Effects of pesticides on Fresh water Fishes

Thesis Submitted to Midnapore City College
for the Partial Fulfillment of the Degree of
Master of Science (Zoology)

Submitted by

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2023

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Ref.No- MCC/DIR-CER(PG)/07/23-8293(B) Date-14/07/2023

Certificate

This is to certify that the project report entitled **"Effects of pesticides on Fresh water Fishes"** submitted by **Mansaram Mandal (PG/VUWGP29/ZOO-IVS-019), Riyanka Khatun (PG/VUWGP29/ZOO-IVS-034), Sibsankar Mandi (PG/VUWGP29/ZOO-IVS-043), SK Saheb Hossain (PG/VUWGP29/ZOO-IVS-046), Soma Roy (PG/VUWGP29/ZOO-IVS-049), Sreyashee Dutta (PG/VUWGP29/ZOO-IVS-052), Subhasree Sarkar (PG/VUWGP29/ZOO-IVS-053), Suvendu Pal (PG/VUWGP29/ZOO-IVS-058), Suvrangshu Bera (PG/VUWGP29/ZOO-IVS-059)** to the Midnapore City College, Midnapore, West Bengal, India during the year of 2023 in partial fulfillment for the award of the degree of M.Sc. in a bona fide record of project work carried out by them under our supervision. The contents of this report, in full or in parts, have not been submitted to any other Institution or University for the award of any degree.

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Declaration

We do hereby declare that the present Master thesis entitled '**Effects of pesticides on Fresh water Fishes**' embodies the original research work carried out by me in the Department of Biological Sciences, Midnapore City College Paschim Medinipur, West Bengal, India under the supervision of Dr. Sangita Maiti Dutta, Assistant professor of Zoology, Department of Biological Sciences, Midnapore City College, Kuturiya, P.O. Bhadutala, Pin-721129, Paschim Medinipur, West Bengal, India. No part thereof has been submitted for any degree or diploma in any University.

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Acknowledgement

We would first like to acknowledge Dr. Pradip Ghosh, Hon'ble Founder Director, Midnapore City College, for providing me the opportunity to study and complete my thesis work in this college. We are gratefully indebted to him for his very valuable comments on this thesis. We would also like to acknowledge Dr. Kuntal Ghosh, Teacher-In- Charge, Midnapore City College for his valuable suggestion and cooperation regarding project work and preparation of thesis.

We would like to thank my thesis advisor Dr. Sangita Maiti Dutta, Assistant professor of Zoology, Department of Biological Sciences, Midnapore City College. The door to Dr. Sangita Maiti Dutta, Zoology office was always open whenever we ran into a trouble spot or had a question about my research or writing. She consistently allowed this paper to be our own work but steered me in the right the direction whenever she thought we needed it.

We would also like to thank the other faculties Prof. Nirmal Kumar Sarkar, Dr. Sabyasachi Pal, Dr. Somanka Sanyal, Dr. Monjit Paul, Mr. Tuhin Khaddar, Dr. Joydeep Das, Mrs. Madhumita Dubey and research scholar, Mr. Raja Saha, Putul Karan and Ms. Amina Khatun, and other non-teaching staffs for their support to carry out this research project. Without their passionate participation and input, the validation survey could not have been successfully conducted.

Finally, we must express my very profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

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Abstract:

Human being uses pesticides for their benefits – for increasing crop yield and for the control of insect vectors of diseases. However, these pesticides are not always useful. Pesticides can be toxic to other non-target and beneficial plants and animals. The adverse effects of toxicants become significant when they affect economically important organisms or affect those organisms which are consumed by economically important animals and human beings and produces stress conditions either in the form of physiological, biochemical, damage to vital organs or even death of living organisms of terrestrial and aquatic environment. The symptoms of toxicity either appear in the animal accumulating the poisonous material or it makes the animal poisonous to other organisms which feed on it. Thus, the concentration becomes bio-accumulated and bio-magnified and the worst sufferers are animals at the top of the trophic structure. *Labeo bata* are highly recommended culturing fish as it is widely accepted as food.

In this present study, an attempt has been made to investigate the chronic toxicity of pesticides on the fish (*Labeo bata*). For this regard fishes are collected from two different sites. Site I is control site where very less human activities are seen, whereas the Site II is pesticide-contaminated water body. Different physic-chemical parameters like pH, DO, BOD, COD, Alkalinity, TDS, and Turbidity has been studied for determine the water quality of both sites. Different physiological biomarkers have been estimated from the collected fish of both sites. ALT, AST, LDH and blood glucose has been found higher in fish of Site II than Site I. Total protein concentration is found higher in Site I organism than Site II. Different antioxidants like Catalase, SOD, GSH has been estimated and found higher in Site II than Site I fish. MDA activity also found higher in Site II animal than Site I. The main focus of our project is to find out the effects of pesticide on aquatic organisms and their mechanism of physiological adaptation in pesticide contaminated aquatic environment.

Key words: Pesticides, Environmental pollution, Fish, Biomarker.

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Chapter -1: Introduction

Introduction

The Food and Agricultural Organization (FAO) of the United National has declared a that world Food production should be increased by 70% in order to full fill the demand of growing population. Based on chemical composition, pesticides are classified into different groups namely: organochlorines, organophosphorus, carbonates, pyrethrin, and pyrethroids. Organophosphates pesticides are used in different parts of the world due to their biodegradability, high effectiveness and low environmental impact. Although other groups of pesticides with a shorter life and comparatively very low mammalian toxicity are obtainable (e.g.-pyrethroids). Pesticides can also be classifying into different categories depending on their chronic impacts as mutagenic, teratogenic, neurotoxic, endocrine disrupting, immunotoxins or immune suppressants, carcinogenic.

Pesticides are frequently used to increase agricultural productivity by controlling certain pests. However, at the same time, serious environmental issues intensify because of pesticides, which alter the ecosystem's structure and functioning through the food chain. Pesticides enter the aquatic environmental through rivers, by direct application spray movement, aerial spraying and washing from the atmosphere by precipitation, degradation and run off from agriculture land, factory effluents and sewage. Pesticides have now become an integral part of our modern life and are used to protect agricultural land, stored grain, flower gardens as well as to eradicate the pests transmitting dangerous infectious diseases. Pesticides affect non-target species including fish as well as other organisms. Chemical substances enter the human body via the food chain since fish being a vital source of animal protein. The intake of pesticides affects the biochemical composition of fishes.

