ASSESSMENT OF PHOTOLYTIC DEGRADATION OF LAMBDA CYHALOTHRIN AND ITS EFFECTS ON FISH – THE MODEL ORGANISM

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A thesis submitted in partial fulfillment of requirement for the degree of Master of Science

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Environmental Science

By

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THESIS ACCEPTANCE CERTIFICATE

It is certified that the contents and forms of the thesis entitled "**Assessment of photolytic degradation of Lambda Cyhalothrin and its effects on Fish – the model organism**" submitted by **Ms. Mehwish Niaz,** registration number 00000278001 has been satisfactory, free of errors and plagiarism for the requirements of the degree of Masters of Science in Environmental Science. It is further certified that necessary amendments as pointed out by the GEC members of the scholar have also been incorporated in the said thesis.

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CERTIFICATE

This dissertation submitted by Ms. Mehwish Niaz is accepted in its present form, by the Institute of Environmental Sciences and Engineering (IESE), School of Civil and Environmental Engineering (SCEE), National University of Sciences and Technology (NUST), Islamabad, Pakistan as satisfying the partial requirement for the degree of Master of Environmental Science.

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DEDICATION

This thesis is dedicated to my family and parents who offered the best support system all through my life. It is also dedicated to my boys, Umar and Usman who made radical changes in their tiny lives to help me complete this degree!

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All praises for Allah, the Most Gracious and Most Beneficent!

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List of Abbreviations

admetSAR	adsorption, distribution, metabolism, excretion, and toxicity structure-activity relationship
APHA	American Public Health Association
CPM	Cypermethrin
DO	Dissolved oxygen
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization
IUPAC	International Union of Pure and Applied Chemistry
LCT	Lambda cyhalothrin
LD_{50}	Lethal dose 50
mRNA	messenger Ribonucleic acid
NCBI	National Centre for Biotechnology Information
NIST	National Institute of Standards and Technology
OECD	Organization for Economic Co-operations and Development
PDB	Protein Data Bank
QSAR	Quantitative Structure-Activity Relationship
RNA	Ribonucleic acid
SMILES	Simplified Molecular Input Line Entry System
USDA	United States Department of Agriculture
UV	Ultraviolet

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ABSTRACT

Natural water reserves and the aquatic life forms within are currently under a serious threat because of the agricultural runoff contamination. Unchecked and excessive pesticide use has challenged the quality of water reservoirs and pose aquatic organisms a serious threat. Lambda cyhalothrin (LCT), a synthetic pyrethroid and a neurotoxin is excessively used on cotton and fruit crops in recent past and it has eventually found its way to natural water reserves. Photodegradation offers a green solution to eliminate toxic compounds from the environment reducing exposure and impacts on the aquatic ecosystems. In current study, it has been attempted to assess possibility of using UV light source to cause LCT to degrade, in order to offer an economic and sustainable solution to pesticide contamination. For assessing impacts on aquatic organisms, grass carp was selected as the test specie. Environmentally relevant concentrations (0.75, 1.0 and 1.25µg/l) of LCT were chosen to spike experimental tank waters to investigate an array of all possible toxic impacts on fish. Grass carp presented red eyes, erratic swimming movements and hypoxia. Fluctuation of physicochemical parameters (pH, DO, EC, turbidity and hardness) within experimental tank water was also observed. Fish serum biochemistry such as glucose, total protein, triglycerides and amylase levels were considerably reduced, compared to the control at all (selected and tested) concentrations. Histopathological studies presented vital organ tissues with severe lesions in microscopic visualization. Gill, kidney, muscle and brain tissues displayed architectural loss, necrosis and damaged cell structures. Genetic alterations and docking simulations were studied in-silico, using web tools. Docking results presented significant bonding between the 3D structures of lambda cyhalothrin and key proteins proving that LCT may cause genetic mutations. Upon analyzing water samples in gas chromatogram, it was observed that UV light did cause lambda cyhalothrin to degrade. The peak areas of samples were compared at the retention time of 6.09 ± 0.079. The results showed that as the initial concentration of lambda cyhalothrin increased, photodegradation/ percent removal of LCT decreased, for all tested UV exposure times. The results of this study indicated extreme toxicity of lambda cyhalothrin where its concentration as low as 0.75µg/l caused extensive histopathological, biochemical and physiological damages in fish. UV light source proved to be an effective technique for photodegrading LCT in an aquatic environment, 11W bulb caused as high as 62% photodegradation of LCT. These findings may be of great help in regulating the use of LCT and design a regimen for safe degradation of this toxin from aquatic environment.

CHAPTER 1

INTRODUCTION

1.1 Background

Water is life! It is the biggest gift of nature. It is remarkable how flowers sprout, dead soil becomes fertile, birds sing happily and humans rejoice right after a pleasant rainfall. The existence of life in all forms depends entirely on water availability and efficient supply. It not only acts as the link between all trophic levels but it also determines the quality and health of that ecosystem. Hence, we take it as the index of health of any biological working environment. This dynamic characteristic of water can be attributed to its unique properties such as high solubility, kinesis and presence or availability in all forms (solid, liquid and gas) in nature.

It's the same properties that make water vulnerable too. On one hand, high solubility of water allows the nutrients to reach in the deepest possible zones allowing life to thrive; and on the other hand contaminants also get transported all the way into deepest areas (Ossai et al., 2020). In some cases, water can act as the gateway for the entry of contaminants too, because of the same property. Hence, it can be inferred that water quality of any system can be crucial in determining its stability and sustainability (Teshome, 2020). This is the reason why water is considered as a health index of any working system.

The quality of water has been severely compromised (with time) because of the rapid and unsustainable technological advancements (Nehra et al., 2021). The incessant abuse of portable water resources; which were once considered a renewable natural resource, quickly converted it into a non-renewable resource! Contamination of surface waters by agricultural pesticides and fertilizers, as well as by industrial metals, is a cause of increasing public concern (Ammar et al., 2017). It was also observed that the developed countries developed contaminations from chemical discharge problems, whereas developing countries from the agricultural sources (Sharma & Bhattacharya, 2017). World population soared exponentially in the recent past; which led the think tanks and scientists to explore more options to assure food security. One of those options was the use of pesticides on agricultural crops in order to obtain healthier and efficient produce. Integrated pest management (IPM) strategies engages use of different pesticides (classes) on agricultural crops (Mbugua et al., 2017). But the most important aspect of pesticide

use is the sustainable agricultural practice, as unchecked use of such chemicals may have negative impacts on non-target organisms.

As reported by USDA, only about >5% of the applied herbicides and ~2% of the insecticides reaches the target pests/weeds, while rest of it may be considered as a possible threat to surrounding ecosystems. Other factors such as the soil character, weather, topography and physicochemical properties of the pesticide also determine the expanse of pesticide residues (Sánchez-Bayo, 2011). This residue then reaches surrounding water bodies either through runoff, leaching, percolation, precipitation, inappropriate waste disposal and/or unsustainable farming activities (Kumar et al., 2010). Pesticide contamination often occurs as transient pulses; with elevated concentrations when a field spraying is followed by significant amounts of precipitation or when pesticides are released as a result of illegal handling and/or the spraying equipment is cleaned (Nørum et al., 2010). The problem is aggravated because of other related concerns such as to improper agricultural practices, lack of information and guidance among the farmers and impractical agricultural infrastructure.

In agrarian economies, major water contamination is caused by the pesticides. Incessant pesticide use has led to contamination of various natural environments as their residues have been detected in various matrices like soil, water and air (Ammar et al., 2017). It has also been reported that this when reaches surrounding water bodies, challenges its physicochemical integrity. Once the health of water has been compromised, it is bound to affect the inhabiting aquatic organisms. These pesticides not only cause immediate and short term damage to the living organisms but also tend to accumulate inside them (Chopra et al., 2011). Once accumulated within a living system, it can travel through the whole ecosystem via food chain. Sometimes, small 'sub-lethal" dose of these chemicals can result in behavioral changes, reproduction impairment, weight loss and inability to cope with various distresses (Bownik et al., 2019).

In aquatic environment, pesticides act as contaminants affecting its overall health (organisms as well as ecosystem) causing aquatic toxicity and sometimes mutations too. The level of harm of any specific pesticide usually depends upon the exposure time, dosage, toxicity potential and persistence of that pesticide. Lethal Dose 50 (LD50) defines toxicity by determining the amount of pesticide required to causes death of an organism within a period of 96 hours. Different

organisms have different LD 50 value, depending upon their ability to cope with toxicity stresses (Bibi et al., 2014).

There are four categories of insecticides used for agricultural purposes; namely pyrethroids, organophosphates, carbamates and organochlorines. Lambda Cyhalothrin belongs to the pesticides' class called pyrethroids. It is widely employed as an insecticide because it has high insecticidal activity . This pesticide is commonly used for cotton, vegetables and orchids. It has also been proven to be an effective insecticide for the locust control. The reason behind consistent use of Lambda Cyhalothrin is that it is highly effective in smaller concentrations, its photo stability and its simple environmental degradation. LCT is fairly photo-stable with natural irradiation and strong adsorption affinity for plants and particulates (Alalibo et al., 2019). The pyrethroids (insecticides) are extremely toxic to fish with 96h LC_{50} values usually below 10g/l. (Kumar et al., 2010).

Lambda cyhalothrin has been reported to be exceptionally toxic to many fish and aquatic invertebrate species. Lambda cyhalothrin (LCT) and cypermethrin (CPM) are micro poisonous to mammals and extremely toxic to aquatic organisms (Xie et al., 2011). The remains of organochlorine have been reported in not only the aquatic and terrestrial environments but also within the organisms living in them. Studies of major rivers and streams report that around 96% of fish, 100% of all surface water samples and 33% of major aquifers contain one or more pesticides at detectable levels (Kumar et al., 2010).

Among the aquatic organisms, fish is most notable with regard to the food chain. It is very rich source of lipids, proteins and nutrients hence important for international nutrient supply. Fish are able to absorb these pollutants directly from the polluted water in their surrounding and indirectly from the food chain (Ammar et al., 2017). The pesticide residue when enters aquatic environment, enters organisms primarily via dermis, breathing through gills and orally by drinking or feeding. This exposure causes biochemical instabilities and significantly damages the physiological parameters. When fish are exposed to this type of chemical pollution, the stress is exhibited in the form of both behavioral and adaptive responses such as disorientation, locomotive strain, aggression and gulping air from water surface. These pesticides are lipophilic in nature; hence accumulate within the fish tissues. The fish exposed to pyrethroid insecticides struggles with the process of natural transmission, blocking in open position, the ionic channels

and induces histopathological, enzymatic and hormonal changes (Kumar et al., 2010). Nearly all of the pyrethroids have been reported to be neurotoxic and repetitive neuron dismissal caused by these pesticides can eventually lead to paralysis. It has also reported that LCT tends to alter the antioxidant system in various organisms causing oxidative stress (Piner & Üner, 2014). This makes humans vulnerable to pesticide ingestion by fish consumption (secondary source). Pyrethroids are metabolized and eliminated at a relatively slow rate by fish than mammals or birds which may explain this compounds higher toxicity in fish than in other organisms (Kumar et al., 2010). Among fish, grass carp is farmed the most for human consumption. Asian countries like China, Iran, Taiwan and Bangladesh have established rich production and breeding of grass carp (Naeem et al., 2011). It is a freshwater fish which is primarily a herbivore feeding on various aquatic herbs. Lambda cyhalothrin is extremely toxic to grass carp and very low quantity and acute exposure can cause irreparable damage to this specie (Ghumman, 2011).

Keeping in view the drastic effects of pesticides in general and lambda cyhalothrin in particular; it is inevitable to devise techniques that help eliminate pesticide residues from aquatic environment. The conventional methods for the removal of pesticides from any environment completely are not efficient; hence focus needs to be shifted towards greener and more sustainable technologies. Ideally, reducing the use of such pesticides at the source can be the start point for its mitigation. Similarly, it is vital to develop bioremediation approaches too, in order to reduce carbon footprint. Apart from these approaches, other biochemical processes may also be explored which may assure efficient and sustainable elimination of pesticides from the areas of interest.

In natural environment, pyrethroids undergo degradation via numerous routes such as biodegradation, hydrolysis, phytoremediation and photo degradation. In contrast to these processes, photochemical degradation has proved to be a much faster, environmentally safe process and does not produce any secondary pollution (Liu et al., 2014). Similarly, in natural water systems, LCT and CPM may be degraded by photochemical processes, which prove to be efficient methods to transform persistent and toxic/poorly biodegradable substances into simpler compounds (Xie et al., 2011). The photo degradation of any chemical follows a pathway in which it breaks down into various other products eventually forming water and carbon dioxide. Many laboratory studies reported using UV lamps for the purpose of photodegrding lambda

cyhalothrin ((Liu et al., 2014; Mbugua et al., 2017; Xie et al., 2011). In one such study, a comparison of photodegradation of five different pesticides was made by using UV lamp source in the presence and absence of a catalyst (Xie et al., 2011). Pesas and Vagi in 2018 compared the use of two different oxidizing agents in the photodegradation of different pesticides and reported that use of either of these did not have any impact on the physicochemical properties of water (Petsas & Vagi, 2018).

It follows a series of steps forming a number of different byproducts. The environmental factors such as dissolved oxygen, temperature, pH, turbidity and many others determine the types of byproduct formed. The process of photo degradation may take place both with and without the catalyst. However, in one such study, the results depicted that the process is slower in the absence of a catalyst and results in an partial mineralization (Petsas & Vagi, 2018). Moreover it was also reported in the same study that the hydroxyl radicals are generated due to advance oxidation process during pesticide degradation process through UV radiations. The process of photo degradation of lambda cyhalothrin was reported to vary with UV exposure time, light intensity and temperature. This means that the reaction dependents on number of photons that have the ability to illuminate the surface; higher number of photons will stimulate more molecules to undergo the reaction and less time will be taken for degradation (Mbugua et al., 2017)

Lambda cyhalothrin is already toxic to fish, but with the added process of photo degradation, where it follows a pathway to break down into various by products; LCT along with these by-products can have deleterious impacts on both fish as well as surrounding water. Various studies have reported a range of LCT degradation by-products depending upon different environmental conditions. Once this particular chemical undergoes photodegradation forming various by-products, the chemistry of surrounding environment also changes owing to the by-products formed (Colombo et al., 2018, Liu et al., 2014). Some of the by-products formed may result in a more toxic environment while others may do the opposite.

1.2 Present Study

This study is designed at evaluating the toxic impacts of lambda cyhalothrin on fish, including wide array of impacts like physiology, behavior, biochemistry, histopathology and changes in gene expression. Grass carp in the most consumed fish around Pakistan and hence any

destructive changes may lead to bioaccumulation and transmittance through the entire food chain. Moreover, an attempt was made to use a green technology i.e. UV light to degrade lambda cyhalothrin within the aquatic environment in order to reduce the toxic exposure.

1.3 Study Objectives:

An experiment based study was devised to be conducted within the Environmental Toxicology laboratory of IESE. It was designed to expose fish to Lambda Cyhalothrin (pesticide) and study all possible toxic impacts on both fish and water; in detail. The objectives of this study are as follows:

i. Assessment of photolytic degradation of lambda cyhalothrin using UV lamp.
ii. Identification of photolytic degradation by-products of lambda cyhalothrin.
iii. Evaluation of physiological and behavioral effects of photolytic degradation of lambda cyhalothrin on fish.

CHAPTER 2

LITERATURE REVIEW

2.1 Current Investigation

Pesticides use has become unavoidable in recent times. The population explosion in past few decades levied pressure on food security thereby indirectly encouraging the use of pesticides. These pesticides (in the form of insecticides or herbicides) attack destructive herbs and insects helping farmers augment their produce. These chemicals have the tendency to enter nearby water systems either through agricultural run-off, drift or unsustainable farming practices. These phenomena result in serious implications such as water contamination and ecological instabilities.

Our fresh water reserves are under serious threats due to non-sustainable use of these toxic compounds. Incessant use of pesticides and other agricultural chemicals has severely compromised the quality of water reserves, threatening the survival of aquatic life forms too. The major routes through which insecticides and pesticides from fields are transported to adjacent water bodies are surface runoff, drains, wind drifts and atmospheric deposition (Nørum et al., 2010). These synthetic pesticides are extremely noxious to fish and other aquatic invertebrates (Singh & Srivastava, 1999). These neurotoxins impair the physiological abilities of aquatic organisms, depriving them of their regular activities. Among aquatic life, fish is of prime importance here as it is an efficient source of protein for humans. Human beings being at the topmost level of food chain, when consume the polluted fish, become the terminal targets of the toxins (He et al., 2017). This study is aimed at investigating the Lambda cyhalothrin degradation potential of UV and also assessing its toxicity to fish (grass carp) through various assays.

2.2 Lambda cyhalothrin LCT (Karate)

Pyrethroids are synthetic pesticides which are generally more effective even in lower concentrations. Lambda cyhalothrin belongs to this pyrethroid class of pesticides and is one of the most effective insecticides used on both food and non-food crops. It was registered back in 1988 by the US environment protection agency (US EPA) Its **IUPAC** name is **1:1 mixture of (S)-α-cyano-3-phenoxybenzyl-(Z)-(1R,3R)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-**

dimethylcyclopropanecarboxylate and(R)-α-cyano-3-phenoxybenzyl (Z)-(1S,3S)-3-(2-chloro-3,3,3-trifluoroprop-1-enyl) -2,2-dimethylcyclopropanecarboxylate (Was) and the chemical structure is as follows:

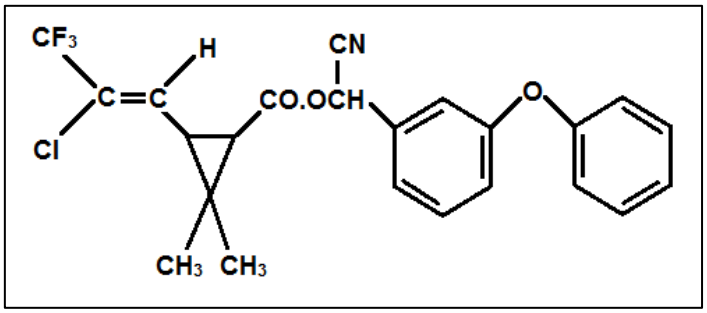


Figure 2.1 Chemical structure of lambda cyhalothrin

Some of the notable physical and chemical properties of lambda cyhalothrin are mentioned in Table 2.1.

Table 2.1 Properties of lambda cyhalothrin

Properties	Values
US EPA PC Code	109704
Molecular Formula	$C_{25}H_{19}ClF_3NO_3$
Molecular Weight	416.3 g/mol
Density	1.12 g/ml
Melting Point	Up to 80^0C
Boiling Point	220^0C
Vapor pressure	1.4×10^{-9}
Water Solubility	0.009 mg/l at 20 ^{0}C
Solubility (other solvents)	450 g/l in xylene

In crop fields, it is applied at the rate of concentration ranging from 0.005 to 0.015kg ai/ha (Singh et al., 2015). It is a synthetic pesticide known to cause water contamination and alters its physicochemical properties too (Alalibo et al., 2019). Due to the ban on organophosphate compounds, the use of pyrethroid insecticides has increased in recent decades (Colombo et al., 2018). There are various techniques to remove toxics and pollutants from water reserves and use

of Ultraviolet light is one of such techniques. Columbo et al. 2012 carried out a study to inspect degradation of lambda cyhalothrin by using UV.

2.3 Grass carp (Ctenopharyngodon idella)

Grass carp (*Ctenopharyngodon idella*) was introduced to Pakistan for the first time, back in 1964 through China (Naeem et al., 2011) and now it's one of the most consumed fish protein in Pakistan (Khalid & Naeem, 2018). It has been reported that exposure to LCT causes extreme stress to fish and also alters its biochemical parameters (Bacchetta et al., 2014). Mode of action adopted by organochlorine pesticides is by blocking the enzyme acetylecholinesterase that results in severe physiological malfunctioning (Banaee, 2013).

Figure 2.2 Grass Carp

2.4 Lambda cyhalothrin and aquatic physico-chemistry

Lambda cyhalothrin is an odorless white/beige solid which is insoluble in water but dissolves in organic solvents such as methanol and n-hexane. For agricultural use, it is usually combined with other agrochemicals in different concentrations (active ingredient) to make a formulation. Once it enters the aquatic medium, it tends to change all of the physicochemical parameters of its environment. The physical properties such as temperature and turbidity vary less. However, the chemical properties such as pH, dissolved oxygen, electrical conductivity and hardness show considerable variations. Physicochemical properties such as dissolved oxygen, temperature and pH are interlinked and any change in one parameter impacts the others too (Sachidanandamurthy & Yajurvedi, 2006).

The toxicity potential of any agrochemical or contaminant towards aquatic ecosystem depends on the physicochemical quality of water (Nkontcheu et al., 2017). Seiyaboh and Izah proved that

anthropogenic interventions resulted in altering physicochemical properties of water reserves including pH, turbidity and dissolved oxygen (Seiyaboh & Izah, 2017).

2.5 Lambda cyhalothrin toxicity and fish

Lambda cyhalothrin, a synthetic insecticide contaminates the physicochemical integrity of receiving water bodies and is toxic to various aquatic life forms (Alalibo et al., 2019). Literature reports detection of lambda cyhalothrin in various water bodies across the world such as 0.00132-0.060µg/L in rivers of Brazil, 0.346µg/L in rivers of Greece, 0.797µg/L in various agricultural zones of the US and 0.983µg/L in various ecosystems of Costa Rica (Vieira & dos Reis Martinez, 2018). Lambda cyhalothrin is highly lipophilic leading to increased absorption through gills, even in lower concentrations. Since fish are unable to metabolize LCT as they lack that enzyme mechanism (He et al., 2008) and get rid of these xenobiotic, they are more sensitive and susceptible to adverse changes within their system as compared to birds and mammals (Kumar et al., 2012). Synthetic pyrethroids (like LCT) are neurotoxins and tend to have higher acute toxicity impacts on the developmental stages of animals and the following figure (Fig. 2.3) explains the neurotoxicity mechanism (Ullah et al., 2019).

