Brain and muscle tissues of the test fish evidenced a highly significant decrease in the activity levels of acetyl cholinesterase under sub-lethal concentration of cartap hydrochloride. Changes in gill, liver and kidney in the fish revealed that they were relatively less affected than brain and muscle tissues under cartap hydrochloride toxicity, and the neural activity in these organs was comparatively less. The decreased activity levels of AChE as observed in the present study may be due to cartap hydrochloride action on the active site of AChE.

Acetyl cholinesterase enzyme involves in the maintenance of the structural and functional integrity of cellular membranes. Of all the pesticide induced biochemical changes inhibition of AChE, the enzyme involved in terminating the action of the neuro-transmitter acetylcholine, is perhaps most often studied. The enzyme acetylcholinesterase (AChE) is highly specific and rapidly hydrolyzes acetylcholine that is formed during the passage of nerve impulse. AChE is often called true cholinesterase. The toxicity of the pesticide is primarily attributed to its ability to inhibit acetylcholinesterase, having an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Soreq and Zakut, 1993).

Direct toxicity of cartap to fish species is not as high as that of other neurotoxic insecticides, with 3-h LC$_{50}$ between 0.02 and 6.8 mg/L (Lannacone et al., 2007 and Zhou et al., 2009). However, cartap affects negatively several species of Hymenoptera and aphid parasitoids used to control a number of crop pests (Ecoli et al., 2010 and Ateyyat, 2012). This insecticide also inhibits hatching of eggs of the nematode Aga- mermis unka, a parasite of the rice pest Nilaparvata lugens (Choo et
and reduces significantly the populations of ladybugs and other predatory insects in cotton crops when applied at the recommended rates, i.e. 20 g/ha (Gour and Pareek, 2005 and Lima, Junior et al., 2010). In rice paddies, cartap hydrochloride reduced populations of coccinellid beetles, carabid beetles, dragonflies and damselflies by 20-50% (Srinivas and Madhumathi, 2005). Pollinators such as honey bees and bumble bees can also be seriously reduced in numbers when feeding on crops treated with cartap hydrochloride, which is included among the most toxic insecticides to bees after neonicotinoids and pyrethroids (Thomazoni et al., 2009 and Marletto et al., 2003). For all its negative impacts on parasitoids and predatory insects it is hard to understand why cartap was the third most common insecticide (19% of all applications) used in IPM programs in Vietnam a decade ago, and is still among the most widely used in rice farms in China (Zhou et al., 2009).

AChE activities in adductor muscle were depressed in freshwater mussels (Elliptio complanata) exposed for 96 h at concentrations as low as 0.1 mg/L and 1.3 mg/L of aldicarb and acephate respectively, while increasing the water temperature from 21 to 30 °C resulted in mortality (Moulton et al., 1996). High AChE inhibition (70%) by acephate was not associated with immobility of Daphnia magna, but increasing the concentration of acephate further had a strong detrimental effect on mobility, suggesting that binding sites other than AChE may be involved in acephate toxicity (Printes and Callaghan, 2004).

Dutta et al. (1995) stated that the inhibitory effects of OP insecticides depend on their binding to the enzyme active site and by their rate of phosphorylation. Acetylcholinesterase (AChE) activity is routinely used as a biomarker of the exposure to certain groups of contaminants (Grue et al., 1997). Murphy et al. (1968) stated that the sensitivity of fish to pesticide exposure, especially organophosphate is dependent
on the level of brain AChE activity. Cholinesterases are useful indicators of pollution because of their high sensitivity and presenting the first detectable signs of sub-lethal stress response in organisms (Stegeman et al., 1992; Chambers and Boone, 2002).

Even low concentration of the toxicant can inhibit AChE (Varo et al., 2003). The inhibition of the acetylcholinesterase by pesticides can affect locomotion and equilibrium of exposed organisms (Saglio and Trijasse, 1998; Bretaud et al., 2000; Jindal and Jha, 2005) and adversely affect various metabolic activities (Pant and Singh, 1983). This enzyme is extremely important for many behavioural activities like prey location, predator evasion and orientation towards food (Miron et al., 2005). AChE is widely used for rapid detection to predict early warning signs of pesticide toxicity (Dutta and Arends, 2003).

In the present study, investigations have been made on activity of AChE in the brain, muscle, heart, liver, kidney and gills of the the fish *Labeo rohita* and changes in behaviour of the fish on exposure to cartap hydrochloride.

After exposure to the toxicant, cartap hydrochloride, AChE activity levels decreased at sub-lethal concentration in all organs, viz gills, gill, bran, heart, liver, kidney and muscle of the fish, *Labeo rohita*. On exposure to sub-lethal concentration the maximum decrease of AChE was noticed in brain and minimum decrease was found in liver and heart.

Inhibition of cholinesterase in either nervous system or flesh has been acknowledged as the adverse effect on the organisms because enzyme activity of the target tissue is known to contribute in the neurotransmission (Padilla, 1995). Cartap hydrochloride has unique mode of action on the insect nervous system at the nicotinic acetylcholine receptors and it has additional effects on (GABA) and H-Glutamate receptor sites, leading to continuous activation of motor neurons and causing
cessation of feeding, tremors of most muscles in the body and later on, paralysis and death (Salgado, 1997; 1998 and Semiz et al., 2006).

With decrease in the activity of AChE is not broken and accumulates within synapses and cannot function in a normal way (Dutta and Arends, 2003). Hence, the altered locomotor behaviour of fish could be attributed to the accumulation of acetylcholine interrupting coordination between the nervous and muscular junctions (Rao et al., 2005). Considering the role of AChE in neurotransmission in both central nervous system and at neuromuscular junctions, the inhibition of AChE activity could be attributed to behavioural changes observed in *Labeo rohita* exposed to pesticide (Kavitha and Rao, 2008).

Most of the observations of AChE inhibition in fish have been emphasized on the brain only as the intoxication of the brain contributes a lot towards behavioral changes (Jaffery and Keizer, 1995). Toni et al. (2010) also reported decrease in flesh ChE activities in fish. The muscle cholinesterases represent the largest pool of cholinesterase in the body. It is also important to control the muscular function; the loss of muscular control can have many problems for fish including loss of swimming control and blockage of the opercular movement. This may result in reduced oxygenation of the blood and consequently lead to hypoxia induced death (Zinkl et al., 1987). This may also attribute towards the changes in behavior of fish exposed to pesticides. The toxicant exposed fish showed erratic, speedy and jerky movements at lower concentration and at the higher concentration fish exhibited hyperactivity, violent behaviour and jumping out of the tanks violently (escape behaviour).

Although catfish brain seemed to be the most sensitive to the exposure of aldicarb, the fish with 90% inhibition of AChE was alive with moderate symptoms of intoxication (Everett et al., 2000), the present study showed less than 90% inhibition
in brain under both sub-lethal concentration of cartap hydrochloride. Significant inhibition of cholinesterase was also observed in heart, liver and muscle in the present investigation. Hence, activity reduction in gills can be attributed to suffocation and reduced respiratory activity which may be a problem in fish as well (Chambers and Carr, 1995). Poisoning of cholinesterase by pesticides in fish to this extent has been considered as a good indicator of intoxication (Coppage and Mathews, 1974; Westlake et al., 1981).

The inhibition observed in the activity of AChE, is in agreement with the findings of other workers (Das and Mukherjee, 2003; Crestani et al., 2007; Joseph and Raj, 2011; Megahed et al., 2013; Namdeo et al., 2013; Ghazala et al., 2014; and Jindal and Kaur, 2014).

Acetylcholinesterase is an enzyme that modulates the amount of neurotransmitter acetylcholine as suggested by O'Biren (1967). Measurement of the activity of this enzyme in aquatic animals not only offers a means of detecting serious pollution by AChE agents but it has the potential for indicating extent of poisoning the animal in actual environment (Coopage, 1933). The extent of brain AChE decrease was proportional to the concentration of the substance (Weiss, 1958). AChE activity coupled with residue analysis was studied by Coppage and Mathews (1974). Coppage et al. (1975) reported that inhibition of 87% of the normal activity was necessary to indicate exposure of fish to anti- AChE–compounds.

