SUB-LETHAL EFFECTS OF CHRONIC PARAQUAT EXPOSURE ON BIOCHEMICAL ALTERATIONSIN INDIAN MAJOR CARP *CIRRHINUS MRIGALA HAMILTON*

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Abstract
Excess eutrophication of aquacultural ecosystems has been a prolonged threat to the fishing industry worldwide. Strategies like manual and mechanical weed removal from eutrophic ponds are costly and ineffective, particularly for larger complicated ecosystems. So the use of effectual herbicides and algaecides has become popular among aquaculturistsas a relatively easy and cost-effective method to minimize eutrophication. Paraquat has been used as a popular aquatic herbicideacross the globe. When utilized in the aquatic environmentParaquat exhibits a high affinity toward the hydrosoil and bottom sediments. Once absorbed at the bottom, Paraquat persists there for longer periods. Hence the study of the effect of Paraquat on a bottom feeder species was important to reveal baseline data.*Cirrhinus mrigala* is an economically important freshwater Indian Major Carp, extensively reared as a bottom feeder in integrated fish farming throughout Southern Asia. As a benthic feeder, this species is highly susceptible to being exposed to Paraquat residues persisting at the bottom of fish ponds. Hence it was chosen as a test animal.The present experiment was designed to study the effects of sub-lethal concentrations of chronic Paraquat exposure on total protein, total lipid, and total glycogen contents in fish*Cirrhinus mrigala*.During the experiment, the fishesin different sets were exposed to $1/20^{th}$ and $1/10^{th}$ concentration of the Median Lethal Concentration (LC_{50}) along with their respective control groups for 30 days using static renewal bioassay apparatus. Post-exposure, vital tissues like gill, muscle, liver, and brain were pooled out separately from live fish and the total protein content was measured by Lowry's method (1951), total lipid content was measured by Barnes and Blackstock method (1973), and total glycogen content was measured by Dezwaan and Zandee's method (1972). Annotated results represented that, the total protein content in the $1/20^{th}$ group showed a significant ($p<0.05$) decrease in the gill and brain tissue while in the $1/10^{th}$ group it showed a moderately significant ($p<0.01$) decrease in the muscle, liver and brain tissue and significant ($p<0.05$) decrease in the gill tissue. The total lipid content in the $1/20^{th}$ group showed a moderately significant ($p<0.01$) decrease in the gill tissue and a significant ($p<0.05$) decrease in the liver and brain tissue while in the $1/10^{th}$ group gill and liver showed moderately significant ($p<0.01$) decrease and the brain tissue showed significant ($p<0.05$) decrease. The total glycogen content in the $1/20^{th}$ group showed a moderately significant ($p<0.01$) decrease in muscle tissue while a significant ($p<0.05$) decrease in gill and liver tissue while in the$1/10^{th}$group it showed a highly significant ($p<0.001$) decrease in muscle tissue, moderately significant ($p<0.01$) decrease in the gill and brain tissues and significant ($p<0.05$) decrease in liver tissue. All activities were compared with their respective control groups to derive the level of significance of the decreased content. In conclusion, alterations caused in the biochemical parameters of fish due to sub-lethal exposure to Paraquat suggest Paraquat as a potential Eco-toxic agent causing severe environmental pollution. The decrease in the level of protein, lipid, and glycogen content can be directly linked with the overall reduction in the weight of marketable fish causing economic losses to fish farmers.

Keywords: Paraquat Dichloride, *Cirrhinus mrigala*, Chronic Toxicity, Biochemical Alterations, Environmental Pollution.

1. Introduction

A recent study conducted by Rivas et.al. (2020) revealed the emergence ofeutrophication as the most common and ubiquitous environmental problem in inland waters. Their study also explained how anthropogenic activities drastically increase the impact, rate, and scope of eutrophication. Beem et.al. (2017) in their study observed that in intensive fish production adding a huge amount of inorganic fertilizers and commercial feeds into the ponds is a very common practice. They concluded from their study that such nutrient-enriched waters often cause eutrophication thus favoring a habitat for extensive aquatic weed growth. Such eutrophication leads to the formation of algal blooms, fish kills, and dead zones (NOAA 2017). Physical and mechanical de-weeding of such ponds is expensive and labor-intensive (Everest and Bayne 2004). Thus for quick and economical weed control, water resource managers popularly use aquatic herbicides in commercial fish ponds (Masser et.al. 2015). But the improper application of herbicides can directly kill the fish (Lembi 2016). The herbicides that are not strong enough to kill the fish can weaken the fish through various modes (Haynk et.al. 2021).So, even though herbicide application can be effective in controlling invasive aquatic weeds, their harmful effects on non-targeted organisms are a topic of thorough evaluation to minimize their adverse effects on aquatic environments (Jiali et.al. 2018). Paraquat is one such extensively used aquatic herbicide. Paraquat Dichloride (1,1'-Dimethyl-4,4'-bipyridinium dichloride) (CAS No = 75-305-73-0) is a highly-toxic, non-selective and second-highest selling herbicide all over world used on a broad-spectrum

of weeds (Conning et.al. 1969, Tomlin 2003, Kanchan et.al. 2015, Arts et.al. 2006).Excess use of Paraquat has caused far-spread residues of it in water as well as land ecosystems which ultimately getintroduced into our food chains (Pateiro-Moure et.al. 2009). Reports illustrating the presence of Paraquat in water bodies across the globe are already present (Ismail et.al. 2011). Such presence of pesticides in non-targeted environments is due to spray drift, leaching, direct application, run-offs, and sewage/factory discharges (Katagi 2010). Paraquat is very persistent in river waters (Wang 1994). Its sediment half-life is in the range of 2 to 820 years depending on various environmental factors (Fernandez et.al. 1998).

Various reports reveal Paraquat's damage to both water and land ecosystems leads to severe acute and chronic poisoning in non-targeted organisms (Moore et.al. 2007, Babatunde et.al. 2001, Leboulanger et.al. 2009, Muangphra et.al. 2014).Effect of Paraquat in two of the Indian Major Carps (IMC)*Catlacatla*(top-feeder)(Bashiniet.al. 2019) and*Labeorohita* (column-feeder) (Bashini and Senthilkumaar 2018)have been studied, but studies related to the bottom-feeder IMC *Cirrhinus mrigala*were scanty. Thus *Cirrhinus mrigala* was chosen as a test animal in this experiment. Report(Cheah et.al. 1998) illustratingthe property of Paraquat to settle down in bottom sediments makes the choice of a bottom feeder fish for this study more relevant.The current investigation was designed to illustrate the sub-lethal effects of Paraquat on biochemical alterations like total protein, total lipid, and total glycogen after chronic exposure of30 days in the test fish. This study is important as a baseline data for non-targeted toxicity and environmental pollution caused due to Paraquat in the bottom-feeder fishes useful to assess environmental risks. This data can also be helpful in comparative analysis of the toxic behavior of Paraquat across different water columns. Alterations in protein, lipid, and glycogen inside the body of quantitatively marketable products like fish, can be directly related to alterations in the total weight of fishcausing economic losses to fish farmers.In an *insitu* study byKathiresan andDeivasigamani (2015) to trace the impact of some herbicides used to control aquatic weeds and their non-targeted effects in fishdemonstrated relatively highest mass mortality (around 50.6%) in *Cirrhinus mrigala*fish with extensive tissue damage in multiple organs 32 days after spraying.Thus the role of the present study in, effectively highlighting the alterations in protein, lipid, and glycogen that actually takes place during such type of mass mortality cases of mrigal carp will also exemplify them as distress indicators in aquacultural farms.

