainbow trout *Oncorhynchus mykiss* and observed the presence of small cytoplasmic vacuoles and nuclear deformities in the epithelium of the proximal tubules of the kidney. Vijayalakshmi and Tilak (1996) investigated the impact of pesticides on the gill morphology of *Labeo rohita* and noticed necrotic changes and alterations in the shape of filaments, as a result of direct contact with the pesticide. Fenvalerate intoxication caused atrophy of the gill filaments, and fusion and atrophy of secondary gill lamellae when exposed to the mixture of monocrotophos and fenvalerate. Noor Alam *et al.* (1996) studied the impact of metacid-50 in the fingerlings of carp *Labeo rohita* and reported histopathological alterations affected adversely respiration and general metabolic, renal, osmoregulatory and digestive activities.

Neskovic *et al.* (1996) conducted studies on histopathological changes in gills, liver and kidney of *Cyprinus carpio* on exposure to roundup. Major alterations included epithelial hyperplasia, sub-epithelial oedema and increase in mucous secretion. Light microscopic studies exhibited severe histopathological changes in the kidney, liver and gills due to the impact of the pollutants in *Channa punctatus* (Anitha and Sree Ram, 1997).
Visoottiviseth et al. (1998) noted various alterations in the histology of the liver of Nile tilapia, *Oreochromis nilotica* upon exposure to triphenyltin hydroxide. The hepatocytes showed variety of changes from congestion and dilation of sinusoidal spaces to vacuolation, pallor of cytoplasm and accumulation of hyaline droplets.

Oliveira-Ribeiro et al. (2000) noted the adverse effects of tributyltin in tropical freshwater fish, *Astyanax bimaculatus*. They investigated the morphological effects on the liver by using light microscopy and transmission electron microscopy.

Bhuiyan et al. (2001) used histology as a tool to detect the alterations in tissues of *Channa punctatus* after exposure to sumithion. Among the different alterations, liver tissue revealed liver cord disarray, vacuolization of cytoplasm, necrosis, hypertrophy of hepatocytes and their nuclei, and at the higher concentration of pesticide, rupturing of blood vessels occurred. According to Schmidt-Posthaus et al. (2001), the polluted river water induced some morphological organ alterations, histological changes in liver like distended sinusoids and separation of liver cells and infections in various tissues (liver, kidney, gills) of brown trout, *Salmo trutta* and rainbow trout, *Oncorhynchus mykiss*.

In secondary lamellae, Santhakumar et al. (2001) noticed gill lesions in perch, *Anabas testudineus* exposed to monocrotophos. Histopathological changes observed in the gills were hemorrhage in the primary and secondary gill lamellae, degeneration and necrosis of epithelial cells. Tilak et al. (2001) noticed histopathological alternations in gills, liver and kidney of *Ctenopharyngodon idella* (Valenciennes) on exposure to fenvalerate like necrosis, vacuolar degeneration and dystrophy. Jiraungkoorskul et al. (2001) investigated the histopathological effects of round up, a glyphosate herbicide on gills of Nile tilapia (*Oreochromis niloticus*). Major alterations observed were the thickening of primary lamellae, oedema with lifting of secondary lamellar epithelium and leukocyte infiltration. While studying the effect of malathion on *Oreochromis niloticus* Elezaby et al. (2001) observed that the pesticide induced many histopathological changes in the liver and gills of the fish which includes hemorrhage, necrosis and destruction of lamellae of the gills and necrosis and lipidosis in the liver of the fish.
Khare et al. (2002) worked on the histopathological alterations in different parts of gills of *Nandus nandus* induced by endosulfan and carbaryl. Sakthivel and Gaikwad (2002) reported the tissue histopathology of *Gambusia affinis* (Baird and Girard) under dimecron toxicity. Long term exposure for 30 days to sublethal concentration 0.0068 mg/l of the pesticide on some tissues like alimentary canal, liver, kidney and gills were studied.

Rahman et al. (2002) observed migration of nuclei, pycnosis and necrosis in the liver of fish, *Anabas testidinieus*, *Channa punctatus* and *Barbodes gonionotus* when exposed for 7 days to sublethal concentrations of diazinon 60% EC. Oliveiro-Ribeiro et al. (2002) studied the toxicity of inorganic mercury and methyl mercury on the liver, gills and kidney of Arctic charr, *Salvelinus alpinus*. Liver was little affected by water-borne and single-tropical-dose contamination of inorganic mercury, but dietary methyl mercury had drastic effects have been noticed with severe necrosis and alterations of cytoplasmic organization.

Fanta et al. (2003) studied the impact of sublethal dose of organophosphate methyl parathion on *Corydorus paleatus* and observed histological changes like cloudy swelling, atrophy, vacuolization, bile stagnation and focal necrosis after contamination. Saravanan et al. (2003) exposed *Oreochromis mossambicus* to sublethal concentration of the insecticide endosulfan for 30 and 60 days and exhibited changes like cell vacuolization, histolysis and necrosis, and changes became prominent after 60 days exposure. Machado and Fanta (2003) reported effects of the organophosphate pesticide, methyl parathion on the branchial epithelium of a freshwater fish *Metynnis roosevelti*. Through light and scanning electron microscopy, shrinking of the branchial epithelium, followed by detachment and hyperplasia were observed.

Cengiz and Unlu (2003) made histopathological studies on the gills of mosquito fish, *Gambusia affinis* after the long term exposure to sublethal concentration of malathion. The various observations included were necrosis and desquamation of secondary lamellar epithelium, lifting up of epithelium, intraepithelial oedema, fusion of adjacent secondary lamellae, hemorrhage at primary lamellae, disorganization and rupture in secondary lamellae, hypertrophy and hyperplasia of epithelial cells.
Histopathological alterations in the gills of *Anabas testudineus* reported by Jain (2004) on long term exposure to sublethal concentration of carbaryl, were complete degeneration of interlamellar region and necrosis in the gill lamellae.

When the zebrafish, *Danio rerio* was exposed to the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most pronounced histological changes included were lipidosis and hypertrophy in the liver hepatocytes (Zodrow et al., 2004). Sakr and Al Lail (2005) reported the histopathological and histochemical changes in the liver of catfish, *Clarias gariepinus* under the effect of fenvalerate. The results showed vacuolization of hepatocytes, blood vessel congestion, necrosis and fatty infiltration.

Shukla *et al.* (2005) noticed that the hepatocytes of *Clarias batrachus* exhibited reduction in their size with peripheral accumulation of cytoplasm on exposure to nuvan. The nuclei of the hepatocytes lost their rounded shape and the cell boundaries became obliterated at some places. They also stated that the hemorrhage in liver was evident with increased volume of sinusoidal space.

Tilak *et al.* (2005) reported that chlorpyrifos intoxication caused vascular degeneration, cloudy swelling, necrosis and degenerative changes in epithelial and pillar cells of the gills of *Catla catla*. They also stated that club shaped lamellae led to progressive degeneration in the gills.

Yildirim *et al.* (2006) noted the histological changes in the liver of Nile tilapia, *Oreochromis niloticus* on exposure to deltamethrin. Vel murugan *et al.* (2007) made studies on lambda-cyhalothrin induced histological changes in the liver of *Cirrhinus mrigala*. The changes observed were hypertrophy of hepatocytes, dilation of sinusoids, necrosis, cloudy degeneration and congestion. The impact of deltamethrin on liver of *Carassius auratus* was responsible for various histological changes like enlargement of sinusoids in the liver of the fish (Staicu *et al.*, 2007).

By doing histological studies, Crestani *et al.* (2007) studied the effect of chomazone on the liver of silver fish, *Rhamdia quelen*, as well as its recovery pattern was also studied. Their studies revealed vacuolization and necrosis in the liver. Similar results were reported (Ayoola, 2008) in *Oreochromis niloticus* under the
effect of glyphosate. It also included extensive pycnosis, involution darkly stained necrotic nuclei and infiltration of leukocytes.

