CHAPTER ONE

INTRODUCTION

A. GENERAL INTRODUCTION :-

India is mainly an agricultural country and 80% of the population belongs to rural areas. Many of them are illiterates, living in primitivity and abominable poverty. The rural people use various pesticides, chemical fertilizers and other agrochemicals indiscriminately, to boost the agricultural production. They use the pesticides and other agrochemicals indiscriminately, unmindful of the concept of time (time of harvest), space (quantity/acre) and quantity. Such pesticides reach water bodies from the soil through thaws, rain and ground waters and often accumulate in them in large quantities. These have serious and adverse ecological consequence, especially on aquatic ecosystem.

Besides this, the vigorous development of industries, especially the chemical industries, discharge out several types of poisonous chemicals as wastes which ultimately find their way to nearby water resources, polluting them very severely. Same is true, if we consider about the domestic sewage.

Poisonous substances in industrial sewer waters, pesticides, chemical fertilizers and agricultural drainage water on reaching water resources have a fatal effects on aquatic ecosystem. Particularly in fish , they destroy the spawning grounds and feeding areas of fishes by destroying the life of food organisms. They affect the vital migration routes in the life of fishes, thus causing

great alterations in the recruitment of new generations of fishes. These poisonous chemicals reduce the resistance capa. ty of fishes to diseases and deteriorate the quality of fish produce. Polluted sewer waters change the hysical, physico-chemical and chemical properties of the water in water bodies, alter the quality and quantity of food organisms and disrupt the biological balance of processes of self-purification in water bodies closely linked with the life activity of single celled organisms such as bacteria, algae and protozoans.

The considerable danger of pesticides is reflected in the fact that they are toxic to hydrobionts even in minute concentrations, especially in the case of chronic exposure, and tend to accumulate in these organisms. Furthermore, pesticides are carried through food chains are particularly harmful to the reproductive system of aquatic animals and pose a potential danger to man when he consumes the flesh of hydrobionts from polluted water bodies.

In addition to the chronic action of small concentrations of toxic substances, aquatic animals are sometime exposed to an accidental discharge of toxicants, resulting in mass mortality of fish and other benifitial aquatic animals. In such cases specialists must quickly determine the cause of fish mortality and implement measures to arrest it. This requires the development of uniform methods for determining the toxicity of the

aquatic medium and methods for the timely diagnosis of toxicosis of fish.

B. REVIEW OF LITERATURE :

i)GENERAL TOXICOLOGY :

For critical evaluation and comparision of the results of present investigation it is necessary to review the work in brief of different research workers in the field of water toxicology.

Toxicity and LC 50 values in fishes have been determined in response to heavy metals and their salts, organic substances including insecticides and pesticides and different industrial effluents by many workers from abroad and in India.

The toxic action of heavy metals and LC 50 values for them at different concentrations on various types of fishes have been worked out by following workers. The lethal threshold and LC 50 values for Cr in fish (Ruesink and Lloyd,1975);action of Zn and LC 50 values for Carp (Tishinova, 1976) and for P.sophore (Khangarot, 1981) toxicity of Pb and LC 50 values for S.gairdneri (Davies et al.,1976), acute toxicity of Cu and LC 50 values in M.bleekeri (Gupta and Rajbanshi, 1981) . Further, the accumulation of metal residues of Hg, As, Pb, Cd and Se in fish body were studied by Walsh et al.(1977) and Labat et al.(1977). The tolerance of Zn and Cu salts on C.carpio and C.idellus have been worked out by Wong et al.(1977).Zn content of gill filaments and gill arches of Zn treated and

untreated dogfish was analysed by Crespo et al.(1979). Transport of nitrite across the gill of S.gairdneri was studied by Bath and Eddy (1980) . LC 50 values for Hg, Cr, Cd and their toxic effects on fish was studied by Kaviraj and Konar (1982) and Saxena et al. (1982). Panigrahi and Misra (1978,1980) have shown the effect of Hg on body of A.scandens and T.mossambica.

The group of toxicants that consists of large number of organic substances includes mainly pesticides. These toxicants have been studied by number of workers in this field. The effect of thiodan on the mortality of C.puntatus was recorded by Lal and Vohra (1974, 1976). Quantitative estimation of biocide residues in few tissues (including gills) of L.rohita and S.fossilis have been worked out by Verma et al.(1978). LC 50 values for 3 herbicides and their toxic effects on cutthroat trout, S.clarki and lake trout, S.namaycush was studied by Woodward and Mayer Jr, (1978). Toxicity of some herbicides to major carp finger lings have been worked out by Singh and Yadav (1978). Similarly acute toxicity of selected rice pesticides to crayfish, P.clarkii were determined for an insecticides, 3 herbicides and 5 fungicides by Chean et al.(1980). The relative toxicity of endosulfan its isomers and formulated products and LC 50 values to the fresh water fish, L.rohita was studies by Rao et al. (1980). Verma et al.(1981) studied LC 50 and acute toxicity of 3 newly formulated pesticides. Verma et al.

(1981) conducted bioassy tests with 23 pesticides using freshwater teleost, S.fossils. They determined the acute toxic range and LC 50 values for 7 organochlorine, 14 organophosphorous and 2 carbamate pesticides.

