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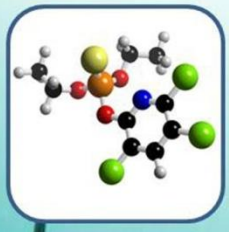
CHLORPYRIPHOS: A THREAT TO FRESH WATER EDIBLE FISHES

DR. P. S. PAWAR

DR. R. N. PATIL



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PREFACE

Aquatic toxicology deals with the study of the effects of toxic substances on aquatic organisms at cellular and molecular level and subsequently on ecosystem. It is a multidisciplinary field which includes toxicology, aquatic ecology and aquatic chemistry. Freshwater, marine water and sedimentsin environment are included in this study.

India is highly populated country. In last few decades the rate of population growth is tremendous. To meet the needs of increased population, development in modern technologies, industrialization and in modernized agricultural practices is also increased. But this modernization brings undesirable and indiscriminate change in the environment. The usage of chemical pesticides in the field of modern agriculture practices has also been increased in order to increase the production to overcome the needs of increasing population. The pesticides are most effective weapons discovered by man to control the pests in agricultural field and in domestic areas. Though they are most effective and definite way to control pests their indiscriminate use has also brings deleterious effect on environment. They persisted in the environment for longer period and unfortunately pollute the streams, lakes and rivers and subsequently they accumulated in the body of non-targeted aquatic organisms. In India huge river basins and prominent agricultural fields are available which are used for cultivating the crops. Farmers applied millions of tons of pesticides to the crops in these river basin in order to protect them from pests. These toxic pesticides pose serious environmental problems particularly water pollution.

After food and air, water is most essential for living organism. Besides it is required in agriculture, industries, recreation, drinking and domestic purposes. Water bodies get polluted by pesticide through surface runoff and spraying. The pesticides accumulate and showed their effects at each level of ecosystem. Presently several types of pesticides are used worldwide, amongst them three classes i. e. organophosphate pesticides (malathion, parathion, chlorpyriphos, dichlorvos, etc.), organochlorine (DDT, aldrin, dieldrin, toxaphene, chlordane, etc.) and carbamates (aldicarb, carbofuran, carbaryl etc.)are widely used by farmers. The organophosphate pesticides are prominently used to control pests because of their low persistence and high toxicity. Organophosphate pesticides inhibit the cholinesterase activity in pests there by resulting into their death. The use of organochlorine

pesticidesis decreased due to their persistence in the environment and low effectiveness as compared to organophosphate pesticides. So, they are replaced by organophosphate pesticides.

Fishes are the good indicator of water pollution and most popular test organism. The fishes and other aquatic organisms are useful highly as nutritious food material to human being from all status and placed an important role in food chain. In population from the huge river basin areas and along the sea coasts in our country, the fishes and other aquatic organisms are supposed to be the best food for people in all categories. The fish accumulates toxic chemicals in their body and shows undesirable deleterious effects at cellular and molecular level. Due to that the quality and nutrition value of fish gets reduced and these toxic substances get entered in to food chain. People consume such fishes from polluted water bodies and health of people is at high risk. Pesticidal toxicity mainly depends upon quality of water, concentration of pesticide and is species specific. The toxicant affects the general metabolism, biochemical composition and normal histological structure of vital organs of fishes.

In toxicity studies standardized tests are used such as toxicity tests for 96 hours (acute test) and for 30 days or more (chronic tests). These tests measure endpoint such as survival of the organisms that are measured at each concentration, along with a control test. In toxicity studies the organisms which are ecologically relevant and sensitive to toxicants and havewell-established available literature background are used. Such organisms can be easily acquired or cultured and maintained in laboratory and are easy to handle. Every organism maintains the 'internal milieu' in their body by adopting various adaptive regulatory mechanisms. The pollutants disturb the regulatory mechanisms which leadsinto behavioural, biochemical, histopathological andphysiological alterations and finally resultinginto death.

- *Dr. Padmini Sandip Pawar*

Dedicated To
My Love
Sandip
and
Shivparvati

Abbreviations

The following abbreviations, Colors and staining intensitieswere used in the tables, captions to figures and contents in thepresent book.

- PAS - Periodic acid - Schiff
- P-PAS - Phenylhydrazine-PAS
- D-PAS -Diastase-PAS
- AB- Alcian blue 8 - GX - 300
- C. I.- Colloidal Iron
- AF - Aldehyde Fuschsin

Colours

- P - Pink or Magenta or Purple
- B - Blue or greenish-blue
- PB - Purple-blue
- BP - Bluish-purple

Staining Intensities

- ++++ - Intense
- +++ -Moderate
- ++- Weak
- + - Poor
- ±- Trace

Introduction:

Water is an essential part of everyday life, but the sources of water are limited. Fresh water is available less than 2.5 percent of earth's total water. The other 97.5 percent is salty which is found in oceans and seas. Out of total fresh water present on earth nearly 80 percent is present in the form of ice and glaciers in the world. So, remaining 0.2 percent of earth's fresh water is available for use (Environmental Protection Agency, 1990). The fresh water is mostly found as groundwater in rivers, lakes, and ponds. If above assumptions are true, the quality of water is highly valuable in our lives because it is essential to all life. We people totally rely on water not only to sustain our lives but to sustain the crops, animals and every living organism. The toxic substances altered quality of water which may be potentially harmful to human being and other living organisms instead of sustaining them (Cook *et al.*, 2008). Now a days human kind has a privilege to get clean pure water and it is possible to expect to turn on the faucet and have clean and pure water due to the technologies. Technologies give more comfort and convenient life to modern society but on the other hand it creates stress on the natural resources as well as on the environment that are now create a major problem. However revolution in science and technology coupled with population explosion and progressive urbanization has been led to introduce a variety of toxic substances in the environment and that substances are now present in our food we eat and habitat where we live and work. To overcome basic needs of population like food, water and shelter, energy crisis and strengthen the defense system numerous countries are now struggling that results into tremendous expansion of atomic and chemical industries in last several decades. The industrial effluent especially from chemical industries, sugar mill effluent, domestic wastes, agricultural wastes are continuously discharged into river, lakes and ponds which are the important water sources. The wastes contain large amount of toxic chemical substances like heavy metals (Cd, Hg, Pb), dyes, ammonia, urea and different types of pesticides. These substances are discharged without any treatment and that deteriorates the water quality very fast.

India is over populated country with high growth rate. The people mainly depend on agricultural products for food but the different types of insect pests, weeds etc. reduce the agricultural production. So, the man has discovered most effective weapon to protect crops and agricultural products from attack of pests and to increase the food production and that is the pesticide. Recently we people use large quantity of pesticides to protect crops from harmful pests and fertilizers to nourish them. These toxic substances may enter into the aquatic system by agricultural runoff and cause serious, detrimental effects on non-target organisms in aquatic ecosystem. These chemicals change the physical, physico-chemical and chemical properties of water and disturb the food web in the aquatic ecosystem.