Fishes are valuable sources of high-grade proteins and fats (FAO 2020). Measurement of total protein content is one of the best tools for the analysis of overall animal physiology (Kapila and Ragathaman 1999).Thus any toxicant-induced alteration in total protein will indicatephysiological stress that can lead to stunted growth or in extreme cases fish mortality. In fishes, the metabolism of lipids is a vital life strategy for development and regeneration (Sheridan and Kittlison 2004). Thus alterations in total lipid levels will indicate a threat to vital processes which can lead to fish mortality. Utilization of stored glycogen indicates the use of energy reserves to compensate for any immediate energy needs (Preiss and Walsh 1981).Thus it can act as the best indicator of stress induced by the toxicant that mobilizes glycogen reserves to generate extra energy required to maintain internal homeostasis.

2. Materials and Methods:

2.1. Obtainment and naturalizationof experimental animals to laboratory conditions.

Healthy fish fingerlings (weight 3.47±0.72 gm. and length 4.41±0.06 cm)were obtained fromthe Government Fish seed Production center, Dhom (Wai), Satara District, Maharashtra State, India. They were transported to the laboratory in jumbo size polythene bags filled with adequate oxygen. Before acclimatization at the laboratory, fishes were disinfected with 0.1% $KmNO_4$ solution and then transferred to glass aquariumswith tap water and accessedwith adequate aeration supportfor acclimatization. With natural photoperiod and normal room, temperature fishes were acclimatizedfor 15 days. During the entire experiment,'Taiyo Discovery commercial fish food' was fed to fish at a 2% rate of their total body weight. Theaquarium water was recycled with fresh tap water every 24 hours to keep it free from fecal matter and food debris. The quality of aquarium water was checked weekly to ensure normal conditions. Decreased or diseased fish were immediately removed and discarded from the experiment. The healthy fishes with good reflexes were finally selected for the study. The selected fish were re-acclimatized into 22-liter capacity transparent plastic containers with adequate aeration support for 7 days. It was these containers in which the fish were to be exposed to the experimental toxicant. 24 hours prior to the test, feeding was ceased to minimize the toxicity of excreta and vomiting in fishes due to the effect of the toxicant.

2.2. Experimental setup:

Chronic Exposure to Paraquat for Biochemical Studies:

Total three sets of experiments viz. total protein, total lipid, and total glycogen were setup simultaneously yet differently but the framework design for all the three experiments were similar. For each setup, the framework design consists of three separate plastic containers, as mentioned above, of 22 liter capacity with adequate aeration supply. Each container was filled with 20-liter tap water. 10 fishwere released in each one of them.All sets were continued for 30 days. In the first of the three containers, fishes were not exposed to any toxicant. So all of them served as control groups for their respective setups. In the second of the three containers, fishes were exposed to $1/20^{th}$(5.25 ppm)concentration of the LC_{50}(105 ppm) dose. In the third container among the three, the fishes were exposed to $1/10^{th}$(10.5 ppm) concentration of the LC_{50} (105 ppm) dose. After every 24 hrs.entire water and toxicant from the sets were replaced with fresh water and toxicant to sustain the optimum toxicity of toxicant throughout the experiment.Dead fishes were discarded immediately. After 30 days live fish from all the containers were sacrificed and their vital organs like gill, muscle, brain,and liver were pooled out separately. Biochemical alterations were studied in all the tissues separately.

2.3. Final analysis of total protein content:

The estimation of total protein was done with Lowry's method (Lowry et.al. 1951). Initially, a standard protein graph was derived by using Bovine Serum Albumin (BSA) as a standard protein.Then, 100 mg of fresh tissue extract was taken in a pre-chilled mortar and pestle and10% Trichloroacetic acid (TCA)was added in it to prepare the homogenate.Further, the homogenate was centrifuged at 10,000g for 15 minutes. The supernatant was discarded and the precipitate was treated with 10 ml of 1N NaOHfor complete protein dissolution. 0.1 ml (1 mg tissue) of this sample was taken in 3 different test tubes (triplicate) and made up to a volume of 10 ml using distilled water.Then 5 ml of Lowry's C solution (Lowry's A + Lowry's B) was added to all the test tubes and allowed to stand still for 10 minutes. Further 0.5 ml of Lowry's D solution (Folin-Catalteu reagent) was added to all the test tubes and all the test tubes were incubated in dark for 30 minutes. Simultaneously, for the preparation of a blank 10 ml of distilled water was added with 5ml of Lowry's C solutionand 0.5 ml of Lowry's D solution followed by 30 minutes of dark incubation. After 30 minutes of dark incubation sample absorbance values were measured at a wavelength of 660nm against the blank by a spectrophotometer. The protein concentration in each sample was obtained from its respective absorbance by referring to both of its synchronization on the standard graph.

2.4. Final analysis of total lipid content:

The estimation of total lipids was done with Barnes and BlackstockPhosphovanillin method (Barnes and Blackstock1973). Initially, a standard lipid graph was derived by using Cholesterol ($C_{27}H_{46}O$) as a standard.Then, 100 mg of fresh tissue extract was taken in a pre-chilled glass homogenizer and 1 ml of chloroform-methanol (Folch's) mixture (2:1) was added to it, to prepare the homogenate. Once homogenized, a 0.2ml of 0.9% sodium chloride solution was added to the homogenate and shaken well to cease the binding of acidic lipids to lipids. This entire solution was then transferred to a separating funnel and allowed to stand still overnight at 40^0C.The next morning a clear biphasic layer was formed in the funnel with lipids in the lower phase. This lower phase was extracted into a separate test tube and 99.5% chloroform was added to it to make up the solution of 10ml. This 10ml mixture was the actual stock sample. 0.1 ml of this stock solution was taken in three separate test tubes (triplicate) and was kept for drying at 40^OC for 2 hours. Thereafter 0.5 ml of Conc. $H_2SO_4$was added to all the 3 testtubes and mixed thoroughly. To acid-digest the sample, all test tubes were corked with non-absorbent cotton wool and boiled in a water bath for 10 minutes followed by cooling of test tubes at room temperature. Finally, 5 ml of phosphovanillin reagent was added to the sample, mixed well, and allowed to stand motionless for half an hour. Simultaneously blank was also prepared using0.1 ml chloroform added with 0.5 ml conc. H_2SO_4 and 5 ml phosphovanillin reagent followed by water bath and cooling protocols similar to the test samples. After half an hour the absorbanceswere measured at 530nm against the blank by using a spectrophotometer. The lipid concentration in all the tissues was derived by comparing their respective absorbance with reference to the standard graph.