Duangsawasdi and Klaverkamp, (1979) stated that the cholinesterase activity in the erythrocytes, gill, heart and serum of rainbow trout was reduced within 3h of exposure to acephate and 1h after exposure to fenitrothion. Chin and Sudderuddin, (1979) stated that large fish survived when AChE inhibition was more than 80%. Kabeer et al. (1979) observed that in Tilapia, a highest level of AChE inhibition was
noticed in brain followed by muscle, gill and liver. According to Rath and Mishra (1980) there was a decline of AChE in brain and an increase trend in liver of fish, *Tilapia mossambica* exposed to dichlorovos. Ujjal Banerjee (1986) reported that the change in concentrations of sodium and potassium in the Central Nervous System due to toxic stress has impaired the ionic balance in nerve membrane. Thus ultimately, the blockage of the nerve conduction was due to non-release of acetylcholine and inhibition of acetylcholinesterase activity finally causing paralysis in the insect body.

According to Sheela Susan Jacob *et al.* (1982) the interference with normal mechanism of nerve impulse and enzyme cholinesterase was because of high toxicity of fenthion. Rao *et al.* (1983) studied the effect of methyl parathion on esterases of freshwater teleost, *Tilapia mossambica* and reported that acetyl cholinesterase activity decreased in muscle, gill, liver and brain tissues. Jarvinen *et al.* (1983) reported that the brain AChE activity was found to be significantly inhibited in fathead minnow exposed to insecticide, dursban.

Brain AChE activity was significantly inhibited when fathead minnow *Pimephales promelas* exposed to chlorpyrifos (Olson and Christensen, 1980). The AChE activity was inhibited when *Cyprinus carpio* was exposed to dimethoate (Manju Tembhre and Santhosh Kumar, 1994). AChE activity was inhibited in various fish with different pesticides (Bhaktavatsalam and Sreenivasa Reddy, 1982) and in *Oreochromis* exposed to phosalone (Devaraj *et al*., 1991).

In the brain AChE activity was significantly greater in fish followed by pigeon and rat. The inhibition of AChE activity by monocrotophos was in the order of rat, pigeon and fish (Yamin *et al*., 1994). Parathion was the most potent inhibitor of AChE in rat followed by pigeon, fish and honeybee (Siddiqui *et al*., 1989).
AChE has a key role in neurotransmission by hydrolyzing the neurotransmitter acetylcholine (ACh) in cholinergic synapses of the nervous system and is the target site of several neurotoxic insecticides (Salgado et al., 1998). Namdeo et al. (2013) observed that 96 h exposure of *Datura stramonium* leaf extract (100 mg/L) produced AChE inhibition. A considerable synergistic inhibitory effect was found in the brain AChE with pre-treatment of *Datura stramonium* leaf extract followed by chlorpyriphos exposure. The study revealed that Chloropyriphos (CPF) produced significant brain AChE inhibition in *Catla catla*. The neurotoxic effect of CPF is potentiated by its biotransformation to a more potent oxon metabolite that inhibits AChE substantially (Salgado et al., 1998).

Leena Muralidharan (2014) observed inhibition of AChE activity. It was due to the high accumulation of fenthion in brain tissue of fish, *Cyprinus carpio*. Ghazala et al. (2014) evaluated the effects of profenofos and carbofuran (commonly used organophosphate and carbamate insecticides) on cholinesterase activity in various organs of *Catla catla* viz., brain, gills, liver, kidney, flesh and blood under the sub-lethal exposure for a period of two months. AChE activities were found to be reduced in all organs. In addition to the brain, gills, flesh and blood, AChE activity was also observed to be reduced in other metabolizing organs i.e., liver and kidney. Maximum inhibition was observed in kidneys and liver at the highest concentration of profenofos. Less inhibition in both the organs was observed, when fish were exposed to various concentrations of carbofuran. A significant difference between maximum inhibition caused by profenofos and carbofuran was observed in the kidney. There was inhibition of cholinesterases in all organs including brain under exposure of both the pesticides.
Jindal and Kaur (2014) studied the activity of AChE in the liver, kidney and gills of the fish, *Ctenopharyngodon idellus* on exposure to chlorpyrifos. Effect of chlorpyrifos on the activity of AChE in liver, kidney and gills of the fish after its chronic exposure caused significant inhibition and recovery in its activity were observed. After exposure to the toxicant, AChE activity decreased at both the concentrations in liver, kidney and gills of *C. idellus*. A significant inhibition in the activity of AChE was observed and it increased with the increase in the exposure to chlorpyrifos in the organs of the fish studied.

In the present study, the AChE activity was inhibited moderately in sub-lethal concentration of cartap hydrochloride. Among the tissues tested the brain AChE was more inhibited than any other tissue AChE. The correlation of the residues and the AChE activity by Coppage and Mathews (1974) also supports the present study. As the exposed fish is continuously bathing in the pesticide medium throughout the exposure period, the accumulation of pesticide residue is a cumulative process; as a consequence the inhibition is also a cumulative process and is time dependent. The more is the exposure period, the more is the accumulation and inhibition of AChE activity.

**IV.5. Histopathological Studies**

Fish are often exposed to highly polluted water, and they cause different disabilities, ranging from biochemical changes in single cells to changes in the whole organism. Histological changes are more sensitive and occur earlier. They provide a better assessment of fish health, as well as the effect of pollution on each biochemical parameter. Histopathological changes have been integrated with the impact of various toxic compounds (Marchand *et al.*, 2009).
IV.5.1. General Histology of fish gill

The gills, which participate in many important functions in fish, such as respiration, osmoregulation and excretion, remain in close contact with the external environment, and particularly sensitive to changes in the quality of the water. They are considered the primary target of the contaminants (Mazon et al., 2002; Fernandes and Mazon, 2003).

Teleosts have five pairs of gill arches. In the front four pairs the slender gill filaments form two lines facing towards the back, and these two lines are joined to each other at the base by a gill septum. Each gill arch supports one set of paired gill filaments. The gill rakers help to make sure that no extraneous material gets into the gill filaments to clog them up. Each paired gill filament in turn supports numerous lamellae (sing. lamella), extending out from both sides of the body filament. It's here in the lamellae that the uptake of oxygen actually takes place. Numerous semicircular secondary gill lamellae are lined up along both sides of the primary gill lamellae. The primary gill lamellae consist of centrally placed rod like supporting axis (SA) with blood vessels (BV) on either side. The secondary lamellae also termed as respiratory lamellae (RL), are highly vascularized and covered with a thin layer of epithelial cells (EC). The region between the two adjacent secondary gill lamellae is known as interlamellar region.

David et al. (2005) stated that the epithelium that covers the gill filaments and lamellae provides a distinct boundary between a fish’s external environment and extracellular fluids and also plays a critical role in the physiological function of the fish gill. The teleost fish gill is covered by a complex epithelium. The gill epithelium is composed of several distinct cell types (Laurent, 1984; Smith et al., 1991), but primarily consists of pavement cells (PVCs) and mitochondrionrich cells (MRCs),
which comprise 90% and 10% of the epithelial surface area respectively. In fish electrolytes are actively taken up by specialized ion-transporting cell in gill epithelia. These are called chloride cells. The epithelial layers on the two sides of the secondary lamellae are separated by widely spaced pillar cells, of characteristic form. These have a central cell body with wide flanges at each end spreading out below the epithelia. The flanges of adjacent pillar cells meet and connect, delimiting an extensive blood space between the pillar cell bodies. The water/blood pathway across which gaseous exchange takes place consists of the epithelium, basement membrane and pillar cell flange (Hughes and Grimstone, 1965).

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, contributing to problems related to respiration and acid-base balances. During pathological studies the variations in the histology are exploited for the evaluation of physiological state of the animal. Histological alterations in marine organisms have been identified as useful biomarkers for environmental contamination (Hinton et al., 1992b; Munday and Nowak, 1997). Histological analysis appears to be a very sensitive parameter and is crucial in determining cellular changes that may occur in target organs, such as the gills, liver and kidney (Dutta, 1996). The histology of control gill and sub-lethal exposures of of 24, 48, 72 and 96 h were shown in Plate.IV.5. I. Fig.A.