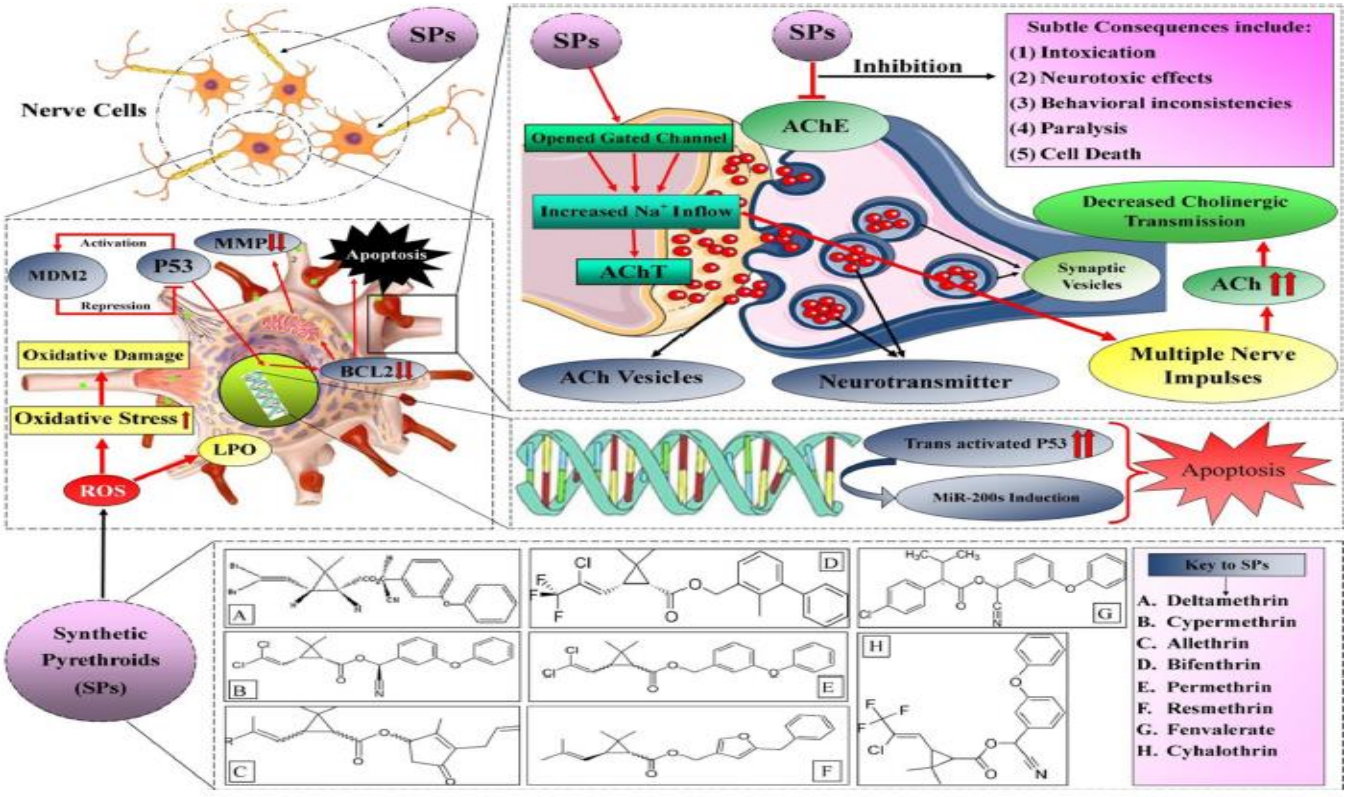


Figure 2.3 Neurotoxicity mechanisms of pyrethroids

Fish is an efficient source of protein and may help prevent malnourishment in countries like Pakistan (Ashraf et al., 2011). Fish are directly in contact with water, hence presence of any such contaminant can have deleterious impacts on their physiology as well as biochemistry (Begum et al., 2003). Bibi et al., 2014 reported that LCT tends to be more toxic towards fish, even in concentration 10-1000 times less than the similar range for birds and mammals. They also reported that lambda cyhalothrin is toxic to grass carp as it alters AChE activity as well as the total protein content in brain, liver and muscle tissues (Bibi et al., 2014). Different kinds of harmful impacts of lambda cyhalothrin on fish species has been reported in literature. The sperm quality of *Oncorhynchus mykiss* (rainbow trout) has been shown to reduce once exposed to lambda cyhalothrin. In *Danio rerio*, LCT exposure severely distresses its endocrine system, particularly the T3 and T4 hormones. Within zebra fish embryos, synthetic pyrethroids have increased capacity of bioconcentration (Wandscheer et al., 2017). In a study where indian carp was exposed to lambda cyhalothrin, histopathological lesions were observed in vital fish organs like kidney, liver, gills and intestines (Velmurugan et al., 2007). Bownik et al. reported that when *Daphnia magna* was exposed to lambda cyhalothrin, it had disapproving impacts on its physiology as well as swimming ability (Bownik et al., 2019).

2.5.1 Lambda cyhalothrin toxicity and fish biochemistry

Biochemical analysis of fish can offer great insight (Agrahari et al., 2007) for ascertaining toxic impacts. Blood in the best indicator for any metabolic defects of the body (Jyothi & Narayan, 1999), hence analysis of haematobiochemical parameters was selected to assess the toxic impacts. Pesticide contamination causes extreme stress for fish, leading to metabolic disturbances of various enzymes, growth retardation as well as sustainability of the organism. The most valuable organs such as liver, kidney, gills and brain get most negatively impacted (Ogamba et al., 2013). Variation in biochemical parameters like glucose, total protein, triglycerides and amylase depict a typical stress response. Hyperglycemia or increased glucose levels are a clear response of fish under stress (Barton & Iwama, 1991).

2.5.2 Lambda cyhalothrin toxicity and fish histopathology

Tissue level alterations as a result of toxic chemical exposure can provide valuable insight to toxicological histopathology (Karbalaei et al., 2021). A study evaluated histopathological alterations within gill and hepatic tissues as a stress biomarker (Jabeen & Chaudhry, 2013).

Another histopathology study concluded that liver was the organ most affected due to stress and toxicity (Camargo & Martinez, 2007). A study by Dohaish concluded that such toxic contaminants not only cause lesions and disturb the histology of vital tissues but also has the capacity to bioaccumulated and travel through food chain (Dohaish, 2018). One more research concluded that histopathological investigation helps catch chemical stressors at a relatively earlier stage. It also identified lesions such as muscle fiber splitting and necrosis as predominant in toxicity exposed fish (Bhuvaneshwari et al., 2015).

2.5.3 Lambda cyhalothrin toxicity and fish relative gene expression

The analysis of gene expressions presents a comparatively reliable method of toxicity assessment as it can identify changes earlier on at transcription level as opposed to the phenotype level (Shen et al., 2020). Lambda cyhalothrin not only causes physiological anomalies, but also leads to behavioral changes, loss of balance, hyperactivity and irregular swimming patterns (Guedegba et al., 2019).

In silico modeling platforms and structure prediction tools not only provide a cost efficient initial pilot study for new medicines and chemical formulations but also help prevent testing on living beings. For molecular and biological exhaustive studies on fish, b-actin has often been used as a control gene for quantifying gene expression (Acharya et al., 2014). Zhang et al., 2011 reported that for the toxicological studies in fish and some other animal species, heat shock protein or HSP-70 can be an effective biomarker (Zhang et al., 2011).

A similar study was conducted where in silico interaction of serum lactate was done with LCT and the resulting simulations presented high affinity among the two (Saxena, 2015). In another study, a QSAR (in silico) model was prepared for forecasting aquatic toxicity of various pesticides, which could prevent in-vivo assays such as lethal dose (LD50) (Agatonovic et al., 2014).

These modern techniques and tools have proved to be very efficient and effective ways to analyze toxicity potentials as well as support in-vivo results. Literature presents various studies where such techniques were effectively applied with reproducible results too.

2.6 Degradation of Lambda Cyhalothrin

With climate change disasters and endangered water reserves, it is now inevitable that we deploy modern techniques to clear out these toxins and reclaim the water reserves. Various eco-toxicological studies conducted in laboratory as well as the fields have revealed a well-established pattern of impacts of lambda cyhalothrin on the water quality. Furthermore, potential toxicity of pyrethroids (different/relevant concentrations) towards aquatic organisms encountered in water courses and sediments has been confirmed (Colombo et al., 2013). Major toxicity of lambda cyhalothrin towards fish is due to its interaction with membrane bound ion channels in neurons and interruption of nerve function by extending the open phase of sodium channel gates, thus leading to hyperactivity of nervous system, subsequently causing paralysis and/or death (Li et al., 2014)

The conventional techniques for pesticide removal have not proven to be considerably efficient which makes is even more challenging task (Colombo et al., 2013). Usually pesticides are perceived in minute concentrations (ng/L or µg/L) as micropollutants and are present as specific components of wastewater, hence difficult to remove by primary and secondary conventional treatments (Petsas & Vagi, 2018). Pesticides of organophosphate class such as Lambda cyhalothrin are generally stable at ambient temperatures (Katagi, 2004). However, lambda cyhalothrin degrades when present within water and soil (Was). Lambda cyhalothrin can be degraded using microbial degradation, hydrolysis and photolysis (Kumar et al., 2010). Natural methods to clean-up pesticides are photodegradation and biodegradation (Fernandez-Alvarez et al., 2007). Sometimes, catalysts are coupled with these techniques in order to amplify the degradation process (Xie et al., 2011). Photochemical transformation of lambda cyhalothrin is another mode of dissipation suggested in literature, however this process in natural waters need to be explored more (Leistra et al., 2004). The degradation products of lambda cyhalothrin vary with the type of technique used and level of pesticide exposure.

Sunlight photodegradation is one of the most destructive pathways for pesticides once they are released into the environment, the ultraviolet component of sunlight is a source of high energy and stimulates photodegradation (Katagi, 2004). Photodegradation is an abiotic process whereby molecules are excited due to high energy photons (UV) and cause an organic reaction (Mbugua et al., 2017). Lambda cyhalothrin present in natural waters can be degraded efficiently by

photochemical processes, converting it into less toxic substance (Xie et al., 2011). Within the laboratory setup, UV is supplied using mercury or xenon lamps of suitable wattage and wavelength. Xie et al., (2011) irradiated an aqueous solution of lambda cyhalothrin in a sunlight simulating reactor which was equipped with xenon lamps, as a source of UV rays. Petsas & Vagi., 2018 employed UV lamps of 18 watts and 365nm wavelength in order to carry out pesticide degradation experiments in the laboratory. Miguel et al., in 2012 carried out similar experiments to inspect photocatalytic degradation of pesticides in natural waters using xenon lamps as a source of UV radiations.

According to Kumar et al., (2010), phodegradation of lambda cyhalothrin followed kinetics of first order and resulted in many by-products. Mbugua et al., also reported that photodegradation of lambda cyhalothrin follows the fort order kinetics (Mbugua et al., 2017). In another photodegradation study, a specialized UV photoreactor was used that operated at a controlled temperature and equipped with a 15W Phillips UV C lamp. In this reactor, photolysis of lambda cyhalothrin was carried out using Fe2+ ions at 254nm UV light (Colombo et al., 2013). Figure 2.4 shows the specialized photoreactor used to carry out this photodegradation study.

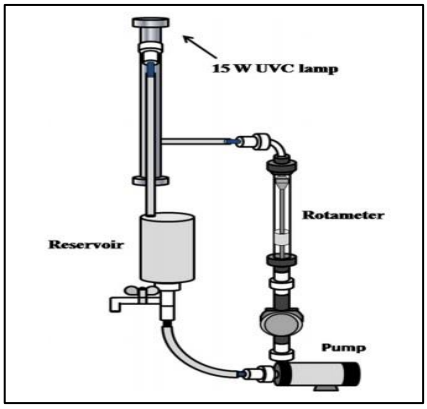


Figure 2.4 Laboratory setup of a photoreactor

In another study, photodegradation of pesticide (Lambda cyhalothrin) was studied using UV lamp. Djouaka et al. employed an 8W UV lamp (Phillips) as a source of UV light to assess if the pesticide residues on vegetables are photodegraded or not (Djouaka et al., 2018).

In some cases, catalysts are also added to stimulate the process of photodegradations. Khan et al., prepared nanocrystallized TiO_2 photocatalyst film in order to degrade lindane which is a persistant organochlorine compound (Kanan et al., 2020). In another study, the presence of

copper as a catalyst resulted in efficient photodegradation of lambda cyhalothrin in an aqueous media (Liu et al., 2007).

2.6.1 Degradation by-products of Lambda cyhalothrin

The derivative products of lambda cyhalothrin through photolysis or hydrolysis have been studied under various studies, but fluctuating environmental conditions may result in different by-products which need to be investigated (Colombo et al., 2018). Usually photodegradation is a series of chemical reactions resulting in subsequent structurally similar by-products. Fernandez-Alvarez et al., in 2017 conducted a photochemical degradation study which resulted in a lot of photoproducts where some of them were identified using the mass spectra information and tallying with the NIST database.

Lambda cyhalothrin is a complex pesticide molecule comprising of a central ester group, an acid group and an alcohol group. Its degradation begins with the cleavage of ester group followed by the rest resulting in different by-products (Premalatha & Miranda, 2019).

The metabolic pathway that lambda cyhalothrin adopts is derived from the degradation technique.

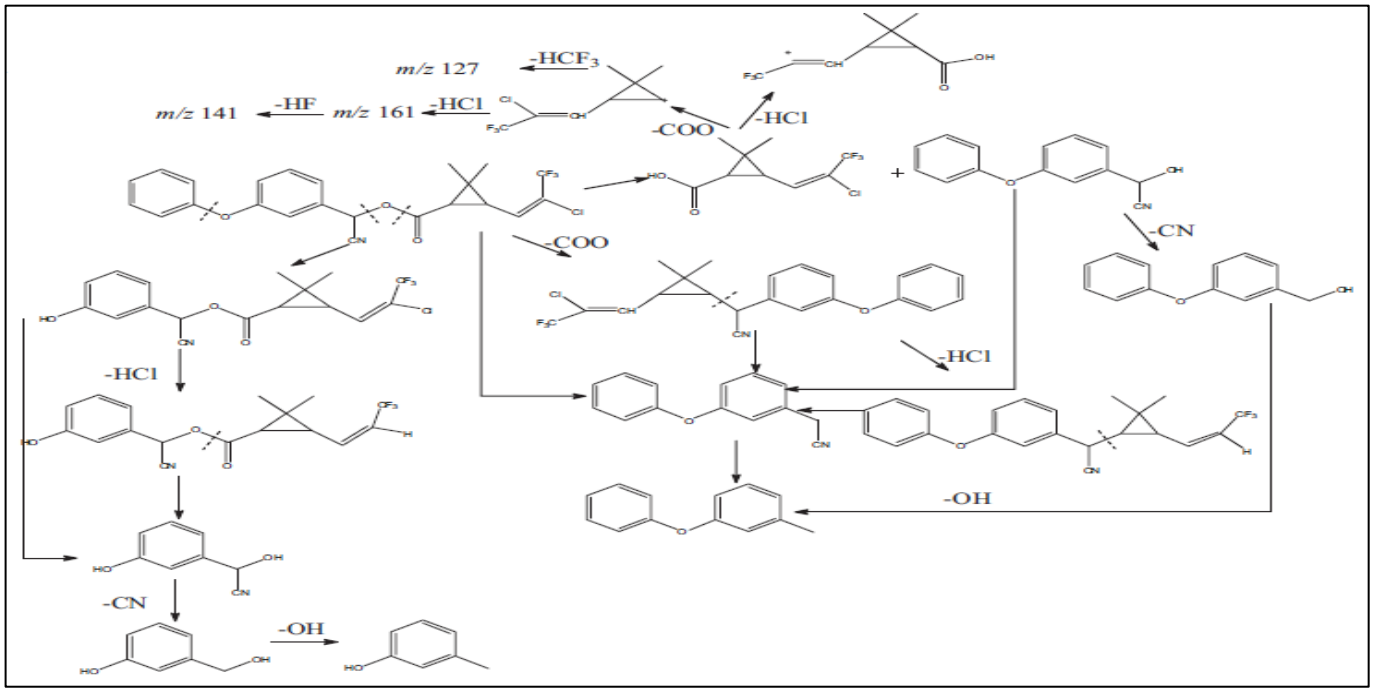


Figure 2.5 Proposed photodegradation pathway of LCT

For photodegradation, the general pathway includes ester bond cleavage, oxidation, dehydroxylation and rearrangement of molecules resulting in CO_2 and H_2O. One of many proposed degradation pathways of LCT suggested by Liu et al., 2011 is attached as follows:

A similar pathway was suggested in another study where the primary position of $OH^{\cdot}$ played major role in determining photoproducts (Xie et al., 2011). Both these pathways result in different photoproducts, determined by other factors such as catalysts, temperature and other environmental variants.

CHAPTER 3

MATERIALS AND METHODS

Toxicity assessment assays were conducted in the Environmental Toxicology Laboratory of Institute of Environmental Sciences& Engineering (IESE) following the protocols mentioned in the OECD and APHA guidelines. The investigational set up comprised of semi static experimental tanks that were equipped with aerators and thermometers, allowing customizable oxygen and temperature controls.

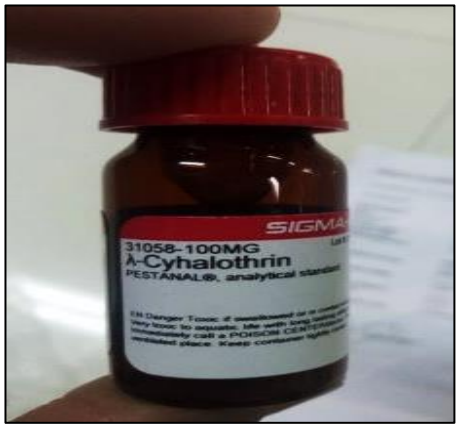
3.1 Test Organism

Grass carp was selected as the test organism. It is herbivore fish which is economical for carrying out bulk experiments and is easily available too. Grass carp has a huge scientific data bank, as it has been used for various experiment based studies in the past. In this study, grass carp was the model organisms for experiments where it was exposed to different concentrations of lambda cyhalothrin and subsequent alterations were observed and recorded. Fish were procured from the Punjab Hatchery, Rawal Town, Islamabad and transported to laboratory in oxygenated polyethylene bags. In laboratory, fish was then transferred to clean experimental tanks. They were acclimatized to the laboratory environment for fourteen days before starting the exposure experiments.

3.2 Chemicals and Instruments

3.2.1 Chemicals

The standard analyte of lambda cyhalothrin was obtained from Sigma Aldrich (31058) which was of ≤95% purity (Fig 3.1 (a). It was stored at -20°C. A commercial formulation of lambda cyhalothrin (EC 2.5) named “Libra” produced locally by Orange Protection was procured from local market, which was used for exposure experiments (Fig 3.1 (b). For chemical analysis, the standard reagent kits were procured from AMP Diagnostic, Austria for indices like glucose, total protein, amylase and triglycerides (Fig 3.2 (a). GC/HPLC grade n-hexane was obtained from Merck for the gas chromatographic analysis (fig 3.3). For fish blood sampling and storage, yellow capped vials containing anticoagulant were purchased from LABOVAC Italiano (Fig 3.4).



(a) Pure Standard **(b) Commercial formulation**

Figure 3.1 Lambda cyhalothrin

Figure 3.2 Standard reagent kits

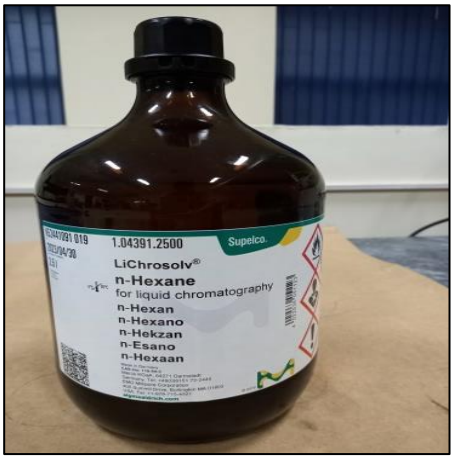


Figure 3.3 n-hexane

Figure 3.4 Fish blood sampling vials

3.2.2 Instrumentation

For studying the dynamics of photodegradation, UV lamps were purchased from local suppliers. Each lamp was of 11W and 254nm and sold as aquarium UV sterilizer (Fig 3.5). An optical microscope Primo star (Zeiss) was used for observing the histopathological changes within fish organs (Fig 3.6).

A chemistry analyzer, AMP Piccos II was used for the biochemical analysis of fish blood (Fig 3.7). Four biochemical indices of fish blood were analyzed for this study; they were glucose, total protein, amylase and triglycerides. Gas chromatographic technique was employed for the optimization and calibration of Lambda cyhalothrin (Fig 3.8).

Figure 3.5 UV lamp

Figure 3.6 Optical microscope

Figure 3.7 Chemistry analyzer

Figure 3.8 Gas chromatogram

3.3 Decontamination of Lab ware

Working with living test organism is a little tricky and requires clean surfaces and environment. Moreover, since this study included RNA, use of clean instruments (glass and plastic ware) was critical in order to avoid any damage to RNA. All of the experimental lab ware was thoroughly cleaned with soapy water, rinsed with distilled water and then oven dried to ensure decontamination.

3.4 Experimental Design

In order to carry out this detailed research, following experimental set up was designed (Fig 3.9).

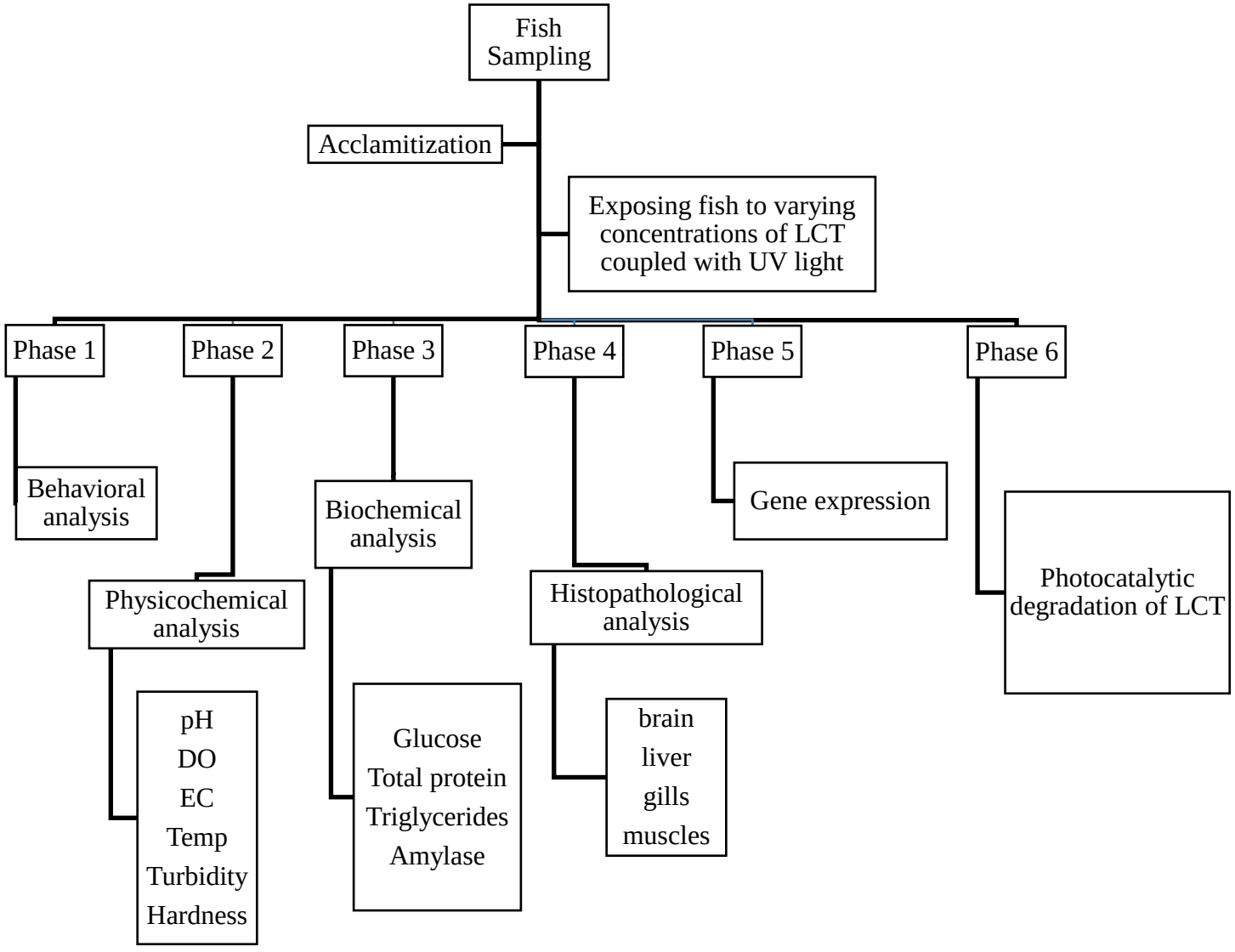


Figure 3.9 Research plan

3.5 Sampling

3.5.1 Sampling site

The study site for this investigation was Rawal Lake (Fig 3.10). It is a freshwater lake situated in North-east of Rawalpindi, while East of Islamabad, Pakistan (33°42’ N, 73°07’ E). It is fed by the Korang river and is 8.8km^2 long lake providing water to the twin cities. A dam has been built on this lake with the storage capacity of about 47,500 acre feet, generating 84,000 acre feet of water on average annual rainfall. This lake is surrounded by some thickly populated areas such as Bani gala, Noorpur Shahan and Bhara Kahu. This population is the source of extensive domestic/municipal waste as well as agricultural wastes. In 2017, severe contamination caused fish death at an alarming rate, at Rawal Lake, as reported by the Dawn (Dawn 16 July, 2017).