2.5. Final Analysis of the total glycogen content:

The estimation of total glycogen content was done by Dezwaan and ZandeeAnthrone Method (Dezwaan and Zandee 1973). Initially, a glycogen graph was derived using glucose as a standard ofglycogen concentration. Then 100 mg of fresh tissue extract was taken in a pre-chilled mortar and pestle and 1ml of30% KOH was added in it to prepare the homogenate. Then 2 ml of ethanol (96%) was added to it with Sodium sulfateas a co-precipitant. Further, this mixture was given a water bath at boiling temperature for a couple of minutes and cooled in an ice bath for 1 hour. The derived product was further centrifugedat 10,000 g for 20 minutes and the precipitate so obtained was mixed with 10 ml distilled water and incubated for 20 minutes at 90^0C to obtain the final sample.0.1 ml (1 mg tissue) of this final sample was taken in 3 different test tubes (triplicate) and distilled water was added to it to make up a volume of 10 ml. Then 5 ml of Anthrone's reagent was added to all the test tubes. Further, all test tubes were corked with cotton plugs and warmed in a water bath for 10 minutes. Finally, all test tubes were cooled down at room temperature and their absorbance values were measured at 620 nm against the blank by using a spectrophotometer. The glycogen concentration in all the tissues was derived by comparing their respective absorbance with reference to the standard graph.

The final data from all three sets of experiments were expressed in the form of arithmetic mean ± standard deviation. The level of significance was derived using the students' T-Test with two-tailed distribution in the two-sample unequal variance (heteroscedastic) method.

3. RESULTS:

ThePhysico-chemical parameters ofwater were recordedusing standard methods in APHA (1998) as follows: Temperature 26.8ºC, pH 6.8-7.1, Dissolved oxygen 5.27-6.62 mg/L, Dissolved Carbon dioxide 15.01±0.16 mg/L, hardness 128.1±3.91 mg/L, phosphates 0.5±0.33 mg/L, nitrates 1.04±0.18 mg/L.

The $1/20^{th}$ and $1/10^{th}$ concentration values derived for the present experiment are 5.25ppm and 10.5 ppm respectively.

The results of the effect of Paraquat dichloride in gill, liver, muscle, and brain tissues of *Cirrhinus mrigala* in the control group, $1/20^{th}$ group, $1/10^{th}$ group after chronic exposure (30 days) in total protein content, total lipid content, and total glycogen content are illustrated in their respective **tables and graphs** as follows:

3.1. 30 days of chronic exposure total protein content results:

The activity recorded in the gill tissue of the control group was 115.25±4.35µg protein/mg tissue. However, in the1/20th concentration group fish, gill exhibited 103.5±3.63 µg protein/mg tissue while in the 1/10th concentration group it exhibited 100.5±7.36 µg protein/mg tissue.

The activity recorded in muscle tissue of the control group was 203.75±3.68 µg protein/mg tissue. However, in the1/20th concentration group, fish muscle exhibited 192.25±6.53µg protein/mg tissue while in the 1/10th concentration group it exhibited 178.75±1.32µg protein/mg tissue.

The activity recorded in the liver tissue of the control group was 142±10 µg protein/mg tissue.However, in the 1/20th concentration group, fish liver exhibited 121.75±2µg protein/mg tissuewhile in the 1/10th concentration group it exhibited 108.66±3.55µg protein/mg tissue.

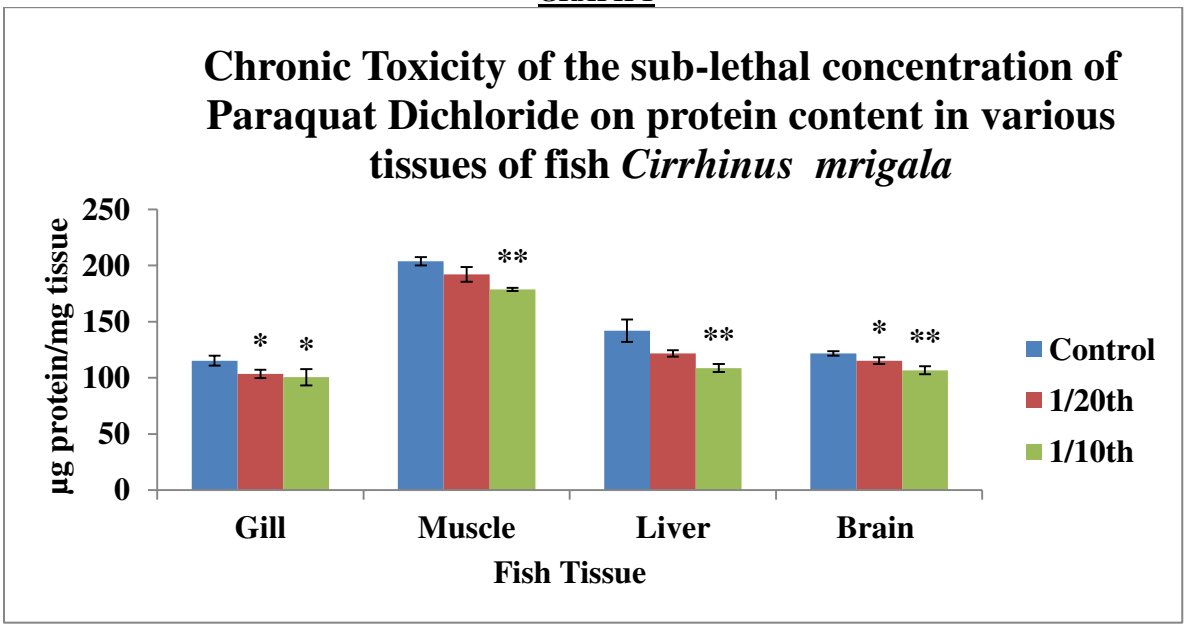
The activity recorded in the brain tissue of the control group was 121.75±1.98µg protein/mg tissue. However, in the 1/20th concentration group, the fish brain exhibited 115.25±3µg protein/mg tissue.While in the 1/10th concentration group it exhibited 106.75±3.5µg protein/mg tissue.

TABLE 1 Effect of Paraquat Dichloride on total protein content in different tissues of the fish *Cirrhinus mrigala* after chronic exposure.