Toxicology:

Toxicology is an important subject of discussion in today's society because of the adverse effects of the increasing use of toxic substances such as chemicals, heavy metals and pesticides. In the ambient environment most of the living organisms exposed to variety of toxic substances either natural or artificial. Such types of exposure cause behavioral, metabolic,cellular and physiological alterations up to death of organism. So, the study of these toxic substances is essential to protect public health against hazards associated with toxic substances in food, air and water. The study of toxic substances regarding its nature, severity, safe concentration and their adverse effects is become valuable for their safe use. According to Trivedi and Shukla (2005) toxicology deals with the study of nature and mechanism of effect of toxic substances on living organism and

other biological system. It also referred as quantitative assessment of severity and frequency of these effects in relation to the exposure period.

Toxicity of toxins or chemicals is evaluated by experiments which find the definite concentration of chemical according to the exposure period to produce desirable effects on organism. Parameters like mortality, behavior, histology, histochemistry, biochemistry, hematology and immunology are evaluatedin the study of toxicity. According to Pandey *et al.* (2005) period dependent safe concentration and lethal concentration would be evaluated in the toxicity tests for the experimental animal. The adverse effects produced by toxic substances in experimental or target animal may be used in the prediction of probable effects in human being. The toxicity tests conducted at various levels according to needs of experimental design.

Acute toxicity:

In acute toxicity, the results illustrated that the detrimental effects of toxic substances into short period (96 hrs.) of exposure in a single dose. The results of acute toxicity tests are represented as LD50 or LC50. Mostly the acute toxicity studies are designed to assess median lethal dose or concentration of the toxicants in single dose and expected 50% mortality in short period. It is usually expressed in mg/L i. E. parts per million or PPM.

Chronic toxicity:

Chronic toxicity is a long term, definite toxicity in which doses of particular toxicants are repeatedly administered in an organism. On the other hand acute toxicity occurs at shorter period but for a higher concentration. In chronic toxicity sublethal concentrations of substances exerts adverse effects on organismsuch as altered behavior, metabolism, physiology, growth and reproduction. The results of chronic toxicity tests may be useful in assessment of water quality guideline and regulations for protection of aquatic communities and ecosystem from the toxicant. According to Trivedi and Shukla (2005) chronic toxicity tests are designed to assess the long term toxicity potential of different toxic substances on non-target organisms.

Pesticide Toxicity:

Use of pesticides to protect the crop from harmful pests is in practice since before 2000 BC and the first known use of pesticide was the elemental sulfur dusting which was used nearly about 4,500 years ago for this purpose. The use of some poisonous plants for the control of various pests have also been mentioned in Rig Veda which is about 4000 years old. Toxic chemicals like lead (Pb), mercury (Hg) and arsenic (As) were used to control croppestsfrom 15th century. In 17^{th} century, the nicotine sulfate used as an insecticide to control different types of pests which was derived from tobacco. Pyrethrum, a type of natural pesticide derived from chrysanthemums and rotenone was introduced in 19^{th} century. In 1950s Arsenic based pesticides were dominantly used. Pesticide means a substance or a mixture of substances or chemicals used to repel pests such as insect vectors, mites, nematodes, molluscs and other organisms which affect the crop yield and human health. Pesticides attract, seduce, and then destroy any pest. It is generally a crop protective agent and is nothing but a class of biocide. Biocide means a substance which kills harmful living organism (Pandey et *al.,* 2005). Pesticides are mainly used in agriculture for the protection of crops from various pests which cause thinning of fruit, premature fall of fruits, defoliation, desiccation of plants, etc. These are used in public places to control various types of disease vectors, to control house hold pests, veterinary pests, garden pests etc. These are also used in paints and glues for the protection of wooden material from termites. These are also used to control weed and unwanted species of plants. Insecticides, herbicides, insect repellents, fungicides, rodenticides,

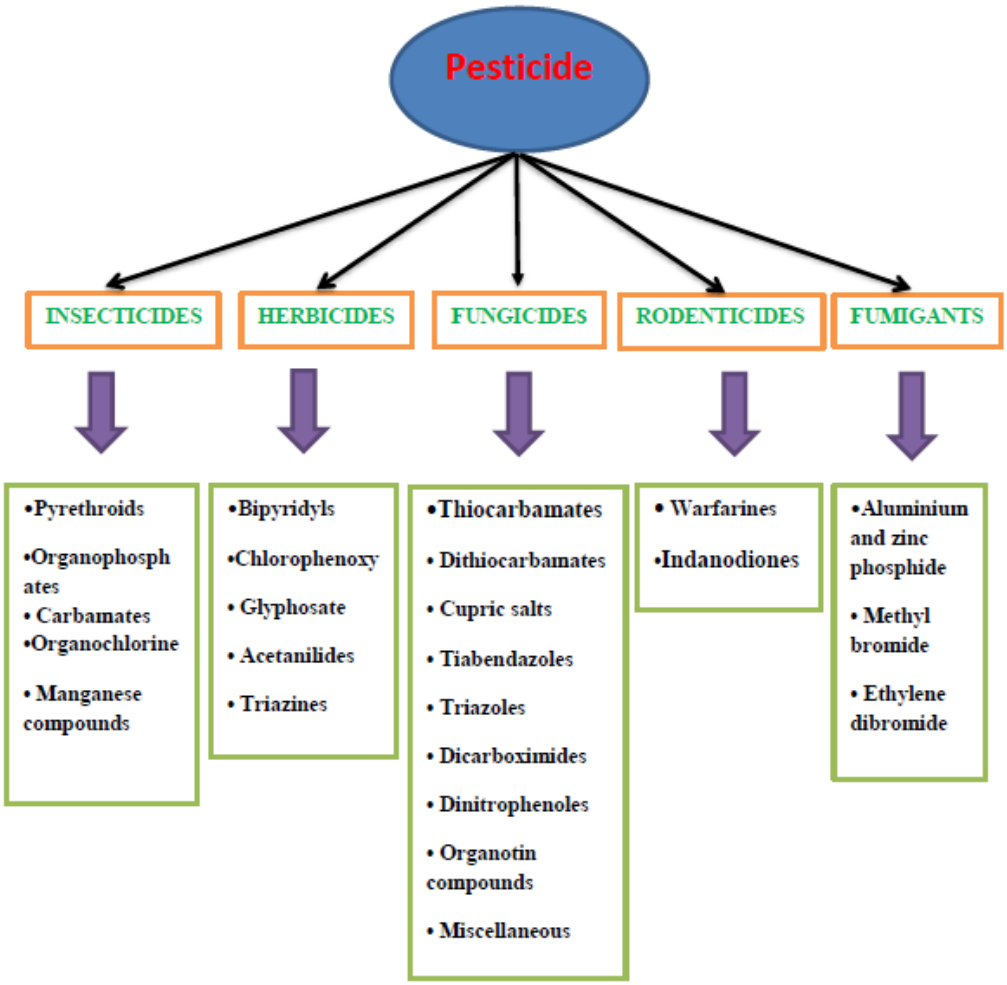
avicides, piscicides, molluscicides, nematicides, termiticides, bactericides, disinfectants, and sanitizers etc. all include under the term pesticide. Though the pesticides are contributed to human welfare as effective weapon against insect pests, they also have some downsides such as fatal to human being and other non-target organisms. Tremendous use of pesticides increase a number of environmental issues. Sprayed pesticides reaches to different vegetation such as air, water and soil and subsequently non target organism other than their target pests. These chemical substances contaminate the environment and water bodies through the various ways that are domestic and agricultural runoff, direct spraying, industrial effluent and direct discharge. Finally the pesticides pollute lakes, rivers, ponds, soil and environment.