The effect of different industrial effluents on various aquatic organisms have been worked out by number of workers. The bioassay studies on the toxicity in carp due to distillary effluent and sewage has been studied by Gill and Toor (1975, 1976). Further, Verma and Dalela (1976) have shown threshold values of toxicity in P.sophore and M.vittatus to distillary wastes, where as the Kraft mill waste has shown little toxic effect on different species of fishes (Grushko et al., 1975). The acid toxicity and LC 50 values were calculated by Ellgaarad and Gilmore (1984) in Blue-gill. Verma et al. (1984) have evaluated the LC 50 values and determined the safe concentration of agricultural and industrial chemicals in larval forms of C. mrigala. The response of teleostean fish and LC 50 values due to textile waste were studied by Verma et al. (1978). The influence of oxygen consumption of L.rohita at different concentrations of industrial effluent has been studied by Hingorani et al. (1979) and they observed hypoxia due to presence of organic and inorganic salts. Studies on effects of effluent from sewage treatment plant on aquatic organisms including fish and physico-chemical parameters including DO, temperature, pH, conductivity and COD has been revealed by Oladimeji and Wade (1984).

Abel (1974) studied the toxicity of synthetic detergents to fish and aquatic invertebrates, Gupta (1986) reported the gill damage in N.notopterus following exposure of phenolic compounds. The critical evaluation of literature shows that, C.pantatus is very rarely used for toxicological studies by earliear workers. Similarly, the malathion and sumithion also very rarely used to study their effects on fish.

ii) HISTOLOGICAL STUDY :-

The general structure and functions of the fish gill has become a matter of great interest in recent years, partly because of an intensive research in fish respiration and particularly because of their interesting adaptation to different types of aquatic habitats such as fresh waters, estuarine waters and marine waters . The gills carry out important functions in fish life which are as follows (1) It is the prime organ of gaseous exchange, (2) It plays an important role in ion regulation, (3) It is involved in nitrogen excretory metabolism, (4) It serves important biosynthetic and metabolic functions. The most important part of the fish gill is epithelial cells which play a role in gas exchange and ion exchange.

The existing literature stated that much more information is available regarding the structure of the gill of fish. General morphological structure of teleost gill is described by number of workers in variety of fishes. (Hughes, 1980; Kendall and Dale, 1979; Laurent and

Dunel 1980; Morgan and Tovell, 1973).

Gill morphology in zebra fish, B.rerio were studied by light and E.M. by Karlsson (1983) after fixation of a gill in a mixture of 1.5% gluturaldehyde and 1.5% paraformaldehyde which gives mucus free surface. He reported different cell types in the branchial epithelium e.g. two types of chloride cells and rodlet type cells.

Mallatt and Paulsen (1986) studied the ultrashtructure of the pacific hagfish, E.stouti and showed that the hagfish gills show unusual features not seen in any other fish gill. According to them the branchial respiratory lamellae of pacific hagfish resembled to the lamellae of lampreys, elasmobranchs and teleosts. Lamellae were lined by epithelium containing pavement cells, undifferentiated basal cells and ionocytes. Ionocytes were identical to chloride cells of teleosts.

The detailed structure of the gills in some common species of teleosts from different waters has been described by Bevelander (1935), Singh (1969), Cockson (1971), Carmignani and Zaccone (1974), Munshi (1980), Ingale (1981) and Nikam (1986).

Several types of intraepithelial cells which belonged to unicellular, multicellular and transitional types have been reported in branchial epithelium of fishes from different habitats. Droscher (1882); Keis and Wilmer, (1932); Bevelander, (1935); Munshi (1960,64) and Carmignani and Zaccone (1974) reported the occurance of bi or trinucleated acidophils. Variations have been reported

regarding the presence or absence of mast cells in branchial epithelium. The occurance of mast cells have been accepted by Munshi (1964) while Ingale (1981) denied this view. Morgan and Tovell (1973), Laurent and Dunel (1980), Lewis and Potter (1982) reported on the constant structure of branchial epithelium. On the other hand Hughes and Grimstone (1965), Munshi and Singh (1968 b), Hughes and Morgan (1973) and Hughes and Munshi (1979) reported many structural changes in general, in the organization of the gill in relation to the enviornment. These authors further reported that thickened epithelium could cut down diffusion of gas from gill into the water and if the water is deoxygenated this might serve as an oxygen conserving device.

Grimstone (1965), Munshi and Singh (1968 b) described two rows of secondary lamellae as transverse folds on the primary gill lamellae. These authors further described the structure of secondary gill lamellae. According to them, each secondary gill lamella consisted of a central core of blood sinuses lined and supported by pillar cells and covered by basement membrane and an outer epithelium.

There is controversy regarding the presence or the absence of pillar cells within the gill lamellae. Hughes and Munshi (1979) and Munshi and Singh (1968 b) reported on the absence of pillar cells in A.cuchia and M.cuchia.

On the other hand Munshi (1960, 1961, 1962 a, 1962 b, 1968); Munshi and Singh (1968 b); Ingale (1981) and Nikam (1986) reported the presence of pillar cells in variety of fishes they have studied.

One of the problems regarding the arrangement of pillar cells within the gill lamellae deserve further attention. Rauther (1937) found the arrangement of pillar cells at random, Acrivo (1938) indicated the arrangement of pillar cells to be in parallel rows in ganoid fishes he has studied. Munshi (1960, 1961, 1962 a , 1962 b, 1968) and Munshi and Singh (1968 b) produce clear evidences about the arrangement of pillar cells in air breathing fishes. Ingale (1981) and Nikam (1986) reported that the pillar cells are arranged one after the other in secondary gill lamellae.