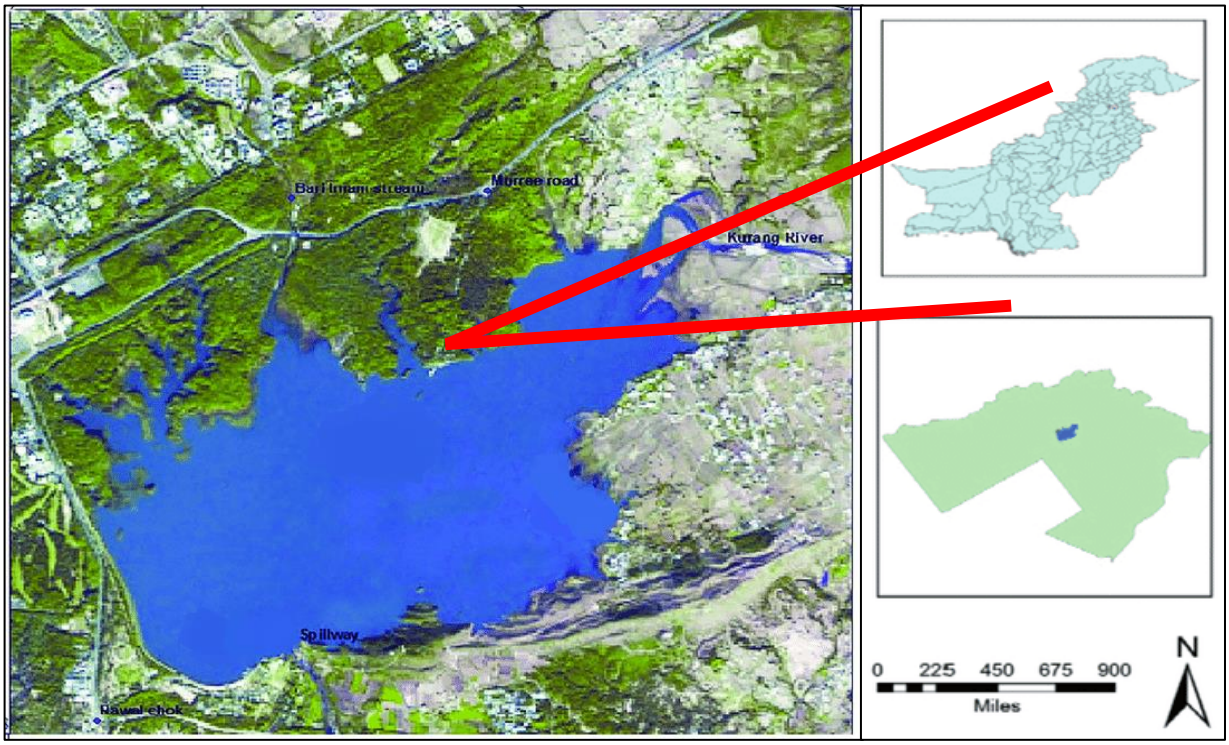


Figure 3.10 Study area

3.5.2 Fish Procurement

This study required fish in bulk; hence it was procured from the Punjab Hatchery, Rawal Town Islamabad. The procured samplings were transported to the Environmental Toxicology Laboratory at IESE, NUST in oxygenated polyethylene bags, and shifted carefully into the experimental tanks (3 X 1.5 X 1.5 ft). The glass tanks were filled with tap water and equipped with aerators/ air pumps to sustain fish inside laboratory.

3.5.3 Acclimatization of Fish

In order to allow fish to adapt to new environmental conditions of the laboratory, the fresh stocks of fish were always acclimatized for about two weeks before carrying out any experiments (Fig 3.11). Since the experimental fish were usually small (0.5–4inch), they are particularly sensitive to changing environment. Fish were carefully monitored for any changes in behavior and fed with pallets of commercial fish feed. The tank water was also changed regularly in order to remove polluted water and keep the fish healthy.

Figure 3.11 Laboratory experimental setup

3.6 Physicochemical analysis of water

According to OECD guidelines, it is crucial to check the water quality carrying fish. Water parameters were carefully monitored for study. A total of six parameters were analyzed with the help of standard procedures (Organization for Economic Cooperation and Development, guideline method, 203, (1992). Water from three different sources i.e. laboratory tap water, lake and experimental tanks were analyzed for all six parameters. For measuring pH and Temperature, Multi parameter analyzer, Consort- C1020 was used. Winkler method was employed for measuring DO, EC (Fig 3.13), Turbidity. Hardness was calculated with the help of titration method.

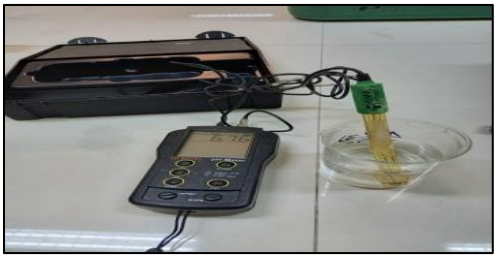


Figure 3.12 Temperature and pH meter

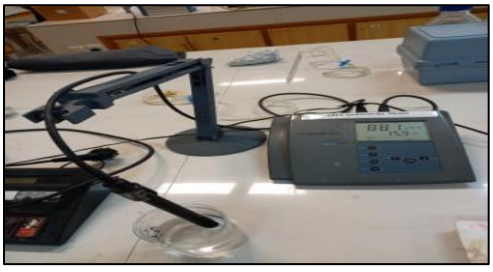


Figure 3.13 EC meter

The following table details standard methodologies which were employed for the physicochemical analysis of experimental tank, hatchery and lake water.

Table 3.1 Instruments and methodologies for physicochemical analysis

Parameter	Instrument/Method used
pH	Multimeter, 156 Hach sension, Germany
Dissolved Oxygen (mg/l)	Winkler Method
Electrical Conductivity (µS/cm)	720 WTW probe
Temperature (°C)	720 WTW probe
Turbidity (NTU)	2100P, portable Turbidity meter, Hach
Chlorine (mg/l)	Colorimeter (Hanna HI 96734)
Hardness (as $MgCO_3$ and $CaCO_3$)	EDTA Titration Method

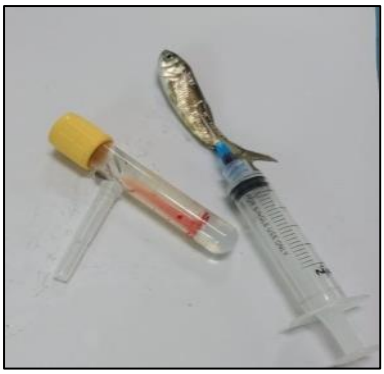
3.7 Photodegradation experiment

For evaluating the degradation of lambda cyhalothrin through UV, each fish tank was filled with 30L of water. Fish were randomly selected and equally divided among exposure and control experiment tanks. Lambda cyhalothrin was added into each tank with specified dosage (0.75, 1.0, 1.25µg/l) and given UV exposure (10, 20 and 30 min) using UV lamp (11W, 254nm). After UV exposure of stipulated time, the tanks were covered with wooden bo in order to reduce sunlight interference (Fig 3.14). Water samples were taken at regular intervals throughout the experiment to determine LCT degradation and by-products.

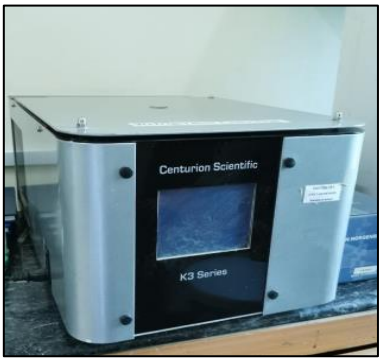
Figure 3.14 Experimental tank covered with wooden box

3.8 Biochemical Analysis

Lambda cyhalothrin is extremely toxic to fish. Biochemical alterations due to lambda cyhalothrin were determined by analyzing indices such as glucose, total protein, triglyceride and amylase. Fish blood was sampled (Fig 3.15a) at stipulated time periods using syringe and stored in yellow caped ETDA tubes containing gel, in order to activate serum separation. It was then centrifuged for 20 min at 4000rpm to ensure blood-serum separation (Fig 3.15b). These samples were then analyzed for biochemical indices using standard reagent kits (Fig 3.15c) and running in chemistry analyzer (Fig 3.15d).



(a) Blood sampling



(b) Centrifuge

(b) Standard reagents

(d) Chemistry analyzer

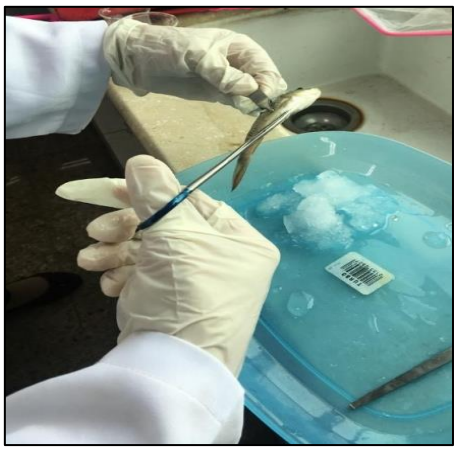
Figure 3.15 Biochemical analysis

3.9 Histopathology of Fish

In order to assess the histopathology damage cause by lambda cyhalothrin, fish was exposed to UV treated/exposed and known concentrations of lambda cyhalothrin by spiking the water of experimental tanks. After exposing for stipulated time period (24, 48, 72 and 96hr), fish were removed from experimental tanks. Fish was then anesthetized using commercial clove oil. After

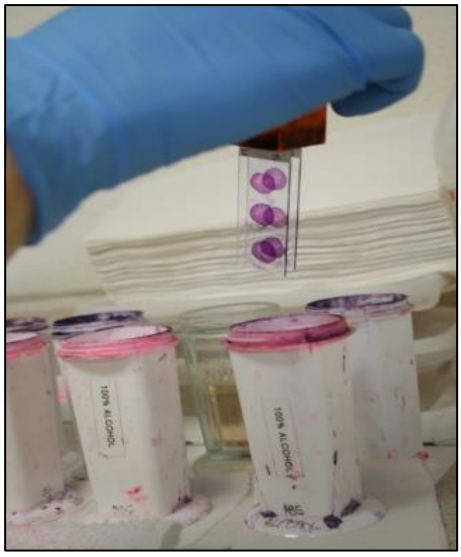
10min, fish was then dissected in order to remove brain, liver, gills and muscles for histopathological studies (Fig 3.16a).

These organs were then rinsed with physiological saline solution, followed by fixing the tissues in Bouin's fluid for a few hours. Tissues were then dehydrated using different concentrations of ethyl alcohol and embedded within paraffin wax (Fig 3.16 b). These waxed tissues were then cut into thin sections of 4-6mm using a rotary microtome. In order to add a contrast to make tissues more visible, the sections were stained with hematoxylin eosin (HE, Fig 3.16c) and finally observed under optical microscope (Fig 3.16d).

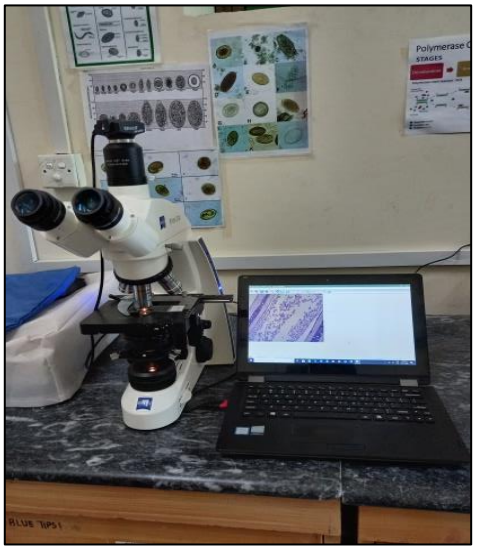


(a) Fish dissection

(b) Organ extraction



(c) Staining



(d) Optical microscope

Figure 3.16 Methodology for histopathology of vital organs

3.10 Relative gene expression

In order to assess the relative gene expression in various organs of grass carp, computational techniques were employed. Lambda cyhalothrin was analyzed first for its toxicity potential. This was done by obtaining **Simplified Molecular Input Line Entry System (SMILES)** through real time data bases such as PubChem (world's largest collection of chemical information) and software such as **EPI Suite**. This code was then entered into another web tool named "admetSAR". It is a very user-friendly interface which helps predict the chemical interactions of chemicals within a biological system. "admet" represents all forms of chemical interactions like a-absorption, d-distribution, m-metabolism, e-excretion and t-toxicity.

The next step is to predict the protein structure best suited for performing docking analysis. **Protein Data Bank (PDB)** is an online reservoir of most of the protein structures. However, if the structures have not already been constructed, we employ many web tools to help predict closest possible structure of the required protein. The chosen proteins were not structured before hence they needed to be structured with the help of various in-silico programs. A total of six proteins of grass carp were selected for primary structure prediction and they are enlisted in Table 3.2:

Table 3.2 Proteins shortlisted for docking

S/No	Protein names
1.	Interleukin
2.	Heat shock protein (HSP)
3.	Cytochrome (CYP1A)
4.	Immunoglobulin (IgM)
5.	B actin

For this, a FASTA sequence of each protein was collected from **NCBI** (National Center for Biotechnology Information). This sequence or amino acid code was then used for structure prediction through four different softwares; **I-tasser**, **Intfold**, **SWISS-MODEL** and **Phyre-2**. These softwares predicted structures in PDB format which was then taken further for **SAVES**

analysis. SAVES analysis helps predict the structure best suited for carrying out docking simulation.In SAVES analysis, the high values of Ramachandran plot determine the best structure to perform docking analysis.

For docking, **"pyrx"** was used which is visual screening software which help create library of compounds against targeted drugs or targeted chemical. The results obtained from pyrx were then exported to software called **"pymol"** for docking analysis. In pymol, the protein (as macromolecule) and lambda cyhalothrin (as ligand) are then superimposed, active sites are nominated and then probable bonding is observed.

3.11 Gas Chromatographic Analysis of degradation of Lambda cyhalothrin

The degradation pathway and by-products of lambda cyhalothrin due to the UV light were assessed using gas chromatography. Gas chromatograph was first optimized before analyzing the samples. Conditioning process was employed for optimizing the GC, which involved altering the temperature of injection port, column and the detector. Final conditions used for running all standards and samples are listed in Table 3.3.

Table 3.3 Operational conditions for gas chromatography

Parameters	Values/ Units
Injector	
Temperature	300 ^{o}C
Column	
Initial temperature	80 ^{o}C
Final temperature	260 ^{o}C
Temperature ramp	30°C/min
Detector	
Temperature	320 ^{o}C

3.11.1 Working standard solutions

A pure (≤95%) analytical standard of lambda cyhalothrin was procured from Sigma Aldrich for making working standards. These standards were prepared in n-hexane. Standard stock solution of lambda cyhalothrin was prepared by carefully weighing 0.01g of pure lambda cyhalothrin standard (powder) and dissolving it in 100ml of n-hexane.

Figure 3.17 Laboratory setup of gas chromatography

Six serial dilutions were prepared by taking 1ml of each solution and mixing with 9ml of n-hexane to make up the final volume of 10ml. The 6^{th} dilution was intermediate solution from which three further dilutions i.e. 0.1, 0.5. 0.75µg/l were prepared, whereas 1, 1.25 and 1.5 µg/l standards were prepared from 5^{th} dilution/ solution.

3.11.2 Extraction of Lambda cyhalothrin from water samples

In this study, water within experimental tanks was spiked with known concentration of lambda cyhalothrin before exposing to UV light to allow photodegradation. Commercial grade lambda cyhalothrin was used for these experimental runs. In order to run the samples through gas chromatograph, lambda cyhalothrin needed to be extracted from the collected samples.

Figure 3.18 Extraction of lambda cyhalothrin from spiked water samples

From the collected water samples during the exposure experiments, 5ml of each sample was taken and extracted with n-hexane three times. In order to remove moisture from the extracted samples, the supernatant was passed through $NaSO_4$ (oven dried at 500^0C for 4hr, Fig 3.18). 1 ml methanol was added to the extract and then finally passed through rotary evaporator to obtain concentrated sample (Xie et al., 2011). The standards were collected in amber vials and stored in refrigerators (-20^0C) to be analyzed through gas chromatography. the detailed process is depicted in Figure 3.19.

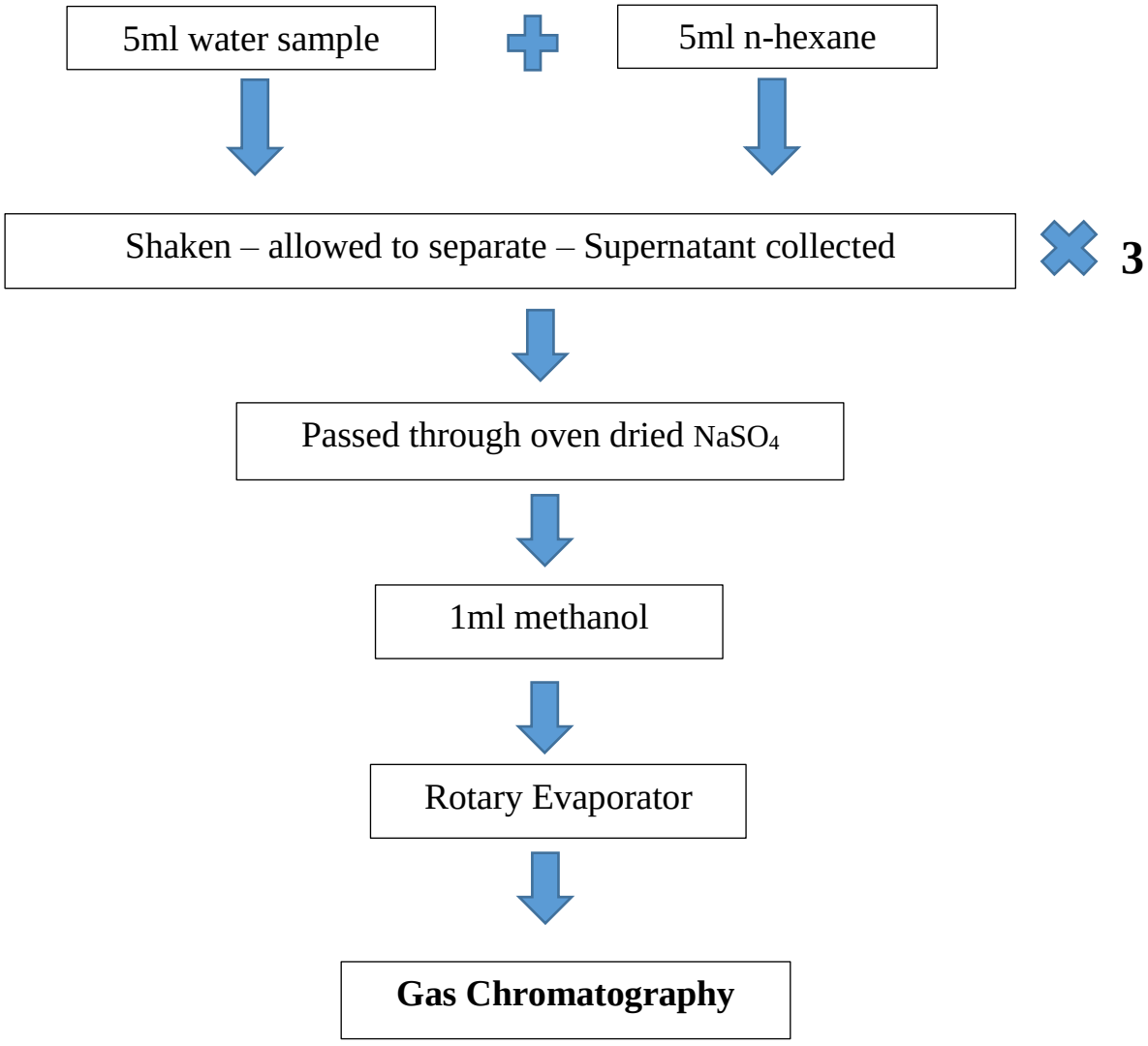


Figure 3.19 Methodology for extraction of LCT from spiked samples

CHAPTER 4

RESULTS AND DISCUSSION

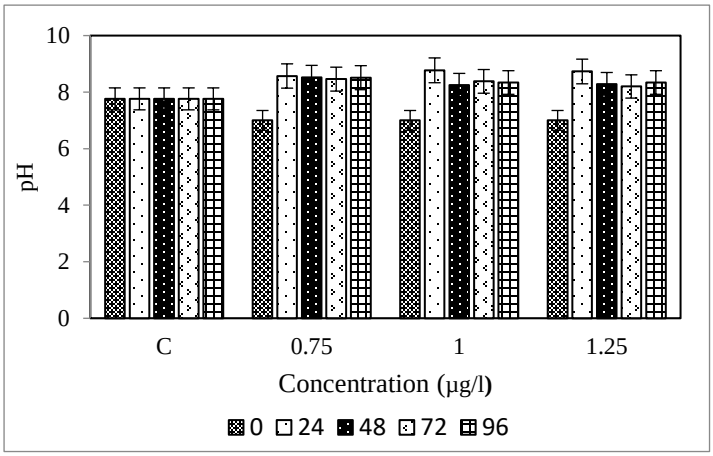
This study is primarily based on exploring the toxicity potential of lambda cyhalothrin for fish and use of UV light source to investigate the degradation of this pesticide into its by-products. The water of experimental tanks were spiked with specified concentrations of lambda cyhalothrin and then exposed to UV light source to induce pesticide degradation. The dynamics of pesticide pathways were determined in water as well as fish by collecting samples are regular intervals. For a more robust study, a total of six variables were selected from the literature of previous studies. These included three different pesticide concentrations and three different UV exposure timings.