Groups	Total Protein Content (µg protein / mg tissue)			
	Gill	Muscle	Liver	Brain
Control Group	115.25±4.35	203.75±3.68	142±10	121.75±1.98
1/20th	103.5±3.63 *	192.25±6.53	121.75±2.78	115.25±3 *
1/10th	100.5±7.36 *	178.75±1.32 **	108.66±3.55 **	106.75±3.5 **

(Values expressed as Arithmetic Mean of (n=3); ±SD),
***= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)**

GRAPH 1



Toxic Effect of the sub-lethal concentration of Paraquat Dichloride on total protein content in Gill, Muscle, Liver, and Brain tissues of the fish *Cirrhinus mrigala* after chronic exposure (30 days). Data expressed in arithmetic mean± Standard Deviation. Error bars represent the SD of 3 individual observations. * indicates p<0.05(significant), ** indicates p<0.01(moderately significant), * indicates p<0.001(highly significant)**

The total protein content in the 1/20th group showed a significant (p<0.05) decrease in both gills (t.stat- 3.58) and brain (t.stat- 3.13) as compared to the control group, while in the 1/10th group it showed a moderately significant (p<0.01) decrease in the muscle (t.stat- 11.06), liver (t.stat- 5.43), and brain (t.stat- 6.45) tissues while in the gill (t.stat-2.98) tissue it showed a significant (p<0.05) decrease as compared to the control group. The post-experimental total protein content in all the four tested tissues was in the order Muscle > Liver > Brain > Gill in the 1/20th concentration group while in the 1/10th group it was in the order, Muscle > Liver > Brain> Gill.

30 days of chronic exposure total lipid content results:

The activity recorded in the gill tissue of the control group was 114.12±3.29 µg lipid/mg tissue. However, in the1/20th concentration group fish gill exhibited 94.08±4.01µglipid/mg tissue while in the 1/10th concentration group it exhibited 84.6±7.22µglipid/mg tissue.

The activity recorded in muscle tissue of the control group was 164.88±11.37µglipid/mg tissue. However, in the1/20th concentration group, fish muscle exhibited 157.32±12.84µglipid/mg tissue while in the 1/10th concentration group it exhibited 155.16±8.38µglipid/mg tissue.

The activity recorded in the liver tissue of the control group was 227.16±13.73µglipid/mg tissue.However, in the 1/20th concentration group, fish liver exhibited 196.56±10.62µglipid/mg tissue. While in the 1/10th concentration group it exhibited 179.04±4.69µglipid/mg tissue.

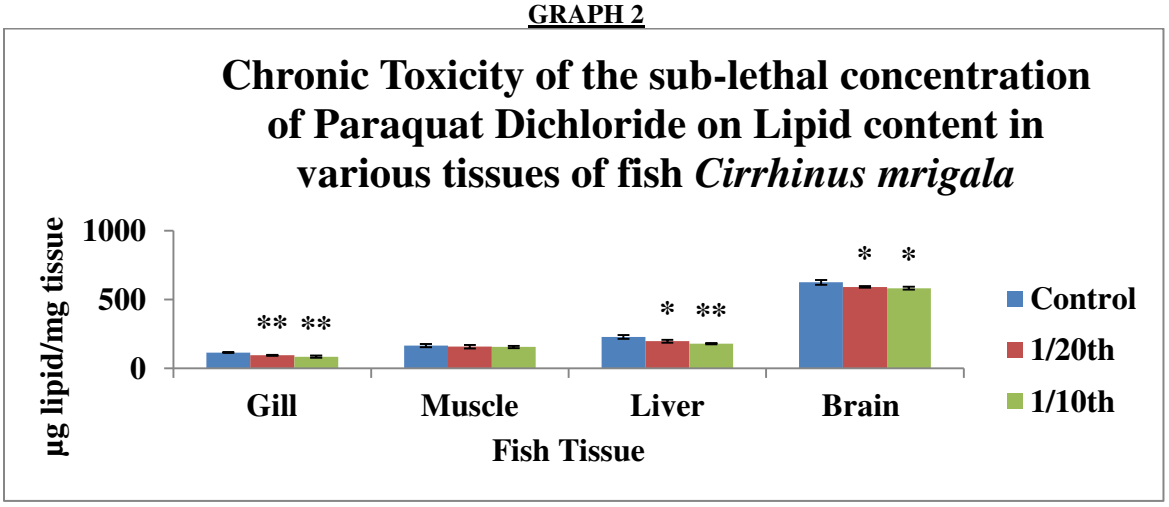
The activity recorded in the brain tissue of the control group was 624.6±16.57µglipid/mg tissue. However, in the 1/20th concentration group, the fish brain exhibited 591.12±5.92µglipid/mg tissue. While in the 1/10th concentration group it exhibited 582.48±10.84 µglipid/mg tissue.

TABLE 2 Effect of Paraquat Dichloride on totallipidcontent in different tissues of the fish *Cirrhinus mrigala* after chronic exposure.

Groups	Total lipid Content (µglipid / mg tissue)			
	Gill	Muscle	Liver	Brain
Control Group	114.12±3.29	164.88±11.37	227.16±13.73	624.6±16.57
1/20th	94.08±4.01 *	157.32±12.84	196.56±10.62	591.12±5.92 *
1/10th	84.6±7.22 *	155.16±8.38 **	179.04±4.69 **	582.48±10.84 **

(Values expressed as Arithmetic Mean of (n=3); ±SD),
***= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)**

GRAPH 2



Toxic Effect of the sub-lethal concentration of Paraquat Dichloride on totallipid content in Gill, Muscle, Liver, and Brain tissues of the fish *Cirrhinus mrigala* after chronic exposure (30 days). Data expressed in arithmetic mean± Standard Deviation.

Error bars represent the SD of 3 individual observations. * indicates p<0.05(significant), ** indicates p<0.01(moderately significant), * indicates p<0.001(highly significant).**

The total lipid content in the 1/20th group showed a moderately significant (p<0.01) decrease in gill (t.stat- 6.68) tissue and a significant (p<0.05) decrease in the liver (t.stat- 3.05) and brain (t.stat-3.29) as compared to control group, while in the 1/10th group it showed a moderately significant (p<0.01) decrease in the gill (t.stat- 6.43) and liver (t.stat- 5.74) tissues while in the brain (t.stat-3.67) tissue it showed a significant (p<0.05) decrease as compared to the control group. The post-experimental total lipid content in all the four tested tissues was in the order Brain >Liver> Muscle >Gill in the 1/20th concentration group while in the 1/10th group it was in the order, Brain >Liver> Muscle > Gill.