The DDT is often banned in India for agriculture used but still it is using in health purposes. These DDT can enter into the food chain and hampered the life cycle of different living organisms. The different types of pesticides have different side effects. In West Bengal different thirty -nine varieties of indigenous fishes are found in the crop field during rainy season. Pathological changes occur mainly in the liver, blood vessels, kidneys, and gills. Liver cells exhibit cytoplasmic granularity, partial loss of liver plate radial orientation, and shrinkage of some liver cell mass. Glomeruli in the posterior kidney show pycnotic changes of cell nuclei, vocalization of cytoplasm, and atrophy of some cells. Gill filaments and lamellae show the precipitated masses that have plugged the central capillaries.

Due to their gills, fish can directly contact the surrounding water, making them susceptible to aquatic pollutants. The pesticides are categorized according to their ability to produce lipid peroxidation and alter body antioxidant status. It is established that stimulation of free radical production, induction of lipid peroxidation, and disturbance of the total antioxidant capability of the body are mechanisms of toxicity in most pesticides. Reactive oxygen species and their highly destructive nature have been known (Butterfield & Lauderback, 2002). The levels of reactive oxygen species (ROS) become elevated causing detrimental effects on cell metabolism, biochemical and other physiological activities. In response to oxidative stress, organisms activate antioxidant defence system consisting of both enzymatic and non-enzymatic components. Reactive oxygen species (ROS) are continually generated as consequences of normal metabolic pathways. However, generation and degradation of ROS levels are controlled by delicate cellular control mechanisms (Halliwell & Gutteridge, 2007). Oxidative stress is caused by an imbalance between the generation of intra- and extracellular ROS and the ability of the antioxidants to scavenge those free radicals (Lushchak, 2011). Exposure to heat causes the production of potent oxidants and free radicals capable of damaging important cell components such as proteins and DNA (Dutta *et al*., 2014, 2018). Oxidative stress is believed to occur when there is an imbalance in the biological oxidant-to-antioxidant ratio and can result in oxidative damage to lipids, proteins, carbohydrates, and nucleic acids. In most cases, the abnormal generation of ROS, which can result in significant damage to cell structure, is considered an important signal of oxidative damage (Barzilai and Yamamoto 2004). Organisms have unique systems for protecting themselves against the damaging effects of activated ROS. For example, superoxide (O^{2-}), the parental form of intracellular ROS, is a very reactive molecule, but it can be converted to H_2O_2 by superoxide dismutase (SOD) and then to oxygen and water by several enzymes including catalase (CAT) (Pi *et.al.,* 2010). Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT considered as an effective method of denoting oxidative stress. Likewise, alterations in lipid peroxidation (LPO) concentration are also used to express severe oxidative damage and are used as a biomarker (Van der Oost *et al.,* 2003). Malondialdehyde (MDA) is a naturally occurring product of LPO which acts as its indicator of stress (Charissou *et al.,* 2004).

In cells, glutathione (GSH) is maintained in reduced form by the enzyme glutathione reductase (GR). GSH can function as an antioxidant in many ways, it can react with singlet

O_2, super oxide and hydroxyl radicals, thereby functions as a free radical scavenger. GSH may stabilize membrane structure by removing acyl peroxides formed from the lipid per oxidation reactions and also act as a prevalent substrate for the enzyme GST. (Price, A., Lucas, P.W. and Lea, P.J.1990).

The effect of organophosphate pesticide malathion on the Acetylcholinesterase (AchE) activities of liver, brain and gill tissues of freshwater stinging catfish Heteropneustes fossilis, highly popular and expensive table fish, due to its higher nutritional quality.

Analysis of biochemical parameters could help to identify target organs of toxicity as well as the general health status of animals. It may also to provide an early warning signal in stressed organism. The source of these parameters is the indicators responding to the environmental effects and can also serve as markers for toxicant exposure and effect in fish. Blood is a pathophysiological reflector of the whole body, and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari *et al.,* 2004). Changes in the biochemical blood profile indicate alterations in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these pollutants (Luskova *et al.,* 2002). The (SGPT, SGOT, Glucose, LDH) can be used to establish the tissue damage of the liver and kidney. Environmental stress caused marked elevations in plasma glucose levels. Serum glutamate pyruvate transaminase (SGPT) or Alanine transferase (ALT) is an essential liver enzyme involve in the normal functioning of the liver. This is an important biomarker to determine the health of the liver as it is an indicative conditions of hepatitis, cirrhosis and inflammation in liver. High levels in blood samples indicate liver injury or damage. SGPT was found increased in *Channa puctatus* (Sulodia and Singh, 2004). Environmental stressor such as contaminants can cause a variety of biological response in fish ranging from the bioindicator technique utilizes a suite of biological responses both as integrators of stress effects and such as sensitive indicators of existing and environmental condition. Serum glutamic-oxaloacetic transaminase (SGOT) or Aspartate transferase (AST) is maximally present in heart followed by liver, skeletal muscle and kidney. Any damage to these organs raises the SGOT level as in myocardial infarction, liver disease such as liver, cirrhosis, viral hepatitis, liver necrosis and skeletal disease. The increase in activity of SGOT might be due to the

disruption of the enzyme by blocking the activity of active site and to cause tissue damage (Verma *et al*., 1981). Normal blood glucose level of fish 40-90mg/dl. The rise in blood glucose is primarily generated by cortisol mediated gluconeogenesis that also inhibits cellular uptake of circulating glucose thus increasing the level in blood circulation. The increased level of glucose is a manifestation for the higher needs of tissues to fuel the metabolic needs of osmoregulation and an important source of energy for maintaining homeostasis in fish in fish during chronic stress. Thereby blood glucose level elevation can be used as an indicator of environmental stresses. So, the increase in level of blood sugar in fish indicates that their habitat may be contaminated by pollutants. The elevated activities of lactate dehydrogenase (LDH) in blood reflect damage to the liver, kidney and muscle tissues. Anaerobic metabolism can be measured by LDH activity and this activity can be impaired after prolonged exposure to xenobiotics. Lactate dehydrogenase (LDH is zinc containing enzyme and is generally associated with cellular metabolic activity. Fish under stress preferentially meet its energy requirements through an anaerobic oxidation process and thus LDH can be used as an indicator in biomonitoring in fish toxicity. The elevated activities of lactate dehydrogenase (LDH) in blood reflect damage to the liver, kidney and muscle tissues. Anaerobic metabolism can be measured by LDH activity and this activity can be impaired after prolonged exposure to xenobiotics. LDH is considered as a useful biomarker to determine the health status of the fish and pollution level of aquatic system. LDH is present in the cytoplasm of different cells of the body. However, an increase in the bloodstream can be related to liver or muscle damage. The study focused on evalution of toxic impact on the basic of results of harmatological, biochemical and histopathological examinations and indices of oxidative strees.