4.1 Physicochemical Analysis of water

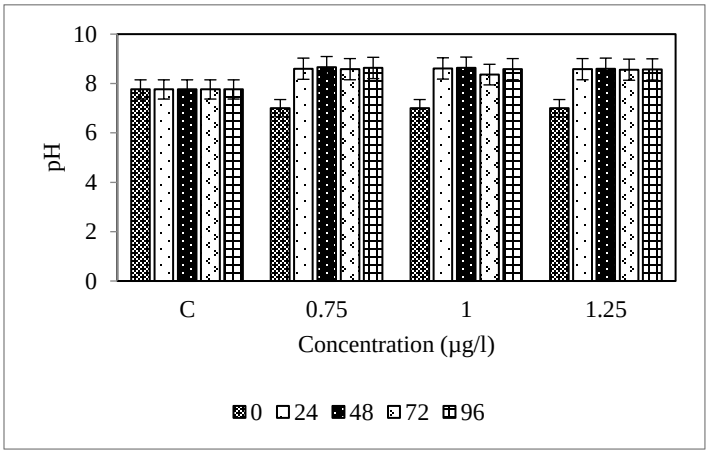
During the experiments, lambda cyhalothrin was introduced into the fish aquaria water and then treated with UV light in order to initiate photodegradation process. Water was sampled from these tanks at regular intervals (24, 48, 72 and 96 hr) to explore photodegradation. Moreover, these water samples were analyzed for the physicochemical parameters (6) too, in order to evaluate the dynamics of photodegradation. The results of all six parameters are as follows:

a) pH:

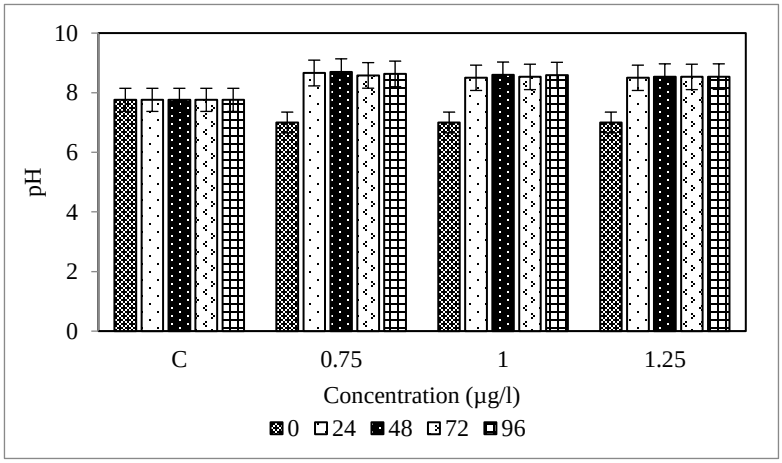
pH is a scale that specifies the acidity or basicity of any aqueous solution. For fish and aquatic life, pH is the prime factor for determining the sustainability of all aquatic life forms. The pH of water is a major parameter to determine the survivability of aquatic life forms, as it determines if water is suitable environment for all organisms to thrive. Different anthropogenic interventions such as urbanization and industrial waste can fluctuate pH values (Qu et al., 2014). Lambda cyhalothrin is an organic compound that mostly rendered the waters of experimental tanks towrds basicity. The trend of changes in pH during the 96hr long experiment was similar for all three concentrations of pesticide as well as all three UV epxosure timings Fig 4.1 a, b, c).



(a) 10min



(b) 20min



(c) 30min

Figure 4.1 Variation of pH of experimental tank water

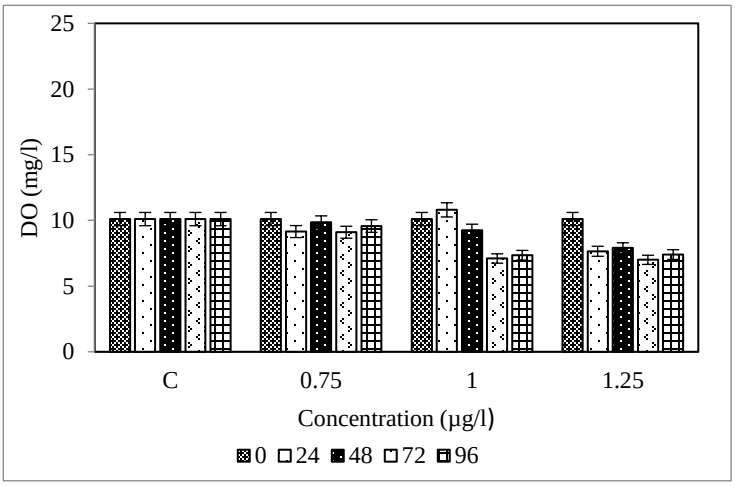
Figure 4.1(a) show the changes in pH over 96hr with 10min of pesticide exposure to the UV light. It can be observed that the changes are not very significant as the values lie between 7 and 8.77. Initially pH increased going from 24 to 48hr and then after 48hr, it remained almost stable with just a few minor changes till 96th hour of the experimental time. In one study, the pH of fish pond varied very little or remained within the range of 7-10 which is suitable for aquaculture (Olukunle & Oyewumi, 2017).

b. Dissolved Oxygen (DO):

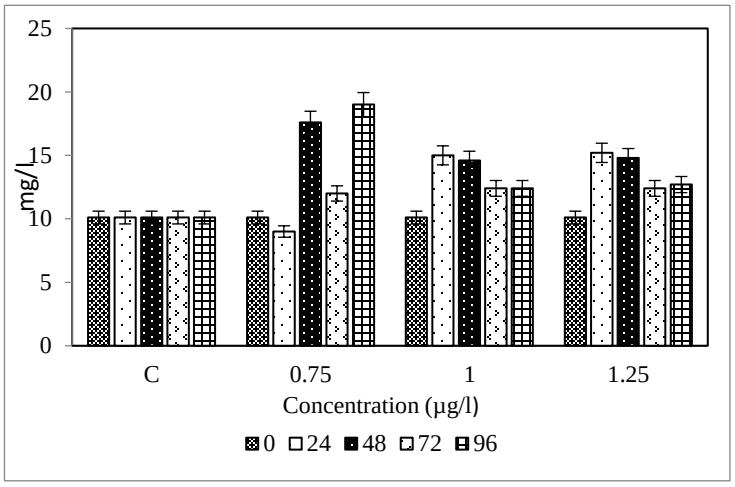
Dissolved oxygen (DO) is the measure of amount of oxygen within a water body and help in determining the quality of water. Fish typically acquire the dissolved oxygen directly from the surrounding water and into their bloodstream with the help of gills. Low levels of oxygen may cause stress leading to fish mortality. The levels of dissolved oxygen in water is primarily determined by temperature. Temperature has inverse relation with dissolved oxygen,

higher the temperatures, lower is the dissolved oxygen Zunaira et al. (2019). (Rehman, Zunaira & Syed, Dr Hussain, Zakir. (2019).

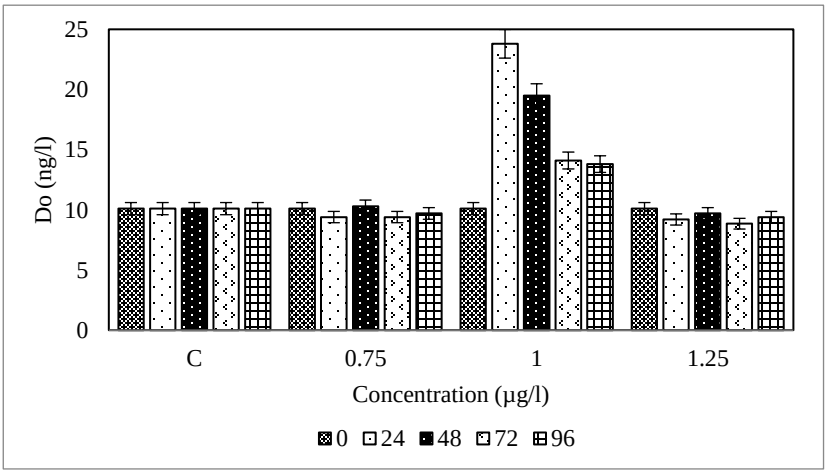
Overall, an irregular trend was observed from 0 to 96hr in all three exposure timings (figure 4.2 a, b, c). At 10mins, less variation was observed, ranging from 7.35 to 10.8, which can be attributed to the varying seasonal temperatures. However, at 20 and 30mins, a bigger range of variation was observed. These experiments were conducted in triplicate batches over a period of few months. The temperature and seasonal changes can account for such changing trend. Exceptionally high levels of dissolved oxygen at 0.75 and 20min can be due to increase in decrease in turbidity or increase pH and temp (Sachidanandamurthy & Yajurvedi, 2006).



(a) 10min



(b) 20min

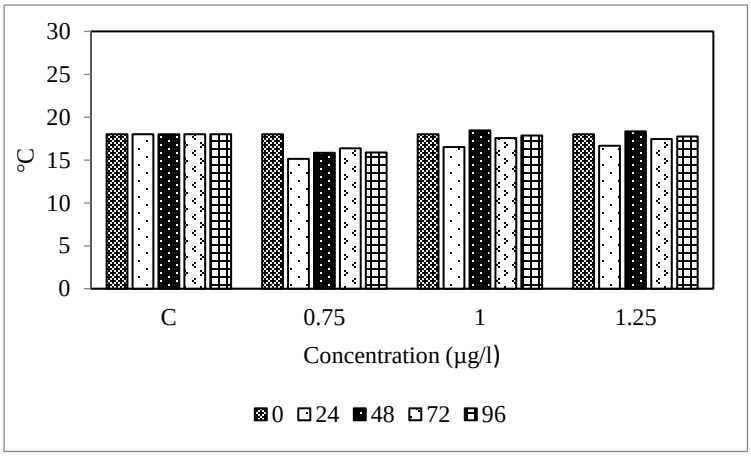


(c) 30min

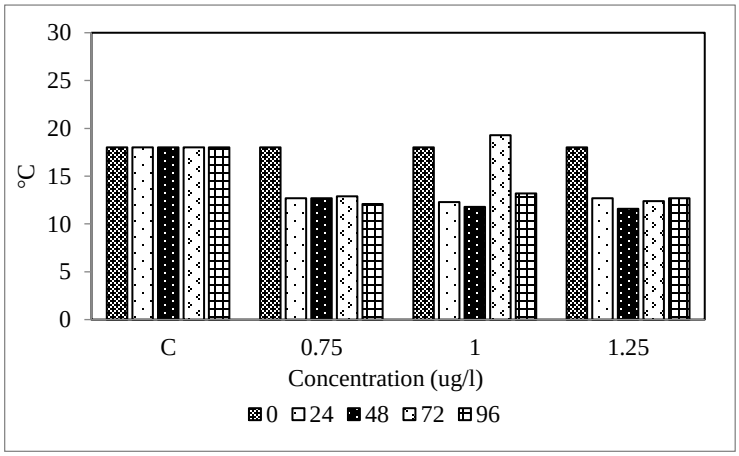
Figure 4.2 Variation of dissolved oxygen of experimental tank water

c. Temperature:

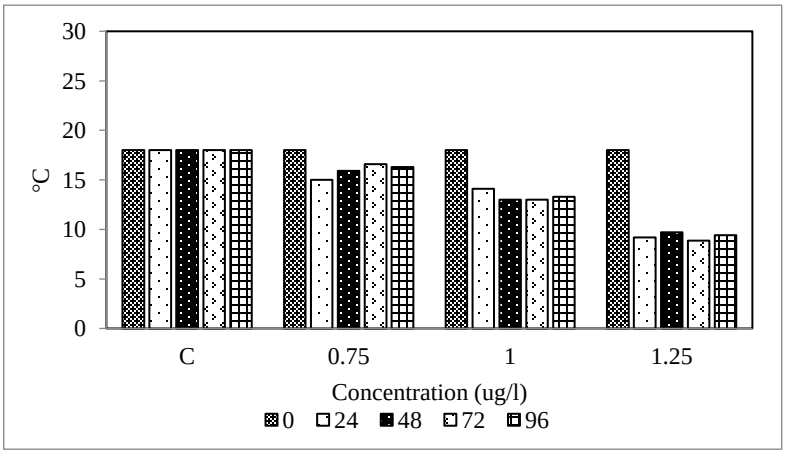
Water temperatures play the most crucial role in determining the sustainability of all aquatic life forms and the ecosystems within. The optimum temperature for healthy fish and their survival ranges from 13-21^{o}C (Viadero, 2005). In these 96hr long experiments, the temperature varied within a very limited range i.e. from 11^{o}C to 18^{o}C. This difference in temperatures may be explained by considering different month (seasons) when the experiments were conducted (fish availability). The temperatures were well within a range suitable for aquatic life to thrive, supported by another study where annual temperature variation range is from 20-28^{o}C (Seginer & Mozes, 2012).



(a) 10 min



(b) 20min



(c) 30min

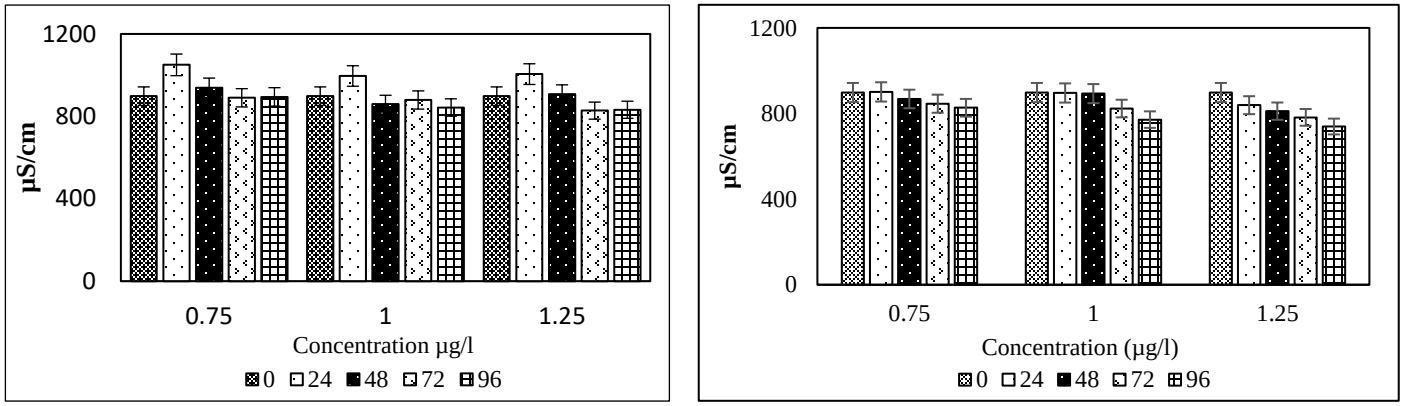
Figure 4.3 Variation of temperature of experimental tank water

d. Electrical Conductivity (EC):

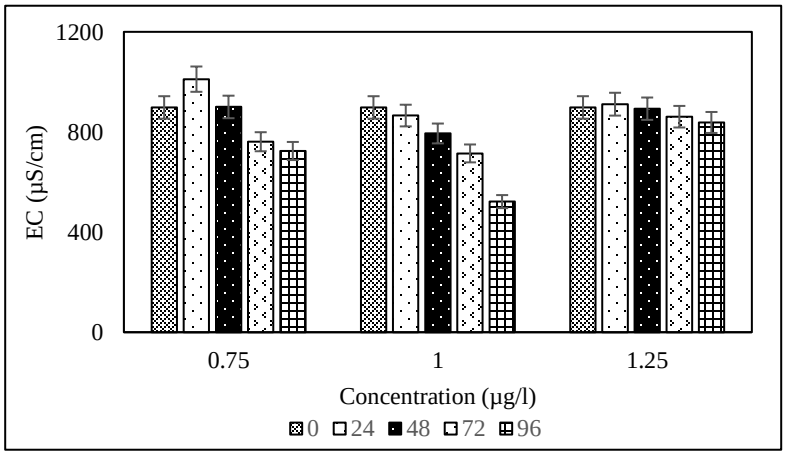
Electrical conductivity is essentially a measure of voltage which is required to get current flowing through any medium. It can also be taken as a measure of the amount of dissolved salts and minerals in water hence can be good indicator of pollutants or contaminants within any water body.

The average of trends depicted by electrical conductivity over the period of 96hr at three different UV exposure times is as depicted in the following figures (Fig 4.4a, b and c). There was a general decreasing trend of electrical conductivity from 0 to 96hrs which could be

However, the electrical conductivity values remained between 500-1100µS/cm which is well within the range of 20-1500µS/cm specified by the FAO for aquaculture (Njoku et al., 2015).



(a) 10min **(b) 20min**

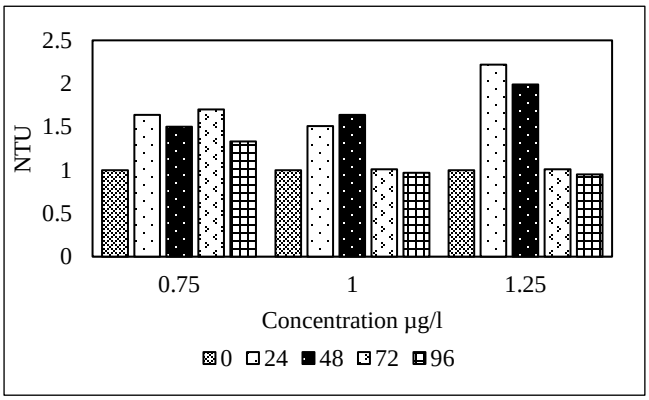


(c) 30min

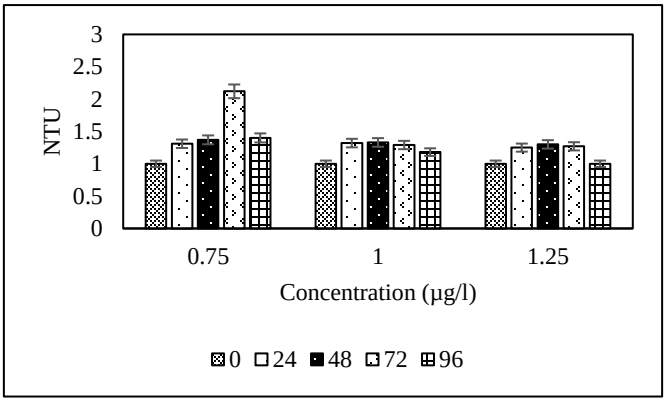
Figure 4.4 Variation of electrical conductivity of experimental tank water

e. Turbidity:

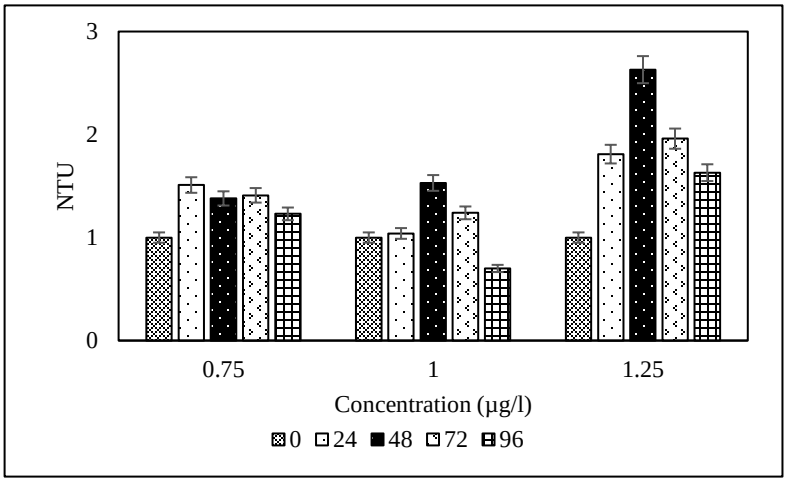
The cloudy and unclear appearance of water is termed turbidity and is mainly because of insoluble suspended particles (Seiyaboh & Izah, 2017). In a study, it was revealed that toxins such as cypermethrin tend to be more lethal to fish in clear waters as opposed to turbid ones (Majumder & Kaviraj, 2021). Following this trend, turbidity in all three exposure times followed a general increasing pattern from start till 72hrs of experiment (Fig 4.5 a, b, c). However, it decreased after that till 96hrs which could be due to reduced number of fish in the tanks, as a pair of fish was removed every 24hr for various toxicity assays.



(a) **10min**



(b) 20min

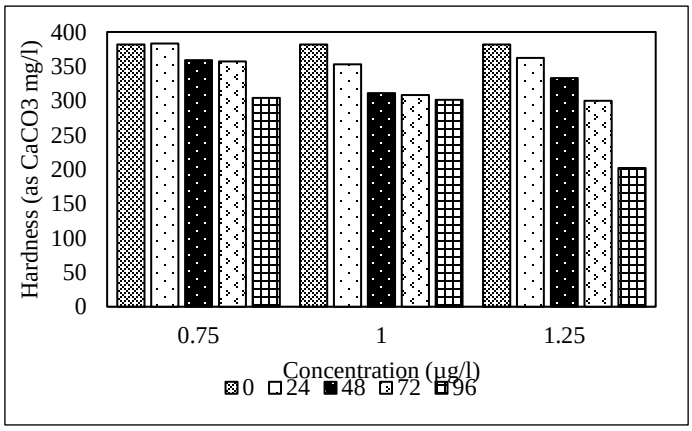


(c) 30min

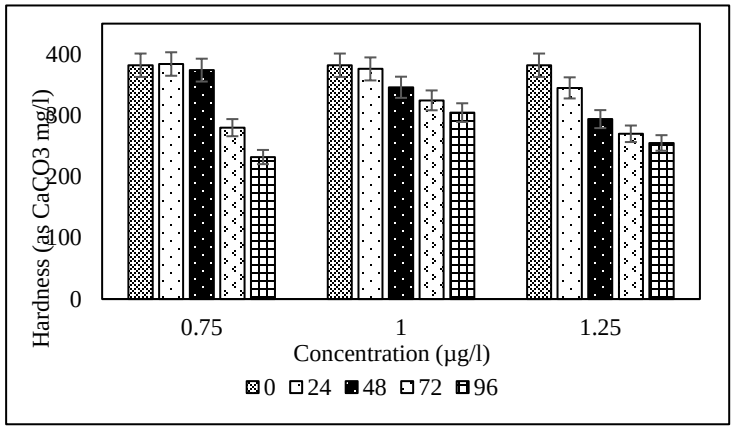
Figure 4.5 Variation of turbidity of experimental tank water

f. Hardness:

The ability of water to endure high concentration of soap is indicated by hardness (Seiyaboh & Izah, 2017). The presence of dissolved calcium and magnesium determine how hard the water is. In this study, hardness presented the most uniform trend, with values fluctuating in a very small range 4.6 a, b and c). In a study, Henderson et al. concluded that hard water from fish tanks slowly evaporate with time, due to which Ca^{++} ions precipitate out as scales, reducing water hardness (Henderson et al., 1959). Decreasing trend of hard water here can also be due to the same reason, as scales within experimental tanks were observed during exposure experiments. The trends can be observed in Fig 4.6 a, b and c.



(a) 10min



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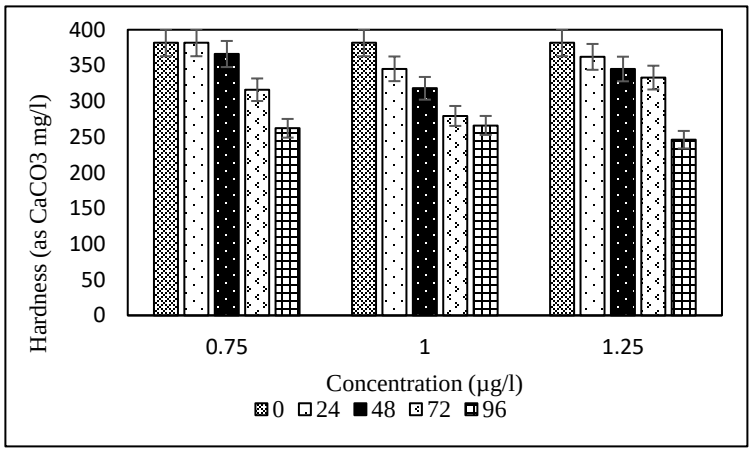


(c) 30min

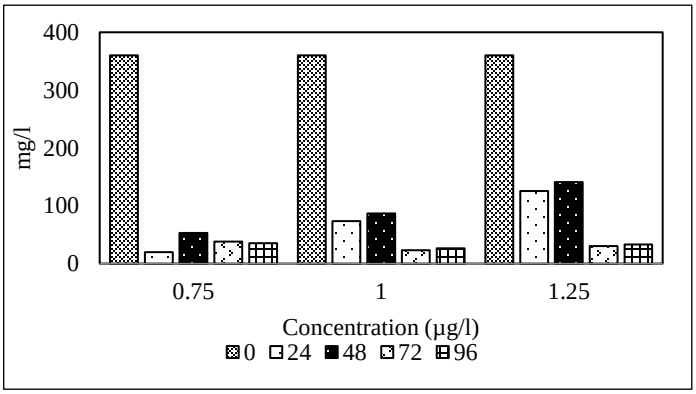
Figure 4.6 Variation of hardness of experimental tank water

4.2 Biochemical Analysis of Fish blood

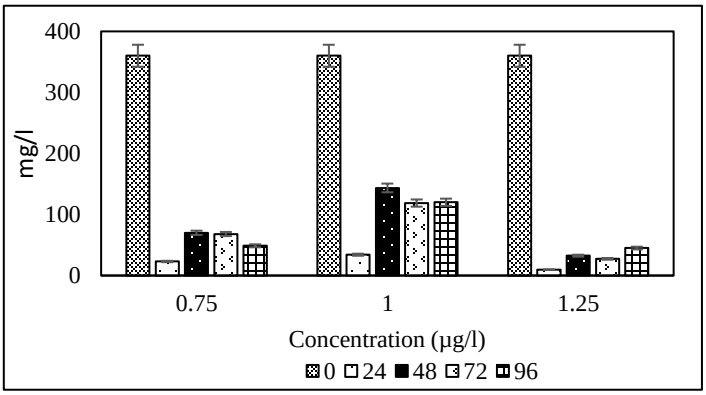
Blood is a patho-physiological gauge of the whole body (Ghayyur, Tabassum, Ahmad, Akhtar, & Khan, 2019). Lambda cyhalothrin being a nurotoxin, can cause significant disturbances within fish body. In order to ascertain toxicity in fish organs and physiology, biochemical analysis can offer great insight (Agrahari et al., 2007).

a. Glucose:

Glucose levels tend to be most sensitive entity of any organism under stress, where greater stress exhibits higher glucose levels (Fırat et al., 2011). Within initial 24 hrs, there was a significant drop in glucose levels as compared to the control. Glucose level then increased from 24 to 48 hrs, indicating that fish is under stress. Hyperglycemia or increase in glucose level may be taken as a physiological stress response of fish in order to cope up for increase in energy demand (Jyothi & Narayan, 1999). After 48hrs, the glucose levels dropped again till 72 hrs, with slight increase all the way upto 96hrs. Similar trend was depicted at all three concentrations of lambda cyhalothri Fig 4.7 a, b and c.