Application of pesticides is necessary now a days and it will increase day by day and it may not be stopped by human in future. But the pesticides adversely affects the wide range of non-target organisms. Balance between different species in ecosystem is destroyed due to effect of pesticides and other toxic substances. These broad spectrum pesticides kill or destroy different types of target organisms similarly they affect non target organisms by interfering with their metabolism, hormone system, nervous system and physiological processes (Sathick, 2012). Numerous non target organisms accumulate pesticides from polluted environment and transfer through food chain. Behavioural, morphological, biochemical and physiological alterations may produce in vital organs that may results even in death of an organism those get exposed to the pesticides. Acute and chronic health effects were seen in people who get exposed to pesticides. According to EPA report (2007) exposure of organism to pesticides may produce wide range of hazards health effects including simple irritation of skin and eyes to complex and severe effects on reproductive, nervous and hormonal system and even cancer problem. There are substantial evidences of associations between organophosphate insecticide exposures and neurobehavioral alterations (Weselak *et al*., 2007; Jurewicz and Hanke, 2008). Limited evidences also exist for other negative outcomes from pesticide exposure such as neurological, birth defects, reproductive and fetal death (Sanborn *et al*., 2007). Miller (2004) stated that as per the report of WHO and UN Environment Program nearly about 3 million workers in the agricultural field suffered every year in the developing world from severe poisoning with different types of pesticides, out of which 18,000 get died. People doing several carriers like pet groomers, veterinarians, gardeners, fumigators and groundskeepers in agricultural fields and comes in contact with pesticides frequently, they may also put their health at high risk from exposure of pesticides. Besides, use of pesticides also declines the number of pollinators and results into loss of biodiversity (Wells, 2007) and loss of habitat for different animals especially for birds (Palmer, 2007). Several insect pests can develop a resistance to the pesticide. Since organochlorine insecticides saturated in fatsand does not excreted, so the organisms tend to retain themalmost indefinately. Biological magnification means the process by which the pesticides or toxicants are more concentrated at each level of the food chain in the ecosystem. In the marine water ecosystem, pesticides are concentrated more in carnivorous fishes, subsequently higher in these fish eating birds and even more in mammals who are at the top of ecological pyramid. Many pesticides may adversely affects the reproduction of fish and fish eating birds. Almost all human being are probably taking pesticides into their body every day with food they eat, water they drinkand the air they breathe**.**

Types of Pesticides:

Pesticides are categorized into three groups ‘Inorganic’, ‘Natural Organic’ and ‘Synthetic Organic’. The inorganic pesticides include fluorides, borates and mercurial, natural organic compounds consists

pyrethrum, rotenone and nicotine (Bogan *et al*., 1961) whereas the synthetic organic compounds contain organophosphates,chlorinated hydrocarbons and carbamates. Pesticides are categorized in different groups viz. herbicides, fungicides, algaecides, rodenticides, insecticides, fumigants, etc. based on their usefulness.



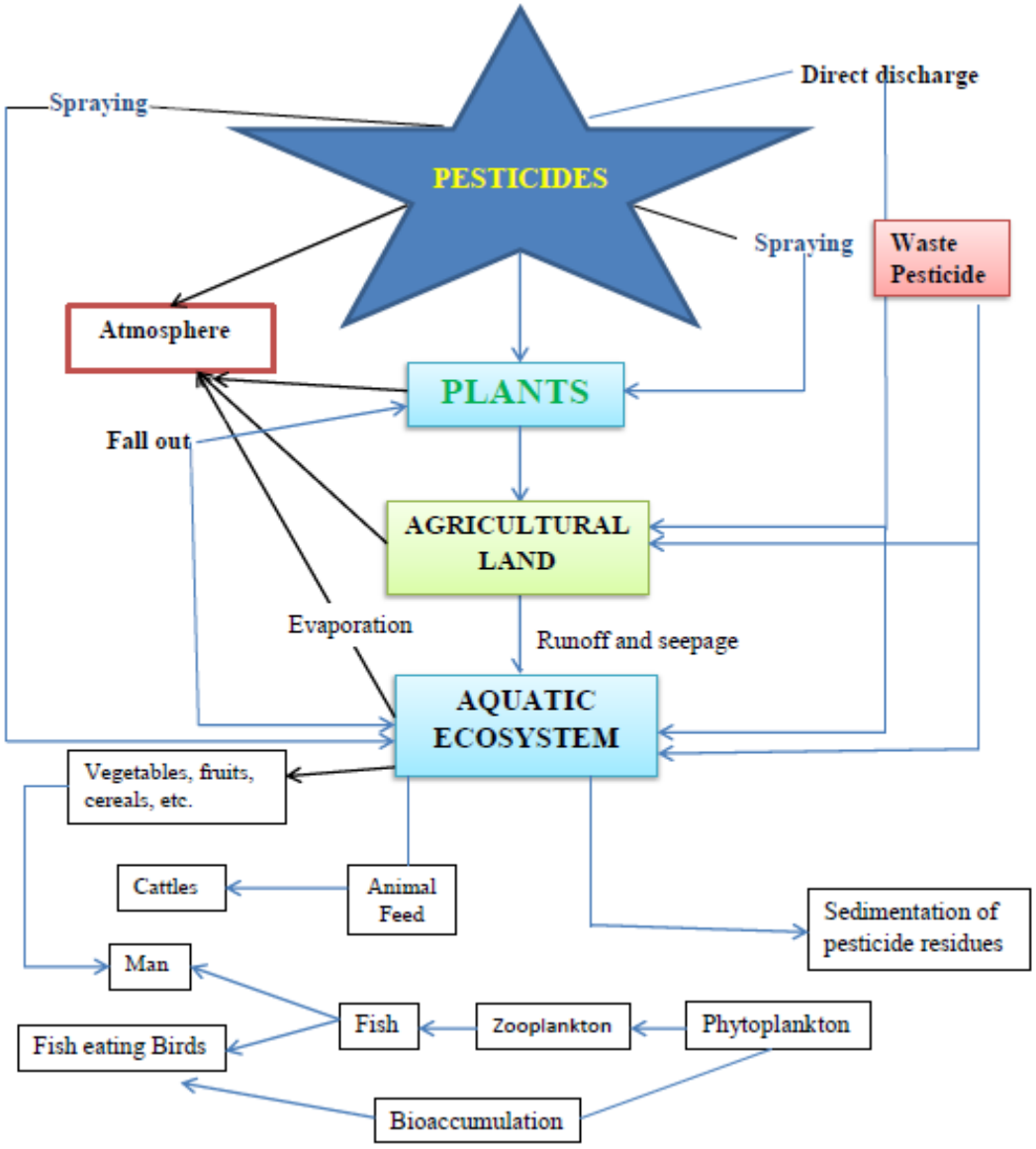
Classification of Pesticide based on their usefulness

Organophosphate insecticides:

Organophosphates are the basis of many insecticides, herbicides, and nerve agents. According to Environmental protection Agency (2012) organophosphate pesticides are highly toxic to non-target organisms, wildlife animals and human being at acute and chronic exposure. Now days organophosphate pesticidesare most widely used to control insect pests. Pandey (2005) stated that people used organophosphate pesticides extensively because of less persistent in the environment than organochlorines. Organophosphates pesticides are readily absorbed by inhalation and ingestion. Skin penetration and subsequent absorption in the organ systemof pesticidevaries with species to species (DuBois, 1971). Organophosphate pesticides affect the nervous system of target organisms by inhibiting acetylcholinesterase activity, which is the enzyme that regulates acetylcholine a neurotransmitter. Azinphos-methyl, tetrachlorvinphos, fenitrothion, azamethiphos, phosmet, diazinon, dichlorvos, chlorpyriphos, malathion, Parathion, and methyl parathion are commonly used in domestic,

agricultural fields, public amusement places and in public health pest control programs such as mosquito eradication (Pesticide Information Profiles, 1995).