The mucous cells have been reported by number of workers (Droscher, 1882; Keis and Wilmer, 1932; Bevelander, 1935; Carmignani and Zaccone, 1974; Laurent and Dunel, 1980; Ingale, 1981; Lewis and Potter, 1982 and Nikam, 1986) along with respiratory epithelium. Zaccone (1972); Singh and Thakur (1975); Gona (1979 a, 1979 b) and Ingale (1981) reported the occurance of mucous cells on secondary gill lamellae and described that occurance of these cells in secondary gill lamellae may be related to more secretion of mucus in diverse ecological conditions to which the fishes are subjected.

An unknown membranous structure consisting of densely packed tubules was found by electron microscopy in a few chloride cells of rainbow trout, S.gairdneri by Abel (1973).

Singh (1972, 1974) reported three kinds of specilized cells namely acidophil cells, the acidophil granular cells and the mucous glands in the gills of marine fish, N.tolu and P.guadrlineatus. The acidophil cells resembled the so called chloride secreting cells. These were present in large number in secondary gill lamellae and near the base. The acidophil granular cells were found in the gills of only one marine fish, N.tolu. He described the structure of the specilized cells in detail.

Sargent et al. (1977) studied the gills of A.anguilla from fresh water and marine water for the structure and functions of chloride cells. According to them the gills of yellow eels in fresh water, contained relatively few chloride cells distributed on both primary and secondary filaments, while in sea water fishes, gills contained many chloride cells located solely on primary filaments. The internal structure of chloride cells from fresh water and sea water gills were generally very similar.

According to Droscher (1882), Owen (1886), and Keis and Wilmer (1932) the number of chloride cells is more in the marine fishes than in fresh water. On the

other hand Saxena (1966), Czolowska (1966), Guha et al. (1967), Zaugg (1970), Zaccone(1973), Singh and Thakur (1975), Das and Srivastav (1978) and Ingale (1981) stated that the chloride cells are more numerous in fresh water fishes than in marine water. Munshi (1964) and Singh (1969) are of opinion that the chloride cells are nothing but mucous cells secreting the chloride ions. Bird and Eble (1979) reported that the chloride secreting cells are clustered at the bases of secondary lamellae in close proximity to afferent lamellar arteries. On the other hand Kapoor (1957) reported the absence of chloride cells in the branchial epithelium of M.armatus. According to him mucus secreted by secretory cells may be concerned with ionic transfer.

iii) HISTOPATHOLOGICAL STUDY :-

Experimental evidences regarding the penetration of poisons in fish from polluted water show that, the penetration takes place through various organs of body, namely gills, mouth, alimentary canal and skin. Pathological, anatomical and histological studies on gills of fish assist greatly in the diagnosis of the nature and intensity of poisoning. They may help in tracing the basic features of pathological processes and their nature, reveal the channel of penetration of poison and provide clues to the nature of the toxicant . Therefore , number of workers have tried to study the effect of different pollutants on the gills of fish.

The fish gill come in direct contact with several types of pollutants in the surrounding water. These pollutants cause several types of alterations in gill structure. The gill histopathology has been studied extensively by number of workers.

Heavy metals (Mn, Ni, Cr, Zn, As, Cd, Pb, Fe, Cu) and their salts constitute the most widely distributed group of highly toxic and long retained stubstances. This group of pollutants is commonly found in sewer waters of different industries. Most heavy metals and their salts are simple inorganic compounds, the toxicity of which is caused by anions, cations or physico-chemical properties of salts (Metelev et al. 1971). The effect of $CdSO_4$, $ZnSO_4$ and $CuSO_4$ on gills has been worked out recently by Labat et al.(1974), Benoit (1975). Acute toxicity due to zinc and copper salt has been studied by Wong et al. (1977). The gill damage due to chlorine in mosquito fish (G.affinis) has been reported by Cohen and Velenzuela (1977). Deung et al. (1978) studied electron microscopically the histopathological changes in the fine structure of gills of C.carssium caused due to $HgCl_2$. Baxter and Cirrie (1978) reported a rapid and pronounced edema in the fillament, enlarged blood channels and leucocyte accumulation in the vascular spaces in a gills of I.metas due to Cu treatment. Crespo et al. (1979) analysed Zn content of gill fillament and gill arches of Zn-treated and untreated dogfish (S.canicula) and found accumulation of Zn in both gill filaments and gill arches.