(a) 10min



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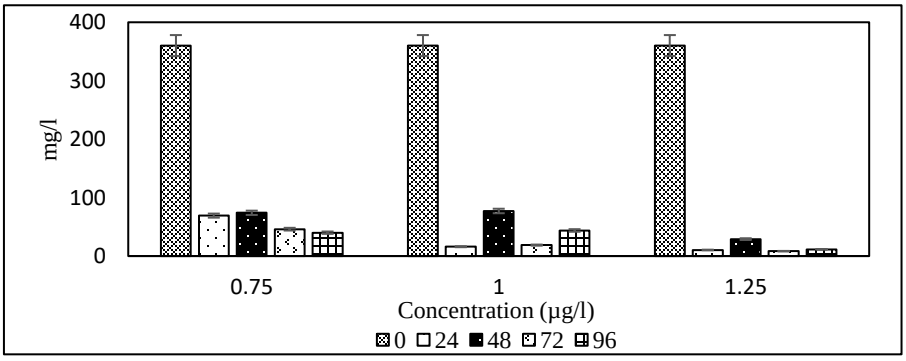


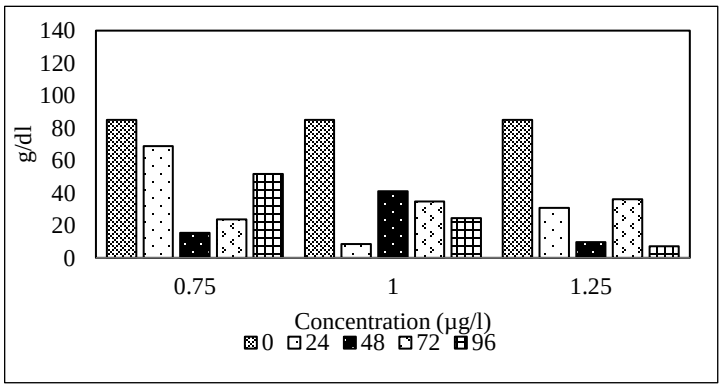
(c) 30min

Figure 4.7 Variation of fish serum glucose

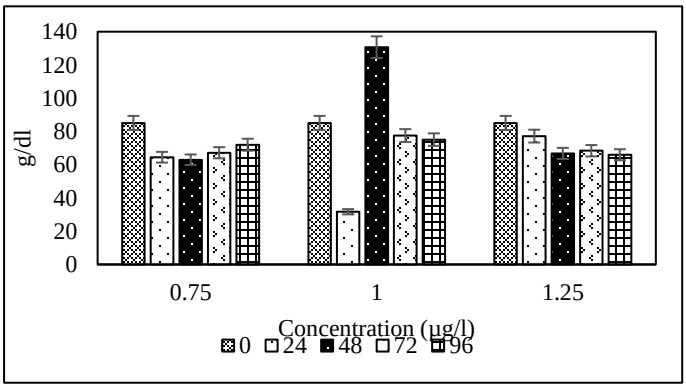
b. Total Protein:

Serum protein is of clinical importance in order to evaluate physiology. Since majority of serum potein is being produced within liver, its level can be taken as an indicator of any probable liver impairment (Fırat et al., 2011). There was a decrease in the level of total protein (hypo proteinemia) within first 24hrs which is a clear response to toxicity. Firat et al., 2011 also reported that dercease in total protein due to pesticide exposition could be attributed to protein metabolism and sysnthesis within liver. Similar decreasing trend was reported by (Khan et al., 2016). Total protein variations are as follows in Fig (a), (b) and (c).

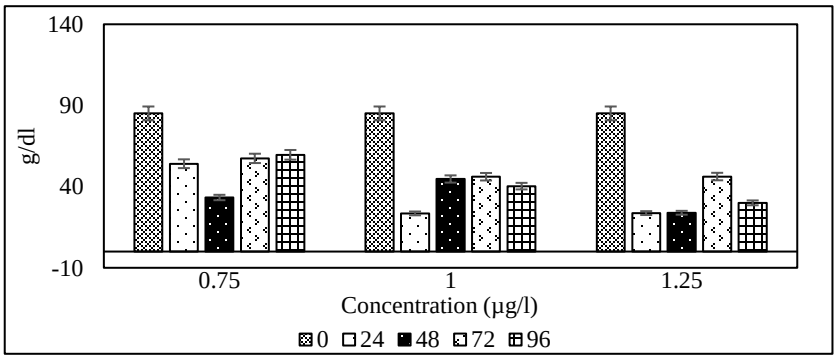
Most of the protein is made up of albumin and globulin and within the body any changes in their concentrations due to toxic exposure impacts total protein levels. In order to meet increasing demand for energy for fish under stress conditions, protein synthesis is increased (Abdel-Daim et al., 2020). A sharp increase in total protein level was observed at 72hrs at 1µg/l concentration of LCT (Fig 4.8b) which could be due to the same reason, i.e. increased protein synthesis.



(a) 10min



(b) 20min



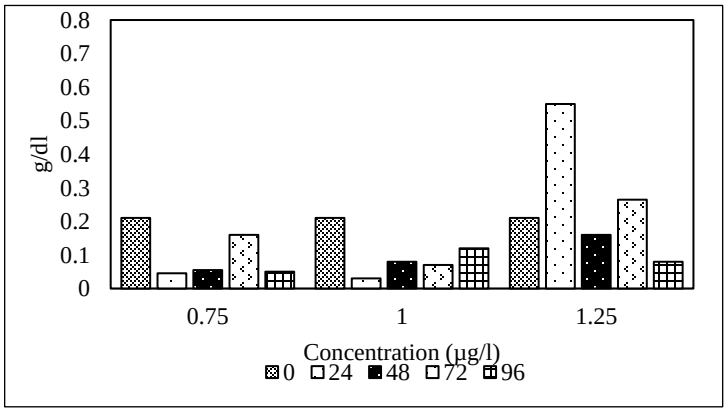
c) 30min

Figure 4.8 Variation of fish serum total protein

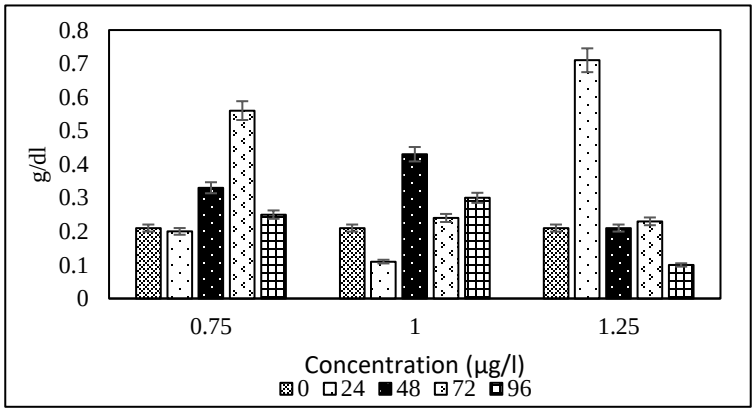
c. Triglycerides:

Triglyceride refers to the fat content withi fish serum. After considerable initial decrease in triglycerides, compared to control, a general increase was observed mostly till 72hrs and then a decrease in last 24 hrs. Increase of triglycerides or hyper triglyceramic conditions of fish under stress could be attirbuted to the damage to vital organs, as reported by Parihar et. al,. 1996. Another study reported that liver cells help release specific enzymes in blood which are responsible for converting triglycerides into fatty acids and glycerol. Once they are damaged due to toxic exposures, triglyceride remians non-metabolized hence causing hyper triglycemia (Prakash & Verma, 2020).

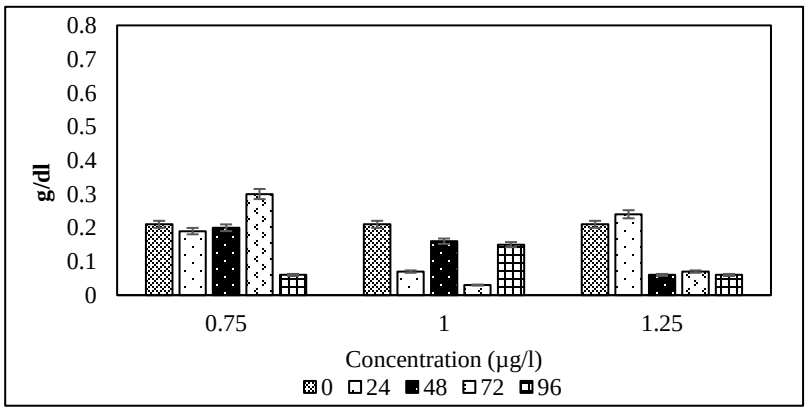
The decrease of triglycerides observed in last 24hrs of experiment can be due to lethal damage to the kidney of fish caused by LCT (or pyrethroids) also reported by (Borges et al., 2007). It was also reported that irregularity witihn serum enzyme activity of liver may also cause hypo triglycemia (Atli et al.. 2015).



(a) **10min**



(b) 20min

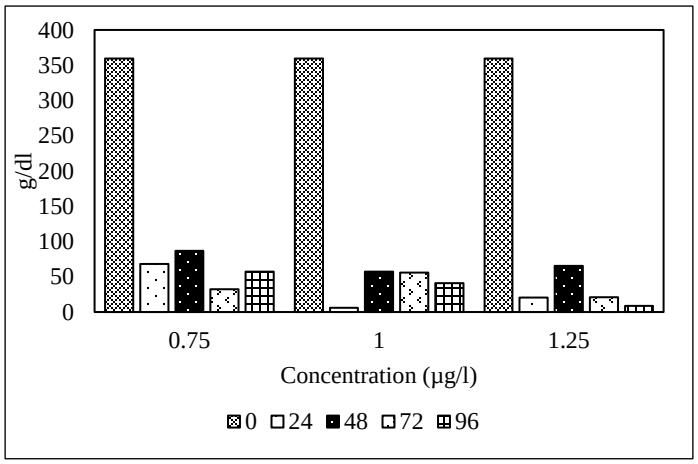


(c) 30min

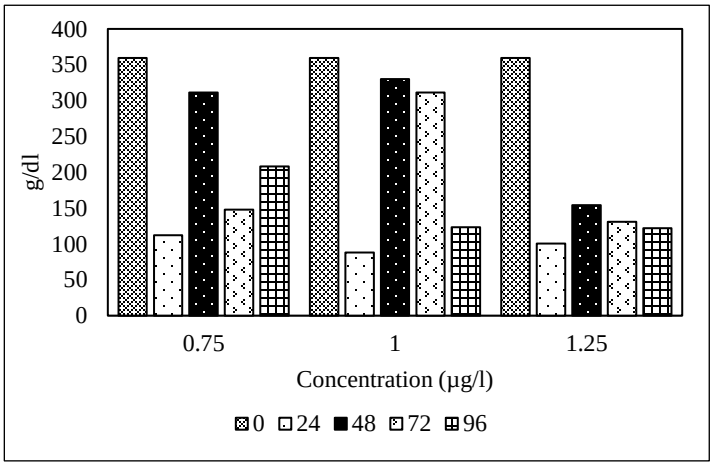
Figure 4.9 Variation of fish serum triglycerides

d. Amylase:

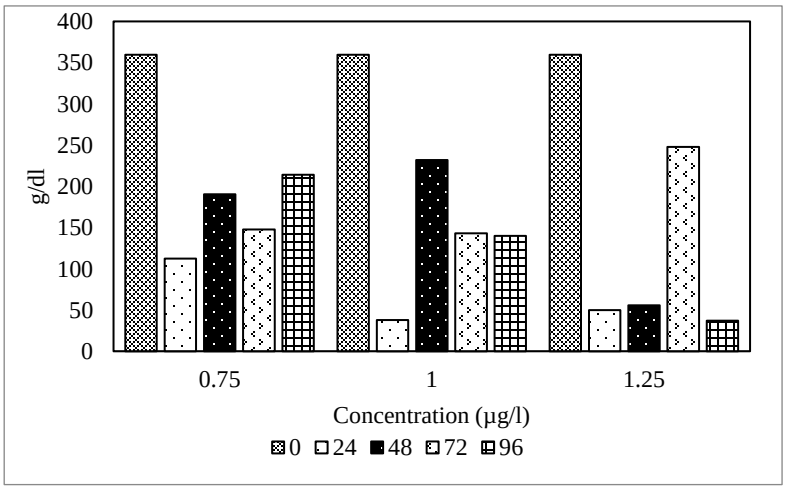
Amylase is an enzyme that helps in breaking doown carbohydrates and complex sugar molecules (Bhilave et al., 2014). Initially there was a sifnificant drop in amylase levels within 24hrs after which an inconsistent pattern was observed till 96 hrs. Asadi et al., 2006 reported that amylase levels are primarily detrrmined by the variations in energy levels on fish body. As fish face stress conditions due to toxic exposure, the energy demand for body increases, causing a decline in maylsase levels and vice versa. This explains the irregular levels of amylase thorughout 96 hr experiments (Asadi et al., 2006). Figure 4.10 a, b and c presents the trend.



(a) 10min



(b) 20min



(c) 30min

Figure 4.10 Variation of fish serum amylase

4.3 Histopathological Analysis of Vital Fish Organs

One of the most reliable techniques for toxicity assessment among fish is histopathological examination (Hadi & Alwan, 2012). Each organ presented significant damage due to varying exposure dosages of UV degraded lambda cyhalothrin. The histopathology of control fish organs exhibited normal histology and was taken as a reference to estimate the extent of damage in exposed organs. Table 4.1 gives the detailed semi-quantitative tally of all four organs along the reference or control organs.

Table 4.1 Semi-quantitative count of Fish organs (Gills, Muscles, Brain and Liver)

Lesions ⬇ / Time ➡	Control	0.75µg/l				1.0µg/l				1.25µg/l			
		24	48	72	96	24	48	72	96	24	48	72	96
GILLS													
Necrosis (N)	-	+	++	++	++	++	++	++	++	++	++	++	++
Lamellar Fusion (LF)	-	+	+	++	++	++	++	+++	+++	++	+++	+++	+++
Cytoplasmic Vacuolation (CV)	-	+	++	++	++	+	++	++	++	+	+	+	+
Lamellar degeneration LD	-	+	+++	+++	+++	+	+++	+++	+++	+	+++	+++	+++
Architectural loss (AL)	-	+	++	+++	+++	+	+	++	++	+	++	+++	+++
Hyperplasia H	-	+	++	++	++	+	+	+	+	+	++	++	+++
MUSCLES													
Degeneration	-	+	++	++	++	++	++	+++	++	++	++	++	++
Necrosis	-	++	++	++	++	++	++	++	++	++	++	++	++
Splitting of muscle fiber (S)	-	++	+++	+++	+++	++	++	+++	++	++	++	++	++
BRAIN													
Structural Damage (SD)	-	+	++	+	+	++	++	++	++	++	+	++	++
Hemorrhage (H)	-	+	++	++	+	++	+	++	+	+	+	++	+++
Necrosis (N)	-	++	+++	++	++	++	++	++	++	++	+++	+++	+++
Vacuoles (V)	-	+	+++	++	++	+	+	++	++	+	++	+++	+++
LIVER													
Pycnotic Nuclei (PN)	-	+++	+++	+++	++	++	++	++	++	++	++	++	++
Vacuolization (V)	-	+	+	++	++	++	++	++	++	++	++	++	++
Necrosis (N)	-	++	++	++	++	++	++	++	++	+++	+++	+++	+++
Bile Stagnation (BS)	-	++	++	++	++	++	++	++	++	++	++	+++	++

Key: (-) No damage; (+) Mild/Moderate damage; (++) Severe damage

Gills:

Control group gills showed normal and symmetrically arranged lamellae without fusion or degeneration (Table 3). No cytoplasmic vacuolization or hyperplasia was observed in control gills. Control gills had healthy cells without architectural loss (Fig 4.11a).

Treated group gills, which was treated with UV degraded lambda cyhalothrin showed significant damage with all three exposure concentrations (0.75, 1 and 1.25 µg/l) of lambda cyhalothrin (Fig 4.11 c, d, e). Lamellar degeneration, fusion and architectural loss were the most pronounced lesion caused by all three concentrations of lambda cyhalothrin. Cytoplasmic vacuolization was more evident in 0.75 µg/l and 1 µg/l as compared to 1.25 µg/l. Least of all lesions was hyperplasia, which was most extensive at highest exposure dosage i.e. 1.25 µg/l. Cytoplasmic vacuolization was more prevalent in lower exposure dosages (0.75 µg/l and 1 µg/l) as compared to higher dosage.

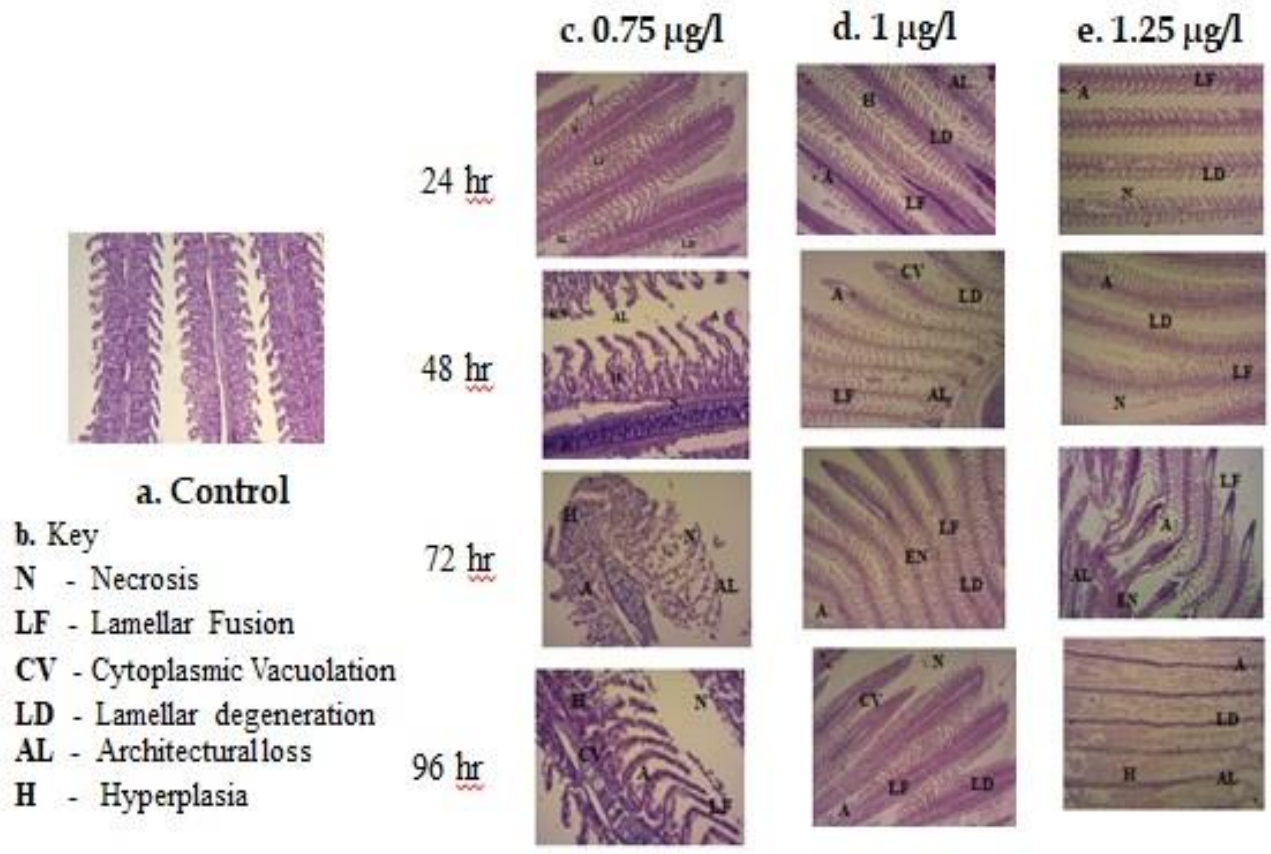


Figure 4.11 Photomicrographs of fish gills

Muscles:

Control group muscles presented normal and healthy muscle fibers/bundles without any sign of necrosis or degeneration. Muscle fibers in healthy unexposed fish were packed tightly together arranged in a homogenous manner (Fig 4.12a).

Treated group muscle histology was significantly damaged. Multiple lesions including necrosis, degeneration and splitting of muscle fibers were observed within exposed fish muscles (Fig. 4.12 c, d, e). The most pronounced effect observed in exposed fish muscles was splitting and degeneration of muscle fibers. At 0.75 µg/l, fibers were damaged the most as compared to other two concentrations. Necrosis and degeneration progressed with time and was amplified at 96hrs. With time, muscle fibers started degenerating and losing the densely packed formation. Necrosis was also observed within fibers, depicting the destructive impacts of the exposed toxin.