IV.5.2. Pathology of Gill tissue under cartap hydrochloride toxicity

The gills being delicate structures are easily affected by contaminated water and rapidly exhibit histopathological effects. This is well reflected in the damage of respiratory epithelium and secondary gill lamellae of exposed fish in the present study. Similar damage of respiratory epithelium and secondary lamellae in the form of
epithelial hypertrophy, hyperplasia, epithelial lifting and necrosis, and curling of secondary lamellae and lamellar fusion have been reported in pesticide-exposed fish gills by several workers (Machado and Fanta, 2003; Velmurugan et al., 2007; Saravanan et al., 2010). Changes in fish gills are among the most commonly recognized responses to environmental pollutants (Au, 2004).

Marked pathological changes were observed in gills of fish, *Labeo rohita*, exposed to sub-lethal concentrations of cartap hydrochloride. In the present study the changes include mild primary, secondary lamellar disorganization, lamellar fusion, congestion of vessels, vascular degeneration and bulging of tip of gill filament were noticed in the 48, 72 and 96 h and no changes were observed in 24 h gill (Plate. IV.5. I. Fig. B, Fig.C, Fig. D and Fig. E).

Histological alterations of the gill in the present study, the fish, *Labeo rohita*, was exposed to sub-lethal (1/10th, static 96 h LC50) concentration of cartap hydrochloride after 24 h, no changes were observed compare to control gill. After 48 h, the primary and secondary lamellar disorganization were noticed as major changes. After 72 h, prominent primary and secondary lamellar disorganization were observed. After 96 h, lamellar fusion and congestion of vessels were noticed.

Alterations like secretion of more mucus, epithelial lifting, hyperplasia and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of some defensive mechanisms. In general, these results increase the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Gupta and Dua, 2002; Fernandes and Mazon, 2003). The epithelial layer of secondary gill lamellae of the fish forms a barrier between the fish blood and surrounding water. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired.
However fish have the capacity to increase their ventilation rate to compensate low oxygen uptake (Fernandes and Mazon, 2003).

Increased mucus secretion, as observed in the exposed carps, has earlier been reported after pesticide exposure in rainbow trout by Altinok and Capkin (2007). Stress arising out of variation in environment and pathologic agents are reported to induce proliferation of mucus cells in gills and increased mucus secretion as defense response (Richmonds and Dutta, 1989). Most part of the gill lesions caused by sub-lethal exposures affects lamellar epithelium; however some alterations in blood vessels may also occur, when fish suffer a more severe type of stress. In this case, damaged pillar cells can result in an increased blood flow inside the lamellae, causing dilation of the marginal channel, blood congestion or even an aneurysm (Rosety-Rodríguez et al., 2002, Gupta and Dua, 2002).

Skidmore and Tovell (1972) stated that the curling of secondary lamellae was due to the reduction in the blood hydrostatic pressure within the pillar cell system and the displacement of lamellae epithelium. The basis of such contention seems to be loss of blood from the nonmarginal space of the secondary lamellae. The lifting of the lamellar epithelium of the gill in *Labeo rohita*, observed in the present investigation, serves as a mechanism of defence, because separation of epithelia from the lamellae increases the distance across which waterborne pollutants must diffuse to reach the bloodstream (Pane et al., 2004). Edema and epithelial lifting as defense response can reduce entry of poisons by diffusion (Barot and Bahadur, 2013). De Boeck et al. (2001) observed that lamellar fusion is a defence mechanism that reduces the branchial superficial area in contact with the external milieu and increases the diffusion barrier to the pollutant copper to *Cyprinus carpio*. Similar information of thickening of gill filament epithelium in fish was reported by several authors.
LEGEND FOR FIGURES

Plate IV.5. I. Gill

Fig.A. Control: Normal gill lamellae of *Labeo rohita*.

- PGL: Primary gill lamellae
- ILR: Inter lamellar region
- SGL: Secondary gill lamellae

Fig.B. Sub-lethal: Gill lamellae of *Labeo rohita* exposed to sub-lethal concentration of cartap hydrochloride for 24 h.

- PGL: Primary gill lamellae
- WC: Water channel
- CA: Central axis

Fig.C. Sub-lethal: Gill lamellae of *Labeo rohita* exposed to sub-lethal concentration of cartap hydrochloride for 48 h.

- MLD: Mild primary disorganization
- SSL: Shortened secondary lamellae
- SLD: Secondary lamellar disorganization

Fig.D. Sub-lethal: Gill lamellae of *Labeo rohita* exposed to sub-lethal concentration of cartap hydrochloride for 72 h.

- PSLD: Prominent secondary lamellar disorganization
- CA: Central axis
- PPLD: Prominent primary lamellar degsorganization

Fig.E. Sub-lethal: Gill lamellae of *Labeo rohita* exposed to sub-lethal concentration of cartap hydrochloride for 96 h.

- CV: Congestion vessels
- LFV: Lamellar fusion vessels
- DPGL: Degenerated Primary Gill Lamella
including Cengiz and Unlu, (2002), Van den Heuvel et al. (2000) and Rosety-Rodríguez et al. (2002). Hyperplasia has been reported as the most remarkable cellular change in the epithelium of secondary gill lamellae of *Labeo rohita* exposed to simazine (Oropesa-Jimenez et al., 2005). In the present study cell proliferation with thickening of the gill filament epithelium is another histological change seen. The proliferated thickening (hyperplasia) of the gill epithelium appears to be a general response to irritation by toxicants. Such types of thickening of gill filament epithelium may lead to lamellar fusion on exposure to all the sub-lethal doses of cartap hydrochloride.

Hyperplasia shown by gills of exposed fish protects the body from diffusion of toxicants by reducing the branchial surface and by increasing the distance between blood and water in which pollutant is dissolved (Oropesa-Jimenez et al., 2005). Gill is the respiratory organ. So contact between gill and pesticide may cause damage the gill tissue and diffusion capacity of gill which in turn has reduced oxygen uptake (Thurberg et al., 1980).

Similar observations have been made by other workers in pesticide exposed fish (Velmurugan et al., 2007; Singh et al., 2009). Respiratory distress is one of the early symptoms of pesticide stress (Murthy, 1986). Decrease in ventilation rate and the consequent fall in oxygen uptake may be due to alteration in energy metabolism under pesticide stress. The formation of an aneurysm is related to the rupture of the pillar cells due to flow of blood or even because of the direct effects of contaminants on these cells (Heath, 1987; Martinez et al., 2004). This is a severe type of lesion. Recovery from it is possible, but it is more difficult than the epithelial changes (Poleksic and Mitrovic-Tutundzic, 1994). In fish, the gill is the first organ to which
the pollutant comes into contact. Hence, it is more vulnerable to damage than any other tissue. The proliferative gill lesions are often observed after exposure of fish to toxicants.

Jiraungkoorskul et al., (2002) reported histopathological changes in the liver and gills of Nile tilapia, *Oreochromis niloticus*, exposed to glyphosate herbicide. In the gills, filamentous cell proliferation, lamellar cell hyperplasia, lamellar fusion, epithelial lifting and aneurysm were observed. Coutinho and Gokhale (2000) found epithelial lifting in the gills of carps (*Cyprinus carpio*) and tilapia (*Oreochromis mossambicus*) exposed to the effluents of a waste water treatment plant. Engelhardt et al. (1981) observed epithelial lifting and lamellar fusion in rainbow trouts (*Oncorhynchus mykiss*) exposed to petroleum residues. Similar alterations were also reported in the gills of fish exposed to metals (Oliveira Ribeiro et al., 2000; Cerqueira and Fernandes, 2002; Martinez et al, 2004) and organic contaminants (Rosety-Rodríguez et al., 2002; Fanta et al., 2003).

Dhanapakiam et al. (2004) observed that in the fish, *Labeo rohita*, exposed to lethal and sub-lethal concentration of tannery effluent after 24 h, the gills showed swelling in the epithelial cells, fusion of secondary lamellae, bulging of tips of gill filaments and atrophy of the lamellae which results in shifting of energy metabolism. The mucus was secreted more than the normal fish. The epithelial cells showed hyperplasia besides a thin coat of mucus over the gills. The increased mucus secretion was also helpful in attenuating the osmotic influence of environmental stress in teleost gills.