Peebua et al. (2008) reported the histological changes in acute and sub-chronic alachlor exposed fish Oreochromis niloticus. Liver showed hydropic swelling of hepatocytes and vacuolization. Lipid vacuoles were observed in hepatocytes in 2\textsuperscript{nd} and 3\textsuperscript{rd} month of subchronic exposure.

Pugazhvendan et al. (2009) studied the effect of malathion toxicity in Ophiocephalus punctatus and noticed severe degenerative histological changes in liver including hypertrophy of cells and their nuclei and necrosis.

Parikh et al. (2010) made histoarchitecture studies on various organs of Oreochromis mossambicus exposed to dimethoate. They reported vacuolar degeneration, swelling in the hepatocytes with indistinguishable cellular outline in the liver of the fish. The alterations found in the kidney of the fish were glomeruli enlargement and oedema in Bowman’s capsules at low concentration, and at high dose of the toxicant, it exhibited vacuolar degeneration accompanied with hemolysis. The gills of the fish revealed curling and clubbing of secondary lamellae (telangiectasis), enlargement of primary lamellae and loss of secondary lamellae.

Prashanth (2011) assessed the histological damage caused to the fish Cirrhinus mrigala exposed to cypermethrin. The alterations include focal necrosis of tubular cells, pycnotic nuclei, degenerated cells extruding into the lumen of tubules, focal degeneration of tubular cells followed by severe necrosis of the whole nephron and leucocytes, and appearance of macrophages surrounding the tubules.

Paithane et al. (2012) studied dimethoate induced histopathological changes in the liver of Channa punctatus and reported cell necrosis, swelling of hepatocytes with nuclear hypertrophy, rupture of sinusoids with hemorrhage at several places and vacuolated hepatocytes. At some places degeneration of nuclei of hepatocytes was also observed. They inferred from their studies that histology of fishes could serve to be sensitive monitoring tool to aquatic health.

Olufayo and Alade (2012) investigated the acute toxicity and histological changes in gills, liver and kidney of catfish, Heterobranchus bidorsalis exposed to
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cypermethrin. The physical reactions observed in the fish were discolorations of the skin, erratic swimming, loss of reflexes, hyperactivities, surfacing, and these effects were toxicant concentration and exposure dependent. The histological examination of the gills, kidney and liver of the fish after 96 hr showed alterations such as RBC’s infiltration, inflammation, vacuolation and necrosis. Deka and Mahante (2012) reported the effect of organophosphorus pesticide malathion on hepato-renal and reproductive organs of Heteropeustes fossilis (Bloch). According to Khan and Sharma (2012), the kidney of Gambusia affinis exhibited various histoarchitectural alterations after exposure to chlorpyrifos which include vacuolated epithelial cells, shrinkage of uriniferous tubules and hypertrophy with pyknotic nuclei of the cells in the renal tubules. These alterations were found to be time and dose dependent.

Banaee et al. (2013) investigated the effect of sub-lethal toxicity of paraquat on the histopathology of gill, liver and spleen of gourami (Trichogaster trichopterus). The changes in gills were characterized by epithelial hypertrophy and oedema in secondary gill lamella. The liver showed hypertrophy of hepatocytes, cloudy swelling and formation of cytoplasmic vacuoles.

Vidhya and Nair (2013) noticed increase in mucus, dilation of primary and secondary gill lamellae, widening of secondary gill filaments, vacuoles, curved secondary lamellae, lamellar necrosis, oedema, ballooning of gill filament and hyperplasia in the gills of Etroplus suratensis exposed to lambda-cyhalothrin.

Theurkar et al. (2014) studied the impact of monocrotophos on the gills of Gambusia affinis. Architectural changes observed were bulging of lamellae, structural disorganization of primary gill lamellae, fusion and destruction of secondary gill lamellae, hyperplasia, excess of mucus secretion and disorganization of gill lamellae. The treated fish depicted disturbance in proper gaseous exchange and osmoregulation. The epithelial lesions observed on the respiratory surface might be due to collapse of pillar cells, increasing number of mucous openings and mucus secretions. They also reported clubbing, hyperemia and oedema.

Subashkumar and Selvanayagam (2014) studied the acute toxicity and gill histopathology of Cyprinus carpio exposed to Zn. They found direct correlation with the toxicant concentration and exposure period in causing histopathological changes,
and alterations like hyperplasia of epithelial cells, epithelial lifting and lamellar fusion which may decrease the contact of toxicants with the vascular system of the gill, resulting in impairment of respiration as well as fish health.

Barbhuiya and Dey (2014) made investigations on histopathological changes in the liver of *Heteropneustes fossilis* exposed to chlorpyrifos. The liver of treated fish showed congestion of central vein, necrosis and pycnosis of hepatocytes, cytoplasmic vacuolization and thrombosis in hepatoportal blood vessel. Haemorrhage around the central vein and haemosiderosis was also observed.

Al-Mamoori *et al.* (2014) investigated the toxic effect of chlorfos on the gills and liver of *Cyprinus carpio*. The histological alterations of gills observed were partial lamellar deformation, marginal dilation and hyperplasia of epithelial cells, while liver appeared with marked focal infiltration of lymphocytes, hepatocytes degeneration, increased sinusoids, and marked degeneration with necrosis especially at chronic exposure. Similar observations have been made by Ghasemzadeh *et al.* (2015) in gill of *Scatophagus argus* exposed to diazinon and Alim and Matter (2015) in *Oreochromis niloticus* exposed to biopesticide.

Focal necrosis, pycnotic nuclei, cytoplasmic vacuolization in tubular epithelium, expansion & dwindling of tubular lumen & shrinkage of glomerulus have been reported in different fishes (*Oncorhynchus mykiss* exposed to diazinon, Banaee *et al.*, 2013; *Labeo rohita* exposed to dimethoate, Dey & Saha, 2014, *Cyprinus carpio* exposed to glyphosate, Ma *et al.*, 2015).

### 2.3. ULTRASTRUCTURAL STUDIES

The ultrastructural studies involve the study at surface morphology and organelle level. The organelle level involves the change in nucleus, mitochondria and rough endoplasmic reticulum etc. (Baker, 1969; Trump *et al.*, 1975; Jindal and Kaur, 2015). For the studies at cellular or tissue level, ultrastructural analysis appears to be a very sensitive parameter (Hinton *et al.*, 1992; Johal *et al.*, 2007). Altered morphology structure is the expression of the disease (Meyers and Hendricks, 1985; Klontz, 1985; Ferguson, 1989; Hayes, 1994).
Scales are widely used for classification, identification and growth studies in fishes (Chu, 1935; Lagler et al., 1977; Hecht, 1980; Johal and Tandon, 1989). Later these have been used as the indicators of toxic substances (Johal and Sawhney, 1997).

Lanzing and Higginbotham (1974) made SEM studies on the structure of scales of *Tilapia mossambica*. They reported that the cerculli have numerous minute processes called as scaler denticals which helped the scales to have firm position in the dermal tissues. They also observed that the denticles appeared to curve towards focus of the scales.

Ishikawa *et al.* (1975) made electron microscopic observations on diethyl nitrosamine exposed fish *Oryzias latipes* and reported an extensive rough endoplasmic reticulum in lamellar pattern, number of mitochondria, several lysosomes in tumour cells and inconspicuous golgi apparatus. Goldblatt and Gunning (1984) studied the ultrastructure of liver and reported that the normal polyhedral hepatocyte has numerous organelles such as mitochondria, peroxisomes, lysosomes and rough and smooth endoplasmic reticulum, whereas toxicant induced liver showed changes such as proliferation of smooth endoplasmic reticulum, cholestasis and 2 types of parasinusoidal cells. From these studies, they inferred that the liver receives nutrient from sinusoidal lumen across a fenestrated endothelium which is separated by space of Disse’s from the plasma membrane.

Macchiarelli and Motta (1986) used SEM technique to describe the microstructure of liver and showed that the peritoneal surface is composed of mesothelial cells possessing microvilli and cilia. They also observed that kupffer cells and sinusoidal epithelial cells showed small proliferations.

