How to Cite:
Naresh, R., & Rajashekhar, A. V. (2022). Study on impact of parboiled rice mill effluents on biochemical constituents of Labeo rohita (Hamilton 1822). *International Journal of Health Sciences*, *6*(S2), 14167–14183. https://doi.org/10.53730/ijhs.v6nS2.8931

Study on impact of parboiled rice mill effluents on biochemical constituents of Labeo rohita (Hamilton 1822)

Ravirarala Naresh
Assistant Professor, Department of Zoology, Govt Degree College for Women, Nalgonda dist, Telangana
Corresponding author email: dravraj@gmail.com

A. V. Rajashekhar
Associate Professor ,Aquatic Biology and Parasitology Lab, Department of Zoology, UCS, Osmania University, Hyderabad, Telangana, India

***Abstract*---**The parboiled rice mill effluents (PRME) are among the major contributors of chemical pollutants that enter into various aquatic bodies and affect the aquatic biota, accounting for several types of chemicals released in the nearby areas. The present investigation (2021-22), fingerlings of *Labeo rohita* was exposed to acute (96 hrs) and chronic (30 days) dose of parboiled rice mill effluents (PRME). The PRME collected from the parboiled rice mills were analyzed for Physico-chemical parameters and chronic and acute toxicity tests in *Labeo rohita* fish. The results showed that the severity in the Physico-chemical parameters and toxicity levels cause moderate to massive destruction in gills, liver and muscle, which is concentration and time-dependent. In acute toxicity experiments (96hrs) the fingerlings of fresh water fish *Labeo rohita* was exposed to different concentrations of (12.5%, 25%, 50%, 75% and 100%) parboiled rice mill effluents. The LC_{50} value for various exposure times was determined and analyzed by using Finney probit method. The value of LC_{50} of untreated (UT) parboiled rice mill effluents after 96hrs was calculated as 88%. During chronic toxicity experiments test fish fingerlings were exposed to three different sub lethal concentrations of ($1/5^{th}$ of 96 hrs LC_{50}, $1/10^{th}$ of 96 hrs LC_{50} and $1/15^{th}$ of 96 hrs LC_{50}) parboiled rice mill effluents. Various tissues viz. gill, liver and muscle were obtained separately from control and effluent treated fishes for biochemical estimations after the chronic exposure (10days, 20 days and 30 days). The tissues were used for biochemical estimation, glycogen content in all the tissues decreased considerably upon the chronic toxicity of PRME when compare to control group. The total protein content decreased in all the three tissues in treated group of

PRME. In general, the total lipid content reduced in all the three tissues after chronic exposure when compared to control group of *Labeo rohita* fishes. The results indicated in the present study that the PRME caused marked depletion in biochemical composition in various tissues of the fish *Labeo rohita* after chronic exposure.

Keywords---acute toxicity test, chronic toxicity, parboiled rice mill, sub lethal concentrations, biochemical constituents, Labeo rohita.

Introduction

The rapid industrialization throughout the world particularly due to the alarming rise in the human population has been responsible for a terrible amount of environmental pollution. It is negatively impact on the environmental quality (Ali, S.H *et al.,* 2018), particularly aquatic life due to the discharge of these voluminous amounts of effluents into natural water bodies, on a daily basis without any pretreatment all over the world (Ganeshwade, R.M *et al.,* 2006; Agrawal, A *et al.,* 2010; Edokpayi, J.N *et al.,* 2017). In developing and under developed countries, effluents from different industries are indiscriminately released into water bodies and even into adjacent fields without any pre-treatment, thus creating serious problems for the non-target beneficial organisms. Fishes are ectothermic aquatic animals. Hence their life depends on different physico-chemical characteristics of the water (Ficke, Myrick, & Hansen, 2007). They make an utmost contribution to their ecosystem by providing necessary nutrients that support the whole ecosystem and maintaining food web organization (Cooke *et al.,* 2016; Dwivedi *et al.,* 2018a). All over the world efforts are being made to use of both the fresh and marine water bodies for fish production. Fishes are very good bio indicator organisms for the assessment of impacts of toxic substances like pesticides, effluents and heavy metals in aquatic ecosystems; hence they can be used in bio toxicity assays to find out aquatic hazards (Xia .C *et al.,* 2018). Due to enforcing of stringent environmental laws and regulations on industrial waste discharge (Maleki. A*etal.* 2005; Ebrahimpour. M *et al.,* 2010), presently more attention is being given to bio assay toxicity testing to detect harmful effects of toxic substances present in the industrial effluents on aquatic organisms. In toxic genomics studies fishes were considered as ideal aquatic organisms due to they have strong power to establish biomarkers of exposure (Prakash and Verma, 2020b). So far, previous studies on fishes clearly revealed that fish can be used as a pollution bio-indicator to measure the health of the environment (Authman MS *et al.,* 2015; Jangu S *et al.*2018).