Marina et al. (2007) studied the histological alterations in the gills of the fish, *Prochilodus lineatus*, caged at different sites at Cambe stream. The commonest anomalies found were considered to be at stage I in severity; these include dilation of
the marginal channel, hyperplasia of the epithelial cells, lifting of the lamellar epithelium, hypertrophy of epithelial cells and lamellar disorganization.

Jayachandran and Pugazhendy (2009) observed excessive mucus secretion, lifting up of the epithelium and lamellar fusion in the gills of fish *Labeo rohita* exposed to atrazine. Saravanan et al. (2010) observed the histopathological changes in the gills of endosulfan treated fish, *Labeo rohita*, such as shapeless primary and secondary lamellae, haematomas and aneuryama. Debris of inter lamellar epithelium, damaged secondary lamellae and red blood cells were often observed between secondary lamellae and primary lamellae.

Anita Susan et al. (2012) observed that fenvalerate caused *Labeo rohita* atrophy and complete fusion of secondary gill filaments, obliterating the interlamellar region in the gills of *Labeo rohita*. In *Catla catla*, degenerative changes in intralamellar cells and interlamellar spaces, club shaped secondary lamellae, vacuolar degeneration within the nucleus, separation of epithelial layer from the central sinus of filaments and severe proliferation of epithelial cells were noticed. The conspicuous change observed in the gill of *Cirrhinus mrigala* was the greater dilation of primary gill lamella compared to secondary gill lamellae.

Das and Gupta (2012) observed that gills of Indian flying barb, *Esomus danricus*, exposed to endosulfan showed various histopathological changes including marked epithelial lifting, lamellar fusion, hyperplasia, hypertrophy, mucus secretion, mucous cell proliferation, vascular congestion and blood sinus constriction after 28 days of toxicant exposure. Jothinarendiran (2012) reported that the intimate contact of gills of *Channa punctatus* with toxicant dimethoate caused histopathological changes. Damage of gill tissue reduced the diffusion capacity of the gill leading the decrease in oxygen uptake (Thurberg et al., 1980). Nikalje et al. (2012) observed shorterning of
secondary lamellae, dilation of primary gill lamellae, destruction of mucous cells, detachment of epithelial surface in primary gill lamellae, disintegration of spongy cartilage and thickening of secondary gill lamellae in the gill of *Labeo rohita* exposed to textile mill effluent (TME).

Maharajan *et al.* (2013) reported that after exposure of profenofos the gill tissues of *Catla Catla* (Hamilton) revealed, rupture of capillaries at the tip of secondary gill lamellae releasing blood cells, hemorrhage between gill filaments, telangiectasis in secondary lamellae and hemocytes accumulated in secondary gill lamellae. Butchiram *et al.* (2013) observed multiple changes, including epithelial hyperplasia, fusion of secondary lamellae, degeneration of primary and secondary gill lamellae and enhanced mucus production, in *Labeo rohita* after exposure to phenol.

Ram Narayan Singh (2014) observed changes in the gills of dimethoate exposed fish, *Cyprinus carpio*. They were epithelial hyperplasia, lifting and degeneration of respiratory epithelium, lamellar bending and curling, fusion and disintegration of secondary gill lamellae. Dey and Saha (2014) reported that the gill of dimethoate-treated fish *Labeo rohita* (Hamilton) gill showed marked histological changes such as detachment of epithelial surface in primary gill lamella from secondary lamella, swelling at the tips of the secondary gill lamellae, hypertrophy and marked hyperplasia, separation of the basement membrane, curling and fusion of adjacent gill lamellae after 10 days.

**IV.5.3. General Histology of Liver**

The surface of liver is covered with serous membrane and some connective tissue extends inward into parenchyma. The normal liver is a large bilobed orange-coloured organ. The parenchyma tissue is composed of parenchymal cells (hepatic cells) and lattice fibers to support the former. Hepatic cells are roundish polygonal
containing clear spherical nucleus and granular cytoplasm. Hepatic cells (hepatocytes) are located among the sinusoids forming cord-like structures known as hepatic cell cords. The bile canaliculus is centrally located in each cord. The cords extended between central and portal zones (Hinton et al., 1972; Kendall and Hawkins, 1975; Hinton and Pool, 1976 and Brusle and Anadon, 1996). Pancreatic tissue can be differentiated from hepatic tissue by its acinar arrangement. In addition, thin septa of connective tissue separate the hepatocytes from the exocrine pancreatic cells (Brusle and Anadon, 1996).

The liver is the central metabolic organ of fish and has numerous anabolic and catabolic functions. In contrast to the heterogeneous physiology of the organ, its structure is very homogeneous. The liver is an organ that stores carbohydrates as glycogen and, especially before spawning, fats. The hepatocytes normally appear compact, may give the impression of being more or less vacuolized under the light microscope, according to the degree of fat storage.

The liver stores nutrients absorbed from the digestive tract, releasing them to other tissues. Hepatic metabolism is essentially related to the anabolism of proteins, lipids, and carbohydrates, and the catabolism of glycogen and nitrogen (Lilian Franco-Belussi et al., 2012). Liver functions also include storage of substances, bile secretion, and detoxification of pollutants and toxic agents (Hildebrand and Goslow, 2006). In addition these functions liver plays an essential role in maintaining body homeostasis (Hildebrand and Goslow, 2006; Rappaport, 1963). The control, normal structure of liver of *Labeo rohita* is shown in Plate.IV.5. II. Fig.A.

**IV.5.4. Pathology of Liver tissue under cartap hydrochloride toxicity**

The changes in the liver of fish *Labeo rohita* exposed to sub-lethal concentration of cartap hydrochloride were characterized by prominent yellowish
brown pigment (48 h), cytoplasmic degeneration and vacuoles (72 and 96 h), disappearance of hepatic cells and change in the shape of the hepatocytes. The decrease in the diameter of the hepatic cell was due to shrinkage of the cell. The nuclei became pyknotic and eccentric. In addition to these changes hypertropic nuclei and hyperplasia of cells and degeneration of cell membrane were also observed in the liver of 48 h, 72 h and 96 h. Relatively less effect were observed in the liver of 24 h (Plate.IV.5.II. Fig.B, Fig.C, Fig.D and Fig.E).

Fish liver is a key organ and controls many life functions and plays a prominent role in fish physiology, both in anabolism (proteins, lipids and carbohydrates) and catabolism (nitrogen, glycogenolysis, detoxication). Fish are especially susceptible to environmental variations and respond more sensitively to pollutants than numerous mammals (Jacques Brusle and Gemma Gonzalezi Anadon, 1995). Since the liver has a primary role in the metabolism and excretion of xenobiotic compounds, in digestion and storing, and also in the production of yolk protein, structural alterations can obviously occur in some toxic conditions.

The Liver is the largest and important organ of the body doing several physiological functions. It has no direct contact with pollutants dissolved in water. Due to its contact with blood it is indirectly affected. The diffusion of organophosphates depends on lipid solubility and the removal by the blood depends on the lipid content of the blood or special carriers (Randall et al., 1996).

This was the reason for some changes in the liver cell morphology being observed first close to blood vessels. Singh (2009) and Dey and Saha (2014) noticed such type of changes in common carp (C. carpio) after dimethoate exposure. The organ most associated with the detoxification and bio-transformation process is the liver.
LEGEND FOR FIGURES

Plate.IV.5.II. Liver

Fig.A. Control: Normal structure of liver of *Labeo rohita*

- N: Nucleus
- HC: Hepatic cell
- EG: Eosinophilic granules

Fig.B. Sub-lethal: Structure of liver of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 24 h.

- HC: Hepatic cell
- EG: Eosinophilic granules

Fig.C. Sub-lethal: Structure of liver of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 48 h.

- DHPT: Degeneration of hepato-pancreatic tissue
- PYBP: Prominent yellowish brown pigment

Fig.D. Sub-lethal: Structure of liver of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 72 h.

- CV: Cytoplasmic vacuoles
- CD: Cytoplasmic degeneration

Fig.E. Sub-lethal: Structure of liver of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 96 h.