Hollander (1986) made microanalysis of scales of poecilid fishes and reported a wide range of structural variation in the dentition on the crust of cerculi. He named the teeth like structures as lepidonts, whose shape, size, and angle in relation to scale body, and the type of attachment have significance for classification purposes.

Using SEM technique, Prasad (1991) studied on the effects of crude oil on the gills of air breathing organs of climbing perch, *Anabas testudineus* and reported that gills showed swelling of epithelial cells, increase in mucous secretion and filling up of...
interlamellar space with mucous cells. Pathological alterations such as lesions in the epithelial layer, hypertrophic mucous cells and sloughing of the epithelial layer were also observed.

Roncero et al. (1992) made ultrastructural studies on the liver of adult trench, *Tinca tinca* L. after exposure to copper sulfate. They observed that lesions were characterized by the accumulation of large amount of haemoglobin pigment in the cytoplasm produced by intense haemolysis, initially in kupffer cells and later in hepatocytes. Massive necrosis of liver parenchyma was also noticed.

Johal and Dua (1994) proposed the use of fish scales as an indicator to pesticide pollution. Further, they reported that upon exposure of *Channa punctatus* to sublethal concentration of organophosphorus pesticide endosulfan, the scale showed disorganization of calcareous material, and thus, resulted in the detachment of scales from the body. Johal (2001) made identical observations on scales of *Channa punctatus* & reported the damage done to the circuli & lepidonts. Coban et al. (2013) stated that scale deformations were in focus region & increased with increasing toxicant concentration.

Pfeiffer et al. (1997) investigated electron microscopic perspectives of gill pathology induced by 1-naphthyl-N-ethylcarbamate in the goldfish (*Carassius auratus* Linnaeus). The studies revealed secondary lamellar fusion, distortion and mucus release. TEM responses included enlargement of sub-epithelial lymphatic spaces and mitochondrial disruption and distortion of the lamellar epithelium. Pillar cells became detached and chloride cells were vacuolated.

Eiras-Stofella and Charvet-Almeida (1998) investigated ultrastructure of the gills of *Prochilodus scrofa* Steindachner (Pisces, Teleostei). Their studies revealed that abundance of mucous secretory cells in between pavement cells of the primary lamellae and branchio-pharyngeal region, indicated the existence of a protection strategy of the respiratory lamellae.

The investigations made by Braunbeck and Appelbaum (1999) showed ultrastructural alterations in hepatocytes and enterocytes of common carp, *Cyprinus carpio*, induced orally by low doses of endosulfan. The liver depicted enlargement of
nucleolus, increase in size of golgi fields and number of rough endoplasmic reticulum lamellae, proliferation of peroxisomes and lysosomes under exposure.

Sawhney and Johal (2000b) made studies on the erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch). A prolonged exposure of malathion decreased RBC count. The scanning electron microscopic study showed various changes in RBCs such as contraction of the cells, oozing out of cytoplasm from the cells with projections, cytoplasmic blebbing and disruption of cell membrane. These changes had a direct relation with the concentration of toxicant and exposure period.

Wilson and Laurent (2002) reviewed the microscopic anatomy of the branchial epithelia from representatives of the major groups of fishes and concluded that chloride cells are generally characterized by a high mitochondrial density and an amplification of the basolateral membrane through folding or the presence of an intracellular tubular system.

Thophon *et al.* (2004) studied the impact of cadmium on the ultrastructural alterations in liver and kidney of white seabass, *Lates calcarifer*. They observed changes in hepatocytes like mitochondrial congestion, swelling and lysis, and rough endoplasmic reticulum showed dilation, fragmentation and vesiculation.

Effect of carbaryl was observed on the erythrocytes of *Channa punctatus* (Bloch) by Johal and Grewal (2004). The erythrocytes exhibited different shapes due to protrusion of cytoplasm, hole like depressions and swelling or shrinkage of erythrocytes.

Segnini de Bravo *et al.* (2005) studied effects of herbicide on the kidneys of two Venezuelan cultured fish: *Caquetaia kraussii* and *Colossoma macropomum*. Kidney tubule alterations included loss of plasmalemma and cell interdigitations, misshaped mitochondria, decrease in rough endoplasmic reticulum and free polysomes, and the presence of autophagic vacuoles. They also correlated these alterations with the fish behaviour.

Jindal and Jha (2005) made SEM studies to see the impact of monocrotophos on the gills of the *Cyprinus carpio*. They reported swollen microridges due to the
presence of mucous and beaded appearance were the main alterations in the primary gill filaments and gill rakers.

Jindal and Rani (2005) reported alterations in morphology of erythrocytes of *Cyprinus carpio* var. *communis* upon exposure to methyl parathion. They observed increase in the erythrocyte count at low toxicant concentration and vice-versa. They also noticed significant changes in the structure of erythrocytes using SEM. Serezli *et al.* (2011) & Gupta & Poddar (2014) also reported similar alterations in morphology of red blood cells of toxicant exposed fishes. Similar observations have been reported by Jindal & Kaur (2014b) in erythrocytes of *C. idellus* exposed to chlorpyrifos.

According to Johal *et al.* (2007), the ultrastructural changes in the gills of *Cyprinus carpio* due to the toxic exposure of monocrotophos were thinning of microridges, upliftment of epithelial cells, development of hyperplasia, decrease in the density of mucous cells serving as first line of defense and also total dystrophy of epithelial tissue.

Giari *et al.* (2008) studied ultrastructural alterations in the hepatocytes and enterocytes of *Dicentrarchus labrax* L. exposed to mercury. These were hydropic cell swelling, alterations in endoplasmic reticulum and mitochondria, and abundance of myelinoid bodies. They also reported significant increase in hepatocyte number. Scaff and Scussel (2008) made ultrastructural and histochemical analysis of channel catfish (*Ictalurus punctatus*) liver treated with fumonisin B1. They reported that the liver presented a several response with an accentuate tissue disorganization, absence of cell limits and intense cytoplasm vacuolization. De Melo *et al.* (2008) used light and transmission electron microscopy to study hepatic changes in *Rhamdia quelen* under the impact of folidol 600 and these include loss of cellular contour, pycnotic nucleus and decharacterization of endothelium and of rough endoplasmic reticulum. Apoptosis was observed as the cytoplasm contracted and the chromatin formed masses concentrated in the edge of the nucleus.

Abdel-Moneim and Abdel-Mohsen (2010) studied ultrastructure changes in hepatocytes of catfish *Clarias gariepinus* from Lake Mariut, Egypt. Fish hepatocytes from the polluted area showed accumulation of the heterochromatin, enlarged
nucleoli, and an extremely folded nuclear envelope. Perichromatin granules got increased and progressively formed small clusters closely associated with patches of heterochromatin. In the cytoplasm, fractionation, dilation, vesiculation of rough endoplasmic reticulum (RER) and elevated amounts of smooth endoplasmic reticulum (SER) tubules were noted.

Mir et al. (2011) investigated ultrastructural analysis of liver of the snow trout, *Schizothorax curvifrons* and reported that the hepatocytes possess a centrally located nucleus with distinct nucleolous, cristae of granular endoplasmic reticulum are arranged in a parallel array and mitochondria are varying in shape from circular to elongate.

Braich and Jangu (2012) reported individual breakage in the lepidonts as well as their uprooting from the circuli, with lesions and cracks on the scale of the fish.

Panagiotis et al. (2014) did scanning electron microscopy of *Carassius gibelio* hepatocytes under toxic conditions. They found damages and holes on the plasma membrane, separation of hepatocytes from the sinusoids by the space of Disse.

Jindal and Kaur (2014b) while working on *C. idellus* exposed to CPF, found phenotypic alterations in erythrocytes. These include spherocytes, acanthocytes, echinocytes, ovalocytes and discocytes, and stated that the change in erythrocyte can be due to altered lipid microenvironment of the cell.

Lee et al. (2014) studied ultrastructural changes in the liver of *Cyprinus carpio* induced by zinc oxide and reported presence of black granules of various sizes in the lysosomes, indistinct nuclear membrane and non-spherical nucleus. They also observed noticed mild changes in the size and number of the lysosomes in the renal tubule and vacuolization in cytoplasm of MRC in gills of *C. carpio*.