Fate of pesticide in the environment:



When a pesticide is sprayed into the agricultural and domestic fields many things happen with it. Cessna (2009) stated that all applied chemicals or pesticides do not reach the destination but they spread into ambient environment and pollute the vegetation.

Environmentalists and scientists are all too aware of long term or chronic effects of pesticides as they seep away to contaminate water resources. Application drift, vapor loss after application and erosion of pesticide treated soil cause the air contamination or pollution inand around the agricultural fields. Pesticide deposition after their application and surface runoff from treated land may cause contamination of soil, vegetation and water bodies (Cessna, 2005), they may pollute surface water and ground water. The pesticides and their

derivatives may be transported long distance. The polluted water bodies with different pesticides can create a significant threat to aquatic ecosystem and drinking water resources.

In aquatic ecosystem fish and aquatic biota may harmed by polluted water. Pesticide runoff into water bodies can be lethal to aquatic life, fish dies after consumed plants from contaminated water bodies. Repeated exposure of pesticide polluted water can causes physiological and behavioral changes, decreased immunity to disease, reduced spawning ground and reproduction that reduce fish population. Pesticide can accumulate in bodies of zooplankton which are the main source of food of fishes and aquatic invertebrates, so fish and aquatic invertebrates accumulate harmful chemical substances. Fish eating birds, predators and human consume those fishes which are harmful to them. People suffering from numerous physiological problems, the pregnant woman is worstvictim. More infant mortality has been reported from localities where high residues of pesticides were found in human milk (Shukla and Trivedi, 2005).

Physicochemical parameters:

Physicochemical properties of water like temperature, pH, dissolved oxygen (DO), hardness, etc. plays an important role in safety of an aquatic ecosystem and supports life of organisms in it. Interaction between the biotic and abiotic components is the key feature of an ecosystem. Any change in the abiotic components will be reflected in the biotic life. According to Kamble (2007) a minute or sudden change in physicochemical properties of water may be lethal to the organism. Quality of water is important in study of wet land habitat because of the chemical and physical factors can positively or negatively affects the productivity of producers and subsequently influences the trophic levels and total biomass of the aquatic ecosystem (Wetzel, 1975). There are numerous toxic substances naturally or artificially introduce in the environment and affect the water quality and its physicochemical properties. So, it is always good to monitor the physicochemical parameters of aquatic ecosystems whenever such ecosystems are studied.

Temperature:

Temperature exerts a major influence on biological activity and growth of an organism. The organisms inhabiting the wet land habitats such as rivers and lakes are governed by temperature. Aquatic organisms such as zooplanktons, phytoplankton, fishes and other species all have preferred range of temperature. As temperature get too far above or below the preferred range, the number of individuals of the species decreases until finally they are none. Temperature influences the chemistry of water and at higher temperature the rate of chemical reaction increases, so the temperature is important physical property of water. Warm water holds less dissolved oxygen than cool water, and may not contain enough dissolved oxygen for the survival of different species of aquatic life. According to citizen's guide meant for understanding and monitoring lakes and streams,the higher temperature increases the toxicity of some toxins. Temperature is an important factor and all life processes are accelerated or slowed down by temperature changes in the environment. It influences the solubility of gases and salts in water. Volume as well as density of water depends upon temperature.

pH:

pH is a numeric scale used to specify the acidity or basicity of an aqueous solution. Water pH affects the toxicity of pollutant which dissociate in to ionized and unionized fraction will be markedly toxic. Change in pH level i. E. hydrogen ion concentration changes the degree of toxicity of the poisonous substances. The pH of water was found decreased after addition of pesticide malathion and sumithion in water (Madnaik, 1988).

Dissolved oxygen:

Oxygen is one of the most essential element for any living organisms. The main source of dissolved oxygen is atmosphere and photosynthetic process of producer organisms. The amount of dissolved oxygen in water depends on surface area exposed, temperature, etc. According to Odum (1971) monitoring the oxygen concentration can helps to understand the health of a water body and it is the most convenient way of feeling pulse of an aquatic ecosystem. Dissolved oxygen (DO) is absolutelyessential for the existance of all aquatic organisms such as fish, clams, crabs, zooplankton, etc. Besides biochemistry, oxygen concentration may also affects the odor, clarity and taste of the water. Dissolved oxygenis an important factor in assessing water quality. Oxygen concentration of water can be reduced due to fertilization of aquatic plants in water contaminated with phosphates and nitrates which are the ingredients of fertilizers present in runoff from agricultural fields. According to Cairn *et al.* (1975) many substances are more toxic when dissolved oxygen of the water is reduced.

Hardness:

Hardness is one of the chemical properties of water that is determined by the presence of calcium, magnesium and other divalent and trivalent metallic elements present in the form of dissolved compounds. Several workers have studied toxicity of pollutants depending upon hardness. Morel *et al*. (1973) suggested that increasing hardness may cause decrease in the toxicity due to the reaction of cations with bicarbonate. There is controversy regarding the hardness of water and its toxicity on fishes. The toxicity of toxicants depend on hardness of water as increasing hardness deceases the toxicity (Bhilave, 2001). According to Henderson (1960) the differences in 96 h LC50 values were not significant, between hard and soft water for ten organophosphorus compounds he studied, while LC50 valuesin hard water was less than one third of soft water for organochlorine compounds. But the Alabaster (1969) reported that LC50 values of several pesticides in test fish were usually higher in hardwater than softwater. Cations in hard water react with certain pesticides and reduce the toxicity of pesticides.

Review of literature:

Though the pesticides have the potential to affect almost all aquatic organisms their impact on fishes are particular. Fish is highly sensitive to contaminated water and it is a good indicator of environmental pollution. A number of workers have been reported that synthetic organic pesticides such as organochlorines, organophosphates and carbamates are extremely toxic to non-target organisms in fresh water fauna and adversely affects the complex food chain and population dynamics (Reddy *et al*., 1991; Chandra, 2001). These investigations particularly explain adverse effects of pesticides on mortality, behaviour, metabolism and physiology of fishes. The knowledge of history is useful to interpret the cellular and biochemical alterations in fish after exposure of pesticide, so it is necessary to review the work of different research workers in brief. Toxicity, mortality and physiological alterations have been evaluated and compared by many research workers. Toxic effect and mortality of two organochlorine pesticides, thiodan and lindane on fingerlings of *Oreochromis niloticus* and *Tilapia zilli* were investigated by Gurure (1987).