Accumulation of cadmium in gills was observed by Establier and Gutierrez (1980). Mercuric chloride and methyl mercuric chloride induce extensive production of mucus in rainbow trout (Lock and Van overbeeke, 1981) and same in P.sonophore due to acute poisoning (Khangarot and Somani, 1980) . Histopathological changes due to toxic levels of Cu and Zn on gills leading to severe damage have been studied in details in the case of teleostean fishes (Kumar and pant, 1981) .Tuurala and Sovio (1982) observed structural and circulatory changes in the secondary gill lamellae of S.gairdneri after the exposure to zinc. Effects of chromium with different pH changes are dealt by Van der Putte et al.(1981) and Leino and McCormic (1984). Similarly, effects of chromium sulphate on gill leading to its damage is worked out by Wong and Lau (1982). Khangarot (1982) had shown toxic effects of Zn at different concentrations during various time period, causing edema, fusion of secondary gill lamellae and degeneration of gill epithelium. Higher concentration induce delamination of gill lamellae and necrosis causing death of fish. Furthermore, Zinc toxicity at electron mocroscopic level on gill of rainbow trout has been studied by Skidmore and Tovell(1972) and Crespo (1981). Effect of CdSo4, $ZnSO_4$ and $CuSO_4$ on gills has been worked out very recently by Wagh et al. (1985), Karlsson et al. (1986) recorded cadmium induced changes in gill morphology of zebra fish (B.revio) and rainbow trout

(S.gairdneri). Gupta and Rajbanshi (1986) studied the effects, produced by Cu ions in gills of freshwater murrel (C.punctatus). O_2 uptake and gill morphological alteration in P.clarhii after sublethal exposure to lead have been carried out by Torreblanca et al. (1987), Youson and Christine (1987) found deposition of aluminium in the gill epithelium of rainbow trout (S.gairdneri). Histopathological changes produced due to sublethal and lethal concentrations of $ZnSO_4$ have been worked out by Rameshkumar et al.(1988).

Histopathological changes due to urea stress in gill lamellae of fingerlings are revealed by Srivastawa and Srivastava (1982). Srivastava and Srivastawa (1984) indicated urea transport through gill. They noted separation of basement membrane, closing of secondary lamellae, degeneration of epithelial cells, erosion and hypertrophid and empty mucous cells.

The toxic effects of pesticides in gills of fishes is worked out by number of workers. Lindane poisoning on brown trout caused rapid fading of the gill colour, disarry of the secondary lamellae and break of lamellar epithelium, resulted in fish death. (Abel, 1976; Drewett and Abel, 1983). Verma et al.(1977) estimated quantitatively the biocide residue in gills of two fishes, L.rohita and L.fossilis. Effect of malathion on gills of catfish have been carried out by Mukhopadhya and Dehadraj (1980). Daleal et al.(1980)extensively studied the effect

of endosulfan and rogar in C.gachua after acute and subacute exposure. They stated that the most conspicuous changes produced by these pesticides is the seperation of respiratory gill epithelium from the basement membrane, pronounced hypermia, necrosis, fusion of adjacent gill lamellae, erosion at the distal end of gill filaments and loss of cell membranes. Sigloch (1981) observed severe lesions in secondary gill lamellae including hyperplasia, seperation of the respiratory epithelium and necrosis in juvenile S.gairdneri, C.carpio, I. indus and different stages of S.gairdneri due to rogor, benomyl, pomuran and domatol. Sastry and Sharma (1981) detected dizion induced histopathological changes in various tissues including gills of O.punctatus. Induction of gill lesions exposed to different insecticides in cichilid fish have been shown by Jauch (1979, 1981) and Eller (1971). Kumaraguru et al. (1982)observed epithelial seperation, necrosis, mucous cell hyperplasia and fusion of adjancent secondary lamellae of rainbow trout (S.gairdneri)exposed to an insecticide (permethrin). Alteration in gill due to effect of BHC has been studied by Gupta and Singh (1982) in fresh water teleost, T.fasciatus. Natrajan (1982) pointed out extensive damage to gill epithelium, particularly, secondary lamellae and chloride secreting cells of air breathing fish, C.striatus due to metasystox (insecticide). Alkylbengene sulfonate was recorded, causing hyperplasia of secondary gill lamellae and marked edema in respiratory epithelium leading to bleeding

(Fukuda 1983). Similar observations using DDT, Lindane and BHC were revealed by studinicka et al.(1983) . Effect of DDT, lindane and toxaphene was investigated on carp fry gill (Lakota et al. 1983) and hydrocarbons on fish gills (Lopez, 1981) were demonstrated. Morphological changes produced in gills of carp (C.carpio) exposed to CH_3Br have been investigated by Segers et al. (1984). The histopathological effect produced by sevin and thiodon on gills of C.striatus included tissue disruption and necrosis (Jaikar and Kulshrestha, 1985).

Nemscok et al. (1987) studied the accumulation of pesticides in gills of carp (C.carpio) and noted the accumulation is related with temperature. Khillare and Wagh (1988) found disturbances in the structure of gill of fish, B.stigma due to chronic exposure of fish to endosulfan, malathion and sevin. They noted pycnotic nuclei of the chloride cells and few cell necrosis with hyperplasia of the cells, Singh and PR. Karpagaganapathy (1988) reported histological changes in gills of B.stigma exposed to dimethoate. Histological changes included thickening of gill rays, fusion of secondary gill lamellae, swelling of secondary lamellae and thickening of interlamellar epithelium. Loss of respiratory epithelium, damage in pillar and red blood cells, reduction in length of secondary lamellae were extensive under lethal concentration.

Abel (1974) carried out the effect of synthetic detergents and reported that the detergents are actualy

toxic to fish. The toxicity depends upon the factors like molecular structure of detergents, water hardness, temperature and dissolved O_2 concentration . Gill damage is the most obivous acute toxic effect and death is due to asphyxiation.