Jindal & Kaur (2015) made SEM studies on the scale of *Ctenopharyngodon idellus* exposed to chlorpyrifos & reported disrupted circuli & disorganized margins of scale at lower toxicant concentration, whereas at higher concentration they found extreme disruption at the base of circuli along with disorganized calcareous material.
2.4. BIOCHEMICAL STUDIES

A number of studies have been made on the toxicity of different pesticides conducted on physiological and biochemical responses fishes by causing variations in proteins, glucose and cholesterol levels. The biochemical parameters are very useful to evaluate the fish health conditions after exposure to pesticides.

A decrease in protein content was reported in the liver of *Channa punctatus* on exposure to malathion and carbaryl (Saxena *et al.*, 1989) and in *Oreochromis mossambicus* treated with endosulfan (Ganesan *et al.*, 1980). Dose-dependent depletion of protein content in *Barsillus bendelisis* exposed to thiodon was reported by Deoray and Wagh (1991).

In various studies with administration of organophosphates, glucose level increased as a general response (Srivastava, 1981; Singh and Srivastava, 1982; Mishra and Srivastava, 1983; Natarajan, 1989; Gill *et al.*, 1990; Balint *et al.*, 1995).

Shakoori *et al.* (1996) studied the effects of sublethal doses of fenvalerate administered continuously for four weeks on the liver and muscles of a freshwater fish, *Ctenopharyngodon idella* and found a decrease in protein in both the tissues.

Das and Mukherjee (2000) worked on the chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* and noticed that level of protein was inhibited in the kidney and liver of the fish.

Tiwari (2004) exposed *Cirrhinus mrigala* to malathion for 7, 14 and 21 d and observed decrease in total proteins, and an increase in free amino acids levels on 7 and 14 day exposure.

Begum (2004) studied the level of some enzymes of protein and carbohydrate metabolism in liver and muscle of *Clarias batrachus* on exposure to carbofuran exposure and reported a delayed decrease in total protein in liver and muscle, and found recovery in protein content by the end of the recovery period.

El-Sayed *et al.* (2007) reported that deltamethrin treatment caused adverse effects in the form of hypoproteinemia, hypoglycemia in *Oreochromis niloticus*. They attributed these alterations to hepatic dysfunction and reflected immunosuppressive effect of the pesticide.
Studies have shown that variation in protein and carbohydrate metabolism have occurred in fishes that are in toxicants induced stress condition (Crestani et al., 2007; Fonseca et al., 2008; Sancho et al., 2010).

Langiano and Martinez (2008) determined the toxicity of roundup to *Prochilodus lineatus* and observed an increase in glucose in fish exposed to 10 mg L$^{-1}$ of the herbicide, indicated typical stress response of the fish against the toxicity.

Saha and Kaviraj (2009) studied the effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish, *Heteropneustes fossilis*. They found that stress on the fish was evident from the rise of glucose and reduction in glycogen level.

Karami-Mohajeri and Abdollahi (2011) reviewed toxic effects of organophosphates, carbamates and organochlorines on cellular metabolism of lipids, proteins, and carbohydrates. Their study indicated that OP and CB pesticides impair the enzymatic pathways involved in metabolism of fats, protein and carbohydrates.

The hepatocytes convert glucose to glycogen. Hepatocytes also synthesise glucose via gluconeogenesis and release stored glucose via glycogenolysis (Gharaei et al., 2011). Because glycogen reserves in the liver of fish under stress are used as emergency energy supplies, changes in the glycogen levels in the set issues could indicate changes in the health status of fish populations (Cicik and Engin, 2005).

Narra et al. (2011) studied the toxic effect of CPF on protein metabolism of *Clarias batrachus*. Total protein and amino acid got decreased in all tissues for 28 days and recovery was observed.

Al-Kahtani (2011) studied effect of on biochemical parameters of *Oreochromis niloticus* and observed total protein, carbohydrate and cholesterol levels in liver, muscle, kidney and gills of the fish. Similar observations have been made by jagadeesan and Darcus (2012) on *Catla catla* exposed to profenophos.

Magar and Shaikh (2013) made investigations on biochemical alterations in liver and gills of *Channa punctatus* treated with malathion and found reduction in proteins and amino acids content during 96 h exposure.
Nagaraju and Rathnamma (2013) made examination on toxic effect of sublethal concentrations of profenofos on protein metabolism in liver, kidney and gills of *Labeo rohita*. They found minimum protein depletion (13.84%) in kidney, and maximum (24.21%) in Liver after 1 d exposure; minimum depletion (9.19%) and maximum (22.38%) in gill after 4 d; and maximum (19.34%) in liver after 8 days exposure of the toxicant.

Binukumari and Vasanthi (2013) studied the toxic impact of dimethoate on the protein metabolism in liver, kidney and gills of *Labeo rohita* and they reported a decline in protein content in all the tissues. Further maximum decrease of protein in gills was attributed to possible utilization of its products for metabolic purpose.

Recent studies indicate that pesticides significantly decrease the total protein in the tissues (Palanikumar *et al.*, 2014; Harabawy and Ibrahim, 2014).

According to Bibi *et al.* (2014) total protein content in different tissues of the fish decreased with increasing concentration of lambda-cyhalothrin and observed highest decline in liver.

### 2.4.1. MARKER ENZYME ACTIVITY

Enzyme inhibition has been suggested to evaluate the impact of organophosphates and carbamates in aquatic ecosystems as biological indicators in prevention of deleterious effects (Rand and Petrocelli, 1985; Heath, 1987; Boudou and Ribeyre, 1989; Dutta *et al.*, 1995 and Luskova, 1997). Evaluation of enzyme activities in the organs of aquatic organs in the diagnosis of the effects of pollutants is one of the emerging areas in toxicological monitoring and remediation programmes (Oluah *et al.*, 2005). Enzyme evaluation is widely used for rapid detection to predict early warning of pesticide induced toxicity (Dutta and Arends, 2003) and it has become an important tool to monitor the environmental exposure of the fish to contaminants (Hinton and Lauren 1990; Deviller *et al.*, 2005; Fernandes *et al.*, 2008; Carrola *et al.*, 2009).

Aspartate aminotransferase and alanine aminotransferase are known to play a important role in mobilising L-amino acid for gluconeogenesis and also function as links between carbohydrate and protein metabolism under altered physiological,

The exposure of methyl parathion to *Tilapia mossambica*, for 48 hr decreased the activity of succinate and lactate dehydrogenase in the gill, liver and muscle tissues (Rao and Rao, 1979). Sastry and Sharma (1981) showed that the activity of alkaline phosphatase, ATPase and lactate dehydrogenase remained unchanged in the brain of *Ophiocephalus punctatus* after 15 days of exposure to an organophosphate pesticide.

Tripathi and Shukla (1990) have shown that an exposure of methyl parathion and endosulfan caused a decline in the efficiency of TCA cycle and the anaerobic glycolytic pathway as reflected by the reduced activity of malate dehydrogenase and lactate dehydrogenase in the liver of *Clarias batrachus*.

Das and Mukherjee (2003) evaluated the toxicity of cypermethrin in *Labeo rohita* fingerlings: biochemical, enzymatic and haematological consequences and reported decreased RNA levels while elevated DNA levels, unchanged acid phosphatase while, depleted alkaline phosphatase. They also reported that brain acetylcholinesterase activity got decreased significantly over a period of 45 days. Lactate dehydrogenase activity in brain and liver was elevated, but inhibited in kidney. Succinate dehydrogenase and ATPase activities were depleted in brain, kidney and liver.

Tripathi *et al.* (2003) studied toxic effects of dimethoate on metabolism and enzyme system of *Channa punctatus*. They concluded that carbohydrate and nitrogenous metabolism were significantly affected due to the hypoxic conditions occurred on exposure to the pesticide, as total protein, nucleic acids (DNA and RNA), glycogen, pyruvate levels and cytochrome oxidase activity got significantly decreased, while total free amino acids and lactate level and lactic dehydrogenase activity increased after the sub-lethal exposure.