Abnormal behavior such as restlessness, sudden quick movement, rolling movement, swimming on the back were observed when the fishes were exposed to diazinon, the affected fish become very weak, settled down at the bottom and died (Rehman, 2002). Study on behavioural responses of fish, *Heteropneustes fossilis* exposed to paper mill effluent was carried by Baruah and Das (2002). They observed that the paper mill effluent

cause numerous behavioural, physiological effects in fishes. Maruthi and Rao (2003) observed that even very dilute sugar mill effluent was also toxic to fish, *Channa punctatus* at higher flow rate. Machado and Fanta (2003), studied the toxicity of methyl parathion on fresh water fish, *Metynnis roosevelti.* Vasait and Patil (2005), observed leaning of body, paler body colour and mouth was opened in the fish, *Nemacheilus botia* exposed to monocrotophos. Kopruku *et al*. (2006) worked on acute toxicity of diazinon and its effects on behavior of fingerlings of European catfish, *Silurus glanis L.*, they observed that thenumberof dead fishes was increased with increasing pesticide concentration and exposure period. Chlorpyrifos is highly toxic and had a detrimental impact on the behavioural response of *Cyprinus Carpio* at sublethal concentration (Halappa and David, 2009). Christopher*et al*. (2010) studied the toxicity stress and mortality of air breathing fish, *C. punctatus* after exposure of three pesticides viz. carbosulphan, glyphosate and atrazine. Dose and dose-time dependent mortality rate, stress signs in the form of behavioral changes were observed by them in response to these pesticides. Srivastava *et al*. (2010) observed the toxicity and behavioural response of fish, *H. fossilis* to dimethoate.

The fish showed behavioural changes like increased opercular movement and abnormal swimming, inactive, static movement, loss of buoyancy and intermittent muscular spasm due to pesticide intoxication. Fish, Nile tilapia *O. niloticus* exposed to different concentrations of ammonia showed changes in swimming behaviour, loss of equilibrium, high gill ventilation changes in skin color and mortality caused by increased concentrations of ammonia (Abdalla and Heba, 2011). As per their observation growth performance of fishes were negatively correlated to the ammonia concentration. Acute toxicity of pesticide metasystox has been studied on fish,*N. botia* by Nikam *et al*. (2011). They evaluated the median lethal concentrations of pesticide for 24, 48, 72 and 96 hrs. and the LC50 values were 10.3, 9.131, 7.884 and 7.018 ppm, and observed altered behavior in exposed fish at the time of experiment. Napit (2013) studied the toxicity of pesticides on fish fauna of Bhopal lower lake. Subhashkumar and Selvanayagam (2014) studied the acute toxicity of Zinc oxide nanoparticles on fresh water fish, *Cyprinus carpio* and predicted the mortality. Fenthion induced toxicity and behavioural responses in fresh water African catfish, *Clarius gariepinus* was studied by Somdare, (2015). He observed behavioural changes like jumping out of water, erratic swimming, increased opercular activity, breathe hardly, increased mucus secretion followed by fatigueness and death after exposure of different fenthion concentrations.

Histological studies:

Histology deals with the microscopic study of the cell and tissue anatomy. Thin sectioned tissue successfully utilize as a diagnostic tool in medical and pathological sciences. The knowledge of histology is essential to compare the normal and abnormal, diseased, degenerated cellular architecture. The cellular and sub cellular constituents of the tissue play an important role in the physiological and metabolic functions. Histological structure of brain, gill and liver in *Eutroplus maculatus* was studied by Gopalakrishanan (1990). Hinton and Lauren (1990) described histology of liver, according to them liver consist of two portions one is parenchyma and other is stroma, the parenchyma comprises various types of cell within the liver and extra cellular spaces whereas stroma consists of blood vessels and connective tissue. Mohammad Rashad (2004) described the general account of histology of liver, kidney, gill, stomach, intestine, and reproductive organs in fishes. In the histology of stomach and intestine of *H. fossilis,* basic pattern of wall layers was similar to other teleost fishes. Histology of esophagus, stomach and intestine of Malaysian river cat fish, *M. nemurus*was studied by Ghada (2012). Sharon and Zilberg (2013), studied histology of differentfishesviz. *Poecillia reticulate, P.*

velifera, Pterophyllum scalare and *Australian Sea Bass*and observed histological structure of detoxifying organ liver and kidney, respiratory organ gill, alimentary canal, reproductive organs, skin and muscle. Bahuguna (2014), studied the histology of gill in fingerlings of *Schizothorax plagiostomus*. Reethamma and Joseph (2014) observed and described the histology of gill, liver, muscle and intestine in *E. maculatus.* Histology of gill and liver in *Lutjanus johni and Lutjanus russelli* was observed by Pilla *et al*. (2014), they described the appearance and pattern of hepatocytes, sonusoides, Kupffer cells and blood vessels in liver and gill arch, primary gill lamellae, secondary gill lamellae, pillar cells and goblet cells in gill. According to Deshmukh *et al*. (2015a) the stomach consists of two parts i. E. cardio-fundic and pyloric. Cardio-fundic region consists of mucosa layer made up by numerous folds covered by the columnar epithelial cells. In cardio-fundic region gastric glands were more in number than pyloric region. Inintestine,proximal region consists of numerous sharp fingerlike villi covered with columnar epithelial cells which comprisesmucous secreting goblet cellsand absorptive cells. In intestine the proximal and middle region contains less number of goblet cells while distal region contains more number of the goblet cells.

Histopathological studies:

Histopathology is a microscopic examination of tissue in order to study the manifestation. It is a clear picture of cytoachitectural changes produced during the chemical intoxication and help in assessing the extent of any toxicant (pesticide) pollution in the ecosystem. According to Jiraungkoorshul *et al*. (2003) histopathological alterations due to lethal and sublethal effects of toxic compounds are very important for detail prediction of health status of fish and for future ecological impact. Histopathological alterations in different tissues of fishes exposed to various toxicants have been studied by number of workers.

Kidney lesions, damage in hematopoietic tissue, degeneration in tubular epithelium and shrinkage of glomerular epithelium were reported in the fish, *C. punctatus* exposed to chronic sublethal concentration of elsan, mercury and ammonia (Banerjee and Bhattacharya, 1994). Ravindar Kumar (1999) extensively studied the effect of ammonia in *C. punctatus*. He reveled necrosis, vacuolization in cytoplasm and connective tissue damage in kidney whereas degeneration of cell membrane in liver. Histopathological changes due to toxic effect of hexachlorocyclohexane on liver of *Labeo rohita* have been studied by Das and Mukharjee (2000), they observed swelling of the hepatocytes with diffused necrosis and marked swelling of blood vessels in the liver. Bhuiyan *et al*. (2001) showed sumithion induced severe degenerative changes including hypertrophy of cell and nuclei, sinusoidal spaces, vacuolation in the cytoplasm of hepatocytes, necrotic patches, ruptured blood vessels, fragmentation and ruptured cell membrane in histological structure of liver of fish, *C. punctatus.* Histopathological effects of herbicide, glyphosate in gill, kidney and liver of Nile tilapia *O. niloticus* were reported by (Jiraungkoorshul *et al*., 2002). They found hyperplasia of lamellar cells, fusion of lamellae, epithelial lifting, aneurism and cell proliferation in gill filament, as well as nuclear pyknosis and vacuolation in hepatocytes in liver and dilated Bowman's capsule and aggregation of hyaline droplets in the renal tubular cells of kidney.