Able and skidmore (1975) reported that the changes produced due to detergent (sodium lauyl sulfate) are in general similar to the changes induced in gills by other toxic agents. They observed lifting of gill epithelium from underlying tissue and invantion of the lymphocytes and granulocytes in subepithelial space.

Lesions associated with chronic exposure to ammonia in the gills have been worked out by Smith and Piper (1972) in trout, by Cruz et al.(1982) in milk fish (C.chanos) and by Yamagata and Makotoniwa (1982) in eel fish. Exposure of fishes to different concentration of ammonia leads to most common histopathological changes such as hypertrophy, lamellar fusion, vaccuolation of lamellae, severe hyperplasia of gill epithelium, blood filled aneurisms in gill tissue, etc. Similarly, Yang and Sen (1986) carried out histopathological study due to acute toxicity of ammonia on common carp (C.carpio). He observed hypertrophy and necrosis of gill tissue and detachment of epithelium. The detachment of gill lamellar epithelium initiated from the proximal part of gill lamellae towards the apparent tip.

Crude oil spill in pleuronectes has been studied by Haensly et al.(1982). Solangi and Overstreet (1982)

reported on the epithelial hypertrophy, fusion of gill lamellae and seperation of gill epithelium after 30 days, in esturine fishes exposed to crude oil. Whereas, Engelhardt et al.(1981) noted similar changes in gills of S.gairdneri due to petroleum exposure. Khan and Kiceniuk (1984) reported increased number of mucous cells, capillary dilation, lamellar hyperplasia and fusion of adjacent fillaments in gills of Atlantic cod (C.morhua) exposed to crude oil for about 12-13 weeks.

Matel (1982) studied the effect of a hypertonic medium on the ultrastructure of gill chloride cells in the P.pungitius inhabiting fresh water. He noticed hypertrophy and proliferation of mature chloride cells and their transformation into the excretory state.

Ahmed (1984) carried out the effect of sublethal and chronic level of methylene blue on pathology of gill in fresh water catfish, M.vittatus.

Mitz and Giesy (1985) carried out biomonitoring study using juvenile channel catfish (I.punctatus) at five sites along a 9-Km section of the Flint River at the Anthony Ragnone waste water treatment plant near Montrose Michigan in relation with survival of fish, its growth and gill histopathology.

Gill damage including acute inflammation, necrosis, seperation of basement membrane from the gill filament, clumping of cells of cartilage axis and loss of epithelial cells and pilaster cells were observed in gills

of N.notopterus, following exposure to phenolic compounds by Gupta (1986).

Cruz and Tamse (1986) analysed the histopathological changes produced by $KMNO_4$ in gills of milk fish (C.chanos) fingerlings.

Tilney and Hoccutt (1986) examined the morphological changes in gill epithelia and ultrastructure due to cold stress of O.mossambicus by using light, scanning and transmission electron microscopy.

iv) HISTOCHEMICAL STUDY :-

The branchial epithelium has been studied extensively by number of workers in variety of fish species from diverse habitat by using both light and electron microscopes. All these studies revealed a little difference, in the structure of branchial epithelium, particularly in the cell types. The cell types of the branchial epithelium has been described in several species of fish. (Morris, 1957; Cockson, 1971; Carmignnani and Zaccone, 1974; Munshi, 1980; Laurent and Dunel, 1980; Lewis and Potter, 1982). This study indicated that the secretory epithelium lies along the gill filament. The branchial epithelium consists of squamous epithelial cells, mucous cells, chloride cells and some nondifferentiated cells. Morgan and Tovell(1973), Laurent and Dunel (1980) and Lewis and Potter (1982) showed constrant structure for branchial respiratory lamellae and

the interlamellar regions of gill filaments. They reported a thick stratified epithelium, lining the filament between the gill lamellae in which two special types of cells namely chloride cells and mucous cells are visualised. However, in some species of fish chloride cells and mucous cells also occur on the basal part of the lamellae. The mucous cells has also been reported in gill lamellae of some species of fish.

In teleost fish the branchial epithelium forming a layer of squamous epithelial cells, connective tissue and basal lamina, acts as a barrier between the oxygen rich water and the blood supply of gill (Cockson, 1970).

Recently, epithelial cells, mucous cells and up to some extent chloride cells have been studied histochemically to understand the chemical moieties e.g. mucosubstances, enzymes, proteins, carbohydrates, lipids and other metabolites, present in them and their possible role in the life of fish.

There are many reports on enzyme activities in variety of fishes from diverse habitats and under experimental conditions. Rana (1971) studied the alkaline and acid phosphatase activity in the respiratory organs of two fresh water fishes and the variations in the enzyme activities was related to the functional capacity of the respiratory organs. Das and Banergiee (1980) demonstrated the changes in activity of lysosomal enzymes, alkaline and

acid phosphatase and β glucoronidase in gills of two fishes, H.fossilis and L.rohita in relation with cadmium toxicity. Similarly, Katoley and Katoley (1988) also demonstrated the alkaline and acid phosphatase activity in gills of mosquito fish, G.affinis exposed to low doses of cadmium chloride. They further reported that the acid phosphatase activity was more in comparison with alkaline phosphatase. Khangarot (1984) showed an increased acid phosphatase activity in the secondary gill lamellae and pillar cells system of C.punctatus exposed to zinc. According to them the increased activity may be related to cellular breakdown with acid phosphatase release from ruptured lysosomes.