- CV: Cytoplasmic vacuoles
- CD: Cytoplasmic degeneration
- FV: Formation of vacuoles
Due to its function, position and blood supply (Van der Oost et al., 2003) it is also one of the organs most affected by contaminants in the water (Rodrigues and Fanta, 1998). In fish liver, the presence of necrosis area is also related with xenobiotic concentration during detoxifying process (Mela et al., 2007). The results of the present study are agreement with earlier reports of Saravanan et al. (2003) in Oreochromis mossambicus, Nagarajan and Aruna Devi (2006) in Labeo rohita and Saravananan et al. (2010) in Labeo rohita. Radhaiah and Jayantha Rao (1992) reported moderate cytoplasmic degeneration in hepatocytes, formation of vacuoles, rupture in blood vessels and appearance of blood cells among hepatocytes, pyknotic nuclei in the liver of Tilapia mossambica exposed to fenvalerate. Similar changes were observed in three Indian major carps Catla catla, Labeo rohita and Cirrhinus mrigala exposed to fenvalerate by Anita Susan (1994) and Vijayalakshmi (1994). Tilak et al. (2001a, 2001b) reported the same degenerative changes in Labeo rohita and Ctenopharyngodon idellus under fenvalerate toxicity.

Loganathan et al. (2006) reported that the histological alterations in the liver of Labeo rohita caused severe necrosis, hemorrhage, distended sinusoids with minor vacoulation and pyknotic nuclei. Vidyulata Devi (2008) also reported the same in C.punctatus under lethal and sub-lethal exposure to indoxacarb. Peters et al. (1987) observed liver cell shrinkage, hepatocytomegaly which is an excessive hypertrophy of individual liver cells, local blood congestions in the liver sinusoids and an increasing dissolution of the liver cell strings (trabeculae). Anomalies such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position, close to the cell membrane, were also described in the siluriform Corydoras paleatus contaminated by organophosphate pesticides (Fanta et al., 2003). Pacheco and Santos (2002) described that increased vacuolisation of the hepatocytes is a signal of
degenerative process that suggested metabolic damage, possibly related to exposure to contaminated water. Histopathological change, bile stagnation in liver, was characterized by the remains of the bile in the form of brownish-yellow granules in the cytoplasm of the hepatocytes (Pacheco and Santos, 2002). This accumulation of bile indicated possible damage to the hepatic metabolism (Fanta et al., 2003).

Camargo and Martinez (2006) observed that the main alterations found in the liver were irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation and the presence of eosinophilic granules in the cytoplasm. Bile stagnation was identified as brownish-yellow granules in the cytoplasm. The majority of the alterations found in the liver of the caged animals belongs to stages I and II. Saravanan et al. (2010) reported that enlargement of liver cells with vacuolation, histolysis and necrosis in *Labeo rohita* after 50 days of exposure to *Azadirachta indica* (A. Juss). Further, the treated fish liver showed shrinkage, disintegration and vacuolation ultimately resulting in necrosis compared to normal hexagonal cells found in the control liver.

Sridhar and Joice (2012) reported that exposure of *Cyprinus carpio* to carbendazim induced obvious histopathological changes in the liver. Such as the hepatocytes lost their normal architecture. The lumen of sinusoid contained mainly erythrocytes and macrophages. The intrahepatic blood vessels were dilated and congested with blood and inflammatory leucocytic infiltrations. They also observed marked cytoplasmic vacuolization, an intense migration of cells, such as red blood cells from sinusoid to hepatic parenchyma if the fish which were exposed to carbendazim for longer duration.

Butchiram et al. (2013) reported that the changes in the liver of fish, *Labeo rohita* exposed to phenol included formation of a number of vacuoles, enlargement of nuclei of some cells, and enlarged sinusoids with numerous blood cells at sub-lethal
consentration, and nuclear and cytoplasmic degeneration and melanomacrophages aggregated at lethal consentration. Mohammad et al. (2013) reported the histopathological changes in the liver of fish, *C. gariepinus*, collected from El-Rahawy drain which includes loss of cellular architecture of liver, vacuolar degeneration, pycnotic nuclei, leukocyte infiltration, hyaline degeneration and focal areas of necrosis of the hepatocytes. Valon et al. (2013) observed that the nuclear karyolysis and karyopiknosis, Hydropic vacuolation (HydVac), inflammation with lymphocytes of portal areas and lytic necrosis were noticed in the Sitnica river fish. Anita Susan et al. (2012) observed a number of intercellular empty spaces, degenerative changes in the peripancreatic tissue and blood congestion (blood appeared as streaks) in the liver of *Labeo rohita, Catla catla* and *Cirrhinus mrigala* exposed to fenvalerate. Deore and Wagh (2012) reported that histopathological impact of lethal and sub-lethal concentrations of mercury chloride and copper chloride in liver of freshwater teleost, *Channa gachua* (Ham) revealed vacuolation in cytoplasm, degeneration of nuclei, vacuolation in stroma, cloudy swellings, pycnotic nuclei, necrosis, rupture of blood sinusoids and disarray of hepatic cords.

Deore and Saha (2014) studied that dimethoate exposure at different doses caused severe pathological lesions in liver tissue of *Labeo rohita*. The fish liver showed dissociated swollen hepatocytes, vacuolisation, extensively degenerated and granular cytoplasm, Karyolysis and pyknosis of nuclei and damage of central veins.

**IV.5.5. General Histology of fish Kidney**

In fish, as in higher vertebrates, the kidney performs an important function to maintain the homeostasis. The kidney, being the only organ for elimination of toxic materials, is affected by contaminants in water (Thophon et al., 2003; Mela et al., 2007). Histopathological alterations can be used as indicators of the effects of various
pollutants on the organism especially fish. Kidney is an important organ of excretion and osmoregulation and is highly susceptible to toxic substance because of its high blood supply. Kidney is a vital organ of body, for it is important to maintain the homeostasis, maintaining volume and pH of blood and body fluids, as well as, erythropoiesis (Hickman and Trump, 1969). It is well known that freshwater teleosts keep up the hypertonicity of their blood mainly with the help of the water-excreting kidney. The kidney may undergo some structural changes affecting other functions, as erythrocytes production, and consequently, condition of fish in general. Silva and Martinez (2007) stated that the kidney histopathology is a general quality indicator of the aquatic environment.

Kidneys are extremely elongated bodies extending along the whole length of the visceral cavity. They are situated on the dorsal side of the body wall above the swim-bladder and are distinct anteriorly but become partly fused in the middle region. Teleostean kidney consists of head and body kidneys. Head kidney is the anterior portion of the kidney and consists of lymphoid tissue. Body kidney comprises of numerous functional excretory units, the nephrons and intestinal lymphoid tissue (haematopoietic tissue). Each nephron consists of a renal corpuscle and a long convoluted uriniferous tubule. The renal corpuscle is made up of a double walled cup called the Bowman’s capsule and a knot of arterioles termed glomerulus. The Bowman’s capsule is lined by an outer parietal layer and an inner visceral layer of epithelial cells.

Renal tubules are thin and short in the neck segment. The proximal convoluted segment is divided into two parts, segment I and segment II. The renal tubules are composed of cuboidal epithelial cells with densely arranged microvilli in the tubular lumen. In segment II, renal tubules are composed of cuboidal epithelial cells.
Cilia and microvilli are found in the tubular lumen. In the distal convoluted segment, epithelial cells have no microvilli. The cells of this segment are stained with eosin more faintly than those of proximal convoluted segment. Thus, it is easy to distinguish between proximal and distal convoluted segments under light microscopy (Oguri, 1982). The normal structure of kidney of *Labeo rohita* is shown in the Plate.IV.5.III.Fig. A.

**IV.5.6. Pathology of Kidney tissue under cartap hydrochloride toxicity**

On microscopic examination of kidney of the fish, *Labeo rohita*, treated with the sub-lethal (1/10th of Static, 96 h LC$_{50}$) concentration of cartap hydrochloride for 24, 48, 72 and 96 h, Prominent tubular yellowish brown pigment (48 h), Tubular Cytoplasmic degeneration (72 and 96 h) necrosis of cell and renal tubules, cloudy swelling in renal tubules, degeneration of cytoplasm within pyknotic nuclei, aggregation of cells and disorganization of connective tissue were observed. The disintegration of cell membrane and hypertrophy of nuclei were also seen (Plate. IV.III.Fig.B, Fig.C, Fig.D and Fig.E).