The histopathological alterations were studied by Ortiz (2003) in gill, liver and kidney of three species of fishes i. E. *Mugil, C. carpio* and *Barbus,* exposed to lindane and reported some degenerative changes in these tissues. Dutta *et al*. (2006) conducted a histopathological study on the effect of endosulfan on testes of bluegill fish, *Lepomis macrochirus* exposed for 24, 48, 72 and 96 hours and found that fragmentation of connective tissue, breakage of primary spermatocyte wall and its separation from the seminiferous tubules, damage and

migration of primary spermatogonia into the lumen and damage to connective tissue and seminiferous tubules. According to Kunjamma (2008), the insecticide chlorpyrifos is potent to cause toxic responses, even structural alterations were observed in the sections of gill and liver of fish *O. mossambicus.* Histopathological effects of sublethal concentrations of dichlorvos on the gill and liver in *Cirrhinus mrigala* were determined by Velmurugan *et al*. (2009). They observed hyperplasia, desquamation and necrosis of epithelia and epithelial lifting, edema, lamellar fusion, curling and collapsed secondary lamellae in gill, as well as in liver cloudy swelling of hepatocytes, congestion, vacuolar degeneration, karyolysis and dilation of hepatocytes. All histopathological observation indicated that dichlorvos causes destructive effect even at sublethal concentration. Jayachandran and Pugazhendy (2009) observed histopathological changes in gill of *L. rohita* exposed to herbicide, atrazine. Contaminated water deteriorates histological alterations in gill of *C. carpio* from the fish farm monitored at environmental conditions were studied by Raskovic *et al*. (2010), they stated that the extent of alterations varied from slight to serious ones which were dependent on environmental condition of pond.

Dimethoate produced toxic effect on *O. mossambicus* that causes functional and morphological alterations to the gonads. In ovary disruption of follicular epithelial cells, vacuolization and stromal hemorrhage and in testis swollen seminiferous tubules, inflammation and necrosis occur (Desai *et al*., 2011). Cypermethrin exposure induced marked abnormalities in the kidney of *C. mrigala* with disruption of tubular organization and lymphocytic infiltration. The pathological changes persisted with vacuolation, clotting of blood and glomerular degeneration (Prashanth, 2011). Shete and Patwari (2012) reported that $CuSO_4$ causes damage not only to the absorptive area of the stomach, but it also disturbs the physiological processes and absorption of digested food in *Macrones cavasius*. As per Bias and Lokhande (2012) Cadmium chloride causes deleterious effects on *O. Striatus* and significant alterations in the normal metabolism and produce histopathological alterations in gill, liver, stomach and intestine of fish.

Sandhya rani and Venkataramana (2012) revealed that the malathion causes dose and exposure period dependent changes in the branchial gills of *Glossogobius guiris*. The changes were drastic such as altered cellular morphology and cell diameter were observed at different concentration. Jafarizadeh *et al*. (2012) reported several histopathological changes such ashemolysis, necrosis and edema in kidney of fish, *Hypophtalmictys molitrix* from different fish farms. Olufayo and Alade (2012) examine histological alterations such as inflammation, vacuolation, and necrosis in gill, liver and kidney of fish *Heterobranchus bidorsalis* when exposed to sub lethal concentrations of cypermethrin. Deka and Mahanta (2012) studied the histopathological alterations produced in liver, kidney and reproductive organs of fish, *H. fossilis* exposed to malathion. Nikalje *et al*. (2012) reportedsevere degeneration in normal structure of gills in fish, *L. rohita* exposed to textile mill effluent for acute and chronic exposure period. A wide range of toxic effects of aluminum have been demonstrated in gill, liver and kidney of fish,*T. zilli* by Hadi and Alwan (2012). They stated that degree of destruction of tissue was proportional to the concentration of the aluminum. Yogita and Abha (2013), observed histopathological alterations in gill and liver anatomy of *C. punctatus* after hilban treatment.

The hilban is highly toxic to the fish at low dose by producing hepatic and respiratory toxicity through damage of cellular morphology of gill and liver tissue. Prolonged exposures of fish, *C. punctatus* to malathion causes destruction in the structure of liver and kidney. In liver, degeneration of cytoplasm, vacuolization and atrophy of hepatocytes whereas in kidney necrosis, swelling of renal tubules, disintegrated cytoplasmic material were seen (Magar and Shaikh, 2013). Cypermethrin and Beta-Cyfluthrin both pesticides strongly disrupt normal

hepatic function in rat (Bhushan et al., 2013). Dimethoate induced histological changes in the intestine of fish, *P. ticto* at acute and chronic level was observed by Ganeshwade *et al*. (2013), they observed necrosis and bulging at the tip of villi which leads to their rupture at acute toxicity and chronic exposure showed disintegrated columnar epithelium, degeneration of mucosal lining,vacuolization in submucosa,broken serosa, etc. Muthukumarvel *et al*. (2013) showed vacuolization, dilated blood vessels, disintegration of cell boundaries and subsequent loss of cell wall integrity and complete damage of hepatocytes in liver of fish,*O. mossambicus* at 30 days exposure. Day and Saha (2014), reported dimethoate induced histological alterations in liver, kidney, brain and gill tissues of the Indian major carp *L. rohita*. Sublethal concentrations of cypermethrincauses deleterious effects on the liver and intestine of fish,*O. mossambicus*, well defined changes in their structure has been demonstrated by Karthigayani *et al*. (2014).

After exposure of carbaryl, the histological examination revealed the necrosis, hepatic cord distortion, and cellular damage in liver of fish,*Clarius batrachus* (Archana Kumari *et al*., 2014). In fish, *C. carpio* gill, liver and brain structure significantly altered on exposure it to quinalphos (Chamarthi*et al*., 2014). Jha *et al*. (2014) assessed the histopathological changes in gill of *C. gachua* after exposed to hostathion. Due to hostathion intoxication the gill epithelium was completely separated from the basement membrane and there was swelling of the secondary lamellae and dilation of vessels. The pillar cell nucleus showed necrosis and vacuolation in the secondary gill epithelium was seen. The gill and liver of *C. gariepinus* juvenile exposed to acute concentration of 2,4-D amine would cause maladaptive histopathological changes which could lead to their death (Makinde *et al*., 2015). Anamika *et al*. (2015) showed vacuolation of cytoplasm, degeneration of hepatocytes, rupture of blood vessels, pycnosis and necrosis of cells in the liver of fish, *C. gachua*exposed to profenofos. Iseni *et al*. (2015) observed histological changes in kidney, liver and gonads of fish, *B. pelopponesius* after exposure of insecticide actara 25WG, due to its toxicity the structure of main organs get damaged. Chandra*et al.* (2015) reported that the pesticide, rogorin causes alterations in gill, kidney and liver of *C. batrachus.*

Congestion and swelling occurs in gill lamellar epithelium, cell degradation, shrinkage of glomeruli and decreased hematopoietic activity in kidney as well as cell wall rupture, necrosis, granulated cytoplasm and vacuolated hepatocytes in liver was observed. The effect of confidor on histology of the gill, liver and kidney of *L. rohita* was studied byVeeraiah *et al*. (2015). Necrosis in the primary gill lamellae, fusion of secondary gill lamellae at certain places whereas shortening and clubbing of the ends of the some secondary gill lamellae; changes in the shape and size of the hepatocytes, rupture and degeneration of hepatic cells, formation of vacuoles in the liver as well as vacuolization, degeneration of cell membrane, damage of haemopoietic tissue and renal tubules and hypertrophy of nuclei were observed in kidney.