Chapter-2: Literature Review

Literature review

Pesticides are widely used in agriculture lands. In public health management to kill vectors of diseases, such as mosquitoes, flies and cockroaches, pesticides are also used (Pan *et al.*, 2020). Pesticides play a significant role in improving food production through control against harmful pests with low labor and efforts while on the other hand are regarded as aquatic pollutants. Most insecticides ultimately find their way into rivers, lakes and ponds (Tarahi Tabrizi, 2001) and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields. The contamination of surface waters by insecticides is known to have ill effects on the growth, survival and reproduction of aquatic animals. These toxicants persist in aquatic environment and cause harmful effects to non-target organisms including fish (WHO, 2020).Different concentrations of insecticides are present in many types of waste water and numerous studies have found them to be toxic to aquatic organisms especially fish species (Talebi, 1998).In the past few years, the increase of mortality among the fish in various streams, lakes and ponds of around the world has drawn scholars' attention to the problems caused by insecticides and pesticides run off associated with intense agricultural practices. Pesticides can adversely effect on the targeted organisms and toxic to non-target organisms also, like fish and affect fish health through impairment of metabolism, sometimes leading to mortality, adversely affecting the complex food-web and population dynamics. Excessive use of pesticides may contaminate the irrigation and drainage systems during agriculture activities (Zubairi *et al.,* 2021). Pesticide can act as stress inducing agents which affect the functional state of tissues and also effects on metabolic dysfunction in to the fishes (Pan *et al.,* 2020). Pesticide can affect the liver, muscle, gill and intestinal tissue of fish. The protein content has been found in decreasing manner in the tissues of pesticide treated fish of freshwater. Pesticide toxicity also effects in the role of biomarkers alterations in fish physiology including behavioural changes such as abnormal feeding behaviour due to the fluctuation in antioxidant enzyme activities, erratic swimming and other internal changes those are hematology, growth performance and DNA damage for the hyperactivity among other alterations. Moreover, pesticides may cause an increase in the production of reactive oxygen species (ROS) in aquatic organisms which may lead to impairment of many cellular functions (Uner *et al.,* 2006). The deleterious effect of free radicals can be prevented or counter balanced by

antioxidant systems (Lushchak, 2011). Some of the most commonly used antioxidant enzyme biomarkers are catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-s-transferase. Catalase, a heme protein is induced by the production of hydrogen peroxide in the cells and catalyzes the reaction, which reduces this compound to water and oxygen (Regoli *et al.,*1998). SOD is the first enzyme that responds to oxidative stress during any stress condition in animals (McCord and Fridovich, 1969). The increaseof SOD and catalase levels in gill and liver in tissues of Brycon cephalus exposed to Methyl parathion (Monteiro *et al*., 2009).

Elevation of lipid peroxidation in tissues after exposure to lethal and sublethal concentrations of malathion in acute and subacute durations, as evidenced by increased MDA production in the study, suggests participation of free radical-induced oxidative cell injury in mediating the toxicity of malathion (Patil, V. K., & David, M, 2013) found that 3,4-dichloroaniline, a chemical intermediate in the synthesis of herbicides, has been significantly induced the increase of MDA content in the liver of Carassius auratus. Exposure to polluted water induced the tissue-specific peroxidative damage in the gill, kidney, and liver of *Anguilla anguilla*, and the most affected tissue was the gill (Ahmad *et al.*, 2004). It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species. Glutathione (GSH) is a low molecular weight scavenger of oxygen radicals (Regoli and Principato, 1995; Regoli *et. al.,*1998) and is often used in biomarker studies, as it is an overall modulator of cellular homeostasis (Ringwood *et al.*,1999).

The increase in GSH has been described as one of the protective mechanisms that fish adopt in the exposure to aquatic pollutants (Ansari et al., 2009). Depletion of GSH may reduce the cellular ability to destroy free radicals and reactive oxygen species, so that it raises the general oxidative potential in the cells. GSH plays a protective role against free radical mediated or peroxidative damage (DeLucia *et al*.,1972).

Acetylcholinesterase is the enzyme responsible for terminating the action of acetylcholine at cholinergic synopsis (Ronald et al., 1999). It acts as a key transducer at cell membrane level, an alteration in the level of its functional activity may result in micro deformation and configurational change. Acetylcholinesterase is the target enzyme for organophosphate pesticides which is inhibited by these pesticides and its inhibition leads to blockage of neurotransmission (O'Brien, 1976) leading to physiological alterations in non-targeted organisms of the aquatic system.

Several of soluble enzymes of blood serum have been considered as a relevant stress indicator. Therefore, activities of serum (ALT, AST, and LDH) have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution . An increase of these enzyme activities in the extracellular fluid or serum is a sensitive indicator of even minor cellular damage (Palanivelu *et al.,* 2005) and indicates stress-based tissue impairment. In *Clarias batrachus,* SGOT has been increased after exposure to Tri chloroform (Shoba Rani *et al.,*1989). According to Patrice, normal blood glucose level of fish is 40-90 mg/dl. According to Batron (2002) the physical size of the fish determines blood glucose levels as the larger of fish body size requires more energy to support their life and as a results equally higher secretion of glucose. Hyperglycemia condition is not only influenced by total length or weight but also depends on the environmental conditions and habitat quality.

Exposure to cypermethrin produced a significant increase in the activities of serum LDH in South American cat fish *Rhamdia quelen* (Borges *et al.,* 2007). Cypermethrin also increase LDH level in *Labeo rohita* (Phillip *et al.,* 1995; Das and Mukherjee 2003). Stress is an energy demanding process and the animal mobilizes energy substrates to cope with stress metabolically (Vijayan *et al.,* 1997). Glucose is one of the most sensitive indicators of the stress state of an organism. Its high concentrations in blood indicate that the fish is in stress and it is intensively using energy reserves i.e., Glycogen in liver and muscles (Vosyliene,1999). The stress hormone cortisol has been shown to increase glucose production in fish, by both gluconeogenesis and glycogenolysis, and likely play an important role in the stress-associated increase in plasma glucose concentration (Iwana *et al.,* 1999). In response to pesticides stress, glucose levels were increased in *Prochidolus lineatus* (Martinez *et al.,* 2004) and *O. niloticus* (Monteiro *et al.,* 2005). A decline in serum total protein level was reported in fish *R. quelen* (Borges *et al.,* 2007) and *O.niloticus* (O¨ner *et al.,* 2008) in response to cypermethrin. Other workers also reported similar hematological changes in different fishes Mystus keletius, Oreochromis mossambicus and Channa punctatus (James et al., 1993; Sampath et al., 2003; Huda et al., 2016). Other workers also reported similar hematological changes in different fishes Mystus keletius, Oreochromis

mossambicus and Channa punctatus (James et al., 1993; Sampath et al., 2003; Huda et al., 2016).

Chapter-3: Aims and Objectives

Aims:

To study the effect of pesticides on fresh water fish (*Labeo bata*).