There are a number of reports on the variation in enzyme activity of the organs of fish exposed to various toxicants (Velisek *et al.*, 2006a; Gabriel *et al.*, 2010; Kumaran *et al.*, 2011). In some of these studies exposure to pesticides caused either a significant increase or decrease in the enzyme activities.
Rao (2006a) studied the toxic effects of novel, an organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Exposure (time) dependent increases in alanine aminotransferase (ALAT), and aspartate aminotransferases (ASAT), acid phosphatase (ACP), and alkaline phosphatase (AkP), activities in plasma and kidney; ACP and AkP activities in gill were noticed. However, significant decrease in ALAT, ASAT, ACP, and AkP activities in liver was observed. Depletion of glutathione (GSH) was observed in the above tissues, thereby enhancing the lipid peroxidation resulting in cell damage.

Rao (2006b) investigated biochemical alterations in euryhaline fish, *Oreochromis mossambicus* exposed to sub-lethal concentrations of monocrotophos. He reported that ALAT, ALP, ACP and AAT activities got increased in plasma and kidney, where as liver and gill showed decreased activity. They also reported decrease in LDH activity, GSH level and increased GST activity in liver.

Velisek *et al.* (2006b) studied the effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). The experimental group showed significantly higher values of plasma ammonia, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, lactate and significantly lower values of alkaline phosphatase as compared to that of control group. Teleangiaictasia of secondary gill lamellae and degeneration of hepatocytes were observed with histopathological examination.

Ramesh and Saravanan (2008) studied haematological and biochemical responses in a freshwater fish *Cyprinus carpio* upon exposure to chlorpyrifos.

According to Gaafar *et al.* (2010) eight weeks exposure to 1/10th of 96 hr LC50 (0.1 ppm) of edifenphos led to adverse effects on AST, ALT, ALP, cholinesterase activity and total protein.

Susan *et al.* (2010b) investigated the variations in the distribution of biochemical constituents in the five major tissues viz., liver, muscle, kidney, brain and gills of the two carps, *Labeo rohita* and *Cirrhinus mrigala* exposed to sublethal and lethal concentrations of the technical grade pyrethroid, Fenvalerate. Elevation or depression in the levels of glycogen, total proteins, free amino acids and enzymes
GDH, AAT and ALAT in different tissues observed were discussed in the light of metabolic stress caused due to the exposure to the toxicant.

Vani et al. (2011) made investigations on deltamethrin induced alterations of hematological and biochemical parameters in fingerlings of *Catla catla* (Ham.) and their amelioration by dietary supplement of vitamin C. The fish exposed to deltamethrin showed significantly lower values of all parameters studied except ALT activity. This might be due to possible disruption of hematopoiesis and proteosynthesis. However, the fish fed with varied concentration of vitamin C in diets neutralized the toxic effect of deltamethrin.

The activities of protease, alanine, aspartate aminotransferases, acid phosphatases and alkaline phosphatases were elevated in the tissues of *Cnesterodon decemaculatus* exposed to polluted water for 28 days and changes in the enzyme activities disrupt physiological and biochemical processes (De la Torre et al., 2005). Other authors have also related the protein reduction in fish after exposure to the toxicants (Glusczak et al., 2007; Fonseca et al., 2008)

Gabriel et al. (2012) investigated changes in metabolic enzymes activities in selected organs and tissue of *Clarias gariepinus* exposed to cypermethrin and reported that the enzyme activities in all the organs were inhibited by different levels of cypermethrin concentrations, with ALP activity being mostly affected. The highest enzyme activity ALP was observed in the kidney, while the lowest ALP in the gill. The activities of the enzymes in all the organs showed toxicant concentration dependent activities. Similar observations have been made in other fishes as reported by Srekala et al. (2013) and Magar and Shaikh (2013).

Venturini et al. (2014) reported increased ALP activity induced by trichlorfon in *Piaractus mesopotamicus*. Exposure to malathion caused altered AST, ALT and LDH activity in liver tissue of *Cichlasoma nigrofasciatum* (Banaee et al., 2015).

### 2.4.2. ACETYLCHOLINESTERASE INHIBITION

The inhibition of acetylcholinesterase by organophosphate compounds has become an indicator of organophosphate pollution in the aquatic environment (Williams and Sova, 1966; Rao, 2008). This enzyme is important for the neurological
functioning of the sensory, integrative and neuromuscular systems in fish. The toxicity of organophosphates is primarily attributed to its ability to inhibit acetylcholinesterase, which plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Soreq and Zakut, 1993). The inhibitory effects of these depend on their binding to the enzyme active site and by their rate of phosphorylation (Dutta et al., 1995). This enzyme is extremely important for many behavioural activities like prey location, predator evasion and orientation towards food (Miron et al., 2005). The inhibition of this enzyme alters respiration (Klaverkamp and Hobden, 1980), swimming (Post and Leisure, 1974) and social interaction (Symons, 1973) in salmonides. AChE is widely used for rapid detection to predict early warning of pesticide toxicity (Dutta and Arends, 2003).

Inhibition of AChE was accompanied by an increase in acetylcholine levels and this condition can lead to increase of catecholamines which can affect the activity of enzymes involved in glycogenolysis and glycogen synthesis. Continuous stress may affect the synthesis site of AChE or decrease the levels of excess AChE. Mortality of fish may be due to inhibition of other enzymes, especially those taking part in carbohydrate and protein metabolisms. The inhibitory effect on AChE activity indicates that insecticides might interfere in vital processes like energy metabolism of nerve cells (Ansari and Kumar, 1984).

Several reports suggest that various organophosphorus pesticides at concentrations close to their LC₅₀ can induce a decrease in the enzyme level to 60-20% of their normal physiological activity in fish. Similarly (Salte et al., 1987) evaluated the effects of these pesticides on fish.

Hegazi et al. (1989) worked on Clarias lazera and reported that sublethal concentration of chlorpyrifos reduced brain acetylcholinesterase (AChE) of the catfish and also reduced liver glycogen and blood glucose level of the fish.

Acetylcholinesterase (AChE) activity is routinely used as a biomarker of the exposure to certain groups of contaminants (Grue et al., 1997). Even low concentration of the toxicant can inhibit AChE (Varó et al., 2003). The inhibition of
the acetylcholinesterase by pesticides can affect locomotion and equilibrium of exposed organisms (Saglio and Triasse, 1998; Bretaud et al., 2000; Jindal & Jha, 2005) and adversely affect various metabolic activities (Pant and Singh, 1983).

Dembele et al. (1999) worked on recovery of acetylcholinesterase activity in *Cyprinus carpio*. They showed that brain AChE activity was almost completely recovered within one day after exposure to carbofuran, and recovered by 11.87% after 15 days exposure to chlorfenvinphos. They also reported that after 96 h exposure period to chlorfenvinphos (0.24 ppb) and carbofuran (3 ppb) no mortality occurred, but the fishes treated with chlorfenvinphos were unable to swim. They concluded that chlorfenvinphos was found to be more potent inhibitor than carbofuran *in vivo*.

Fulton and Key (2001) reviewed acetylcholinesterase inhibition in estuarine fish as an indicator of organophosphorus insecticide exposure, and suggested that brain AChE inhibition levels of 70% are associated with mortality in most species.

De la Torre et al. (2002) examined acetylcholinesterase activity in caged *Cyprinus carpio* and field-captured *Cnesterodon decemmaculatus* from a highly polluted peri-urban water body and reported that decline in AChE activity could be interpreted as a secondary outcome of conformational changes in the enzyme due to binding toxicant with a large number of functional sulfhydryl groups.

Varo et al. (2003) studied the acute toxicity of dichlorvos both in vitro & in vivo on AChE activity of sea bass (*Dicentrarchus labrax*). The studies revealed that in vivo exposure to the toxicant of the fish was relatively resistant and being able to tolerate high percentage of head AChE inhibition without lethal effects. Prolonged exposure to 0.108 mg L$^{-1}$ of the toxicant resulted in gill damage and AChE inhibition. 50% recovery was observed on 7.8 days for both the tissues, whereas it took 20.7 and 20.3 days for total recovery in brain and gills respectively.