The activity of carbonic unhydrase has been demonstrated in acidophils by Maetz (1953). Watson et al.(1982) studied in vitro, the effect of acephate (an organophosphorus insecticide) on activity of carbonic unhydrase in the gill of rainbow trout (S.gairdneri) and pointed out that the toxic action of the insecticide may be related to inhibition of carbonic unhydrase activity in the gill which results into disturbances of respiratory capacity and salt balance.

Flemming et al. (1962) reported the presence of acetylcholinesterase in the gill of brakish water, Fundulus species. Koundinya et al. (1978) demonstrated that sumithion inhibited acetylcholinesterase activity in gill with concomitant increase of acetylcholine content. They

are of view that lethal exposure decreased sorbitol dehydrogenase activity but increased lactic dehydrogenase activity. Similarly, Ahamad and Sailatha (1980) studied the impact of malathion on acetylcholinesterase activity in gill of T.mossambica. Acorrding to them inhibition of acetylcholinesterase activity leads to accumulation of acetylcholine content. Verma and Tonk (1984) also, reported reduction in acetylcholinesterase activity in gill of H.fossilis due to biocides.

Increased level of lactate dehydrogenase activity has been noticed in gill of carp (C.carpio) exposed to paraquate (Asztalos and Nemscok, 1985). According to them the increased activity may be related to tissue necrosis.

By exposing the fish, C.batrachus to organophosphorus pesticides, Ghosh (1987), reported increased lactate and succinic dehydrogenase activities while decreased pyruvate dehydrogenase activity in gill.

Zaccone et al. (1986) by employing enzyme histochemistry of frozen gill tissue of the catfish H.fossilis demonstrated the effect of detergent on oxidative enzymes activity in the respiratory epithelium. According to them the most conspicuous changes are the significant alteration of mitochondrial enzymes of the epithelial cells and increase in the lactate dehydrogenase activity.

Comparatively there are many reports on the ATPase activity in the gill tissues of different fishes.

Rana (1977) demonstrated the presence of ATPase in two fresh water fishes. He is of the view that the enzyme activity depends on the functional state of the fish. Na^+ K^+ and Mg^{++} dependent ATPase activity in the gills of rainbow trout has been found to be inhibited by insectcides and herbicides (Davies et al., 1972). According to them insecticides are more toxic and more effective ATPase inhibitor than the herbicides. Similarly, Na^+, K^+ activated ATPase in the gills of rainbow trout (S. gairdneri) has been found to be inhibited by oral administration of DDT (Campbell et al., 1974; Timothy and Johnson, 1974). The subcellular localization of Na^+, and K^+ ion activated ATPase was demonstrated by fractionation studies (Dendy et al., 1973 a, 1973 b). They found cytochrome oxidase in mitochnodrial fraction, phosphoglucomutase appeared in soluble fraction and monosamine oxidase in the nuclear fraction. Similar reports were also made by Kamyia (1972) in A.japonica and further he has discussed the role of Na^+, K^+ ATPase in relation of NaCl excretion. $Cl^-/HCO3^-$ dependent ATPase in the gills of C.ceratus has been found to be inhibited by thyocyanate (De Renis and Bornoncin, 1977). The activity of ATPase has been found to be increased after the transfer of fresh water fish to marine water (lozyka, 1976). $HCO3^-$ activated ATPase was shown to be present in the gill of rainbow trout in both the fresh water and sea water adapted fishes (Kirschner et al., 1974), Hanke et al. (1983) found an increment of ATPase concentration or

its activity due to short time exposure to the pollutants and reduction or inhibition after a long time exposure. Verma and Tonk (1984) detected significant reduction in ATPase activity by chlorodane and aldrin in gill of fish H.fossils.

Tondon and Dubey (1983) showed an elevated activity of fructose 1,6-diphosphate aldolase by malathion and dimecron in the gills of C.batrachus.

Tort et al.(1985) determined the ATP and lactate concentration in gill tissues of S.canicula after short term and long term subacute zinc exposure. They detected increased level of lactate and decreased level of ATP after short term exposure while no significant changes were observed after long term exposure. The histochemical localization of Na^{+} and adenosine triphosphate in chloride cells of A.japonica was examined by means of E.M.(Shirai, 1972) According to him the amount of Na and ATPase activity in the chloride cells of sea water eel was greater than those of the ordinary epithelial cells.

Singh (1972) studied the various chemical moieties by histochemical methods from the chloride cells of marine teleosts. The histochemical studies of these cells show that they were sudanophilic which indicates the occurance of phospholipids. This was also confirmed by Baker (1946). Change in lipid composition due to dodecylbengenesulfonate in gill of round crucian carp has been studied by Nakanish (1986). Phleger (1975) have demonstrated the synthesis of lipid by A.rostrata gill.

Comparatively little attention has been paid to carbohydrate content and its metabolites in gill of fishes. Carmignani and Zaccone (1974) reported on the absence of glycogen in branchial epithelial cells of young individuals of T.moromorata and T.ocellata. Parveen and Vasantha (1988) studied the impact of endosulfan on total sugar and glycogen content in gill of fishes. They found decrease in total sugar and glycogen and suggested that the carbohydrates which are the ready made source of energy may be utilized under pesticide stress. Increased level of glucose and concomitant decreased level of glycogen have been noted by Diwa et al.(1979) in the gills of two fishes H. fossilis and C.bactrachus.