Objectives:

1. Ecological impact assessment for large scale use of chemical pesticide through estimating different Physico–chemical parameters of water bodies.
2. To find out the metabolic responses in *Labeo bata* in stressful environment through biochemical analysis of different metabolic enzymes like SGPT, SGOT, LDH in response to pesticide stress.
3. Determine the glucose level in response to pesticide stress.
4. Comparatively analyze the protein concentration of fish of different habitat.
5. To study the antioxidant responses by estimating the activity of different antioxidant.
6. To identify a suitable biomarker for evaluating environmental stress.

Chapter- 4: Materials and Methods

Materials and Methods:

A. Selection of Study Sites

Study Site-I

One Eco restored (SI) water body measuring of 4 acres, situated at Gurguripal eco park about 10 km away from Midnapore Sadar Block has been identified as a control Site. This water body is a perennial virgin water body because it has been developed through watershed management of an eco-degraded forest in the lateritic waste land and is devoid of any usual human activities.

Study Site-II

This is human used water body (SII) surrounded agricultural field where all kinds of domestic activities like cleaning of cloths, bathing of human beings and domestic animals, and also draining of agricultural runoff takes place. So pesticides used in agricultural field must contaminate this water body.

B. Analysis of physicochemical parameters of water:

The water samples were collected at monthly intervals from March 2023 to May 2023 with the help of indigenously deigned water sampler from different sampling sites of study area. Collected water was analyzed by following standard methods (APHA, 2005; Trivedy and Goel, 1984).

i. **Temperature (^oC):** Water temperature was measure by thermometer.

ii. **pH:** pH was measured by pH meter in laboratory and also by pH paper at site.

iii. **Turbidity (NTU)**: It reduces the light penetration and partly depends on the light flux mainly on the optical properties of water. It can be obtained by immersing Secchidisc in water body and observing its visuality.

iv. **Alkalinity** (mg/L as $CaCO_3$): Alkalinity was measured by titrametric method using 0.1 N HCl and phenolphthalein and methyl orange as indicators.

v. **Dissolved Oxygen** (mg/L): It was measured by Winkler's Iodometric method. The manganous sulphate reacts with alkali i.e. KOH to form a white precipitate of manganous hydroxide which in the presence of oxygen, gets oxidized to brown color compound. In the strong acid medium manganic ions are reduced by iodide ions which get converted to iodine equivalent to original concentration of oxygen in the sample. This iodine has been titrated against thiosulphate using starch indicator.

vi. **Chemical Oxygen Demand** (mg/L): By the use of $K_2Cr_2O_7$ in the presence of H_2SO_4 as oxidizing agent.

vii. **Biochemical Oxygen Demand** (mg/L): It was measured by the degradable organic material present in a water sample and five days incubation method.

C. Selection of target fish species *(Labeo bata)*

Labeo bata, has been selected for its easy availability in a wide range of habitats such as streams, rivers, lakes and ponds and it's also for its economic importance accepted as food.

D. Collection of samples:

The Fish were collected once in a month from February 2023 to May 2023 from two contrasting water bodies; one eco restored natural wetland (Site I) and another human used water body nearby agricultural field (Site II), by Fishing net by professional fisherman.

E. Preparation of tissue homogenate:

Immediately after collected fish, liver and gills was taken, weighted and homogenized in 0.1M phosphate buffer was adjusted so as to obtain a 20% (w/v) homogenate. The homogenization was performed with the use of the Telflon homogenizer of the Potter-Elvehjum type. Next the homogenates were centrifuged at 10000 RPM for 30 min at 4^0 C in order to obtain supernatants, which were used to measure the enzyme activity.

F. Assay of Total Protein contents:

Total protein content of liver and gill were estimated by the standard method (Lowery et al., 1951). The blue colour product was measured after 30 minutes at 750 nm against the black. Bovine serum Albumin (BSA) was used as standard. Protein concentration was calculated and the Protein concentration were expressed as µg/mg wet tissue.

G. Determination of activity of different enzymes:

i. Assay of catalase activities by gel zymography:

Catalase activity was measured spectrophotometrically by the decrease in absorbance at 240 nm due to H_2O_2 consumption according to method of Aebi (1974). The reaction volume and reaction time were 1 mL and 1 min, respectively. The reaction solution contained 80 mM phosphate buffer, pH 6.5 and 50 mM H_2O_2 (Ni *et al.,* 1990). CAT activities are given as nmol/min/ mg protein.

ii. Assay of Super Oxide Dismutase (SOD) activities gel zymography:

Superoxide dismutase (SOD) activity was assessed by the ability of the enzyme to inhibit auto-oxidation of pyrogallol (Marklund and Marklund, 1974). Specific SOD activities are given as nmol/min/ mg protein.

Briefly, fish tissue was homogenized Phosphate buffer (pH 7.0) and, centrifuge (10000 r.p.m, 4°C, 5 min.), supernatant have been taken and processed according to procedure.

iii. Estimation of Malonaldehyde (MDA):

MDA was conducted following the protocol as in Buege and Aust, 1978. The tissue homogenate was precipitated with 5% TCA solution and then vertexed. Then centrifuged at 8000- 10000 rpm for 10 minutes and collected the supernatant 0.3% TBA was added and incubated for 30 minutes. To reduce interference caused by a yellow - orrange coloured produced by some carbohydrates, the mixture is heated at 80^0 C instead for 100^0 C. Finally, the MDA is measured at 520-540 nm in spectrophotometer.

iv. Estimation of Glutathione (GSH):

GSH has been estimated as initially described by Ellman (1959) and modified according to the method of Davila *et al.,* (1991). The homogenate was added with equal volume of 20% Tri-chloro acetic acid (TCA) containing 1Mm EDTA to precipitate the tissue protein. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 200xg. The clear and transparent supernatant (200£gl) was then transferred to a new set of test-tubes. The Ellman (5,5*f Wdithiodis*-2-nitrobenzoic acid) (0.1mM) reagent was prepared in 0.3M phosphate buffer containing 1% sodium citrate. This Ellman reagent (1.8ml) was added to the 200 £gl supernatant to make a total volume 2 ml. After completion of the total reaction, solutions were read at 412nm. Absorbance values were compared with a standard curve generated from known GSH concentration to compute tissue GSH levels.

iv. Estimation of Acetylcholine esterase (AChE):

The enzyme activity was measured by the method of Hestrin (1949) modified by Augustine 1952. Acetylcholine esterase assay system comprised of 1.0 ml of M / 15 phosphate buffer (pH - 7.2) ,1 ml acetylcholine (0.004 M ,pH - 4.0) substrate buffer mixture (1:9 dilution) and 0.2 ml of homogenata and intubate for 13 minute at 37°C. Addition of 2.0 ml alkaline hydroxylamine solution was use for termination of the reaction.The solution was mixed thoroughly and 1 ml of HCl (2:1) was added to the control tubes .the colour of devolope by addition of 1 ml of farric chloride 10 % in is tubes and the OD value at 540 nm was recorded after thorough mixing .In

this assay, mixing solution in every step in very essential to avoid trapping of air bubbles.