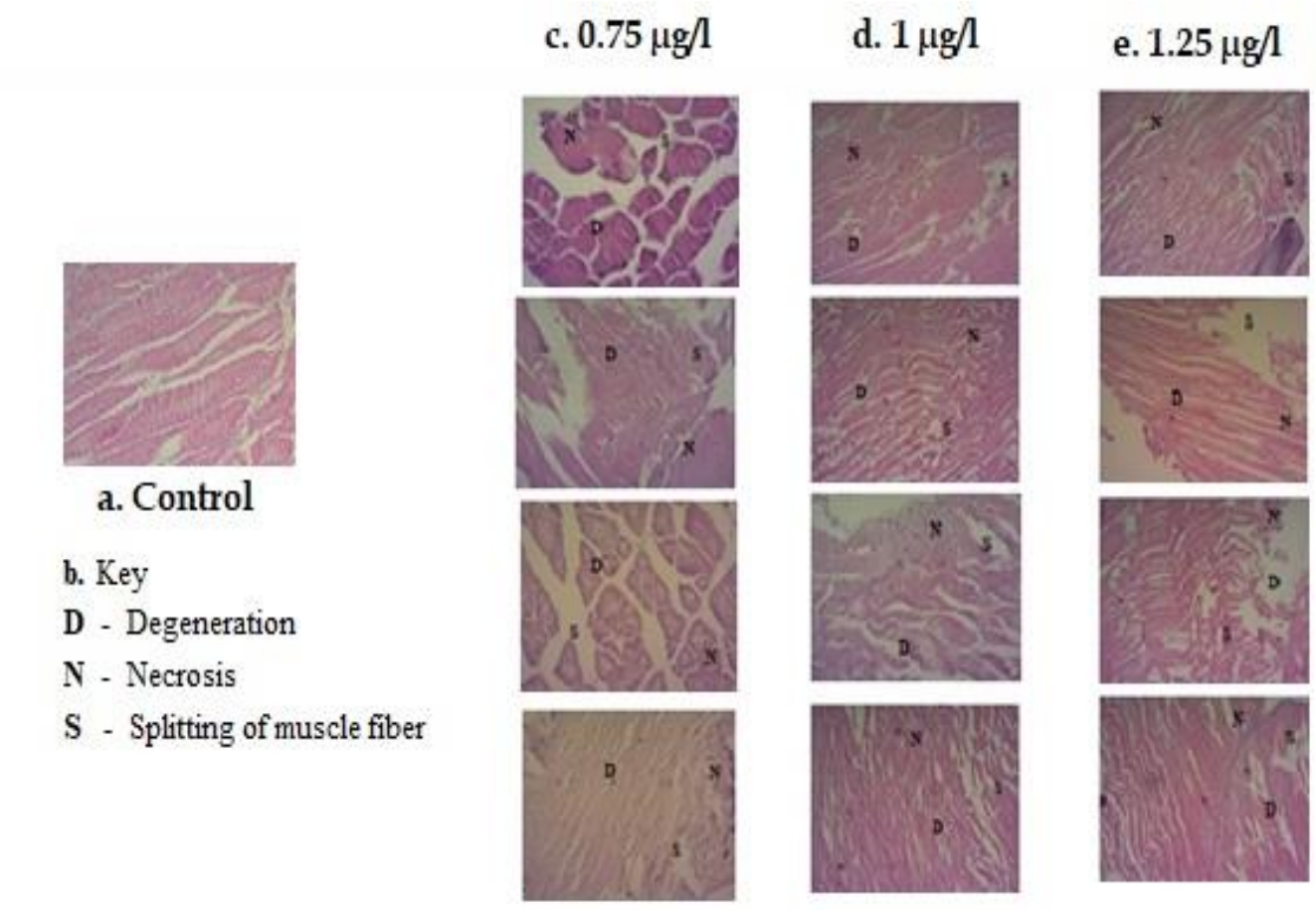


Figure 4.12 Photomicrographs of fish muscles

Brain:

Control group fish brain cells displayed normal healthy cells. There were no recognizable signs of damage (Fig. 4.13a).

Treated group brain cells presented serious degenerative changes owing to the neurotoxic nature of lambda cyhalothrin. Lesions observed in treated group included necrosis, structural damage and vacuolization within the cells (Fig 4.13 c, d, e). Severe necrosis and vacuolization of brain cells were observed in the fish that was given highest dose exposure i.e. 1.25 µg/l. structural damage was not a very persistent lesion as it only appeared mildly in all three exposure dosages. Hemorrhage was the most persistent lesion caused by the neurotoxin with maximum damage at highest exposure dosage.

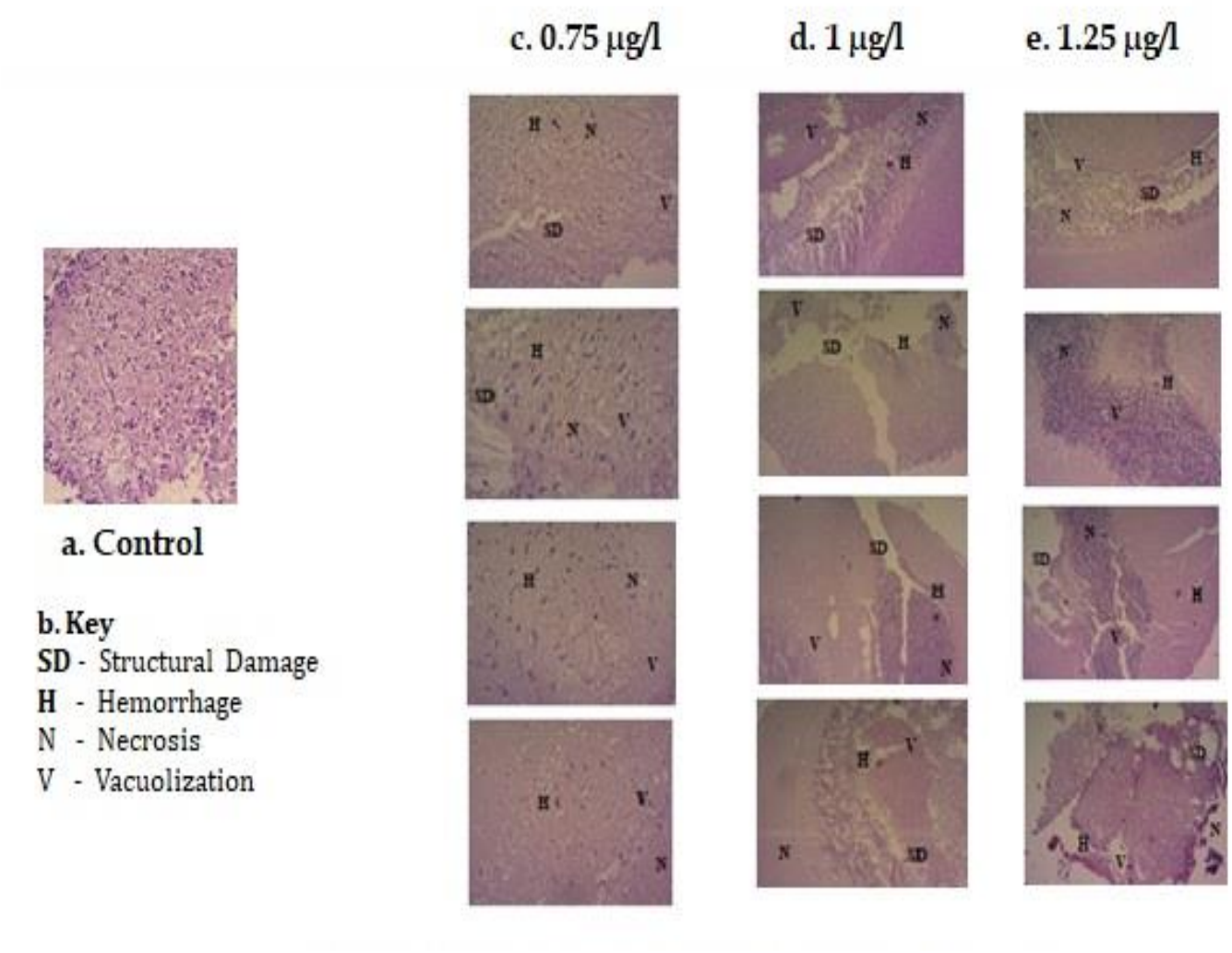


Figure 4. 13 Photomicrographs of fish brain

Liver:

Control group liver histology appeared normal without any pathological changes. The hepatocytes appeared healthy positioned amid blood capillaries also known as sinusoids (Fig. 4.14a).

Treated group hepatocytes displayed serious histological alterations due to toxins. Common lesions among all three concentrations were appearance of pycnotic nuclei and vacuolization within the cytoplasm (Fig. 4.14 c, d, e). Necrosis and bile stagnation were most pronounced at highest exposure dosage i.e. 1.25 µg/l. At lowest dosage of 0.75 µg/l, the most recurrent anomaly was pycnotic nuclei. In some Pictomicrographs, blood congestion was observed depicting severe damage to the hepatocytes.

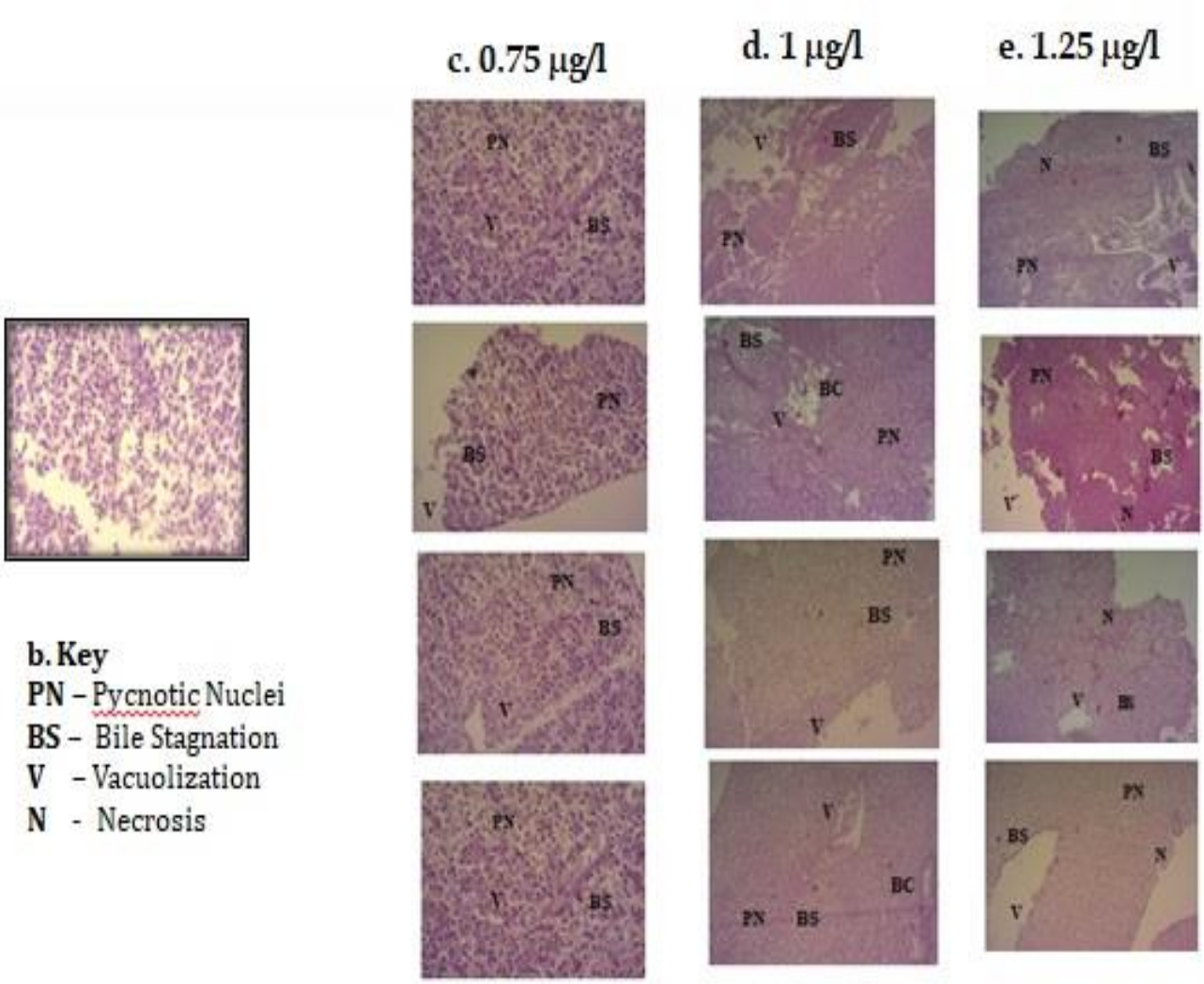


Figure 4.14 Photomicrographs of fish liver

Discussion:

Gills are characteristic fish organ which are actively involved in respiration, acid-base balance, excretion and osmoregulation. With large surface directly exposed to the water, gills are more prone to contaminants (Peebua et al., 2006). With exposure to toxins, the defense mechanism activates resulting in hyperplasia and architectural loss (Camargo & Martinez, 2007). All these lesions have been reported in several other studies too, supporting the results of current study (Arulra j et al., 2019; Fernandes et al., 2020).

Fish is an efficient source of protein where fish **muscles** being the direct and rich source. In order to assess the vulnerability of humans to pesticide contamination, it is important to evaluate the extent and nature of damage caused to fish muscle as a result of being exposed to pesticides. Similar alterations were observed in another study with co-exposure to pesticide and heavy metals (Arulraj et al., 2019).

Brain is the controlling center of vertebrates. Fish when lives in contaminated waters, the pollutants reach deep with organs such as brain through blood circulation(Lakshmaiah, 2016). The synthetic pyrethroids such as lambda cyhalothrin usually obstruct the normal neuronal physiology by disturbing the ion exchange channels (Sabra & Mehana, 2015). Structural damage and vacuolization of brain cells is one of many histological responses towards exposure to toxins (Lakshmaiah, 2017).

Histopathological examination of **liver** is considered to be an accurate method for toxicological studies (Hadi & Alwan, 2012). Liver is the organ largely affected by contaminants and its physiology gets compromised while carrying out detoxification (Camargo & Martinez, 2007). Liver tends to accumulate toxins, making it more prone to atrophy as compared to other organs (Kaoud & El-Dahshan, 2010). Vacuolization and necrosis were some of the distinct anomalies found in treated group livers which are similar to the results described by (Bhuvaneshwari et al., 2015).

4.4 Relative expression of mRNA

The changes in relative expression of mRNA of grass carp after exposure to lambda cyhalothrin was done in-silico using various online tools and web programs.

4.4.1 Properties of lambda cyhalothrin as a phytochemical

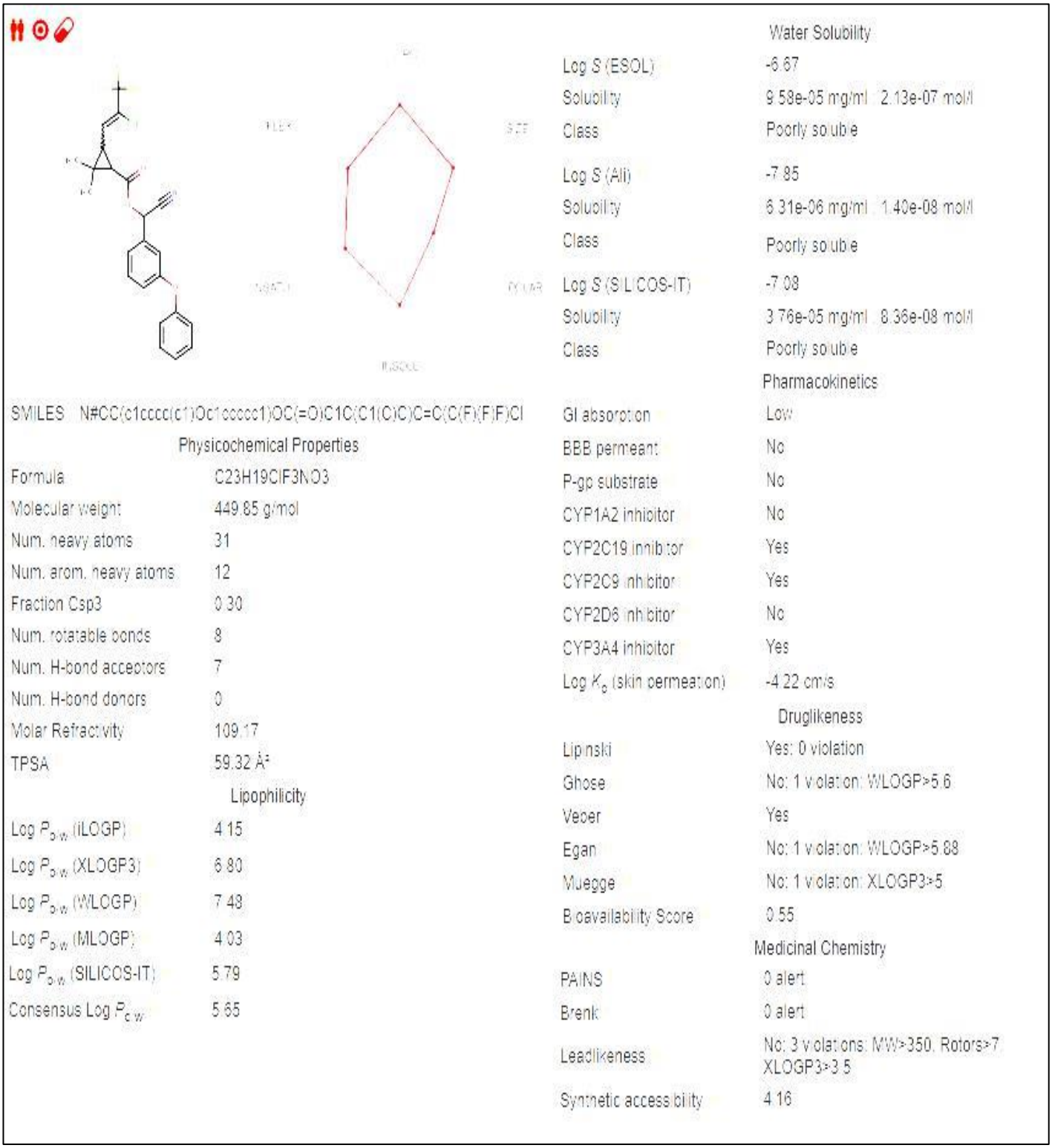
In order to ascertain toxicity of lambda cyhalothrin, SMILES or Simplified Molecular Input Line Entry System were obtained from Pubchem. These sodes are specific to every chemical.

This was then entered into admetsar and the results specified that lambda cyhalothrin is an extremely toxic chemical as it tends to cross blood-brain barrier and also gets absorbed within the human intestines with a high prbability. The admet predicted profile also predicte high probability of LCT being toxic to aquatic organisms such as fish as well as crustaceans. The positive value and high probability of human intestinal absorption and blood brain barrier (table 4.2) confirms the toxic nature of lambda cyhalothrin. The detailed results as in the following Table 4.2.

Table 4.2 Toxicity of LCT within a biological system

S/No	Parameter	Value	Probability
1.	Human intestinal absorption	+	0.9790
2.	Blood brain barrier	+	0.968
3.	CYP3A4 substrate	+	0.6663
4.	CYP2C19 inhibition	+	0.7440
5.	CYP2D6 inhibition	-	0.9002
6.	CYP1A2 inhibition	+	0.6010
7.	CYP inhibitory promiscuity	+	0.8107
8.	Crustacean aquatic toxicity	+	0.9200
9.	Fish aquatic toxicity	+	1.0000

The SMILE (Simplified Molecular Input Line Entry System) code of lambda cyhalothrin was then entered swissADME which predicted the detailed properties of lambda cyhalothrin and its interactions within a biological system as a chemical. The parameters include physicochemical properties, lipophilicity, water solubility, pharmacokinetics, druglikliness and medicinal chemistry.



SMILES N#CC(c1cccc(c1)Oc1ccccc1)OC(=O)C1C(C1(C)C)C=C(C(F)(F)F)Cl

Physicochemical Properties	
Formula	C23H19ClF3NO3
Molecular weight	449.85 g/mol
Num. heavy atoms	31
Num. arom. heavy atoms	12
Fraction Csp3	0.30
Num. rotatable bonds	8
Num. H-bond acceptors	7
Num. H-bond donors	0
Molar Refractivity	109.17
TPSA	59.32 Å²
Lipophilicity	
Log $P_{o/w}$ (iLOGP)	4.15
Log $P_{o/w}$ (XLOGP3)	6.80
Log $P_{o/w}$ (WLOGP)	7.48
Log $P_{o/w}$ (MLOGP)	4.03
Log $P_{o/w}$ (SILICOS-IT)	5.79
Consensus Log $P_{o/w}$	5.65

Water Solubility	
Log S (ESOL)	-6.67
Solubility	9.58e-05 mg/ml 2.13e-07 mol/l
Class	Poorly soluble
Log S (Ali)	-7.85
Solubility	6.31e-06 mg/ml 1.40e-08 mol/l
Class	Poorly soluble
Log S (SILICOS-IT)	-7.08
Solubility	3.76e-05 mg/ml 8.36e-08 mol/l
Class	Poorly soluble
Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	Yes
CYP2C9 inhibitor	Yes
CYP2D6 inhibitor	No
CYP3A4 inhibitor	Yes
Log K_p (skin permeation)	-4.22 cm/s
Druglikeness	
Lipinski	Yes: 0 violation
Ghose	No: 1 violation: WLOGP>5.6
Veber	Yes
Egan	No: 1 violation: WLOGP>5.88
Muegge	No: 1 violation: XLOGP3>5
Bioavailability Score	0.55
Medicinal Chemistry	
PAINS	0 alert
Brenk	0 alert
Leadlikeness	No: 3 violations: MW>350, Rotors>7, XLOGP3>3.5
Synthetic accessibility	4.16

Figure 4.15 admetSAR analysis of lambda cyhalothrin

These softwares clearly establish extreme toxicity of lambda cyhalothrin and is a step forward to assess how it will reflect on genetic make-up of grass carp.

4.4.2 Predicting proteins' primary structures

PDB or the protein data bank did not contain structures of any of the specified proteins of grass carp. Therefore, the structures were prepared through four different softwares i.e. Swiss-model, I-Tasser, Intfold and Phyre-2. All four web tools predicted different structures, which were then further taken to SAVES analysis in order to select the best one. The six shortlisted proteins and their SAVES analysis tables are attached as follows:

1. **Interleukin:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	**Swiss**	**272**	**90.8: 8.4: 07**	**89.8438**	**8: 16: 23**	**4.1**	**4: 2: 2**
Model 2	Phyre-2	95	88.4: 11.6: 0	89.5979	5: 13: 28	4.6	3:2:3
Model 3	Intfold	165	83.6: 12.1: 4.2	84.2105	6: 19: 24	pass	6:0:2
Model 4	i-tasser	165	68.5: 26: 5.5	83.6257	8: 17: 22	7.3	7:2:0

2. **Heat shocl protein HSP 70:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	Swiss	1290	86.8: 12.5: 0.7	94.7692	8: 15: 26	6.1	4: 3: 2
Model 2	Phyre-2	615	85.4: 14.3: 0.3	74.2765	6: 18: 24	5.9	4: 1: 3
Model 3	**Intfold**	**742**	**89.4: 10: 0.7**	**70.3869**	**6: 12: 30**	**5.5**	**4:2:2**
Model 4	I-tasser	742	69.9: 27: 3.1	82.868	9: 17: 22	pass	6: 1: 1

3. **Cytochrome CYP1A:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	**Swiss**	**131**	**90.1: 9.9:0**	**100**	**5: 14: 29**	**2.2**	**0: 5: 3**
Model 2	Phyre-2	65	89.2: 10.8:0	62.6866	5: 14: 28	10.8	3: 1: 4
Model 3	Intfold	131	88.5: 9.9: 1.5	75.9124	6: 12: 30	4.9	5: 1: 2
Model 4	I-tasser	131	82.4: 17.6: 0	89.781	6: 16: 25	5.6	5: 2: 1

4. **Tumor necrosis factor TNF:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	Swiss	176	80.7: 18.7: 0.6	89.071	8: 16: 23	5.8	5: 2: 2
Model 2	Phyre-2	176	65.3: 24.4: 0	47.449	7: 16: 24	7.5	5: 3: 1
Model 3	**Intfold**	**511**	**88.8: 9: 2.2**	**61.9691**	**6: 12: 30**	**5.3**	**4: 2: 3**
Model 4	I-tasser				8: 19: 22		6: 2: 1

5. **Immunoglobulin IgM:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	**Swiss**	**352**	**84.4: 14.2: 1.4**	**71.3855**	**8: 17: 21**	**pass**	**6: 1: 2**
Model 2	Phyre-2	361	87:12.2:0.8	34.5088	7: 17: 24	10.3	4: 4: 1
Model 3	Intfold	384	84.1: 14.3: 1.6	42.7885	7: 12: 29	6.7	5: 3: 1
Model 4	I-tasser	384	62.8: 35.1: 2.1	71.772	10:19: 19	6.3	6: 3: 0

6. **Beta actin:**

Model	**Software used**	**No. of amino acid residues in final structure**	**Ramachandran plot – residues in favoured : allow: outer region**	**ERRAt (quality factor A)**	**Whatcheck: error: warning: pass**	**PROVE % error**	**Pro-check Error: warning: Pass**
Model 1	Swiss	230	95.7: 4.3: 0	94.4223	8: 13: 26	1.3	1: 3: 4
Model 2	Phyre-2	230	84.3:14.8 : 0.9	57.9592	7: 15: 24	11	4: 3: 2
Model 3	**Intfold**	**233**	**96.6: 3.4: 0**	**89.1051**	**6: 10: 31**	**3.3**	**2: 2: 4**
Model 4	I-tasser	233	85: 15: 0	94.071	8: 15: 24	6.2	5: 2: 1

Through SAVES analysis, structures best suited for docking simulations were selected based on Ramacgandaran plot values. Higher Ramachandaran values suggested structure best suited for molecular docking.