3.3 30 days of chronic exposure total glycogen content results:

The activity recorded in the gill tissue of the control group was 7.8±0.89µgglycogen/mg tissue. However, in the1/20th concentration group fish gill exhibited 5.2±0.43µgglycogen/mg tissue while in the 1/10th concentration group it exhibited 2.92±0.22µgglycogen/mg tissue.

The activity recorded in muscle tissue of the control group was 8.12±0.18µgglycogen/mg tissue. However, in the1/20th concentration group, fish muscle exhibited 7.27±0.29µgglycogen/mg tissue while in the 1/10th concentration group it exhibited 5.56±0.07µgglycogen/mg tissue.

The activity recorded in the liver tissue of the control group was 43.34±6.18µgglycogen/mg tissue.However, in the 1/20th concentration group, fish liver exhibited 24.13±5.16µgglycogen/mg tissue. While in the 1/10th concentration group it exhibited 22.08±6.76µgglycogen/mg tissue.

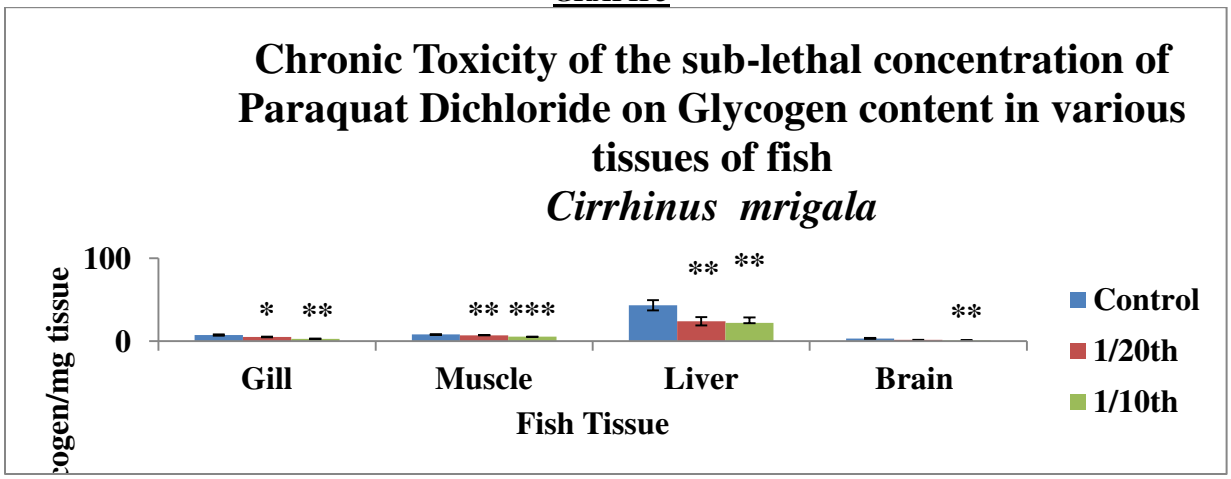
The activity recorded in the brain tissue of the control group was 3.3±0.53µgglycogen/mg tissue. However, in the 1/20th concentration group, the fish brain exhibited 1.85±0.03µg glycogen/mg tissue. While in the 1/10th concentration group it exhibited 0.8±0.33 µgglycogen/mg tissue.

TABLE 3 Effect of Paraquat Dichloride on totalglycogencontent in different tissues of the fish *Cirrhinus mrigala* after chronic exposure.

Groups	Total glycogen Content (µgglycogen / mg tissue)			
	Gill	Muscle	Liver	Brain
Control Group	7.8±0.89	8.12±0.18	43.34±6.18	3.3±0.53
1/20th	5.2±0.43 *	7.27±0.29 **	24.13±5.16 **	1.85±0.03
1/10th	2.92±0.22 **	5.56±0.07 ***	22.08±6.76 **	0.8±0.33 **

(Values expressed as Arithmetic Mean of (n=3); ±SD),
***= p<0.05(significant), **= p<0.01(moderately significant), ***= p<0.001(highly significant)**

GRAPH 3



Toxic Effect of the sub-lethal concentration of Paraquat Dichloride on totalglycogen content in Gill, Muscle, Liver, and Brain tissues of the fish *Cirrhinus mrigala* after chronic exposure (30 days). Data expressed in arithmetic mean± Standard Deviation. Error bars represent the SD of 3 individual observations. * indicates p<0.05(significant), ** indicates p<0.01(moderately significant), * indicates p<0.001(highly significant).**

The total glycogen content in the 1/20th group showed a moderately significant ($p<0.01$) decrease in muscle (t.stat- 22.62) and liver (t.stat-4.12) tissue and a significant ($p<0.05$) decrease in the gill tissue(t.stat- 3.94) as compared to control group, while in the 1/10th group it showed a highly significant ($p<0.001$) decrease in the muscle (t.stat- 13.85) while a moderately significant ($p<0.01$) decrease was seen in the gill(t.stat-8.53), liver(t.stat-4.01), and brain(t.stat- 6.90), tissue as compared to the control group. The post-experimental total glycogen content in all the four tested tissues was in the orderLiver> Muscle > Gill > Brainin the 1/20th concentration group while in the 1/10th group it was in the order, Liver> Muscle > Gill> Brain.

DISCUSSION:

Most chemical pesticides function as metabolic depressors causing anthropogenic pressure on bio-active molecules like protein, lipids, and glycogen (Agrahari and Gopal 2009).

Protein content analysis reveals the underlying condition of internal cells and tissues hence itis a reliablediagnostic tool for determining the health and physiological status of an organism(Manoj 1999). Proteins are good buffering and efficient protective agents, they are also good sources of nutrition and energy (Inyanget.al. 2010).Paraquat induces the formation of excessive Reactive Oxygen Species(ROS) due to redox cycling and cellular NADPH oxidation(Suntres 2002). These ROScauseexcessoxidative stress at cellular as well as molecular levels leading to enormous damage.Protein also serves as an alternative energy source during stress (Magdy et.al. 1993). In the present study protein reserves in the fish body might be utilized to compensate for the stress caused by Paraquat toxicity. Besidesthe effect of the toxicant that leads toInhibition of protein synthesis, damage to protein metabolism, changes in homeostasis balance, anddisturbances in cell building architecture that ultimately cause a decrease in total protein content have been demonstrated in many previous studies(Das and Mukherjee 2000, Gluth and Hanke 1984, Singh and Khare 1999, Desai 2002, Singh et.al. 2010). An increase in the breakdown of proteins is a functional adaptive response to deal with the requirement of extra energy to compensate for homeostasis imbalance caused due to such toxicant stress (Reddy and Bhagyalaxmi 1994). Thus to meet such extra energy demand proteins are used as an alternate energy source (Umminger1977).Besidesthe toxicant injury caused to the tissue and impairment of its functions might cause degradation and utilization of proteins for metabolic purposes (Tiwari and Singh 2005, Birkener et.al. 2000, Grucka-Mamczar et.al. 2005). A similar decrease in total protein content was demonstrated by Ogamba et.al.(2011) in African catfish *Clarias gariepinus* exposed to sub-lethal doses of Paraquat dichloride.