There are stray reports on protein content and their metabolites in gill of fishes. Bhasker et al.(1982) studied the protein metabolism in the gill of T.mossambica exposed to alkaline water and found elevated level of structural and soluble protein fractions . According to them the elevated level of these proteins is related to the gill hypertrophy. Decreased level NH_3 and urea has been reported in the gill of T.mossambica exposed to methyl parathion, by Rao et al. (1981). According to them the change in tissue level in NH_3 suggest the possibility of NH_3 recycling to counteract the methyl parathion toxic stress. Ghosh (1985) studied the effect of chromium on free amino acid content of gill in C.puntatus and reported that, the increment in free amino acid content might be due to enhanced proteolysis or/and due to stepped up protein

synthesis. Gupta et al. (1987) investigated the effect of starvation on total protein and free amino acid in gill of C.punctatus and found out that the total protein decreases while free amino acid content increases in gill during starvation.

Munshi (1980) is of the view that the acidophil granular cells of the gill epithelia are diastase resistant, PAS positive while those belonging to the connective tissue system are PAS negative. However, he further claims that the granular cells become PAS positive after extraction of lipids. According to him the granules are composed of tyrosine rich protein, and carbohydrates, proteins and lipids firmly bound with each other. These cells do not respond to $AgNO_3/HNO_3$ test for chloride. (Singh and Munshi 1968 ; Munshi and Singh, 1968 a). According to Munshi the acidophil cells do not show metachromasia with toludine blue but react strongly towards PAS reaction. The PAS positive material has been identified as mucoprotein complex as per the suggestion of Pearse (1961, 1968).

Several diverse types of mucosubstances have been reported in the gill components of different fishes from diverse habitat and under experimental conditions. There are good many reports on the mucous glycoproteins and mucopolysacchrides in the different gill components. The presence of sulphated mucopolysaccharides have been noticed in few mucocytes of the branchial epithelium of X.maculatus . Similarly, Porcelli and Novelli (1970) also

reported the presence of sulphated mucosubstances in the muciparous cells of developing branchial epithelium of S.fario. Cockson (1971) investigated gills of T.shirana chilwae histochemically and reported on the presence of carboxylated mucopolysaccharides but absence of sulfated mucosubstances. According to him neutral mucopolysaccharides, mucoprotein and protein carbohydrate complexes were probably present.

Wasserman et al. (1972) isolated an acidic glyconsaminoglycans from the soft tissue of the gills of carps (C.carpio) . According to them the major part glycosminoglycans was the mixture of chondroiotin sulphates and of glucosamine containing substance which belong to heparin sulphate.

Histochemical studies of Zaccone (1973) revealed gradual increase in the number of mucous elements in the epithelium of the branchial arches and branchial chamber with only small number in the epithelium of the branchial filament.

Carmignani and Zaccone (1974) reported considerable quantity of sulfated mucosubstances and neutral mucopolysaccharides in the epithelial cells of gills in the young forms of T.ocellata and T.marmorata which were found to be absent in the epithelial cells of gills of adult specimens of these fishes. They further claimed that the mucous cells contained only sulphated mucopolysaccharides however, hyaluronic acid were found

to be absent. According to Narsimham and Parvateswarao(1974) neutral mucosubstances were the most abundant type in the gill of T.mossambica.

Yamada and Yakote (1975) studied histochemically the mucosubstance in both the sexes of adult A.japonica and found presence of neuraminic acid containing mucosaccharide with vicinyl hydroxyl sulfate and carboxyl grouping.

Bird and Eble (1979) reported on the presence of acidic mucosubstances in the mucous cells of gill filaments, sulfated mucopolysaccharides in the epithelial basement membrance and high concentration of carbohydrate polycarboxylates in chloride cells of A.rostrata.

Vinnikov et al. (1979) examined the mucus from the gill of A.guoldenstaedti and A.stellatus under E.M. and reported Na^+, K^+ and Mg^{++} ions, Higher concentration of these electrolytes have been reported in sea water fish than in the fresh water fish.

Pism et al.(1980) demonstrated the mucopolysaccharides in the chloride cells of M.capito and L.reticulatus adapted to both fresh water and salt water. Ingale (1981) studied extensively the nature of mucosubstances in the different gill components such as general respiratory epithelial cells, mucous cells and basement lamina of variety of fishes from fresh water, esturine water and marine water. According to him fish gills reveals species diversity in having different mucopolysaccharid moieties in their general epithelial

cells and mucous cells. In general, the various cellular elements show characteristic reactivities denoting the occurance of neutral and/or acid mucosubstances in varied amounts in them.

Plonka and Neff (1969) have isolated and characterised the nature of mucosubstances in the gills of twelve brook trout, S.fontinalis exposed to acidic water and made comparision with that of equal number of control trout. Similarly, Lock and Van Overbeeke (1981) studied the effects of mercuric chloride and methylmercuric chloride on the activity of mucous cells in the gill epithelium of rainbow trout. They used three parameters to measure this activity i.e density of mucous cells, mucous content and the mucus release into the water.

C. REASONS FOR UNDERTAKING THE PRESENT STUDY :

An insight into the reasons why the present problem was undertaken for investigation can be obtained from the abcve critical review of the work done on fish gill. This review brings out the following reasons led to the selection of present study.