4.4.3. Molecular docking and analysis

For docking simulations, an online web tool called “pyrx” was used that helped build the library. All selected protein structures were run with lambda cyhalothrin (ligand in this case) one by one

to create library and software compatible structures. Pyrx output files were then run through "pymol" which presented results as follows:

(a) Lambda cyhalothrin – interleukin interaction:

The resulting simulation showed two clear bonds (yellow dotted lines in Fig 4.16) between molecular structure and the ligand (lambda cyhalothrin). This means that lambda cyhalothrin is going to affect the bonding, structure and interactions of this protein with fish body.

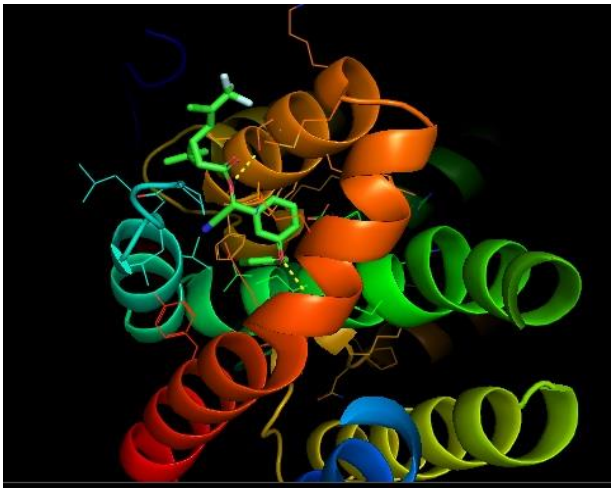


Figure 4.16 Lambda cyhalothrin and interleukin docking

(b) Lambda cyhalothrin-heat shock protein interaction:

Docking simulations of lambda cyhalothrin and heat shock protein presented three distinct bonds (yellow dotted line Fig 4.17) showing ability of lambda cyhalothrin to alter protein structure as well as functioning.

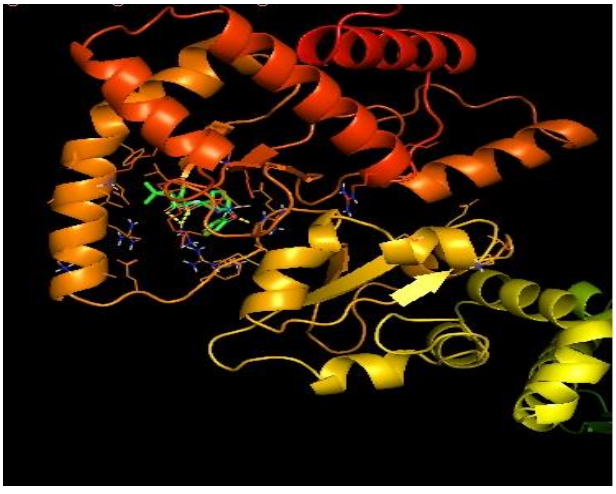


Figure 4.17 Lambda cyhalothrin and heat shock protein docking

(c) Lambda cyhalothrin-cytochrome interaction

No bonding was observed within the structures of LCT and cytochrome in docking simulations which may indicate that lambda cyhalothrin did not cause any damage to this protein.

(d) Lambda cyhalothrin-tumor necrosis factor interaction

Simulations [resented 2 distinct bonds betweem LCT and TNF (Fig 4.18) proving the toxic genetic impacts of this insecticide.

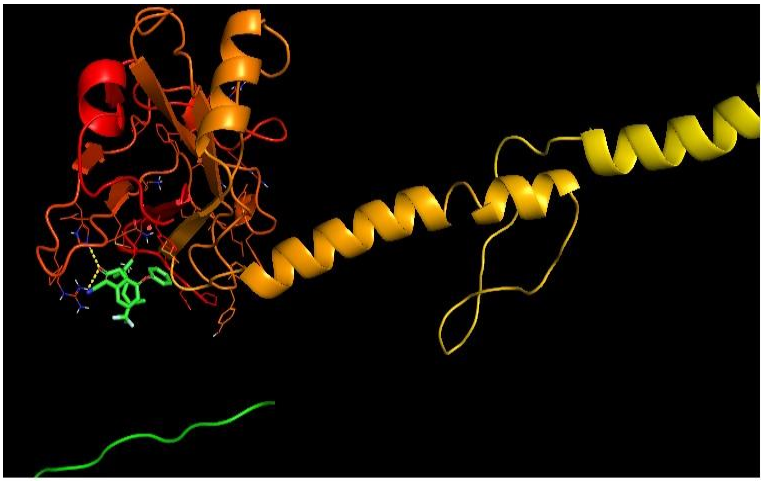


Figure 4.18 Lambda cyhalothrin and tumor necrosis factor docking

(e) Lambda cyhalothrin-immunoglobulin interaction:

Presence of two dotted lines between lambda cyhalothrin 3D structure and protein indicates that protein's structure is compromise due to LCT exposure (Fig 4.19).

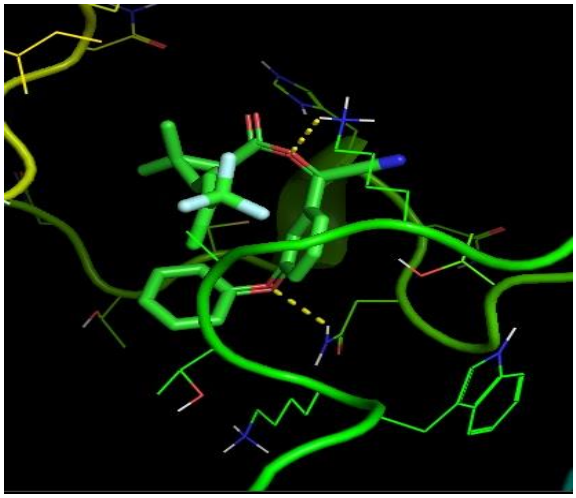


Figure 4.19 Lambda cyhalothrin and immunoglobulin docking

(f) Lambda cyhalothrin-beta actin interaction:

Three distinct dotted yellow lines between 3D models of lambda cyhalothrin and beta actin revealed toxicity potential and damage to protein structure and function (Fig 4.20).

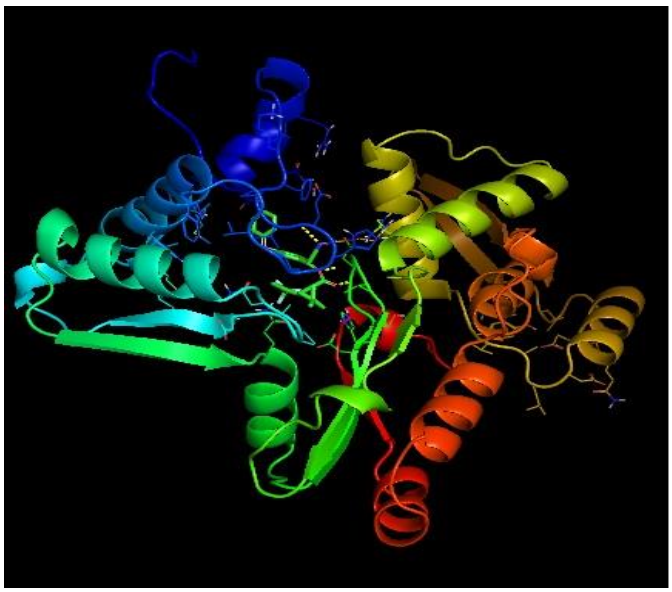


Figure 4. 20 Lambda cyhalothrin and beta actin docking

The simulations are a clear proof of the toxicity potential of lambda cyhalothrin. Moreover, it also concludes that fish living in LCT contaminated waters are at a risk of genetic mutations, as the structures of proteins are compromised due to this toxic exposure.

4.5 Analysis of photodegradation of Lambda cyhalothrin

Literature reports the detection of these hazardous agrochemicals in µg/l or ng/l hence are micropollutants which cannot be removed with simplae primary or secondary treatment plans (Petsas & Vagi, 2018). The most efficient method for removal of persistant and toxic chemicals such as lambda cyhalothrin from natural waters is through photodegradation (Xie et al., 2011). In this study, UV lamps (11W, 254nm) were used as a source of UV light for photodegrading lambda cyhalothrin. A uniform and inverse trend of photodegradation was observed at three different concentrations of lambda cyhalothrin.

4.5.1 Retention time of lambda cyhalothrin

Pure standards of six successive concentrations of lambda cyhalothrin were prepared in pure GC/HPLC grade n-hexane in order to determine its retention time. The resulting chromatogram (Fig 4.20) presented sharp peak at 6.0903 ± 0.079507 minutes, which was then taken as its retention time.

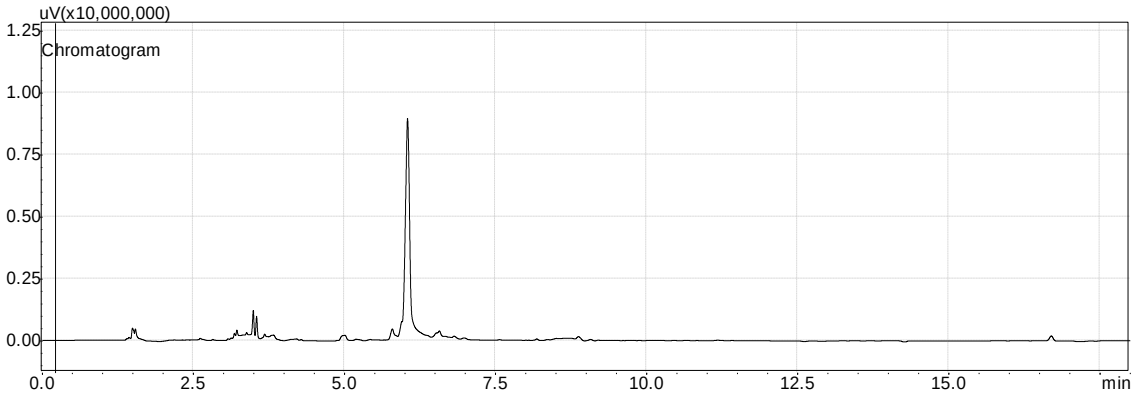


Figure 4.21 Chromatogram for retention time of lambda cyhalothrin

This retention time was then taken as reference in order to obtain the standard calibration curve. A series of four consecutive concentrations, starting from 0.5 to 1.5 µg/l of pure lambda cyhalothrin standards were prepared in n-hexane and the run through GC, using the same retention time as reference. The following standard calibration curve was otained by running these pure standards (Fig 4.21).

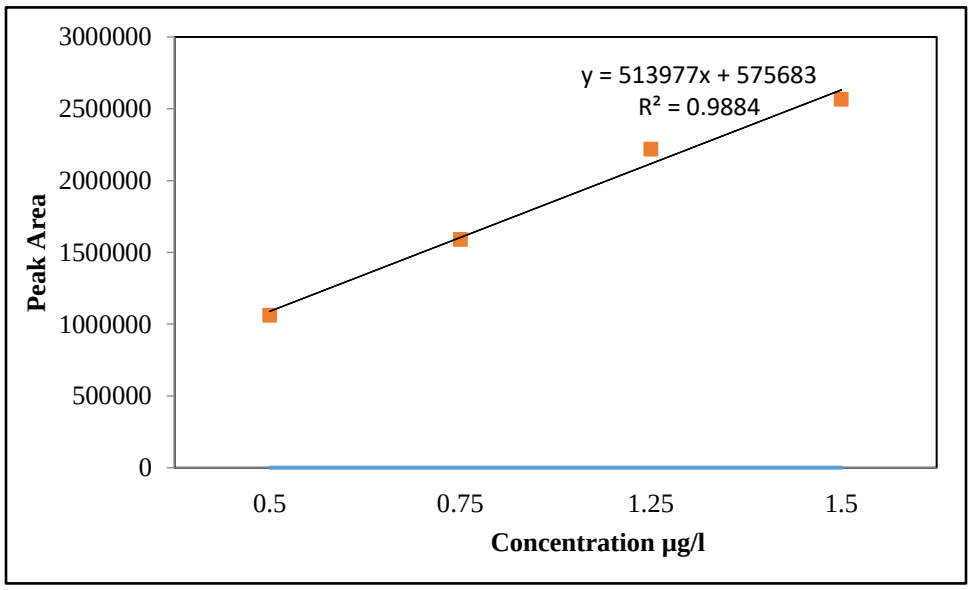
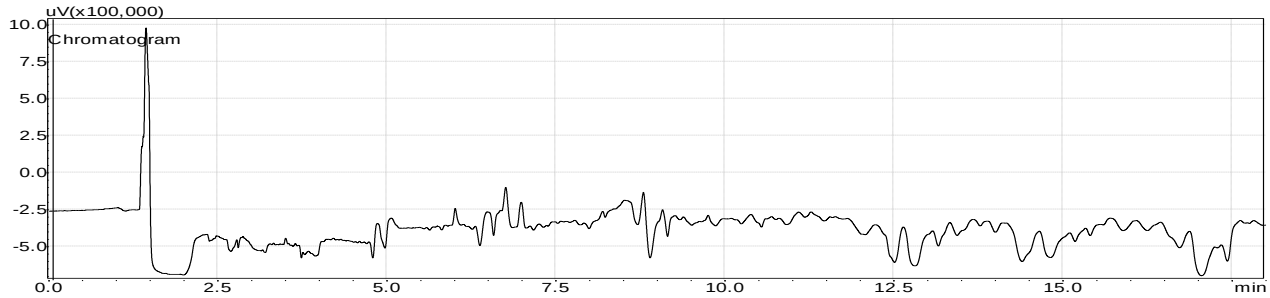


Figure 4.22 Standard Calibration Curve

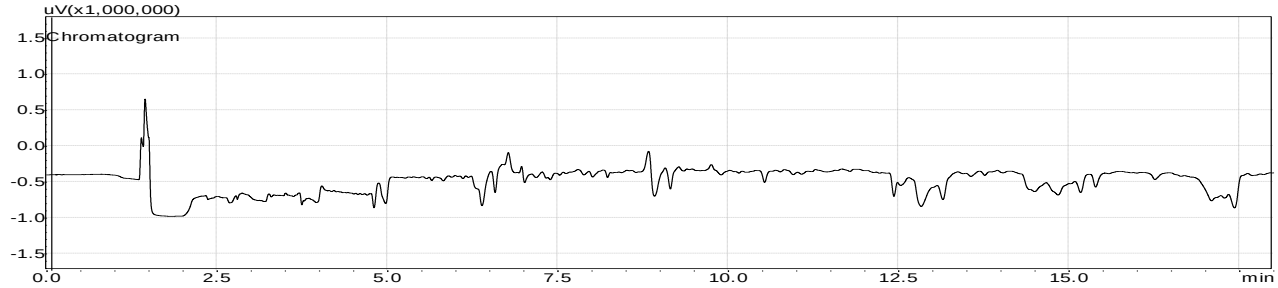
Once calibration curve was established, all of the samples were passed through in order to determine extent of photodegradation. The chromatograms are attached as follows:

4.5.2 Photodegradation Chromatograms

a. 1.25 µg/l (30min):



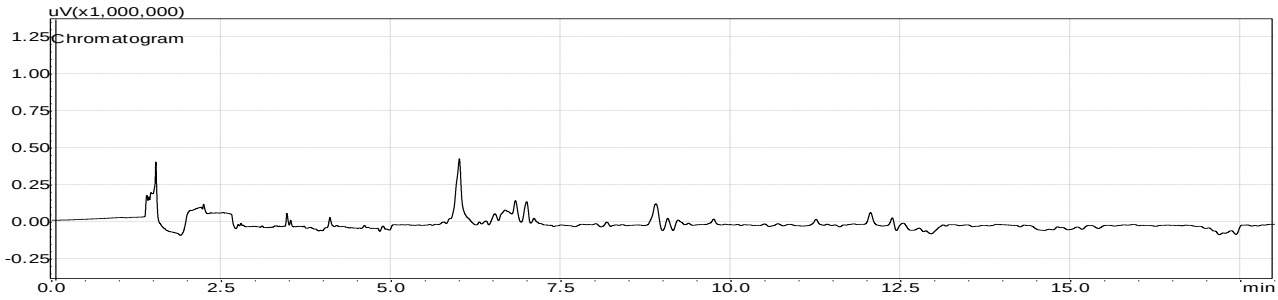
(a) 0 hr



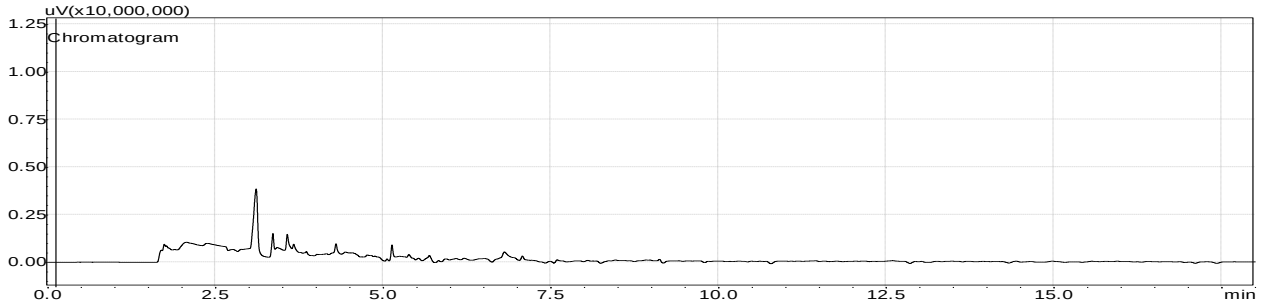
(b) 96 hr

Figure 4.23 Photodegradation chromatogram (1.25 µg/l - 30min)

b. 1.25 µg/l (20min):



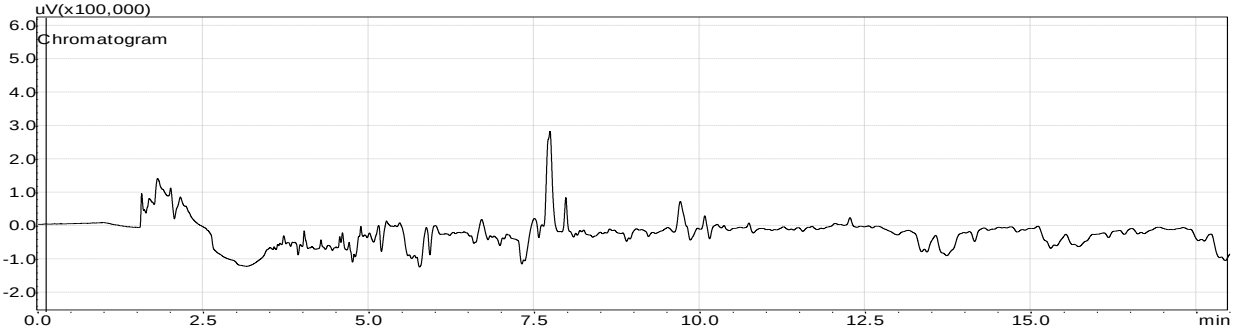
(a) 0 hr



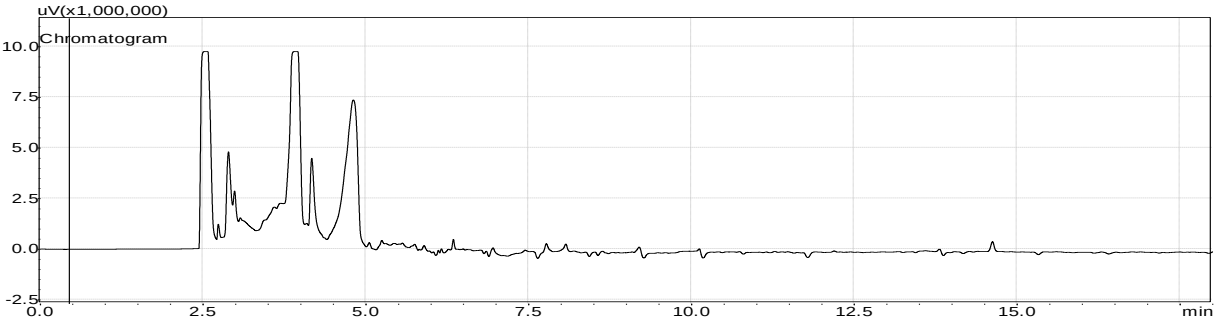
(b) 96 hr

Figure 4.24 Photodegradation chromatogram (1.25 µg/l - 20min)

c. 1.25 µg/l (10min):



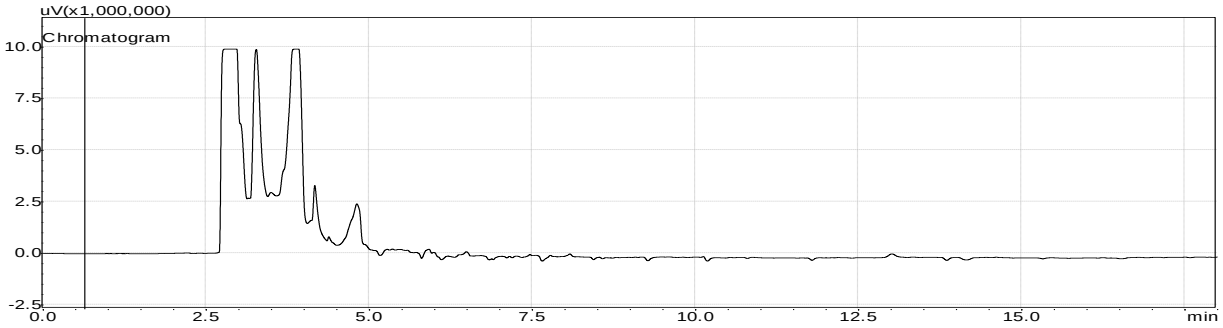
(a) 0 hr



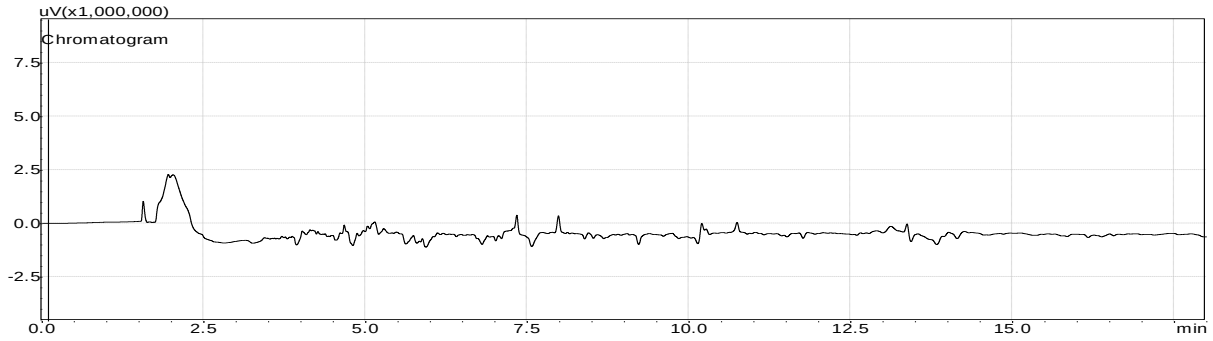
(b) 96 hr

Figure 4.25 Photodegradation chromatogram (1.25µg/l - 10min)