Kidney damage was due to elimination of undetoxified toxicant molecule through urine. Cytoplasmic granules may be formed inside the cells or the reabsorption of plasma proteins lost in the urine, indicating damage in the corpuscle (Takashima and Hibya, 1995).
LEGEND FOR FIGURES

Plate IV. 5.III. Kidney

**Fig. A. Control:** Normal structure of kidney of *Labeo rohita*.
- BC: Bowman’s capsule
- G: Glomerulus
- UT: Uriniferous tubule

**Fig. B. Sub-lethal:** Kidney of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 24 h.
- DCS: Distal convoluted segment
- G: Glomerulus
- BC: Bowman’s capsule

**Fig. C. Sub-lethal:** Kidney of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 48 h.
- DG: Damaged glomerulus
- PTYBP: Prominent tubular yellowish brown pigment
- DUT: Damaged uriniferous tubule

**Fig. D. Sub-lethal:** Kidney of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 72 h.
- TCD: Tubular cytoplasmic degeneration
- DHT: Damage of haematopoietic tissue
- DCS: Distal convoluted segment

**Fig. E. Sub-lethal:** Kidney of *Labeo rohita*, exposed to sub-lethal concentration of cartap hydrochloride for 96 h.
- DRT: Damage of renal tubule
- DB: Damage of Bowman’s capsule
- DHT: Damage of haemopoietic tissue
- TCD: Tubular cytoplasmic degeneration
The present observations are in agreement with the findings of Tilak et al. (2001a and b) and Koteswara Rao (2003), Pallavi Gupta and Neera Srivastava, (2006); Chhaya Bhatnagar et al. (2007); and Manosathiyadevan et al. (2009), (2009a) and Manosathiyadevan and Divyavlayudhan (2012). They observed renal damage, rupture in the glomeruli and reduced renal tubules and reduced lumen in *Labeo rohita*. Epithelial swelling, swelling of mitochondria in the renal tubules and necrosis were also reported in animals administered with methothrin and pyrethrin (Grue et al., 1983). Nutan Kumari et al. (1989) reported shrinkage of glomerulus of kidney in *Channa punctatus* exposed to cadmium chloride. Dubale and Punita Shah (1981) observed that the proximal tubules of the kidney of *Channa punctatus* were fully damaged after exposure to cadmium chloride. Severe necrosis, vacuoles around renal tubule of the kidney and haemorrhage were observed in the kidney of fish, *Cirrhinus mrigala* exposed to fenvalerate (Anita Susan and Tilak, 2003).

According to studies by Mohamed (2009), it was shown that the exposure of fish, *Tilapia zillii* and *Solea vulgaris*, to agricultural and industrial chemicals resulted in several pathological changes in different tissues of fish. Similar alterations in histopathology were also reported in the *Oreochromis spp.*, exposed to hexavalent chromium (Abbas and Ali, 2007). Cengiz (2006) observed degeneration in the renal tubule, pycnotic nuclei in the hematopoietic tissue and degeneration of glomerulus. Similar alterations in the kidney have also been reported in Nile tilapia exposed to ammonia (Benli et al., 2008). Camargo and Martinez (2006) reported that the most important change found in the glomerulus of *P. lineatus* kidney was glomerular expansion, resulting in reduction of Bowman’s space. In the tubules, the most frequent alterations were cloudy swelling, occlusion of tubular lumen and hyaline droplet degeneration.
Dhevakrishnan and Zaman (2012) observed that the histopathological studies of the kidney of the fish, *Labeo rohita* collected from more polluted region showed degeneration and atrophy of renal tubules, degeneration in glomerulus, and disorganization of glomerulus, severe necrosis and highly pycnotic nuclei. These changes were induced by untreated industrial effluents discharged into the more polluted region. Anita Susan *et al.* (2012) observed cloudy swelling of renal tubules, cellular hypertrophy, granular cytoplasm, moderately degeneration of proximal convoluted and secondary convoluted tubules and hemorrhage in the kidney of *Cirrihinus mrigala, Catla catla* and *Labeo rohita* exposed to fenvalerate. Butchiram *et al.* (2013) observed degeneration of proximal and distal convoluted tubule, vacuolization of renal interstitial tissue and deformation of the nuclear membrane of some cells at sub-lethal concentration, and occlusion of tubular lumen, cloudy swelling, and hyaline droplets degeneration at lethal concentration. Dey and Saha (2014) reported that the kidney of fish, *Labeo rohita* treated with dimethoate, showed large space between tubules and tissue, shrunk, fragmented glomeruli with large Bowman’s space, enlarged lumen, disintegrated and vacuolated renal tubules, increased cytoplasmic granules, necrotic changes, hyperplasia and karyolysis.

Thus, the exposure of fish, *Labeo rohita*, to pesticide cartap hydrochloride lead to irreparable architectural changes in various vital organs gill, liver and kidney, making the fish less fit for better survival. These histopathological changes can alter various physiological activities of the fish such as release of various enzymes and the metabolic processes as evidenced from Chapter-IV.

In conclusion, the present study showed that the histopathology is a useful biomarker for environmental contamination, since all the three organs, gill, liver and
kidney, are slightly affected by pesticidal pollution. The histopathological alterations resulting from an exposure to cartap hydrochloride may affect the functional efficiency of gill, liver and kidney leading to malfunctioning of several organ systems of the fish. This in turn may cause the death of the fish.

**IV.6. Residue Analysis**

The results of the thin layer chromatography Rf values of cartap hydrochloride in four different solvent systems were given in Table.IV.6.45. The results of the high pressure liquid chromatographic (HPLC) analysis in the tissue of gill, brain, heart, liver, kidney, and muscle tissues of the fish, *Labeo rohita* were given in Table.IV.6.47, Table.IV.6.48, Table.IV.6.49, Table.IV.6.50, Table.IV.6.51 and Table.IV.6.52; and Fig.IV.6.48, Fig.IV.6.49, Fig.IV.6.50, Fig.IV.6.51, Fig.IV.6.52 and Fig.IV.6.53. Under exposure to sub-lethal concentration, the order of accumulation of residues of cartap hydrochloride was

Liver > Gill > Kidney > Muscle > Brain > Heart

Under sub-lethal exposure to cartap hydrochloride for 24 h, 48 h, 72 h and 96 h, it was observed that the liver tissue accumulated more residue than the other tissues and least amount of residue was found in heart. The other peaks which appeared in the HPLC analysis may be the metabolites of the parent compound cartap hydrochloride or other pesticide residues with similar reports. In TLC confirmation tests (Fig.IV.6.46) also, the residues of cartap hydrochloride appeared as blue spots, prominently in liver sample and less prominently in other samples.

The variations in the residue analysis are attributed to factors like difference in uptake rate and lipid content of respective animal tissue. The chemical structure, solubility, fish interaction and metabolic pattern are responsible for pesticide uptake. The results of the present study revealed that prolonged exposure to sub-lethal
concentrations led to increase in the accumulation of residue. This is in agreement with the earlier reports by Bradbury et al. (1987), Tripathi (1992); Tilak et al. (2003 and 2004); Rose et al. (2013) and Enbaia et al. (2014). The accumulation is a factor responsible for changes in biochemical actions or pathological changes and also disturbance of overall biochemical cyclic reactions which were cumulative causing lethal actions even when the concentrations are at sub-lethal.

According to Tilak et al. (2001), in the freshwater fish *Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*, *Aplodcheilus punchex* and *Ctenopharyngodon idellus*, exposed to fenvalerate to both lethal and sub-lethal concentrations, the pesticide residues bioaccumulated in the lipid tissues of the fish. Among the above-mentioned species, *Labeo rohita* showed more accumulation followed by *Catla catla* and *Cirrhinus mrigala*. Tilak et al. (2003) exposed the freshwater major carp, *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla*, to sub-lethal concentrations of chlorpyrifos for eight days, and noticed that *Labeo rohita* tissues bioaccumulated more amount of pesticide, compared to *Catla catla* and *Cirrhinus mrigala*. They also observed that in all the three fish, the brain tissue accumulated more residue than the liver. According to Bagheri (2007) residues of OP insecticides in the fish species and the water depend on the physiochemical characteristics of water, time of consumption, pH of water and the ambient temperature.