Lipids are a diverse group of ubiquitous compounds that play key roles in cellular life processes, physiology, and pathology (Naudi et.al. 2015). In fish, the lipid is a crucial source of energy that undergo quick breakdown, re-synthesis, and rapid internal conversions in response to diverse condition(Chetty and Indira 1994).Cell membranes are formed by a fluidic lipid-bilayer (Bloom 1991). Peroxidation of lipious cell membranes is a key mechanism of Paraquat-induced toxicity. It triggers apoptosis, decreased production of glutathione, and transcription of inflammatory mediators that lead to aggregation of platelets, the attraction of inflammatory cells, and fibrogenesis (Gawarammana and Bukley 2011). ROS produced by Paraquathaphazardly attack cellular macromolecules including lipids disrupting the vital physiological cell processes,inducing stress, and causing severe damage (Suredaet.al. 2006,Tejada et.al. 2007).Side-chain oxidation and protein-lipid adduction are cumulative effects of excess oxidative stress that cause alterations in both lipids and proteins through direct as well as indirect pathways (Grimsard et.al. 2008, Davies (2016).In the present study decreased levels of lipid suggest the utilization of lipid reserves to fulfill the extra-energy demand required to neutralize such oxidative stress and to sustain internal homeostasis (Srinivasa and Ramanna 1989). The utility of lipids for the provision of extra water needed to maintain the osmotic concentration of body fluids is also a potential reason for decreased total lipid contents (Gopal et.al. 2008). Shivnandan and Binkumari (2021) demonstrated the effect of Malathion causing a decrease in total lipid content in freshwater fish *Labeorohita.*Bantu et.al. (2018) also demonstrated a similar decrease in total lipid content in snakehead fish *Channapunctatus* due to chronic exposure to the pesticidechlorantraniliprole.

Glycogen is an osmotically neutral branched polymer of glucose that isstored in cells during plenty of availability of nutrition so that it can be used in times of stress and need(Preiss and Walsh 1981). Stress has been observed as one of the primary factors that affect glycogen metabolism (Van 2009). He also observed that during stress glycogen in peripheral tissues decreases as a result of increased glycogenolysis as well as due to a reduction in glycogen synthesis.The toxicity of Paraquat is based on redox cycling and the generation of high intracellular oxidative stress (Dinis-Oliveria et.al. 2008). In the present study, decreased levels of total glycogen content in various tissues are an indication of the stressful situation caused due to Paraquat toxicity. The decreased glycogen was potentially utilized to compensate for the excess energy neededto get through oxidative stress induced by Paraquat. Paraquat also induces hypoxia (Xie et.al. 2013). During such hypoxic conditions, fish utilize anaerobic respiration to break down stored glycogen required to compensate for energy needs.Menezeset.al. (2015) demonstrated a similar decrease in total glycogen contents in various tissues of silver catfish exposed to herbicide2,4-Dichlorophenoxyacetic acid. Abbas et.al.(2007) also demonstrated a decrease in total glycogen content in various tissues of Nile Tilapia exposed to Thiobencarb herbicide.

Such biochemical alterations indicate stabilization of internal organ systems for activation of all detoxification mechanisms to survive in toxic environments. The subsequent breakdown of metabolites and synthesis of new products that areuseful in coping with altered states is a representation of the metabolic compensation involved. A decrease in vital body-building metabolites like total protein and energy storage metabolites like total lipid and total glycogen can be directly linked with a decrease in the overall marketable weight of fish leading to economic losses to fish farmers. These losses might seem little when considered for a single fish but if compounded for millions of tonnes of production that comes out of such fish farms and pesticide-affected water bodies, then

such losses can cause a highly significant economic loss to the entire industry at global level. Over it, the threat to the health of consumers is also an uncompromisable loss.The effect of Paraquat causing a loss in fish weight has already been demonstrated(All-Jafiri et.al. 2013). A study by Babatundeet.al. (2009) also showed a significant ($p<0.05$) decrease in the growth rate of commercially important cultured fish *O.niloticus*(Nile tilapia) exposed to sub-lethal concentrations of Paraquat. Thus both of these studies support the fact that the use of Paraquat as an aquatic herbicide can cause commercial losses directly by impacting the palatable weight of fish.

CONCLUSION:

The findings of the present study revealed that chronic sub-lethal exposureto aquatic herbicideParaquat dichloride can cause a significant decrease in the biochemicalparameters like total protein content, total lipid content, and total glycogen content in vital tissues offreshwater Indian Major Carp *Cirrhinus mrigala*. Such a prominentdecrease in vital biomolecules canbe directly linked with a decrease in the marketable weight of the fish causing economic loss to the fish farmers. Long-term consumption of pesticide-affected fish might have far-reaching effects on the health of consumers too. Thus this study contributes to baseline data about Paraquat's toxicity in bottom feeder fishes and represents *Cirrhinus mrigala* as good bio-indicators of environmental toxicity.

REFERENCES:

1. Rivas, G. Pérez, R.Tundisi, JVammen, K.Örmeci, B. Forde, M. (2020). Eutrophication: A growing problem in the Americas and the Caribbean. Brazilian Journal of Biology. 80:3.
2. Beem, M. Shelton, J. Murphy, T. (2017).Aquatic Weed Management Control Methods. Oklahoma State University Extension.
3. NOAA. (2017).what is eutrophication? National Ocean Service website. https://oceanservice.noaa.gov/facts/eutrophication.html.
4. Everest, J. Bayne, D.(2004). Weed Management in Lakes and Ponds. The Alabama Cooperative Extension System. ANR- 48.
5. Masser, M. Murphy, T. Shelton, J.(2013). Aquatic Weed Management: Herbicides. SRAC Publication No. 361 Revision. 1-8.
6. Lembi, C.(2016). Aquatic Weed Control. North Central Regional Extension Publication no 241; 2961.
7. Huynh, K.Kurobe, T.Stillway, M. Lam, C. The, S. (2021). What Does Not Kill Can Weaken: How Herbicides Impact Fish In The San Francisco Estuary. Frontier Young Minds. 9:612966.
8. Jiali, J.Tomofumi, K.Ramírez-Duarte, W.Bolotaolo, M. Lam, C. Pandey, P. Hung, T.Stillway, M. Zweig, L. Caudill, J. Lin, L.Swee, J. (2018) The Sub-lethal effects of herbicides penoxsulam, imazamox, fluridone and glyphosate on Delta Smelt (*Hypomesustranspacificus*), Aquatic Toxicology. 197:79-88.
9. Conning, M. Fletcher, K. Swan, A. (1969).Paraquat and related bipyridyls. Brazilian MedicalBulletin. 25(3):245–249.
10. Tomlin, C. (2003). The Pesticide Manual, 13th Edition. The British Crop Protection Council, Alton, Hampshire, UK.
11. Kanchan, T.Bakkannavar, M. Acharya, R. (2015). Paraquat Poisoning: Analysis of an Uncommon Cause of Fatal Poisoning from Manipal, South India. Toxicology International. 22(1):30-4.
12. Arts, J.Schuit, G.Schipper, A. van der Kleij, A. (2006).Case report of Paraquat poisoning. European Journalof Hospital Pharmacy. 12:22-4.
13. Pateiro-Moure M, Nóvoa-Muñoz C, Arias-Estévez M, López-Periago E, Martínez-Carballo E, Simal-Gándara, J. (2009). Quaternary herbicides retention by the amendment of acid soils with a bentonite-based waste from wineries. Journal of Hazardous Materials. 164: 769–775.
14. Ismail, S.Sameni, M.Halimah, M. (2011). Evaluation of herbicide pollution in the kerianricefields of Perak, Malaysia. World Applied Sciences Journal. 15: 5–13.
15. Katagi, T. (2010).Bioconcentration, bioaccumulation, and metabolism of pesticides in aquatic organisms. Reviews of Environmental Contamination and Toxicology. 204:1-132.
16. Wang, Y. Yen, J.Hsien, Y. Chen, Y. (1994). Dissipation of 2,4-D glyphosate and Paraquat in river water. Water Air Soil Pollution. 72: 1–7.
17. Fernandez, M. Ibanez, M. Pico, Y. Manes, J. (1998). Spatial and temporal trends of paraquat, diquat, and difenzoquat contamination in water from marsh areas of the Valencian Community (Spain). Archives of Environmental Contamination and Toxicology. 35: 377.
18. Moore, N.Viarengo, A.Donkin, P. Hawkins, J. (2007)Autophagic and lysosomal reactions to stress in the hepatopancreas of blue mussels. Aquatic Toxicology. 84(1):80-91.
19. Babatunde, M.Oladimeji, A.Balogun, K. (2001). Acute Toxicity of Gramoxone to *Oreochromisniloticus* (Trewavas) in Nigeria. Water Air Soil Pollution. 131(1):1–10.
20. Leboulanger, C.Bouvy, M. Pagano, M. (2009). Responses of Planktonic Microorganisms from Tropical Reservoirs to Paraquat and Deltamethrin Exposure. Archives of Environmental Contamination and Toxicology. 56(1):39–51.
21. Muangphra, P.Kwankua, W.Gooneratne, R. (2014).Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. Environmental Toxicology. 29(6):612–620.
22. Bashini, M. Jose, D.Senthilkumaar, P. (2019). Impact of Herbicide Paraquat on the Indian Major Carp *Catlacatla*. Immunological Tools for Biodiversity and Status.94-109.
23. Bashini M andSenthilkumaar P. (2018). Impact of herbicide Paraquat on the Indian Major Carp, *Labeorohita*. Journal of Emerging Technologies and Innovative Research. 5-12: 459-466.

24. Cheah, Uan-Boh, Kirkwood, Ralph, Lum, Keng-Yeang. (1998). Degradation of Four Commonly Used Pesticides in Malaysian Agricultural Soils. Journal of Agricultural and Food Chemistry.46(3), 1217–1223.
25. Kathiresan, M. andDeivasigamani, S. (2015). Herbicidal control of water hyacinth (*Eichhorniacrassipes*) and its impact on the aquatic environment. Indian Journal of Agronomy, *60*(4), 606-609.
26. FAO. (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome: FAO.
27. Kapila,M. andRagathaman, G. (1999). Mercury, copper, and cadmium-induced changes in total protein level in muscle tissue of an edible estuarine fish, *Boleophthalmusussumieri* (CUV). Journal of Environmental Biology.20:231-4.
28. Sheridan, A. andKittilson, D. (2004). The role of somatostatins in the regulation of metabolism in fish.Comparative Biochemistry & PhysiologyPart B. Biochemistry and Molecular Biology. 138: 323–330.10.
29. Preiss, J.and Walsh,A. (1981).The comparative biochemistry of glycogen and starch. In: Ginsburg V, Robbins P, editors. Biology of Carbohydrates. John Wiley and Sons New York. 199–314.
30. Lowry, H.Rosebrough, J. Farr, L. Randall, J. (1951).Protein measurement with the Folin phenol reagent. Journal of biological chemistry. *193*: 265-275.
31. Barnes, H.Blackstock, J. (1973). Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphos-phovanillin method for 'total' lipids. Journal of Experimental Marine Biology and Ecology. 12: 103-118.
32. Dezwaan, N.Zandee, I. (1973).The utilization of glycogen and accumulation of some intermediates during anaerobiosis in *Mytilusedulis (L)*. Comparative Biochemistry & Physiology. 43 B: 47-54.
33. APHA AWWA, WEF. (1989). Standard Methods for the examination of water and wastewater 20th.
34. Agrahari, S. and Gopal, K. (2009). Fluctuations of certain biochemical constituents and markers enzymes as a consequence of monocrotophos toxicity in the edible freshwater fish, *Channapunctatus*. Pesticide Biochemistry and Physiology, 94: 5-9.
35. Manoj, K. (1999). Mercury, copper, and cadmium-induced changes in the total protein levels in muscle tissue of an edible estuarine *fish Boleopthalmusdessumuri.Cuv*. Journal of environmental biology. 20: 231-234.
36. Inyang, R. Daka, R. and Ogamba, N. (2010). Effect of sub-lethal concentrations of diazinon on total protein and transaminase activities in *Clariasgariepinus*. Current Research Journal of Biological Sciences.2(6):390-395.
37. Suntres, E. (2002). Role of antioxidants in paraquat toxicity. Toxicology. 180: 65-77.
38. Magdy, A. Salah, El-Deen&Rogers, W. (1993). Changes in Total Protein and Transaminase Activities of Grass Carp Exposed to Diquat, Journal of Aquatic Animal Health. 5:4, 280-286.
39. Das, K. and Mukherjee, C. (2000). Sub-lethal effects of quinalphos on selected blood parameters of *Labeorohita*(Ham.) fingerlings. Asian Fish Science. 13: 225-233.
40. Gluth, G. and Hanko, W. (1984). A comparison of physiological changes in carp (*Cyprinuscarpio*) induced by several pollutants at sub-lethal concentration II- the dependency on the temperature. Comparative Biochemistry and Physiology. 79C: 39-45.
41. Singh, S. and Khare, A. (1999). Effect of pesticide on protein metabolism in liver of *Clariasbatrachus*. Recent Advances in Applied Environmental Zoology. 1: 21.
42. Dessai, S. (2002). Toxicological effect on some biochemical parameters of freshwater fish *Channapunctatus* under the stress of nickel. Journal of Environmental Biology. 23(3): 275-277.
43. Singh, A. Singh, S. Bhartiya, P. and Yadav, K. (2010). Toxic effect of phorate on the serum biochemical parameters of the snake-headed fish *Channapunctatus* (Bloch). Advances in Bioresearch. 1(1): 177-181.
44. Reddy, S. Bhagyalakshmi, A. (1994). Changes in oxidative metabolism in selected tissues of the crab (*Scylla Serrata*) in response to cadmium toxicity. Ecotoxicology and Environmental Safety. 29:255–264.
45. Umminger, L. (1977). Relation of the whole blood sugar concentration in vertebrates to standard metabolic rate. Comparative Biochemistry and Physiology. 55:457-460.
46. Tiwari, S. and Singh, A. (2005). Possibility of using latex extracts of *Neriumindicum* plant for control of predatory fish *Channapunctatus*. Asian Fisheries Science. 18: 161-173.
47. Birkner, E. Mamczar, G. Machoy, Z. Tarnawski, R. and Polaniak, R. (2000). Disturbances of protein metabolism in rats after acute poisoning with sodium fluoride. Fluoride. 33: 182-186.
48. Grucka-Mamczar, E. Birkner, E. Zelejska-Fiolka, J. and Machoy, Z. (2005). Disturbances of kidney function in rats with fluoride-induced hyperglycemia after acute poisoning by sodium fluoride. Fluoride. 38: 48-51.
49. Ogamba, E.Inyang, I. Azuma, I. (2011). Effect of Paraquat Dichloride on some metabolic and enzyme parameters of *Claris gariepinus*. Current Research Journal of Biological Sciences. 3(3): 186-190.
50. Naudí, A. Cabré, R. Jové, M. Ayala, V. Gonzalo, H. Portero-Otín, M. Ferrer, I. Pamplona, R. (2015).Lipidomics of Human Brain Aging and Alzheimer's disease Pathology. International Review of Neurobiology. 1-57.
51. Chetty, N. and Indira K. (1994). Alterations in tissue lipid profiles of *Lamellidensmarginalis* under ambient ammonia stress. Bulletin of environmental contamination and toxicology. 55:693-698.
52. Bloom, M. Evans, E. Mouritsen, G. (1991). Physical properties of the fluid lipid-bilayer component of cell membranes: a perspective. Quarterly Reviews of Biophysics. 24:293.