Venkateswara et al. (2003) observed a 90% inhibition of AChE activity in the brain and gills in 24 h and a complete recovery within 23 days in *O. mossambicus* after exposure to LC$_{50}$ and multiple exposures to sublethal concentrations of profenofos.
According to Sevgiler et al. (2004), exposure of *O. niloticus* to sublethal concentration of etoxazole, a new organofluorine pesticide, led to sharp depletion up to 80% in AChE activity. Significant inhibition in cholinesterase activity in liver tissues of *Oreochromis niloticus* following single and multiple exposure of chlorpyrifos and carbosulfan was reported by Chandrasekara and Pathiratne (2005) and Joseph and Raj (2011).

Uner et al. (2006) investigated the effects of organophosphorus pesticide diazinon on acetylcholinesterase activity and its relationship to lipid peroxidation (LPO) in the brain of *Oreochromis niloticus*. The inhibition of AChE activity in the brain of the fish was correlated with increased MDA levels after 7 and 15 days diazinon exposures and reported that repeated exposure of diazinon on *O. niloticus* in sub-lethal concentrations was a significant factor affecting brain AChE activity and this effect was accompanied with induction of LPO.

Guimarães et al. (2007) evaluated the effect of trichlorfon on acetylcholinesterase activity in *Oreochromis niloticus* and results indicated a significant decrease in the muscular AChE activity in the treated individuals.

Crestani et al. (2007) investigated the effect of clomazone herbicide on Ache activity of silver catfish (*Rhamdia quelen*) and recovery pattern and reported that exposure of the fish to clomazone concentration used in rice field decreased significantly in brain and muscle AChE activity for all periods of exposure, except in brain tissue after 192 hr.

Glusczak et al. (2007) studied acute effects of glyphosate herbicide on metabolic and enzymatic parameters of silver catfish (*Rhamdia quelen*) and showed that in 96 hr glyphosate changed AChE activity, metabolic parameters and TBARS production.

Kavitha and Rao (2008) studied the impact of chlorpyrifos on AChE interaction in *Gambusia affinis*. They reported that AChE got inhibited after 96 hr exposure and also affected antioxidant enzymes & behaviour. They also reported that swimming speed and AChE activity can be recovered, but comparatively needs a longer time.
Patil and David (2009) made investigations on hepatotoxic potential of malathion in *Labeo rohita*. They found inhibition in AChE activity and attributed to decreased ionic composition in liver after 5 & 15 d exposure and got increased after 25 d exposure due to rapid detoxification of the pesticide.

Acetylcholinesterase activity was measured as a marker of chlorpyrifos toxicity in *Oreochromis niloticus*. Its activity was found to be decreased after 30 days of chlorpyrifos treatment. When the fish were transferred into pesticide- free water after treatment, significant recovery of the enzyme activity was observed (Oruç, 2010).

Modesto and Martinez (2010) evaluated effects of roundup transorb on acetylcholinesterase of the neotropical fish *Prochilodus lineatus* and found that after 96 hr the enzyme activity in brain got reduced by 11.5% & exposure to higher concentration by 17.88%. They inferred that this rate of enzyme inhibition in the fish was not a life threat situation but it caused generation of ROS resulting in oxidative stress.

Moraes *et al.* (2011) studied toxicological responses of *Cyprinus carpio* after exposure to herbicide containing imazethapyr and imazapic and observed that after 7 d exposure to the toxicant, enzyme activity increased significantly in brain but inhibited after 30 d. Besides this, herbicide residues were found after 10 & 35 days. The altered AChE activity indicated that the fish adapted to its physiological conditions.

Oruc (2012) studied the oxidative stress responses and recovery patterns along with activity of acetylcholinesterase in the liver of *Oreochromis niloticus* exposed to chlorpyrifos-ethyl. He suggested that the toxicant induced oxidative stress in the tissue and this effect could not be related with anti-acetylcholinesterase activity of the pesticide. The high concentration of chlorpyrifos enhanced lipid peroxidation in the liver. However an adaptive response occurred after exposure to low concentration of chlorpyrifos.

Assis *et al.* (2012) investigated comparative effect of pesticides on brain acetylcholinesterase in tropical fish pirarucu (*Arapaima gigas*), cobia (*Rachycentron...
canadum) and Nile tilapia (Oreochromis niloticus) and reported comparable sensitivity between purified and non-purified enzymes suggesting them as biomarkers for organophosphorus and carbamate detection in routine environmental and food monitoring programs for pesticides.

Srivastava et al. (2013) studied inhibition and recovery of acetylcholinesterase activity in the gills of Cirrhinus mrigala exposed to nuvan. They stated that at 4 hr exposure, the AChE activity decreased up to 68.81% at 5 mg/l and 77.88% at 15 mg/l of the toxicant. The inhibition of the enzyme activity was found to be associated with influence on transmission of nerve impulses, resulting in improper functioning of gills. The study also showed that the activity of the enzyme in the gills remained significantly lower and the gills are unable to attain their normal metabolism even after long recovery periods.

Capkin et al. (2014) determined the effects of carbosulfan exposure on erythrocyte and liver acetylcholinesterase activity in rainbow trout (Oncorhynchus mykiss). A higher degree of enzyme inhibition was observed in the erythrocyte as compared with liver. They also found a positive correlation between the enzyme inhibition and the time of exposure.

Jindal and Kaur (2014a) examined the inhibition and recovery response of AChE of chlorpyrifos on different organs of Ctenopharyngodon idellus exposed to chlorpyrifos. The study revealed that the exposure to the pesticide influenced the behavioural activities of the fish. The effect on AChE activity in the organs of the fish can be used as an early biomarker of toxicity of chlorpyrifos. They also reported total recovery upto 95-99% after 2 months.

Rajni and Revathy (2015) studied the effect of combination of chlorpyrifos & cypermethrin on AChE activity in Danio rerio and found inhibited AChE activity increased with pesticide concentration and exposure period.

2.4.3. ANTIOXIDANTS

Oxidative stress occurs when there is critical imbalance between oxidants and antioxidants due to the depletion of antioxidants or excessive accumulation of the reactive oxygen species leading to damage (Fang, 1975; Sies, 1986; Di Giulio et al.,
Xenobiotics, such as pesticides, leads to the generation of ROS and altering free oxygen radicals scavenging enzyme systems (Livingstone, 2001) and cause oxidative stress. Reactive oxygen species can react with biological macromolecules and produce lipid peroxidation, damage DNA and protein oxidation, resulting in oxidative stress (Livingstone et al., 1993; Nordberg and Arnér, 2001; Monterio et al., 2006). Antioxidants are essential to maintain the redox status of the cells and serve as important defense against oxidative stress. Antioxidants of fish may be useful biomarkers in toxicological evaluations (Bainy et al., 1996; Ahmad et al., 2000).

Enzymes associated with antioxidant defence mechanism are altered under the influence of pesticide (Winston and Di Giulio, 1991).

The treatment of menadione led to a decreasing CAT activity in Ameliurus nebulosus (Hasspieler et al., 1994).

Banerjee et al. (1999) studied biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers and suggested that OFR scavenging enzymes were induced while combating oxidative stress in a differential manner in organochlorine, organophosphate and carbamate poisoning.

Oruc and Uner (2000) investigated the effects of the herbicide 2, 4-D and the insecticide azinphosmethyl on hepatic antioxidant enzyme activities and lipid peroxidation in tilapia. They reported that combined treatment of the pesticides exerted synergistic effects in the activity of SOD, while antagonistic effects were found in the activities of GPx & GR. The results indicated that Oreochromis niloticus resisted oxidative stress by antioxidant mechanism and prevented increases in lipid peroxidation.