V. Metabolic Enzymes:

Blood was collected from the caudal vein by using syringe and shifted to clotted tubes. Blood plasma was obtained by the centrifugation of blood samples in a cooled centrifuge (4°C, 837x g). Biochemical indices were analyzed in blood plasma included Glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) which were determined using biochemical analyzer and commercial kit as AUTOSPAN® Glucose GOD-POD, End point assay and kinetic assay of ARKRAY Healthcare Pvt. Ltd. SGOT(ASAT) KIT Mod. IFCC Method, LDH (P-L) KIT DGKC Method, SGPT (ALAT) KIT Mod. IFCC Method Manufactured by Coral Clinical Systems (A Division of Tulip Diagnostics Pvt.Ltd).

Chapter -5: Result

Results:

A. Ecological parameters of water of two study sites during the study period

a. Temperature (^{0}C)

At study site-I (S-I), water temperature revealed a range of variation with a minimum of 20.5^{0}C in February'2023 and that of a maximum of 28.5 ^{0}C in May, 2023 and at study site-II (S-II), water temperature showed a range of variation with a minimum of 21^{0}C in February'2023 and that of a maximum of 30^{0}C in May,2023 (**Fig 1a**).

b. pH

At S-I, pH displayed a variation with a minimum of 6.6 and that of a maximum of 6.8 and at S-II pH displayed a variation with a minimum of 7.6 and that of a maximum of 8.2 throughout the entire study period from February'2023 to May,2023 (**Fig 1b**).

c. Alkalinity (mg/L)

At S-I, alkalinity of water revealed a wide range of variation with a minimum of 65 mg/l and that of a maximum of 85 mg/l and at S-II minimum of 115 mg/l and that of a maximum of 125 mg/l throughout the entire study period from February'2023 to May,2023 (**Fig1c**).

d. Turbidity (NTU)

At S-I, Turbidity showed a wide range of variation with a minimum of 76 NTU and that of a maximum of 98 NTU and for SII minimum of 105 NTU and that of a maximum of 125 NTU throughout the entire study period from February'2023 to May,2023 (**Fig1d**).

e. Dissolved Oxygen (mg/l)

At S-I, Dissolved Oxygen (DO) displayed a wide range of variation with a minimum of 6 mg/l and that of maximum of 7.3 mg/l and for SII minimum of 4.54 mg/l and that of a maximum of 6.3 mg/l throughout the entire study period from February'2023 to May,2023 (**Fig1e**).

h. Biological Oxygen Demand (mg/L)

At S-I, Biological Oxygen Demand (BOD) showed a wide range of variation with minimum of 1.7 mg/l and that of maximum of 2.8 mg/l and for SII minimum of 2.6 mg/l and that of maximum of 4.1 mg/l throughout the entire study period from February'2023 to May,2023 **(Fig 1f**).

i. Chemical Oxygen Demand (mg/L)

At S-I, Chemical Oxygen Demand (COD) displayed a wide range of variation with a minimum 50mg/l and that of maximum of 75 mg/land for SII minimum of 65 mg/l and that of maximum of 118 mg/l throughout the entire study period from February'2023 to May,2023 **(Fig 1g)**

2. Biochemical analysis of metabolic enzyme:

The value of ALT of S-II fish was found 1.54 higher than S-I fish (**Fig 2a**). The value of AST of S-II fish was found 1.82 higher than S-I fish (**Fig 2b**). The value of LDH of S-II fish was found almost 1.96 folds than S-I fish (**Fig 2c**). The level of glucose of S-II fish was found 0.96 folds than S-I fish (**Fig 2d**).

3. Total protein concentration:

The total protein concentration of S-II fish was found 0.63 lower and 0.44 lower than S-I fish in liver and gill respectively (**Fig 3).**

4. Antioxidant enzyme activity:

4.1. Super Oxide Dismutase (SOD) activity:

SOD activity of S-II fish was found almost 1.24 folds and 1.14 folds than S-I fish in liver and gill respectively (**Fig 4a)**.

4.2. Catalyze activity:

Catalyze activity of S-II fish was found almost 1.38 folds and 1.88 folds than S-I fish in liver and gill respectively **(Fig 4b)**.

4.3. Estimation of Malonaldehyde (MDA):

MDA activity of S-II fish was found almost 1.35 folds and 1.18 folds than S-I fish in liver and gill respectively **(Fig 4c**)

4.4. Estimation of Glutathione (GSH):

GSH activity of S-II fish was found almost 0.84 lower than S-I fish muscle **(Fig 4d).**

4.5. Estimation of Acetylcholine esterase (AChE):

AChE activity of S-II fish was found almost 0.59 folds lower than S-I fish brain tissue **(Fig 4e).**