Tilak et al. (2004) also observed that the residues of chlorpyrifos accumulated more in brain than in liver of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Mohammed Sweilum (2007) studied the effect of sub-lethal toxicity and bioaccumulation of dimethoate and malathion in Nile tilapia *Oreochromis niloticus*. Pesticide residues in the liver, gills and muscles of fish increased with increased
pesticide concentrations in fish ponds. Their bioaccumulation in the liver was higher than in gill or muscle, which had the lowest residues for these pesticides.

Essumang et al. (2009) observed pesticide residues in the water and fish (Lagoon tilapia) samples from lagoons in Ghana. Afful et al. (2010) identified and quantified an organochlorine pesticide residues namely gamma-HCH, delta-HCH, heptachlor, aldrin, gammachlordane, p,p’-DDE, alpha-endosulfan, dieldrin, endrin, endrin-aldehyde, endosulfan-sulfate, p,p’-DDT, endrinoctone and methoxychlor in six fish species namely Heterotis niloticus, Channa obscura, Hepsetus odoe, Tilapia zilli, Clarias gariepinus and Chrysichthys nigrodigitatus collected from Densu basin, Ghana.

Suneetha (2012) reported organochlorine and nitrogen containing pesticide residues in Labeo rohita. They traced the residues of DDT, endosulfan, p-methyl, cypermethrin, deltamethrin, atrazine and isoproturan in fish hatchery of Pakistan. Similar findings were also reported by Mahboob et al. (2011) in Cirrhinus mrigala. Suneetha (2012) observed residues in Labeo rohita exposed to sub-lethal concentration of endosulfan after 15 days of exposure. It was observed that liver tissue accumulated more residue than the other and minimum was noticed in muscle.

Liver is the main detoxifying tissue containing relatively high levels of detoxifying enzyme. It is also the first organ to face the effect of pesticides being carried through the portal circulation which might have been the cause of the greater accumulation of larvin. Mono oxygenase enzymes are found in high concentration in the liver and many tissues such as gonad, kidney intestine, gill and heart (Lindstrom Seppa et al., 1981). These enzymes decrease the lipid solubility of organic contaminants thereby facilitating excretion of the pollutants (Verma and Gupta, 1976). The rapid loss of dimethoate from liver was reported by Ghausia Begum et al. (1994).
Table IV. 6. 45.

**Pesticide Standard Confirmation and the Tissue Chromatogram**

<table>
<thead>
<tr>
<th>Layer</th>
<th>Silica gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>Hexane + Acetone and water (90+5+5 ( V/V ))</td>
</tr>
<tr>
<td>Front</td>
<td>10 Cm</td>
</tr>
<tr>
<td>Impregnated reagent</td>
<td>0.3 Per cent Silver nitrate</td>
</tr>
<tr>
<td>Time</td>
<td>30 minutes</td>
</tr>
<tr>
<td>UV light exposure</td>
<td>10 minutes</td>
</tr>
<tr>
<td>Hours and Exposure</td>
<td>24, 48, 72 and 96 h</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>R(_f) Values of standard in different solvent systems</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent System</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Hexane + Acetone</td>
</tr>
<tr>
<td>Acetone + Water</td>
</tr>
<tr>
<td>Hexane + Acetone</td>
</tr>
<tr>
<td>Acetonitrile + Water</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>100xRf</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill</td>
<td>86.2</td>
</tr>
<tr>
<td>Brain</td>
<td>52.4</td>
</tr>
<tr>
<td>Heart</td>
<td>48.6</td>
</tr>
<tr>
<td>Liver</td>
<td>89.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>74.4</td>
</tr>
<tr>
<td>Muscle</td>
<td>56.8</td>
</tr>
</tbody>
</table>
Fig.IV.6.46. Standards (A) and Chromatogramme (B) of cartap hydrochloride in thin Layer chromatography (TLC).
Fig. IV.6.47. A. Calibration curve of cartap hydrochloride.

![Calibration curve](image)

B. RP-HPLC chromatogram of cartap hydrochloride standards

![RP-HPLC chromatogram](image)

Rose *et al.* (2013) assessed the levels of OCs in water, sediment, invertebrates (crayfish shrimps and crabs) and twelve species of fish, and reported that the most bioaccumulated OCs in the fish and invertebrates were beta-HCH and p, p’DDE. Choudhury *et al.* (2013) studied the presence of pesticide residues in fish samples *Puntius sophore, Amblyparyngodon mola, Cirrhinus mrigala, Catla catla, Labeo rohita, Labeo goniuf, Cyprinus carpio* and *Labeo calbasu*
Akan et al. (2013a and b) detected eleven organochlorine pesticide residues in all the fish *Clarias gariepinus, Heterotis niloticus, Oreochromis niloticus* and *Tilapia zilli*. Endosulfan was the most abundant pesticide residue found in tissues of all the fish species. He also reported some organophosphorus pesticides, dichlorvos, diazinon, chlorpyrifos and fenitrothion in the flesh, liver, stomach and gills of four commercially species, *Clarias gariepinus, Heterotis niloticus, Oreochromis niloticus* and *Tilapia zilli* from Alau Dam, Borno State.

Table IV.6.46. Retention time and Sensitivity of cartap hydrochloride.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>t_R (min)</th>
<th>Equation</th>
<th>R^2</th>
<th>LOD (µg/L)</th>
<th>LOQ (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cartap hydrochloride</td>
<td>3.0</td>
<td>y = 139.3x + 3475</td>
<td>0.944</td>
<td>0.1</td>
<td>0.012</td>
</tr>
</tbody>
</table>

The linear correlation coefficient was 0.944. Limit of detection (LOD) of cartap hydrochloride was calculated at a single-to-single ratio of 3, while the limit of quantification (LOQ) was obtained at a single-to-single ratio of 10. The LOD and LOQ for cartap hydrochloride were 0.1 (µg/L) and 0.012 (µg/L) respectively. The mobile phase and flow rate conditions were suitable for estimation of cartap hydrochloride residues in the freshwater fish, *Labeo rohita*. In the present study the residue concentrations of cartap hydrochloride in the blood was not detected.

Enbaia et al. (2014) studied the estimation of organochlorine pesticide residues in Libyan fish Round Sardinella (*Sardinella aurita*), European Pilchard (*Thunnus thynnus*), Yellow Fin Tuna (*Thunnus albacares*) and Bogue (*Boops boops*).
Table IV.6.47. Gill tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Concentration (µg.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.089 ± 0.032</td>
</tr>
<tr>
<td>48</td>
<td>0.123 ± 0.041</td>
</tr>
<tr>
<td>72</td>
<td>0.241 ± 0.018</td>
</tr>
<tr>
<td>96</td>
<td>0.386 ± 0.021</td>
</tr>
</tbody>
</table>

Fig IV.6.48. Gill tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Table IV.6.48. Brain tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Concentration (µg.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.047 ± 0.28</td>
</tr>
<tr>
<td>48</td>
<td>0.112 ± 0.14</td>
</tr>
<tr>
<td>72</td>
<td>0.184 ± 0.23</td>
</tr>
<tr>
<td>96</td>
<td>0.263 ± 0.45</td>
</tr>
</tbody>
</table>

Fig IV.6.49. Brain tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Table IV.6.49. Heart tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Withdrawal time (h)</th>
<th>Concentration (µg·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.007 ± 0.18</td>
</tr>
<tr>
<td>48</td>
<td>0.032 ± 0.29</td>
</tr>
<tr>
<td>72</td>
<td>0.046 ± 0.41</td>
</tr>
<tr>
<td>96</td>
<td>0.098 ± 0.36</td>
</tr>
</tbody>
</table>

Fig IV.6.50. Heart tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Table IV.6.50. Liver tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Withdrawal time (h)</th>
<th>Concentration (µg.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.092 ± 0.24</td>
</tr>
<tr>
<td>48</td>
<td>0.254 ± 0.35</td>
</tr>
<tr>
<td>72</td>
<td>0.412 ± 0.18</td>
</tr>
<tr>
<td>96</td>
<td>0.624 ± 0.24</td>
</tr>
</tbody>
</table>

Fig IV.6.51. Liver tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Table IV.6.51. Kidney tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Withdrawal time (h)</th>
<th>Concentration (µg.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.009 ± 0.36</td>
</tr>
<tr>
<td>48</td>
<td>0.048 ± 0.27</td>
</tr>
<tr>
<td>72</td>
<td>0.187 ± 0.19</td>
</tr>
<tr>
<td>96</td>
<td>0.352 ± 0.45</td>
</tr>
</tbody>
</table>

Fig IV.6.52. Kidney tissue (Mean ± SD) concentrations of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Table IV.6.52. Muscle tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.