Histochemical studies:

Histochemical studies are useful to understand about the presence of chemical moieties like mucosubstances, enzymes, proteins, carbohydrates, lipids and other metabolites at a particular histological site and their role in the tissues of an animal. Histochemical techniques help to analyze not only the location of protein, carbohydrate and lipid but also molecular changes at cellular level (Pathan *et al*., 2009). There are reports on the histochemical investigation by number of such chemical moieties by some workers. Sing (1972) studied the various chemical moieties by histochemical methods from the gill of teleosts. According to Dubale and Awasthi (1982) pesticide rogor influences the fluctuations in the activity of acid phosphatase after 16 and 24 days of treatment.

They furthur observed the intense staining reactivity in the cytoplasm at the end of 48 days in the kidney of fish, *H. fossilis*. A study on characterization of mucosubstances in the gill of *Channa punctatus* due to two organophosphate insecticides malathion and sumithion intoxication was carried out by Madnaik (1988). Bias and Tazeen (1999), reported decrease in protein, glycogen and lipid content in *Mystus vittatus* due to severity of the action of pesticide aldrex. Parasher and Banerjee (2002), reported histochemical alterations by lead nitrate at lethal concentration on the gill of *H. fossilis*. They investigate the number of chloride cells in epithelial lining of both primary and secondary lamellae. Rawat *et al*. (2002), observed relationship between liver glycogen and endosulfan toxicity in a catfish, *H. fossilis* exposed to different concentration. Sakr *et al*. (2002) found that different concentrations of hostathion induced gradual and marked reduction in glycogen and protein content in liver cells of *C. gariepinus.* They suggested that carbohydrate depletion might be due to disturbed role of golgi apparatus in polysaccharide synthesis under the insecticide stress which in turns utilizes the reserved glycogen for energy needs. Sonawane *et al*. (2004) observed significant depletion in protein, glycogen and free amino acid in blood, muscle, liver and kidney of fish, *Lepidocephalis thermalis* exposed to sublethal concentration of sugar mill effluents.

According to them the decrease might be because of glycogenesis, gluconeogenesis for energy production and proteolysis due to impact of effluent stress in tissue. Sakr and Jamal Al Lail (2005) reported decrease in glycogen and total protein content in liver of fish,*C. gariepinus* exposed to fenvalerate. According to them the reduction in glycogen inclusion became more pronounced after 10 days exposure period similarly total protein content were found noticeably decreased in cytoplasm and nucleus in liver cell at the same exposure period. After chronic exposure of fish, *Rasbora daniconius* to paper mill effluent the glycogen, protein and lipid content was decreased which might be due to the toxicity stress (Pathan *et al*., 2009). The loss of protein, carbohydrate and acid mucopolysaccharide in the liver of *C. carpio* due to endosulphan toxicity was noticed by Hundet and Prabhat (2013). According to them carbohydrate metabolism was less affected in comparison with that of protein and acid mucopolysaccharide. Stoyanova *et al*. (2015) observed increased hepatic glycogen content in fish, *Aristichthys nobilis* with increasing insecticide thiamethoxam concentrations. They observed that the PAS reaction intensity was increased in dose dependent manner.

Biochemical studies:

Pesticides can damage the physiological system affecting at organ or cellular level or even at molecular level which cause changes in biochemical composition of organ. Extensive biochemical studies have been carried out on the effect of pesticide on fishes. Change in lipid composition due to dodecylbengenesulfonate in gill of carp has been studied by Nakanish (1986). Begum and Vijayaraghavan (1995) studied the acute effect of dimethoate on some aspects of carbohydrate metabolism of hepatic tissue in cat fish, *C. batrachus* after 1, 2, 4 and 8 days of exposure to sublethal concentration and found that the glycogen content was depleted, whereas the lactate level was increased. The activity level of glycogen phosphorylase in the hepatic tissue was also increased. These results suggested that the carbohydrate metabolism was adversely affected in the hepatic tissue by the organophosphate pesticide dimethoate.

The effects of sublethal doses of fenvalerate administered continuously for four weeks on the blood, liver and muscle of a freshwater fish, *Ctenopharyngodon idella* was studied by Shakoori*et al*.(1996). At the end of treatment the glycogen and protein content was decreased while glucose and free amino acid content was increased. Niveditha *et al*. (1998) studied adaptive changes in the levels of carbohydrate metabolites, glucose,

glycogen and lactic acid, in the freshwater edible fish, *S. mossambicus* exposed to a carbamates, ziram (fungicide). An increase in the blood glucose level and a decrease in the liver glycogen and blood lactic acid level was noted in treated fishes. But the muscle and heart glycogen content was found to be increased. The fish, *O. mossambicus* also exhibited decline in protein, lipid and glycogen content in liver when exposed to a lethal concentration of 0.16 ppm for 96 hours and a sublethal concentrations of 0.08, 0.04 and 0.02 ppm of agrofen for 60 days (Verma and Panigrahi, 1998). A short term exposure of four days to the pesticide, sevin caused a reduction in protein content of the tissues of gut and kidney in the fish,*O. mossambicus* (Chopra *et al*., 2001). Durga Prasad and Veeraiah (2002) investigated the effect of cypermethrin on protein metabolism of the fish, *Labeo rohita* and showed decreased protein level in muscle, gill, liver and kidney while increased free amino acids level in all tissues. Khan (2003) conducted a comparative study between malathion and biosal, a neem based phytopesticide. The study clearly revealed that the chemical pesticide caused more serious reduction in protein content in the liver and kidney of *Calotes versicolor* than the phytopesticide. Kumar and Saradhamani (2004) conducted a study on changes induced in the total protein content of muscle, liver, kidney and brain of the freshwater fish, *C. mrigala* exposed to pesticide avanut. Total protein content in muscle, liver, kidney and brain was decreased,wherein significantdecline in higher concentrations was observed in kidney in comparison with other tissue.