(b) 1.0 µg/l (30min):



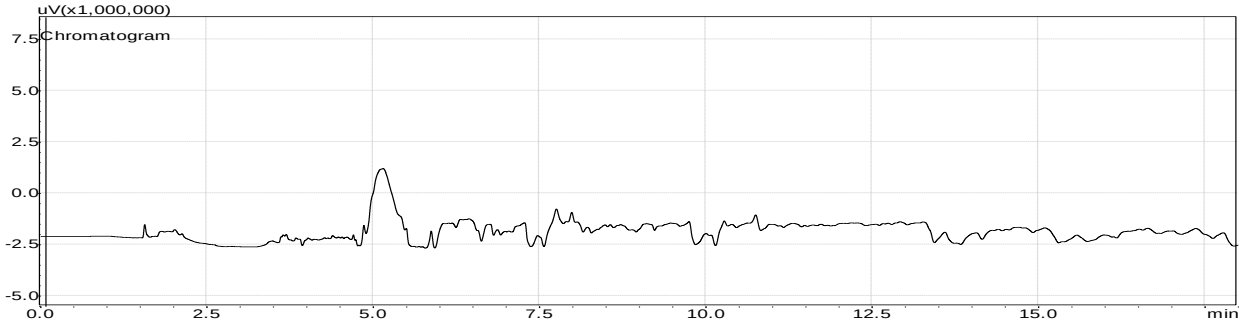
(a) 0 hr



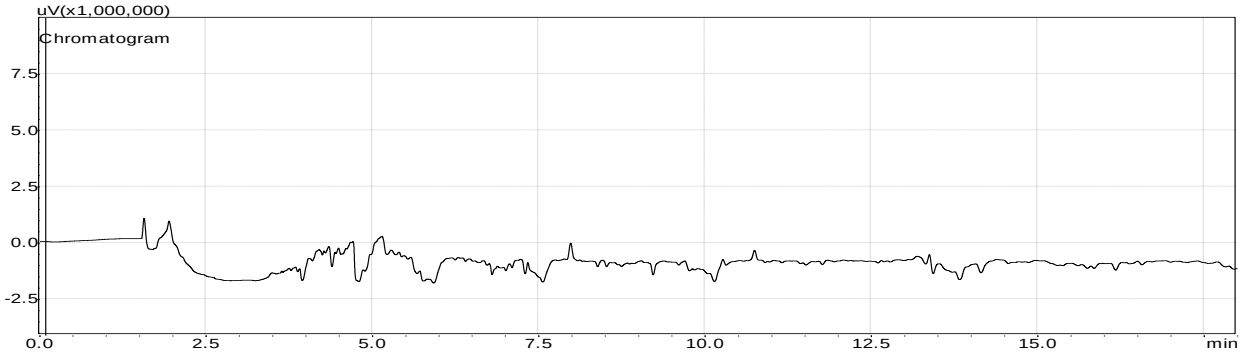
(b) 96 hr

Figure 4.26 Photodegradation chromatogram (1.0 µg/l - 30min)

(c) 1.0 µg/l (20min):



(a) 0 hr

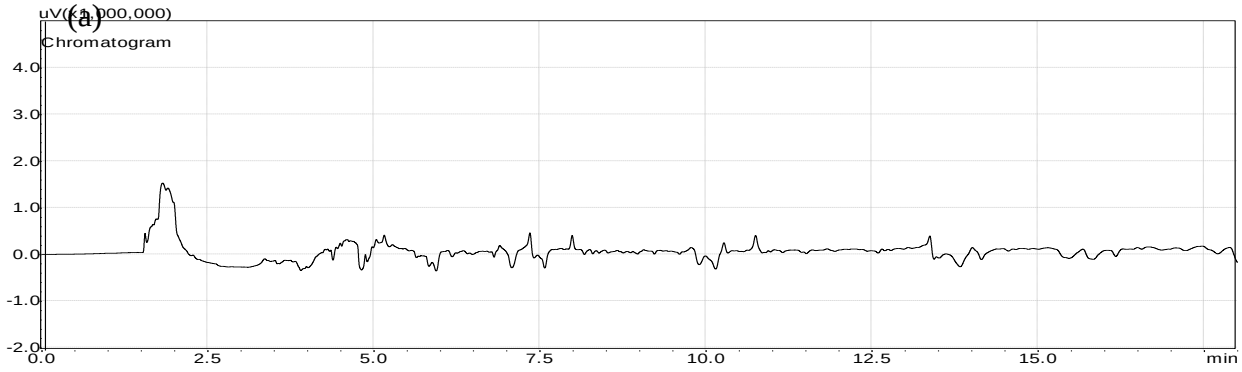


(b) 96 hr

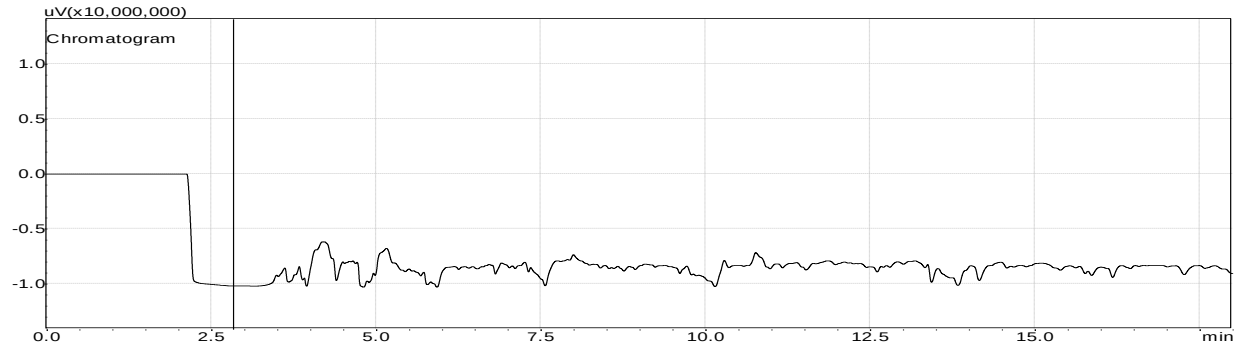
Figure 4.27 Photodegradation chromatogram (1.0 µg/l - 20min)

(d) 1.0 µg/l (10min):

(a)



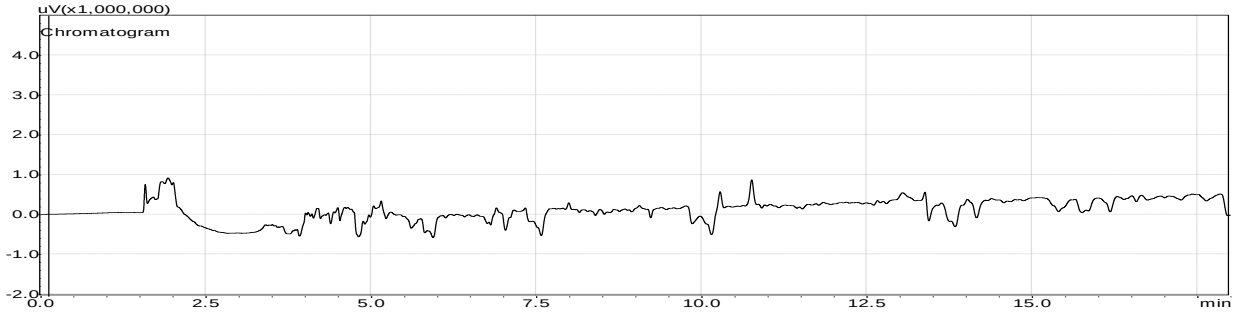
(b) 0 hr



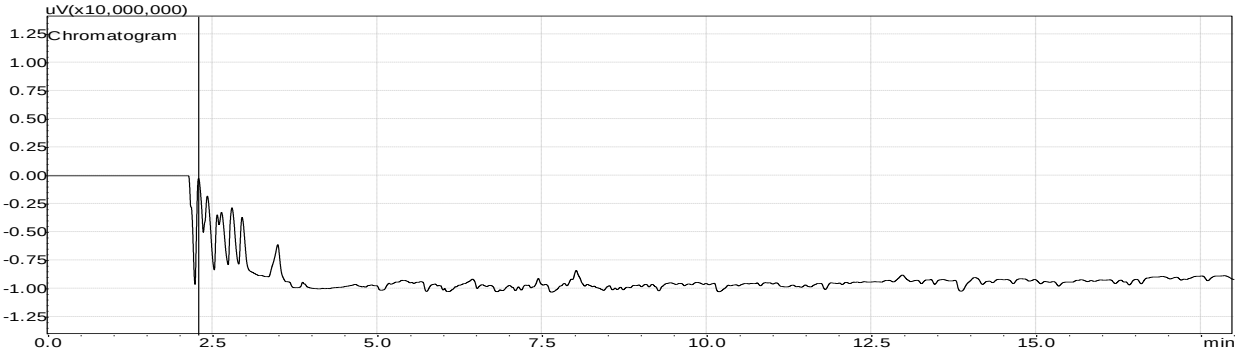
(b) 96 hr

Figure 4.28 Photodegradation chromatogram (1.0 µg/l - 10min)

(e) **0.75 µg/l (30min):**



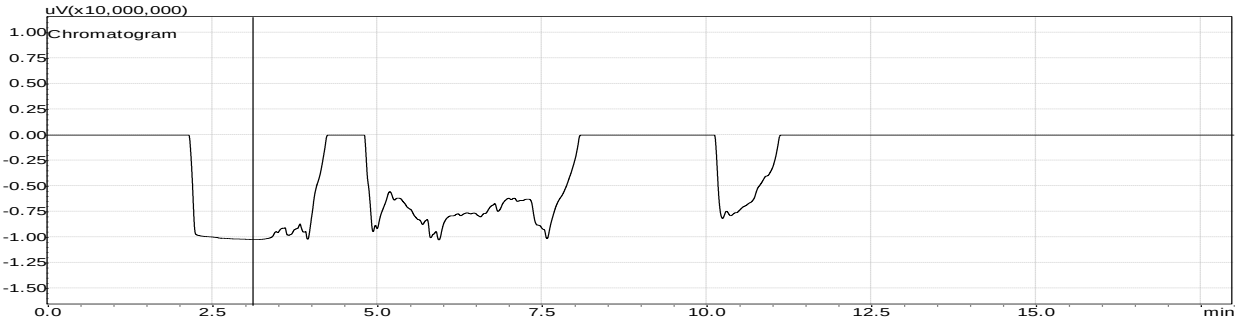
(a) 0 hr



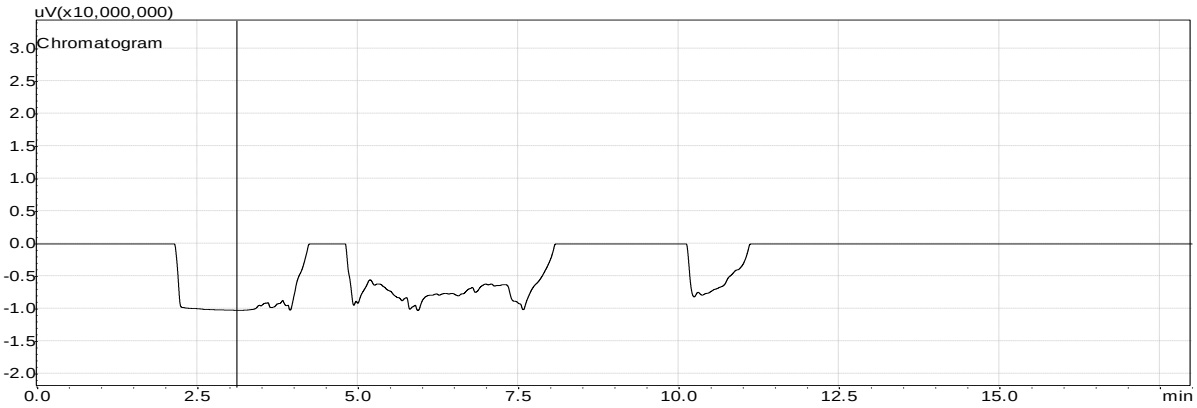
(b) 96 hr

Figure 4.29 Photodegradation chromatogram (0.75µg/l - 30min)

(f) **0.75 µg/l (20min):**



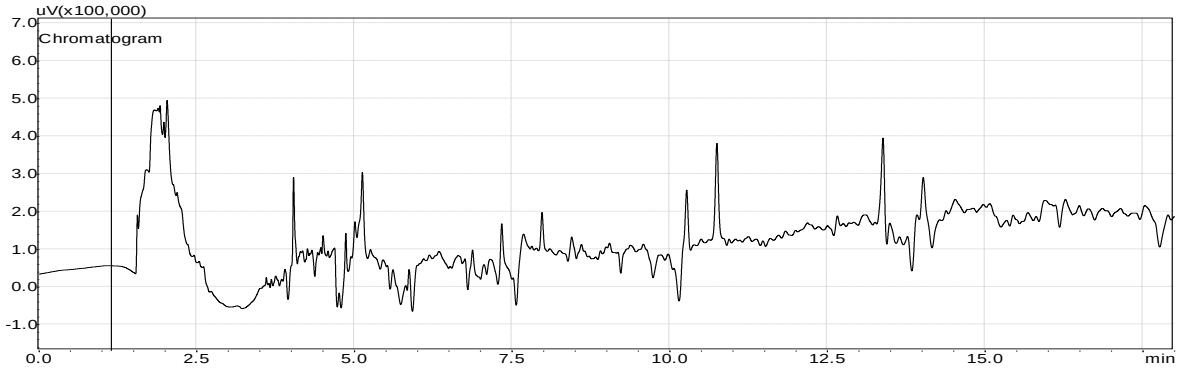
(a) 0 hr



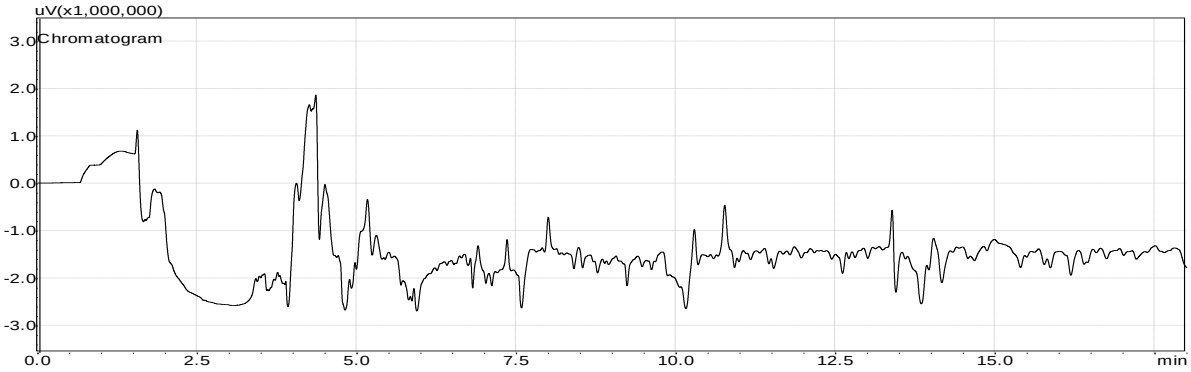
(b) 96 hr

Figure 4.30 Photodegradation chromatogram (0.75 µg/l - 20min)

(g) 0.75µg/l (10min):



(a) 0 hr



(b) 96 hr

Figure 4.31 Photodegradation chromatogram (0.75 µg/l - 20min)

4.5.3 percent removal through photodegradation

Once the calibration curve was established, the response factor was calculated for every concentration using the following formula (Zafar et al., 2020):

Response Factor = Peak Area of Standard / Amount of standards

Based on this calculation, the average response factor was calculated, which was then further used to calculate the amount of analyte using the following formula:

Amount of Analyte = Peak Area of sample / Response Factor

Table 4.3 details the amount of analyte calculated with different concentrations of LCT as well as varying UV exposure times.

Table 4.3 Amount of Analysis

Time (min)	Concentration µg/l		
	0.75	**1**	**1.25**
10	0.28	0.47	0.6
20	0.33	0.48	0.70
30	0.48	0.65	1.05

The amount of analyte presented in Table 4.3 designates the concentration of lambda cyhalothrin left within tank water after dedicated UV exposure time (10, 20 and 30 min). After determining the amount of analyte, the following formula (Weng et al., 2018) was used for calculating the amount of LCT that was degraded because of UV lamp.

$$\mathbf{R\ (\%) = C_0 - C_t / C_0\ X\ 100\%}$$

R represents the pesticide residue (%), C_0 the initial concentration and C_t the final concentration of the analyte. Table gives the percent removal of pesticide through photodegradation.

Table 4.4 Percent removal (%)

Time (min)	Concentrations µg/l		
	0.75	**1**	**1.25**
10	62	53	50
20	56	52	20
30	36	20	16

Table 4.4 shows a decreasing trend of photodegradation with respect to time. More exposure to UV resulted in lesser degradation of lambda cyhalothrin and vice versa. However, as the initial concentration of LCT increased, the degradation amount also increased. A matching degradation pattern was reported by (Mbugua et al., 2017). The photodegradation trend is expressed in Figure 4.32.

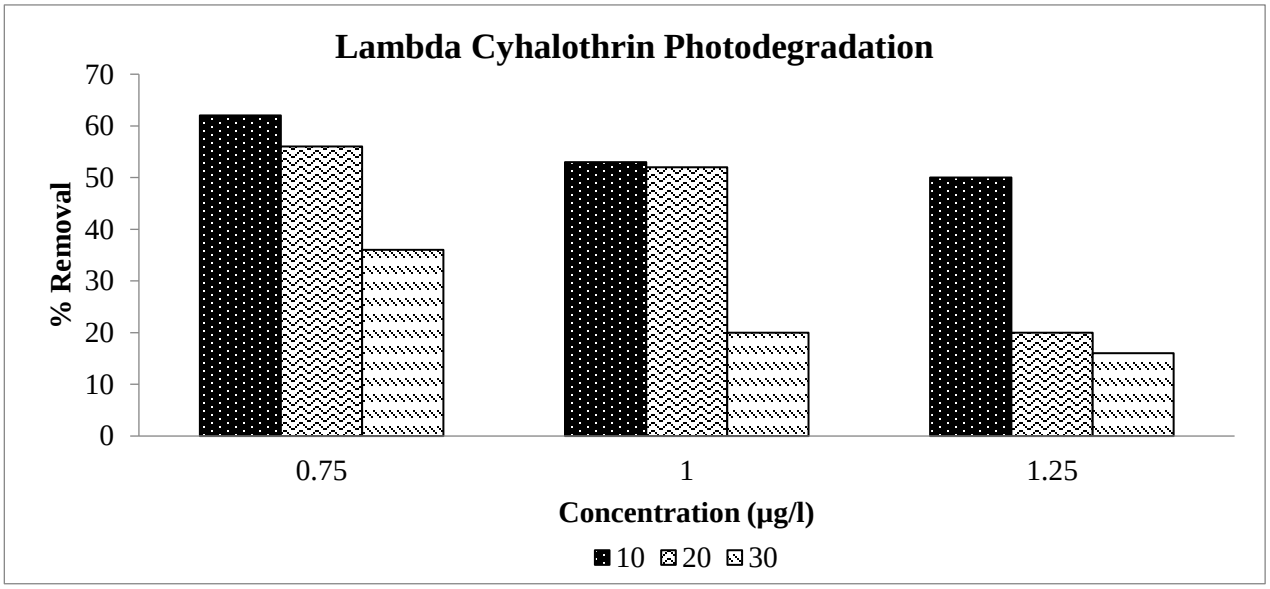


Figure 4.32 Photodegradation of lambda cyhalothrin

4.5.4 Photolytic degradation by-products of lambda cyhalothrin

During the GC run off all photodegraded samples, consistant sharp peaks were observed at specific retention times (Table 4.5), which could account for the degradation by-products of lambda cyhalothrin. Since GC could only identify halo-group compounds, these by-products can be suspectet to contain halo group attached to its structure. The sharp peaks and their corresponding retention time is as follows:

Table 4.5 Probable photodegradation by-products

Peaks	Retention Time (sec)
Peak 1	20.45
Peak 2	22.15
Peak 3	38.35

Probable halo-group substituted by-products of lambda cyhalothrin reported in literature are as follows:

Table 4.6 Photodegradation by-products

S/No.	Name	Retention Time (sec)	Reference
1.	3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylic acid	6.42	(Fernandez-Alvarez et al., 2007)
2.	[3-(2-Chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropyl](3 phenoxyphenyl)cetonitrile	13.25 – 14.70	
3.	α-cyano-3-hydroxybenzyl-3-(3,3,3-trifluoroprop-1-en-1-yl) 2,2-dimethylcyclopropanecarboxylate	15.779	(Xie et al., 2011)

These may be the possible by-products of UV photodegradation of lambda cyhalothrin corresponding to three repeated peaks observed in the chromatograms of samples run through GC.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From current study, we may safely conclude that:

1. Lambda cyhalothrin is an effective insecticide, yet extremely toxic to aquatic organisms (fish) even in concentration as low as 0.75 µg/l.
2. LCT in an aquatic environment alters the physicochemical properties of water (pH, EC, DO, turbidity and hardness) fluctuating with time depending on surrounding environment.
3. Fish presents behavioral disparities such as hyper excitability, erratic swimming movements, schooling, red eyes along with sensitivity towards sunlight after being exposed to lambda cyhalothrin.
4. Fish exposed to lower concentrations of lambda cyhalothrin (0.75 µg/l) causes dangerous histopathological lesions in vital organs. Gills, brain, muscles and liver histology presented structural deformation and necrosis leading to physiological disorders.
5. Biochemical response of fish after exposure to lambda cyhalothrin confirms that fish goes under stress. Serum glucose, total protein, triglycerides and amylase presented considerably low values as compared to the control with variations over time, indicating coping mechanism of fish
6. Changes in the relative gene expression of fish were also observed as a result of toxicity of lambda cyhalothrin. Docking simulations presented significant bonding between 3D structures of proteins and lambda cyhalothrin, ratifying that such toxic exposures can cause genetic mutations in fish body
7. UV lamps (11W) prove to be an effective method for carrying out photodegradation of toxic compounds such as lambda cyhalothrin. Maximum photodegradation of 62% was obtained at 0.75 µg/l and 10 minutes of UV light source.

5.2 Recommendations

Following recommendations are presented in light of present study:

1. Photodegradation with the help of UV light source may be optimized with detailed kinetic studies to advance this green technology in order to develop a workable pollutant remobal technique.
2. Due to overuse of pesticides, development of resistance of fish at various life stages may be explored more in order to regularize agrochemical use.
3. Toxic impacts at gene level may be expllored in depth in order to ascertain exact changes in gene expression.
4. The photolytic degradation by-products of lambda cyhalothrin need detailed investigation so that it can be established whether overall toxicity is reduced or increased after photolysis.
5. Standardize agrochemical use keeping in view pesticides resistance within fish at various life stages.

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