<table>
<thead>
<tr>
<th>Withdrawal time (h)</th>
<th>Concentration (µg.g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0.027 ± 0.23</td>
</tr>
<tr>
<td>48</td>
<td>0.083 ± 0.35</td>
</tr>
<tr>
<td>72</td>
<td>0.214 ± 0.28</td>
</tr>
<tr>
<td>96</td>
<td>0.324 ± 0.19</td>
</tr>
</tbody>
</table>

Fig IV.6.53. Muscle tissue concentrations (Mean ± SD) of cartap hydrochloride in fish, *Labeo rohita* after exposure to sub-lethal concentration for 24, 48, 72 and 96 h.
Organochlorine pesticides, endosulfan, heptachlor, methoxychlor, dieldrin, residues were found in those fish. Akan et al. (2014) reported the presence of organochlorine and organophosphorous pesticide residues, dichlorodiphenyl dichloroethylene, (o,p’-DDE), 4,4’-DDD, 4,4’-DDT), dichlorvos, diazinon, chlorpyrifos, fenitrothion, Alpha BHC, Gamma BHC, metoxichlor, lindane, endosulfan sulphate, dieldrin and aldrin in organs of liver, gills, stomach and flesh of *Tilapia zilli*, *Clarias Gariepinus*, *Hetrois niloticus* and *Oreochromis niloticus* from Lake Chad.

The results of the present study indicate that prolonged exposure to sub-lethal concentration of cartap hydrochloride in freshwater fish, *Labeo rohita*, leads to increased accumulation of residues. This is in corroboration with the earlier reports of carbamate residues. Thus the uptake and persistence of cartap hydrochloride not only depends on a number of physical and chemical conditions, but also varies according to the biological conditions. Lipid and water contents are different among fish (FDA, 1999). Even for the same fish species, the lipid content could vary due to seasonal or physiological changes (Mendez and Gonzalez, 1997). Besides, pesticides may differ in their hydrophilic or hydrophobic characteristics. Residue levels in fish vary with species due to differences in lipid content, biology (trophic level habitat and reproductive season), exposure, detoxification, capability, and ecology (Nowak and Julli, 1991). The differences in residue levels may also be a reflection of different exposure regimes and innate individual differences in metabolism.

Over the years, pesticides have been determined by many conventional as well as modern day methods like spectrophotometry (Randhir Kumar and Banerjee, 2012), Polarography (Lin et al., 1999), Gas chromatographic technique, using various detectors or in combination with MS (wong et al., 2012). Extensive research has been
carried out on the analysis of pesticide residues in foodstuffs and environmental matrices (Wong et al., 2012; Curl et al., 2003).

Pesticides, organochlorines, organophosphates and carbamates have emerged as human-made potential threat to the biota and its environment and their use has been clearly identified as a principal driving force behind the drastic reduction of biodiversity in different parts of the world (Wahid Abdul, 2004). The fish, *Labeo rohita*, is one of the non-target organisms to be affected by the pesticides that are used in the fields. The aquatic organisms that inhabit different aquatic bodies are facing the problem with the invasion of pesticides used in high quantities for agricultural practices. The fish affected by the pesticide could pose a health problem to the people who consume the fish from the contaminated environment. The movement of Pesticides in the environment and the organisms is shown in the Fig.IV.6.54.

The results of the present study revealed that prolonged exposure to sub-lethal concentrations of cartap hydrochloride in *Labeo rohita* leads to increased accumulation of residues. This is in corroboration with the earlier reports of OP residues. A thorough literature search revealed that repeated or continuous exposure to low concentrations of pesticides can lead to high residue concentrations without mortalities. Thus the uptake and persistence of larvin depends not only on a number of physical and chemical conditions, but also varies according to the biological conditions. The fish diseases are prevailing, locally fish farmers are spraying OP compounds for control which has to be monitored curtailed and regulated.
Fig.IV.6.54. The movement of Pesticides in the environment and the organisms (Adopted from Sharma and Kaur, 1995).
All systemic compounds have effects with time of exposure. However, only the persistent chemicals (fipronil, neonicotinoids, cartap and some OPs) have cumulative effects over time, since the non-persistent compounds are quickly degraded in soil and water. For risk assessment of these compounds it is important to understand their chronic impacts. Unlike traditional protocols based on acute toxicity, the persistent activity of the parent and toxic metabolites requires that exposure time must be taken into consideration (Halm et al., 2006). Concerns about the impacts of dietary feeding on honey bees and other non-target organisms are thus justified (Alix and Vergnet, 2007; Cresswell, 2011; Rortais et al., 2005), because the accumulation of small residue levels ingested repeatedly over time will eventually produce a delayed toxic effect (Tennekes and Sanchez-Bayo, 2012).

The above is also relevant to the impact of small residues of those systemic insecticides that have cumulative effects (e.g. neonicotinoids, fipronil and cartap) on aquatic ecosystems. Because of the short life-cycle of many zooplankton species, the negative population parameters that result from sub-lethal and chronic effects on such organisms can lead their local populations to extinction (Stoughton et al., 2008). Immediate reductions in populations and species may not always be apparent due to the small residue concentrations and the delayed effects they cause. For example, in recent surveys of pesticide residues in freshwaters of six metropolitan areas of USA, fipronil appears regularly in certain states (Sprague et al., 2008). Imidacloprid was detected in 89% of water samples in agricultural areas of California, with 19% exceeding the US Environmental Protection Agency’s chronic invertebrate Aquatic Life Benchmark of 1.05 μg/L (Starner and Goh, 2012). There is already a widespread contamination of waterways and estuaries with persistent systemic insecticides. The first consequence of such contamination is the progressive reduction, and possible
elimination, of entire populations of aquatic arthropods from the affected areas. As time is a critical variable in this type of assessment, it is envisaged that should this contamination continue at the current pace over the years to come the biodiversity and functionality of many aquatic ecosystems will be seriously compromised (Miranda, et al, 2011). Secondly, as these organisms are a primary food source of a large number of vertebrates (e.g. fish, frogs and birds), the depletion of their main food resource will inevitably have indirect impacts on the animal populations that depend on them for their own survival. The case of the partridge in England is an example of how a combination of herbicides and insecticides can bring the demise of a non-target species by indirectly suppressing its food requirements (Potts, 1986). Therefore, warnings about the possible role of environmental contamination with systemic and neonicotinoids in steeply declining populations of birds, frogs, hedgehogs, bats and other insectivorous animals are not far fetched and should be taken seriously (Tennekes, 2010b).

Chen and Chang (2013) developed the granular formulations of butachlor, phorate, chlorpyrifos, carbofuran, terbufos, terbufos, disulfoton, cartap and diazinon which are frequently used in Taiwan. The concentrations of the active ingredient are from 7% to 26% which are higher than those used before. The carrier was provided by Oil-Dri corporation of America. The stabilizer was also applied on the formulation using glycols. The results showed that the granules of butachlor, phorate, carbofuran, terbufos and disulfoton are chemically stable in the aging test. The granules of chlorpyrifos, cartap and diazinon, however, are not stable.

The present study has brought some light on the direct, sub-lethal and indirect effects that systemic insecticides have on species populations and ecosystems. Some long-term impacts have been known for some time, but it is the rapid increase in
the usage of systemic products that poses a new challenge to the ecological risk assessment of agrochemicals. Indeed, current risk protocols, based on acute, short-term toxic effects are inadequate to cope with the chronic exposure and cumulative, delayed impacts of the new compounds. Awareness of the increasing contamination of the environment with active residues of these chemicals should help regulators and managers to implement new approaches for risk assessment of these substances.

Repeated exposure to cartap can result in reduced fish egg production and hatching, nest and brood abandonment, lower resistance to disease, decreased body weight, hormonal changes, and reduced avoidance of predators. The overall consequences of sub-lethal doses of pesticides can be reduced adult survival and lowered population abundance.