Peña-Llopis et al. (2001) exposed Anguilla anguilla to 41.8 mg/l of the herbicide molinate for 96 hr to evaluate glutathione-dependent resistance. They observed intercorrelated GSH & GR enzyme activities. The study indicated that eels which were able to induce GR activity, increase GSH and maintain the GSH : GSSG ratio in the liver showed an extended survival under the oxidative stress generated by molinate than those that lost glutathione homeostasis.
Elia et al. (2002) evaluated biochemical responses of bluegill sunfish (*Lepomis macrochirus*) to atrazine induced oxidative stress and found increased GST activity in gills, increased activity of SOD, GSH, GSSG and LPO in liver of the fish. The GST activity was higher except in spring, and GST activity was associated with toxicant level and was probably metabolic adaptations of the fish to higher levels of pollutants.

Sayeed et al. (2003) studied oxidative stress response of *Channa punctatus* exposed to deltamethrin. They reported that single exposure for 48 hr caused induction of various antioxidant enzymes and non-enzymatic antioxidants in kidney and liver, and certain antioxidants were found to be depleted in gills.

Zhang et al. (2004) studied the effects of chronic exposure of 2, 4-dichlorophenol on the antioxidant system in liver of *Carassius aurotaurus*. Their results showed that after 40 d exposure, the activities of catalase, selenium-dependent glutathione peroxidase and the content of oxidized glutathione were induced. Besides this, they also found good dose effect ed relations between 2, 4-DCP level and CAT activity, GSSG content and Se-GPx activity.

Oruc et al. (2004) made investigations on tissue-specific oxidative stress response in *Oreochromis niloticus* and *Cyprinus carpio* exposed to 2, 4-D and azinphosmethyl & their combination for 96 hr. SOD, GPx and CAT activities were assessed in kidney, brain and gill. Individual and combined treatments caused an elevation in CAT and GPx activities in kidney of *C. carpio*. The study also revealed that fish exposed to pesticides develop tissue-specific adaptive responses to protect cells against oxidative stress. *C. carpio* was found to be more sensitive than *O. niloticus*.

In aquatic ecosystems, pollution is described as an enhancer of intracellular formation of reactive oxygen species (ROS), which can give rise to oxidative damage, as described in flathead grey mullet (*Mugil cephalus*), flounder (*Platichthys flesus*) and Nile tilapia (*Oreochromis niloticus*) (Ferreira et al., 2005; Figueiredo-Fernandes et al., 2006).
Rao (2006a) studied toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of *Oreochromis mossambicus* and found induction in hepatic glutathione-s-transferase levels indicating the protection against the toxicity of xenobiotic induced lipid peroxidation. The study also revealed that RPR-V affected the intermediary metabolism of the fish and the increase of biomarker enzymes in plasma might be due to the necrosis of liver.

Monocrotophos exposed fish, *Gambusia affinis*, exhibited significant induction in antioxidant enzyme activities and gradually restored to the control levels (Kavitha and Rao, 2007). They also said that the MCP besides its inhibitory effect on target enzyme AChE activity resulted in induction in antioxidant enzyme activities as a characteristic of oxidative stress, could be used as biomarker.

Crestani *et al.* (2007) studied the effect of clomazone herbicide on biochemical and histological aspects of silver catfish (*Rhamdia quelen*). AChE, TBARS, catalase assessed in liver, brain and muscle of the fish and histological analysis showed vacuolation in the liver after herbicide exposure. Giordano *et al.* (2007) suggested that OPs may lead to promote higher reactive oxygen species levels and induced lipid peroxidation. They also observed the cytotoxicity and ROS production induced by OPs.

Miron *et al.* (2008) and Jin *et al.* (2010) observed an increase in lipid peroxidation in *Leporinus obtusidens* exposed to clomazone & *Danio rerio* exposed to atrazine respectively. Similarly Isik & Celik (2008) made investigations on the effects of methyl parathion and diazinon as inducers for oxidative stress on certain biomarkers in liver and gills of *Oncorhynchus mykiss*. They reported that administrations of the pesticides promoted MDA content in some of the tissues and found a fluctuating trend in GSH-Px, GST, SOD and GR activities after 24, 48 and 72 hr.

Kavitha and Rao (2008) evaluated toxic effects of chlorpyrifos on antioxidant enzymes in mosquito fish, *Gambusia affinis* and found decreased levels of SOD, CAT and GR activities, and induction in lipid peroxidation in exposed fish. They also observed that the antioxidant levels were restored to near control by 16–18 days.
Oropesa et al. (2009) assessed the glutathione and malondialdehyde levels in common carp after exposure to simazine and reported an increase in tissue reduced glutathione and malondialdehyde levels in the fish. Similarly Mansour and Mossa (2009) and Lushchak et al. (2009) observed decreased level in GST activity after exposure to chlorpyrifos and roundup in the liver of the fish.

Slaninova et al. (2009) reviewed oxidative stress in fish induced by pesticides and stated that anticholinergic activity of organophosphates leads to the accumulation of ROS and resulting lipid peroxidation. They also added that oxidative damage from fenpyroximate actuation is related to the disruption of mitochondrial redox respiratory chain.

The chronic effect of PCZ (Propiconazole), a triazole-containing fungicide commonly present in aquatic environment, on GSH-related antioxidant system and oxidative stress indices of rainbow trout (Oncorhynchus mykiss) were investigated by Li et al. (2010a). GSH levels and GH-related enzyme activities, including GPx, GR and GST, were quantified in three tissues- liver, gill and muscle. The levels of LPO and CP were also measured as makers of oxidative damage.

Nwani et al. (2010) investigated toxicity of atrazine on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish Channa punctatus (Bloch). In fish exposed for 15 days to different sub-lethal concentrations of the toxicant, induction of oxidative stress in the liver was evident by increased lipid peroxidation levels. The antioxidants SOD, CAT and GR responded positively in a concentration dependent pattern, thus suggesting the use of these antioxidants as potential biomarkers of toxicity associated with contaminations exposure in freshwater fishes.

Toni et al. (2011) worked on the assessment of oxidative stress and metabolic changes in Cyprinus carpio exposed to different concentrations of tebuconazole and reported that fish exhibited significant increase in TBARS levels in all concentrations used while the enzymatic and non-enzymatic antioxidants were decreased. Among the metabolic parameters, glycogen and glucose increased and protein levels decreased.

Prusty et al. (2011) worked on the effect of short term exposure of fenvalerate on biochemical responses in Labeo rohita (Hamilton) fingerlings. Significant
alteration in SOD activity of liver and gill was observed. Catalase activity in gills of fishes was also affected significantly.

Amin and Hashem (2012) studied deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (Clarias gariepinus) and role of alpha-tocopherol and showed that 48 hr exposure to 0.75 μg/l of toxicant significantly increased lipid peroxidation in the liver, kidney and gills, while catalase activity got decreased.

Stara et al. (2012) studied the effect of chronic exposure to simazine on oxidative stress and antioxidant response in Cyprinus carpio and showed the impact of the increased production of ROS leading to oxidative damage to lipids, proteins and inhibition of antioxidant capacity. Activity of the antioxidant enzymes SOD, CAT, GPx and GSH in groups with high concentration of the toxicant increased at 14 and 28 days, but decreased after 60 days exposure.

Sharma and Ansari (2013) investigated effects of deltamethrin on CAT, LPO and GSH in tissues of zebrafish Danio rerio. An inhibition of catalase activity and GSH level & enhancement in lipid peroxidation continues at all exposure periods was observed. They also found toxicity time as well as concentration dependent.

Ural (2013) studied chlorpyrifos-induced changes in antioxidant status of liver, kidney and gills of Cyprinus carpio carpio. The study demonstrated that CPF had a negative effect on the the antioxidant enzyme activities of the fish and the toxic effect was neutralised by the administration of lycopene.

Yonar et al. (2014) made investigations on malathion induced changes in oxidative/antioxidant status of Cyprinus carpio carpio and reported the malathion-induced toxicity was ameliorated by protective role of propolis.

Toxicants entering into aquatic environment exert their effect through altering redox cycling in fish. The antioxidant defense as well as oxidative damage is a common effect in fish exposed to xenobiotics in aquatic ecosystem (Ekambaram et al., 2014). They also inferred that the formation of damaged products through stress can persist even after the stress has been stabilized by continuous exposure leading to
various molecular changes in the fish which will lead to their survival or death process and depend on the intensity of damage caused by the pollutants at one point of time.