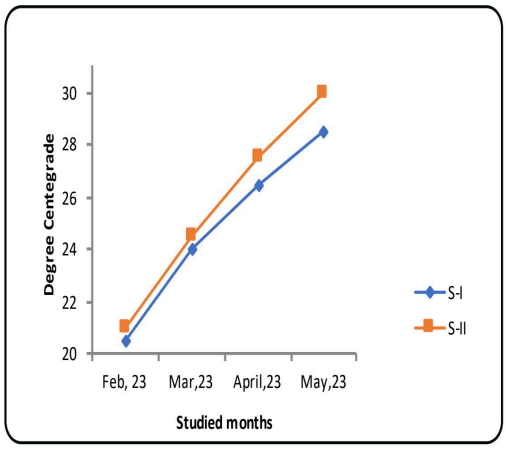

Fig. 1a: variation of temperature

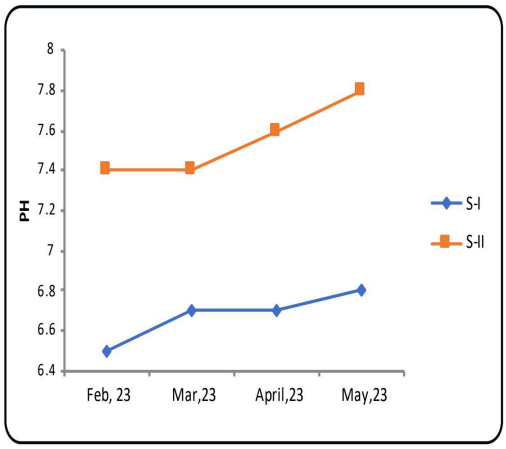

Fig.1b: variation of pH

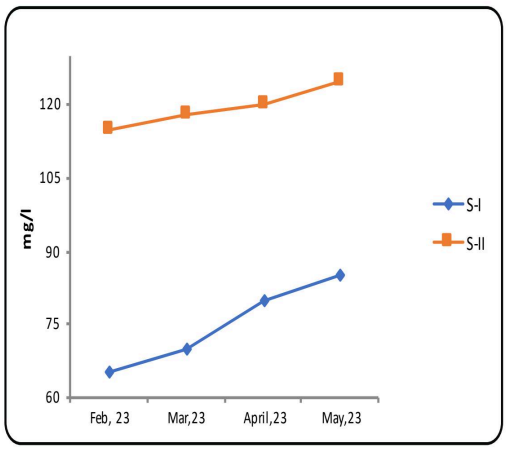

Fig 1c: Variation of Alkalinity

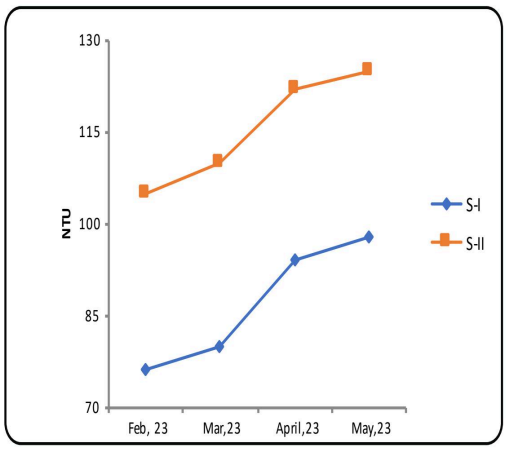

Fig 1d:variation of turbidity

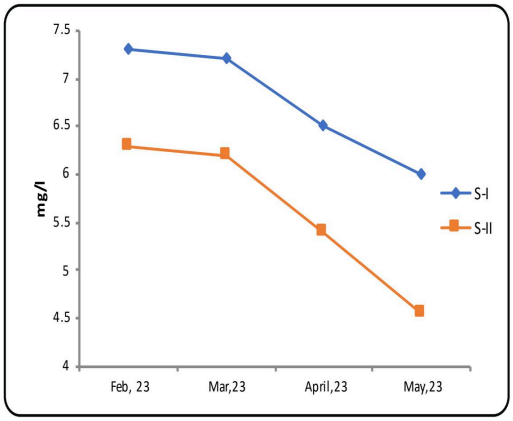

Fig 1e: variation of DO

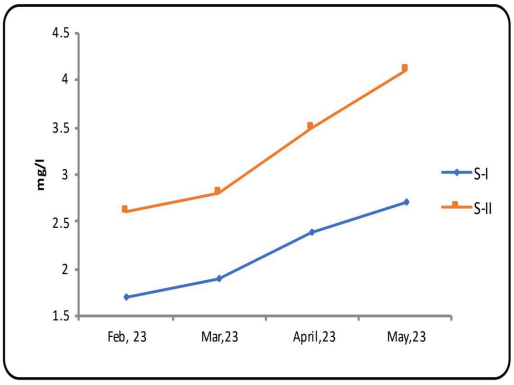

Fig 1f: variation of BOD

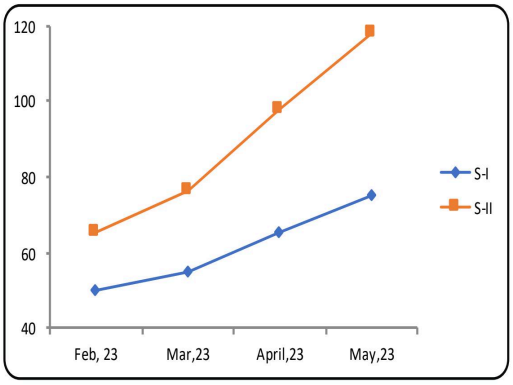

Fig 1g: variation of COD

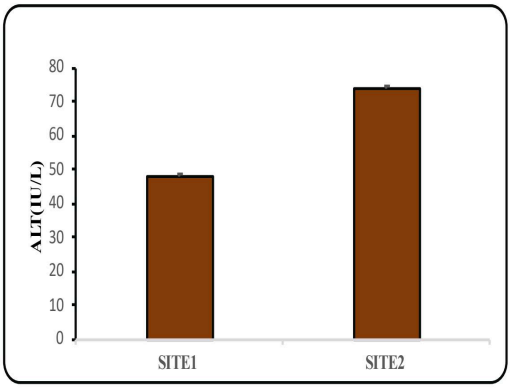

Fig 2a: : Activity of ALT(Alanine amino transferase) in blood of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean ± SE.

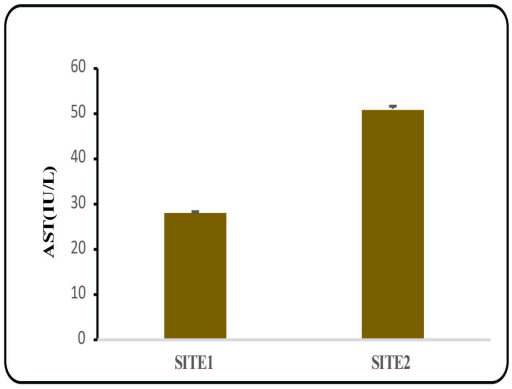

Fig 2b: : Activity of AST(Aspartate amino transferase) in blood of*Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean ± SE.

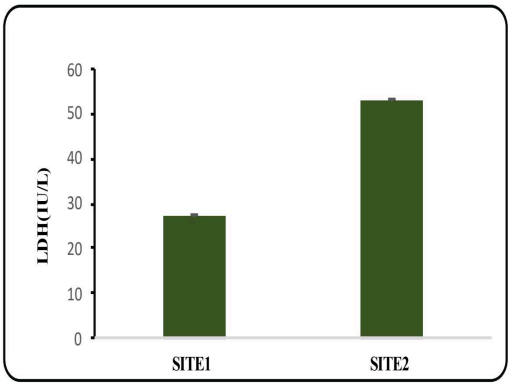

Fig 2c: : Activity of LDH(Lactate dehydrogenase) in blood of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean ± SE.

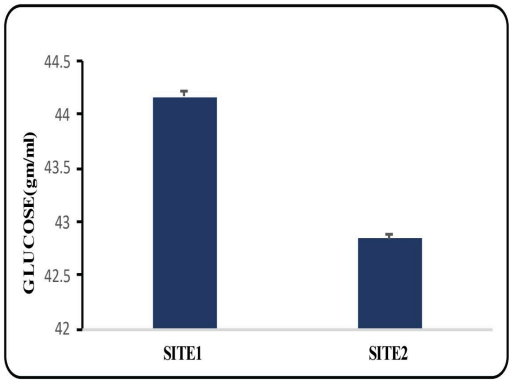

Fig 2d: : Amount of Glucose in blood of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean ± SE.