Tripathi and Verma (2004) stated that endosulphan is toxic to the fish,*C. batrachus*. Sublethal concentration of pesticide decreases the activity of citrate synthase and glucose-6-phosphate dehydrogenase in the brain, liver and skeletal muscle of fish as well as the lactate dehydrogenase activity in brain also reduces in response to endosulphan. Naveed *et al*. (2006) studied the toxicity of lihocin on the biochemical composition of *C. punctatus*, and found decreased level of glycogen and pyruvate whileincreasedlevel of glucose and lactic acid in liver, brain and kidney due to the influence of pesticide. Muley *et al*. (2007) showed that the industrial effluent alter the biochemical composition in gill, liver and kidney of fish, *L. rohita* at lethal and sublethal concentrations. According to them decrease in the glycogen, protein and lipid content in gill, liver, muscle and kidney might be due to utilization of these moieties for production of energy to cope up with toxic stress of the intoxicant.

The biochemical components like glycogen, lipid and protein in fish,*Arias dussumieri* decreased after exposure of dimethoate. The percent decrease in glycogen was greater followed by lipid and then proteins. It might be due to glycogenolysis, lipolysis and proteolysis to meet the energy demands (Rathod *et al*., 2009). Sing *et al*. (2010a) evaluated effect of sublethal doses of cypermethrin on biochemical profile of the fish, *C. fasciatus*. After exposure significant decrease was observed in the level of total protein, nucleic acid and activity of enzyme acetylcholinesterase, succinic dehydrogenase and increase in free amino acid and lactic dehydrogenase in muscle, gonad, liver and nervous tissue of fish. Sing *et al*. (2010b) studied toxicity of phorate on fish, *C. punctatus*. They examine toxic effect of pesticide on fish metabolism. The metabolism was collapsed in terms of decrease in level of serum protein, cholesterol and glucose, fall in the biochemical contents indicated the rapid utilization of stored energy to counteract pesticidal stress. When freshwater fish, *P. ticto* was exposed to lethal and sub lethal concentrations of dimethoate for 96 hrs. and 60 days duration, significant decrease in glycogen and slight decrease in protein was observed whereas cholesterol and ascorbic acid content was increased to both concentrations and durations of exposure (Ganeshwade, 2011). Khatani (2011) indicated the toxic effect of the insecticide, abamectine on the biochemical constituents of the tilapia fish, *O. mossambicus.*

According to him the changes in protein, carbohydrate and lipid in the insecticide treated fishes affect the nutritive value of fish. Soorena *et al.* (2011) assumed that herbicide; atrazine has strong effect on the blood biochemical indices of the grass carp. They showed that total plasma protein, albumin, glucose, cholesterol and triglycerides decreased significantly with increased pesticide concentration and exposure period. Vasantharaja *et al*. (2012) revealed significant changes in the biochemical constituents in cypermethrin exposed fish, *C. mrigala*, where total proteins and glucose were decreased while free amino acids increased in gill, liver and kidney of fish. Magar and Dube (2013) exposed the fish, *C. punctatus* to sublethal concentration of malathion for 96 hrs. and observed that there was decrease in protein, lipid and glycogen content in cardiac muscle tissue. Changes in glycogen content of the liver, muscle and gonad of the fish, *N. botia* exposed to meothrin was studied by Chaudhary and Yadav (2013). According to them the glycolysis in all tissues was maximum so the glycogen loss in the liver, muscle and gonad might be due to toxic action of pesticide on tissue energy level. This was supported by Satyavardhan (2013), who mentioned that fenvalerate and malathion causes reduction in total protein and glycogen in gill, liver, kidney, brain and muscle of exposed fish, *C. idella.* Chamarthi*et al*. (2014) noticed that the quinalphos had altered the biochemical metabolism in the fish, *C. carpio* by changing the level of total protein, soluble proteins, structural proteins, and free amino acids in different tissue at different concentrations of pesticides.

Various biochemical parameters like serum total protein, albumin, creatinine, bilirubin and urea in the cat fish, *H. fossilis* after treatment of nuvan were studied by Shaikh and Gautam (2014). They found significant alterations in all biochemical parameters that were dose and time dependent, serum total protein and albumin decreased significantly whereas serum creatinine, bilirubin and urea increased with increasing concentration of pesticide and time. Suneetha (2014) studied changes in total proteins and protein metabolic enzymes such as AAT and ALAT of vital organs viz. brain, liver, gill, kidney and muscle of *L. rohita* after exposure of two pesticides endosulphan and fenvalerate. The percent decrease in total protein and increase in activity of AAT and ALAT was, more pronounced in fenvalerate than endosulphan. Justin and Josef (2015) analyzed acetamipride toxicity on biochemical biomarkers in liver, brain and gill of *O. mossambicus*. Alterations in biochemical parameters like protein and carbohydrates indicated the susceptibility of organ to pollutant followed by altering their function. They assumed that decreased protein content induce proteinaemia and may be correlated with reduced protein synthesis, that reflects changes in normal metabolic activities. Tissue biochemical changes in *L. rohita* after sub lethal exposure to phorate 10G were evaluated by Patel *et al.* (2015), they showed sensitivity of crude protein and fat against pesticide. Protein and fat content decreased while moisture and ash content increased with increased concentrations and exposure period.

Reasons for undertaking the present research work:

It is cleared from the foregoing account that pesticide toxicity to fishes is a field of rushed ongoing research. A careful perusal of available relevant literature would reveal that toxic effects of pesticideson fishes occasionally show a general pattern while it show new reactions, manifestations or a peculiar alteration which were totally unrelated to the previous recorded literature. The pesticide formulation, the experimental animal and the experimental condition are held responsible for this differentiation in such experimental results. So, the present problem was undertaken from above critical review of the work done on fish. The fresh water fish, *Channa gachua* having its abundance in Krishna river basin around Karad city, its food value and its economic importance, the protection of fish is must from the pesticide contamination.

CHLORPYRIPHOS:
A THREAT TO FRESH WATER EDIBLE FISHES

About The Author

Dr. Padmini S. Pawar is currently working as an Assistant Professor at Y. C. Institute Of Science, Satara. She has completed M. Sc. (Zoology), B. Ed. and Ph. D. from Shivaji university, Kolhapur and has more than 4 years of teaching experience. She has presented research papers in number of National and International Conferences and has also published number of research papers in peer reviewed and UGC approved international journals. Her areas of interest are ecotoxicology and animal physiology.

Dr. Rajaram N. Patil is Ex. Head, Department of Zoology and Vice Principal in S. G. M. College, Karad and had more than 32 years of teaching experience at UG, PG and M. Phil. He had worked as Member of B.O.S. in Zoology and Faculty of Science; Chairman, B.O.S. in Zoology, Member of Faculty of Science in Academic Council, B.U.T.R., R.R.32(5), Shivaji University, Kolhapur. He is Recognized Research Guide for M. Phil., Ph. D. and M. Sc. in Shivaji University, Kolhapur. He has published 4 books and 18 papers in National and International Journals and awarded by number of institutions.

About the Book:

This book essentially indicate the truth of pesticidal pollution and its impact on non target organism and subsequently whole food chain by employing standard methodologies and different concepts. This book is thoroughly engage with the important aspect of toxicology and helps the researchers and academics to evaluate effect of toxicants on non target organisms. And also helps society to learn and literate about safe use of pesticide, its toxicity, physiological impacts and mortality. This book covers basic lines of behavioural and physiological abnormalities due to the pesticides on fishand gives indication that this toxicant causes mutagenicity and carcinogenicity. Simplicity and clarity is the morality of this work and book.

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