Al-Ghanim (2014) studied the effect of an organophosphate insecticide diazinon on the activity of acetylcholinesterase and lipid peroxidation of *Cyprinus carpio* and found that induction of oxidation stress in various tissues was evidence of increased LPO levels which seems to be associated with the concentration of the toxicant.

Sharma and Ansari (2014) studied the impact of azadirachtin on some biomarkers of oxidative stress in *Danio rerio* and they found reduction in the CAT activity which was related to the occurrence of increased lipid peroxidation (LPO) in the fish exposed to the insecticide. Exposed fish also showed reduction in GSH level.

Zeid and Khalil (2014) made investigations on effects of acute fenitrothion insecticide exposure on oxidative stress in *Oreochromis niloticus* and concluded that oxidative stress evoked by FNT could be responded of its genotoxicity which was proven by determined clastogenic effect resulting from over production of reactive oxygen species or depletion of endogenous antioxidants.

Enhanced level of LPO has been reported in liver, kidney and gills of fish exposed to different pesticides (*Danio rerio* exposed to atrazine, Blahova et al., 2013; *Carassius auratus* exposed to sencor, Husak et al., 2014; *Carassius carassius* exposed to endosulphan, Dar et al., 2015).

Devan et al. (2015) made investigations on oxidative stress and antioxidants responses induced by quinalphos in *Cyprinus carpio*. They found an increase in SOD, CAT and GST activity and LPO level in liver of the fish.

### 2.5. PESTICIDE RESIDUE ANALYSIS

There are few reports regarding the residue analysis of conservative pesticides in biota from aquatic environment (Ramesh et al., 1990; Rajendran et al., 1992; Shailaja and Singbal, 1994; Shailaja and Nair, 1997).

Linn (1968) made observations on the toxicity of chlorpyrifos on caged and released sunfish (*Lepomis cyanellus*) in rice fields in California and concluded that application rates of 0.028 kg/ha had negligible effects on fish survival, whereas 0.056 kg/ha appeared to cause mortality of sensitive fish species.
Neely and Blau (1977) studied the disposition of chlorpyrifos in a pond environment. Bioconcentration factor (BCF) for chlorpyrifos in invertebrates and fish ranged from 42 to 5100 ml/g, depending on the species, exposure concentration and exposure conditions. The model estimated the BCF in fish to be 700 ml/g and predicted the maximum concentration would occur in 37.5 days under conditions of a declining water level in the pond.

Surface water contamination may have ecotoxicological effects for aquatic flora and fauna as well as for human health if used for public consumption (Forney and Davis, 1981; Mulla and Mian, 1981; Leonard, 1988; Miyamoto et al., 1990). In addition, many pesticides eventually end up in ground water and their transformation products may remain for years (Belfroid et al., 1998).

Contamination of ground water resources by pesticides has brought increased environmental concern (Foster et al., 1991; Schiavon et al., 1995; Guzzella et al., 1996; Papadopoulou-Mourkidou et al., 2004).

Data from the National Contaminate Biomonitoring Program (USEPA, 1992) showed that chlorpyrifos was found in nine out of sixteen wild channel catfish samples with concentrations above the detection limit (2.5 ppb).

Walker and Livingstone (1992) explained that xenobiotics which are fat soluble are readily taken up from water into tissues of aquatic organisms. Priya & Maruthi (2012) has also opined that the detectable level of residue varies on the fat content of the examined tissue.

Sharma (1994) made gas chromatographic analysis of BHC residues in certain tissues of a freshwater teleost Channa punctatus (Bloch.). He noticed that residues of BHC isomers accumulated in the gills, intestine and liver of the exposed fishes.

Dutta et al. (1994) observed the accumulation of malathion in different organs of Heteropneustes fossilis. For this, quantitative estimation of malathion has been carried out on gill, ovary, kidney, liver and muscle tissues. After the 10 days exposure to a sublethal concentration of malathion, the gills showed the maximum residual accumulation.
Most of the applied pesticides are subject to many transport and conversion products. Thus, they do not remain at their target site but often enter aquatic environment via soil percolation, air drift or surface runoff affecting abundance and diversity of non-target species producing complex effects on the ecosystems and altering trophic interactions (Miliadis, 1994; Rand et al., 1995).

Goel (1995) estimated the accumulation of methyl parathion in the kidney of freshwater fish *Channa punctatus* by employing high performance liquid chromatography (HPLC) technique.

The pesticides are generally analysed by spectrophotometry (Janghel et al., 2007), thin layer chromatography (Rathore and Begum, 1993; Patil and Shingare, 1994), high performance liquid chromatography and high performance liquid chromatography-mass spectrophotometry (Debayle et al., 2008), gas chromatography (Abhilash et al., 2009) and gas chromatography-mass spectrophotometry (Thanh et al., 2008).

Satyanarayan and Ramakant (2004) and Satyanarayan et al. (2005) assessed the bioaccumulation kinetics and organ distribution of chlorinated pesticides in *Puntius ticto* and *Cyprinus carpio* respectively. The fishes were exposed to test water containing sublethal concentration of aldrin, dieldrin, BHC and DDT. Variability of these pesticides was evaluated in different tissues like gills, muscles, intestine, kidney and liver of the fishes.

Pesticide residues have a complex environmental fate in aquatic ecosystems (Carvalho et al., 2002; Daam and van Den Brink, 2011).

According to Sankararamakrishnan et al. (2005), high concentration of malathion and dieldrin in the surface water could be attributed to the agricultural runoff resulting from the extensive agricultural activity in the banks of rivers.

Agarwal (2009) worked on the bioaccumulation of pesticides and reported that their metabolites and residues in the environment not only remain where they are applied but instead partition occurs between the major environmental compartments in according to their physicochemical properties and may thereby transported several kilometers from the point of their original release.
Akhtar et al. (2009) conducted investigation to determine the residual concentration of five pesticides in Ganga river fishes. He found that total HCH concentration was above the MRL values in comparison to other four pesticides, and concluded that the pesticide pollution may be due to untreated sewage sludge of the river.

Afful et al. (2010) conducted pesticide residue analysis on seven banned pesticides in *Channa obscura* and recorded the highest residue concentration of 35.2 g/kg. Upadhi and Wokoma (2012) analysed some pesticide residues in surface water, sediment and fish tissue of Elechi creek, Niger Delta, Nigeria and their study showed evidence of the identification of pesticides such as 2, 4-diamine, diazinon, paraquat, endosulfan, lindane and propoxur at low concentrations.

Higher pesticide accumulation in liver and least in muscles has been reported in different fishes (*Heterotis niloticus, Oreochromis niloticus* exposed to various organophosphates, Essumang et al., 2009; *Catla catla, Labeo rohita, Cirrhinus mrigala* exposed to chlorpyrifos, Tilak et al., 2004; *Clarias gariepinus, Heterotis niloticus, Oreochromis niloticus, Tilapia zilli* exposed to different organophosphates, Akan et al., 2013). Rao et al. (2003a) also reported higher bioaccumulation of chlorpyrifos in viscera as compare to head in *Oreochromis mossambicas*.

Akan et al. (2013) detected pesticide residues in fish samples and found endosulfan as the most abundant pesticide residue in the tissues of the fish species with a value of $8.98 \pm 0.02 \mu g/g$ in the liver of *Oreochromis niloticus*.

Nagaraju and Rathnamma, (2014) using gas liquid chromatography-flame ionization detector (GLC-FID) analysed residue of carbamate pesticide in *Labeo rohita*. The order of tissues where the carbosulfan bioaccumulated was in the order of: gill < kidney < liver < muscle. They suggested that prolonged exposure to carbosulfan to the fish lead to increased accumulation of pesticide residues in tissues.

Mena et al. (2014) worked on pesticide residue analyses and biomarker responses of native Costa Rican fish belonging to Poeciliidae and Cichlidae families to assess environmental impacts of pesticides in Palo Verde national park.