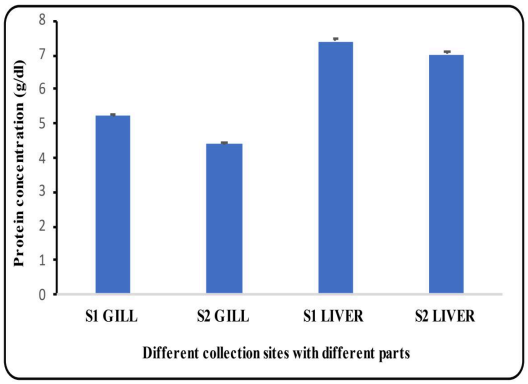

Fig.3: Estimation of protein value by Lowry et al.,1957. The value in the bar diagram denoted mean ± SE.

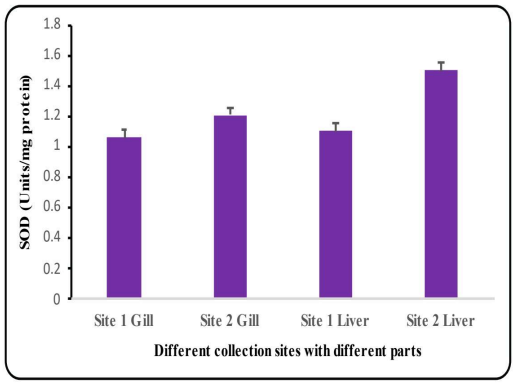

Fig 4a: Activity of SOD in gill and liver of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean± SE.

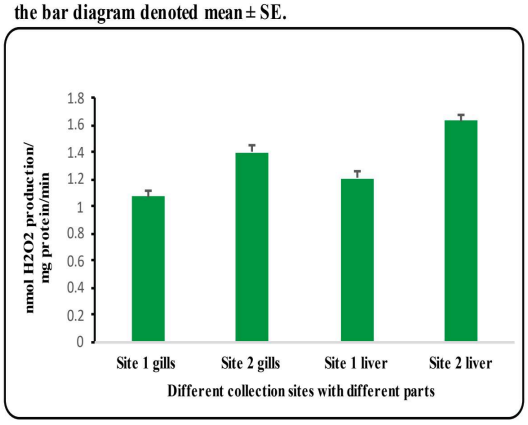

Fig 4b: Activity of CAT in gill and liver of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean± SE.

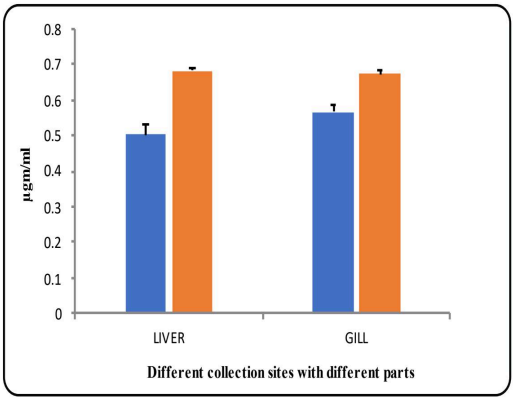

Fig 4c: Activity of MDA in gill and liver of *Labeo bata* of S-1 and S-2. The value in the bar diagram denoted mean± SE.

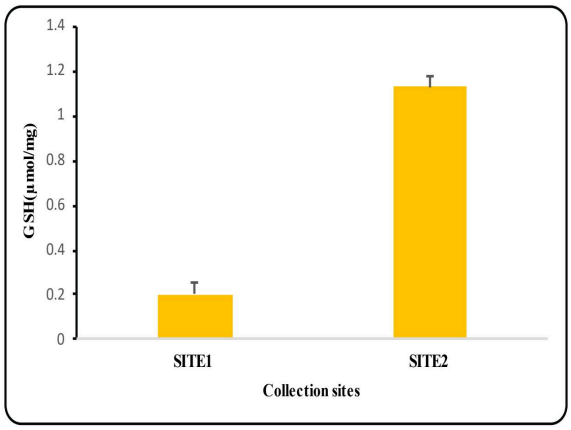

Fig. 4d: Amount of GSH in gill and liver of *Labeo bata* of site-1 and site2. The value in the bar diagram denoted mean ± SE.

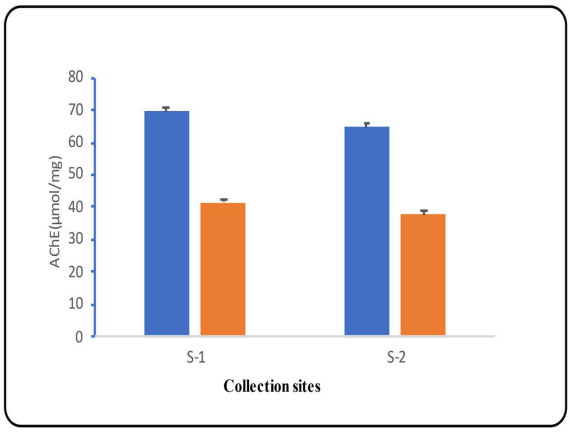

Fig.4e : Enzyme activity of Acetyl choline esterase (AChE) in brains of Labeo bata of S-1& S-2. The value in the bar diagram denoted mean ± SE.

Chapter-6: Discussion

Discussion

The present ecological study of two water bodies has shown distinct seasonal dynamics of abiotic parameters. The maintenance of the balance in the different physico-chemical parameters comes into play by the involvement of several organisms residing in that habitat. Pollution has been increased day by day with the increasing population, the agricultural activity, pest control activity, gardening activity etc. When pesticides are mixed in water bodies by drainage, rain fall, and other sources, it causes serious and harmful damages in higher organisms. Majority of pesticides are lipophilic in nature cannot dissolve in water. So, they enter into the aquatic organisms directly by oral apertures, gills and dermal contact. Those pesticides con increase the production of ROS by the formation of free hydroxyl radicals which lead to lipid peroxidation. Thy can also modulate the activity of metabolic enzymes. Habitat of an animal is important as they fully dependent upon it (Parrino *et al.,* 2018). Hematological parameters act as biomarker of fish health status and help to monitor conditions like habitat change, nutritional changes and environmental changes (Gabriel *et al.,* 2004). The present results are in agreement with the findings of Jee et al. (2005) who found that an increase in activities of serum ALT, AST, and LDH in Korean rockfish (Sebastes schlegeli) exposed to cypermethrin. Exposure to cypermethrin produced a significant increase in the activities of serum ALP and LDH in fish Rhamdia quelen (Borges *et al.*, 2007) and Labeo rohita (Das and Mukherjee 2003). Poor ability to utilize dietary glucose might be caused by low hexokinase activity and lack of the inducible enzyme glucokinase. The possible inhibition of insulin release by somatostatins released due to high blood glucose concentration or relatively low number of insulin receptors in fish compared to mammals (Kamalam *et al.,* 2017).