1. The existing literature on the LC 50 values of fishes shows that it has been studied in number of fishes for organic and inorganic pollutants, metals, detergents, etc. however, the LC 50 value of C.puntatus for

malathion and sumithion (organophosphorus pesticides) have not yet studied. Hence, it was decided to study the LC 50 value of above fish for malathion and sumithion and to study the behaviour of fishes under experimental conditions.

2. A critical review on the gill of fishes shows that most of the work has been carried out to understand the histological structure of gill in normal fish and the histopathological changes brought about therein by variety of pollutants. Comparatively less attention has been paid to the histochemistry of mucosubstance.

3. There are few reports on the gill of fishes concerning the nature of mucosubstances at different cellular sites in some of fishes from different habitats, however, to my knowledge, no systemic, histochemical study of mucosubstances has been carried out in gills of fishes exposed to some pollutants, particularly to pesticides.

4. In some of earlier researches, gills of a given fish/fishes have been studied sperately for the characterization of mucosubstances at different cellular sites such as epithelial cells, mucous cells and basement membrane or for understanding histopathological changes produced by some of pollutants. To my knowledge, however, no attempts have ever been made to understand the effects, produced by

pollutants, particularly pesticides on histology and histochemistry of mucosubstances in fish gill, simultaneously.

5. At present chloride cells have been reported in good many numbers of teleosts. However, in the typical fresh water teleosts, the presence of these cells have been denied by some workers. Some of the authors are of the view that the chloride cells are nothing but mucous cells since they actively secrete chloride ions. Some one described the presence of acidophilic, nonmucoid granular cells at the base of the gill lamellae of certain teleostan fishes and suggested that these cells may be responsible for chloride excretion and therefore, termed 'chloride secreting cells'.

6. A critical review on the gill of fishes shows that no body has studied the effect of two pesticides of the same group on histology and histochemistry of mucosubstance in the gills of same fish. Hence, it was thought desirable to study and compare the effect of malathion and sumithion on histology and histochemistry of mucosubstances in gills of fish C.puntatus

7. The existing literature revealed six types of mucous cells in gills of fishes from different environment. However, all these types have not been reported in one and same fish from particular environment. With this

view in mind in the present investigation it was thought desirable to study the types of mucous cells in gills of fresh water teleost C.punctatus.

8. The existing literature revealed diverse nature of mucosubstances in the epithelial as well as mucous cells in gills of different fishes from same as well as different habitats. Therefore, it was decided to study the nature of mucosubstances in fresh water teleost fish C.punctatus.

9. Based on the aformentioned critical points of evaluation of the present information, it was decided to study and to get an insight into the histological architecture, the histopathological changes and characterisation of mucosubstances in the gill of C.punctatus due to malathion and sumithion intoxication.

D. PLAN OF THE PROPOSED WORK :

a) CHOISE OF THE FISH :

For the present investigation the fish C.punctattus was selected. The fishes of this species were collected locally from river Krishna, keeping in mind their availability throughout the year and being sturdy these were considered appropriate to study the effect of the two pesticides on their vital organ, gill.

b) CHOICE OF THE PESTICIDES :

For the present investigation two pesticides of organophosphorus group Viz. malathion and sumithion were selected to investigate their effect on histomorphology and histochemistry of mucosubstances in the gills of fish C.punctatus.

c) CHOICE OF TECHNIQUES :

i) LC 50 - The LC 50 values were calculated by method given by Metelev et al.(1971).

ii) As the present investigation aims at detailed study of histology of gill in normal fish and alteration occuring in different cellular sites of gill after exposing the fish to different concentrations of malathion and sumithion, Standard histological techniques were employed.

iii) A series of histochemical techniques which are well established and recommended are employed in the present investigation. The main aim of selection these methods is to characterize histochemically the nature of mucosubstances. These histochemical techniques are currently used to identify glycogen, neutral mucosubstances, acidic mucosubstances such as sulfomucins, sialomucins, hyaluronic acid and some atypical mucosubstances.

d. CRITICAL EVALUATION OF DISSERTATION :

The present investigation entitled 'effect of

malathion and sumithion on histology and histochemistry of mucosubstances in the gills of fish, C.punctatus' was carried out to augment our knowledge about histology of gill in control fish, histopathological changes produced in gill after exposing the fishes to different concentrations of both the pesticides, nature of mucosubstances at different cellular sites of gills such as epithelial cells, mucous cells and to compare the effects produced by malathion and sumithion on histomorphology and nature of mucosubstances in gills as well as to compare the results obtained in the present investigation with that of existing literature on gill of fishes.

e) PRESENTATION OF THE DISSERTATION :

It was thought to divide the present dissertation into four chapters. The first chapter being on introduction, giving general introduction, review of existing literature on histology, histopathology and histochemistry of mucosubstances in gills of C.puntatus and reason that stimulated to undertake the present investigation. The second chapter deals with material and methods employed in the present work. Chapter thrid will include general observations, determination of LC 50, fish behaviour, histological, histopathological and histochemical observations on the nature and distribution of mucosubstances in epithelial and mucous cells in gills of C.punctatus, respectively. The fourth chapter will be on

discussion on the results obtained in the present investigation and the existing literature on gills of fishes. The last chapter will be followed by summary and concluding remarks. The references cited in the various chapters will be listed at the end of the dissertation.