53. Gawarammana, B. & Buckley, A. (2011). Medical management of Paraquat ingestion. British Journal of Clinical Pharmacology. 72(5); 745–757.
54. Sureda, A. Box, A. Ensenat, M. Alou, E. Tauler, P. Deudero, S. Pons, A. (2006). Enzymatic antioxidant response of a labrid fish (*Corisjulis*) liver to Environmental caulerpenyne. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 144:191–196.
55. Tejada, S. Sureda, A. Roca, C. Gamundí, A. Esteban, S. (2007). Antioxidant response and oxidative damage in brain cortex after high dose of pilocarpine. Brain Research Bulletin. 71:372–375.
56. Grimsrud, A. Xie, H. Griffin, J. and Bernlohr, A. (2008). Oxidative stress and covalent modification of protein with bioactive aldehydes. Journal of Biological Chemistry. 283, 21837–21841.
57. Davies, J. (2016). Protein oxidation and peroxidation. The Biochemical Journal. 473; 805–825.
58. Srinivasa, R. and Ramana, R. (1989). In vivo modification of lipid metabolism in response to phosphamidon, methyl parathion, and lindane exposure in the prawn *Metapaeneusmonoceros*. Bulletin of Environmental Contamination and Toxicology. 43: 603-610.
59. Gopal, S. Reddy, M. Ramodu, M. Indira, P. (2008). Levels of lipid metabolites in *Cyprinuscarpio (L.)* on sub-lethal exposure to synthetic detergent Linear Alkyl Benzene Sulfonate (LAS). Asian Journal of Environmental Science. 3-1: 17-22.
60. Sivanandan, M. Binukumari. (2021). Acute and sub-lethal intoxication of malathion in an Indian Major Carp, *Labeorohita*: hematological and biochemical responses. Environmental Analysis Health and Toxicology. 36(3).
61. Bantu, N. Zenebe, H. Krishna, C. Gopala, K. Rathnamma, Abaynew, Zenebe, T. Mulgeta. (2018). Effect of an insecticide chlorantraniliprole on biochemical characteristics of snakehead fish, *Channapunctatus*. Innovative Journal of Agricultural Sciences. 6-1: 1-7.
62. Preiss, J. Walsh, A. (1981). The comparative biochemistry of glycogen and starch. In: Ginsburg V, Robbins P, editors. Biology of Carbohydrates. John Wiley and Sons New York. 199–314.
63. Van Cromphaut, J. (2009). Hyperglycaemia as part of the stress response: the underlying mechanisms. Best Practice and Research: Clinical Anaesthesiology. **23**, 375–386 (2009).
64. Dinis-Oliveira, J. Duarte, A. SánchezNavarro, A. Remião, F. Bastos, L. Carvalho, F. (2008). Paraquat Poisonings: Mechanisms of Lung Toxicity, Clinical Features, and Treatment. Critical Reviews in Toxicology. 38(1), 13–71.
65. Xie, H. Tan, J. Wang, R. Meng, X. Tang, X. Gao, S. (2013). Expression and significance of HIF-1 in pulmonary fibrosis induced by paraquat. Experimental Biology and Medicine. 238(9), 1062–1068.
66. Menezes, C. Fonseca, B. Leitemperger, J. *et al.* (2015). Commercial formulation containing 2,4-D affects biochemical parameters and morphological indices of silver catfish exposed for 90 days. Fish Physiology and Biochemistry. 41: 323–330.
67. Abbas, H. Authman, M. El. Badawi, A. Abumourad, I. (2007), Studies On The Effect Of Thiobencarb Herbicide On Some Biological, Physiological, Biochemical, Histological And Genetic Aspects Of Nile Tilapia, *OreochromisNiloticus*. Egyptian Journal of Aquatic Biology and Fisheries. 11(1): 123-150.
68. Al-Jiffri, O. Al-Sharif, M. Abd El-Kader, M. Ashmawy, M. (2013). Weight reduction improves markers of hepatic function and insulin resistance in type-2 diabetic patients with non-alcoholic fatty liver. African Health Sciences. -13(3):667-72.
69. Babatunde, M. Balogun, K. Oladimeji, A. Auta, J. Abolude, S. (2009). The growth response of fingerlings of *O. niloticus* in sub-lethal test to Paraquat. Fisheries Society of Nigeria. 181-184.