In the present study an increase in superoxide dismutase activity was observed in S-II of *Labeo bata*. Similar result was reported by Farombi and Adelowo in 2008 that SOD activity was increased in liver and kidney of *Clarias gariepinus* treated with herbicide butachlor. SOD activity also found altered in muscle of common carp *Cyprinus carpio* treated with pesticide simazine (Stara *et al.,*2012). In *Channa striatus*, time dependent elevation of SOD activity is also observed (Desai et al., 2013) when they exposed to 2, 4-D pesticide. According to Hemalatha *et al.,* in 2016 reported sublethal effect of quinalphos on SOD activity of freshwater fish *Cyprinus carpio.* The present study an increase in catalase activity was observed in S-II fish of *Labeo bata*. Similar response was observed in liver

CAT activity of rainbow trout (*Oncorhynchus mykiss*) exposed to sublethal concentration of carbosulphan (25 µg L-1) for a period of 60 days. Several studies indicated that organophosphates induce lipid peroxidation, and what follows—acute tubular necrosis, which accompanies organophosphate toxicity, is connected with ROS generation and lipid peroxidation process (Poovala *et al.,* 1998). Increase in catalase activity suggests the higher occurrence of H_2O_2 which definitely suggest the oxidative stress is occurring in S-II fish than S-I fish. In the present study, increasing rate of SOD (Fig:4a) and catalase (Fig:4b) activity favored the inactivation of hydrogen peroxide and superoxide anion radicals.

Organophosphates caused a significant increase in MDA (an end product of lipid peroxidation) level (Mashali *et al.,* 2005). The increased of MDA level (Fig 4c) serves as an index of peroxidative damage in different tissues. Glutathione is regarded as the cellular protectant. In the cases of GSH increased, cellular system experiences oxidative stress. GSH level has been found less in the tissue of S-II fish than S-I fish. Due to the increased of GSH (Fig 4d) restrict its utility in other protective metabolic processes. This increase in GSH utilization, produced oxidized glutathione (GSSG) and induced GR activity for reduced equivalents and normal redox homeostasis in the living cells (Oliva et al., 2010).

Generally, the results of SGPT, SGOT, LDH and Glucose (Fig:2a,2b,2c,2d) may indicate degeneration changes and hypofunction of liver as the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes are released from the cells into the blood serum. Therefore, increases in these enzyme activities in serum is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of liver damage by pesticide and metals, which gives an indication of the hepatotoxic effect of toxicants (Harvey *et al.,* 1994). The decrease in total proteins could be attributed in part to the damaging effects of pesticide on liver cells as confirmed by the increase in the activities of serum SGPT and SGOT observed in this study. LDH considered as a useful biomarker to determine the health status of the fish and pollution level of aquatic system. The elevation of LDH level indicated anaerobic metabolism of pesticide treated fish and thus the aerobic oxidation through the Krebs cycle was adversely affected due to cumulative toxic effect of cypermethrin (Das and Mukherjee 2003).

AChE is a stress indicator in all types of pollutants in aquatic systems (Das, 1998). Brzezinski and Ludwicki (1973) proposed that inhibition of AChE is accompanied by an increase in acetylcholine levels. This condition can lead to increase of catecholamines, which can affect the activity of enzymes involved in glycogenolysis and glycogen synthesis. Thus, increase in the levels of catecholamine may produce hyperglycemic condition as was also observed in the present study amply reflected by increase in blood glucose level in the exposed animals (Fig 4e).

Chapter-7: Conclusions and expected outcome

Conclusion:

Exposure of aquatic as well as terrestrial organisms to pesticides for the long term means an incessant health risk for the inhabitants. So, directly and indirectly, human populace is at elevated risk by consuming the toxicities fish species. This clearly reveals that the individual should take the required preventative measure in the application of pesticides to guard the fish population and also to other aquatic fauna.

Thus, it is likely that numerous methods based on molecular biology techniques will update toxicological applications, making them more affordable and removing the need for animals to identify ecological stresses. Researchers have identified several different effects of pesticide toxicity in fish species, including oxidative damage, the AchE movement's resistance, changes in histopathology, embryonic and developmental changes, carcinogenicity, and mutagenesis. These effects may occur at different levels when exposed to pesticides chronically.

Due to pesticide use and the unfavorable effects on non-target aquatic organisms, such as fish species, it is essential to develop strict laws and regulations that forbid the random application of this pesticide. Additive reactions to organophosphate compounds may have hazardous or deadly consequences in fish species because pesticides in the environment contain some other harmful substance component, i.e., compounds of organophosphate.

Therefore, it is crucial to regularly monitor the level of pesticide residues in food sources and to keep an eye on people in order to gauge how much pesticide exposure each person has received. To determine the concentration and exposure time of these pesticides as well as to significantly cause lethal and sub-lethal effects on the organisms, more experimental work should be done.

From the experimental results of different enzyme assays and it has been cleared that animals which were already located in the SII are adapted to stressful condition for a long time. So their antioxidant system is more active than the antioxidant system of animals from control Site. As the SII site is congested water body, there are no outlets and run off. At the summer time when the water body continuing drying, water become more alkaline and dissolved oxygen was continuing decreased. Animals in this site are facing stress than Site I. The blood levels of SGOT, SGPT and may increases as due to the cellular damage

in the liver and that high levels of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals. From the observations of present study, it can be concluded that the response of antioxidant enzyme (SOD) confirms that the fishes are under severe oxidative stress. The enzymatic and the non-enzymatic antioxidant machinery are interacting in a concentrated manner to eliminate ROS and prevent damage to cellular components. This suggests that Methanol at lethal and sublethal levels is capable of causing oxidative damage in *labeo bata*.

Chapter-8: Future Scope

FUTURE SCOPE

- Through this project work increased public awareness about the impact of pesticides on freshwater fishes can drive positive change. Education programs, outreach initiatives, and community engagement efforts can raise awareness among farmers, stakeholders, and the general public about the importance of responsible pesticide use and its potential consequences on aquatic ecosystem.
- Implementing effective regulations, adopting sustainable practices, and promoting awareness, we can work towards minimizing pesticide impacts and safeguarding the health of freshwater ecosystems and fish populations.

Chapter-9: References

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Thank you