Fishes were exposed to toxicants, they generally produce detoxifying enzymes, but the production of these enzymes is only possible in acute toxicity exposures. The inability of the fish to detoxify the chemical and excrete the resultant metabolite of detoxification, in addition cause direct damage of epithelial cells of gills by the toxicant, possible damage of liver cells (Omoregie*et al.,* 1998), and internal asphyxiation (Duffus, 1980) may lead to speed and high mortality recorded in the acute toxicity studies of this nature. Under stress conditions, fish need more energy to detoxify the toxic substances to overcome stress conditions. Fish have a very little amount of carbohydrates for the detoxification process hence the body

depends on the next alternative source of energy to overcome stress condition. Protein is the next alternative source of energy to meet increased energy demand under stress. The depletion of protein content in liver, muscle and gonads occurs due to the degradation of protein and utilization of protein metabolites for metabolic purposes (Malla Reddy and Bashamohideen, 1995).

Fishes are the most significant biomonitoring agents for assessing hazardous components accumulated in contaminated aquatic ecosystems. They help not in the understanding of the nature but also observe the changes of aquatic ecosystems in effective manner and compared to other aquatic animals, they are extremely susceptible to environmental changes, particularly aquatic pollution. Biochemical factors have been more popular in recent years, clinical diagnostics to evaluate the impact of environmental stressors and determine pollution toxicity. Biochemical tests and clinical diagnostic tests are gradually becoming more common for determining fish health

Labeo rohita is an indigenous major carp and imported as an alien species in numerous Nations. As it is a great source of protein, column-feeding fish, commercial fish with significant demand in the market due to its excellent nutritional content and taste. Inland fisheries culture has become a very important sector of both fisheries as well as agriculture and increases the economy of rural agriculture sector and helps to solve the country's malnutrition problems. The present study was designed to examine the harmful effects of parboiled rice mill effluent on various biomolecules of fresh water major carp *Labeo rohita* in the gills, muscle and liver.

Parboiling is the process of partially boiling paddy inside the husk. It is a paddy pre-milling technique that originated in India (V. Subrahmanyan 1971). Parboiling is a hydrothermal process that converts the crystalline structure of starch in paddy rice into an amorphous form (Rao, R.S.N. and Juliano, S.O. 1970). Parboiling of paddy causes gelatinization of the starch during boiling, and cooling, the amylase molecules of starch are re-associated with one another, forming a densely packed structure during cooling.

Parboiling is a partial boiling of paddy which consists of three steps which are Soaking, heating (wet or dry, atmospheric or in modern methods by high pressure steaming), and drying rough or brown rice (*Miah, M. A. et al., 2002;* Behera and Sutar, 2018*)*. These steps make it easier to process rice by hand for improving nutrient quality, modifying its texture, and increasing resistance to weevils (Kik, M.C.and Williams, and R.R.1945). The first step in the parboiling process is to soaking of paddy in hot or cold water for 24–48 hours, until the kernels are saturated with moisture. Soaking is the crucial processing step in the rice parboiling process (Priestly, R.J. 1976). During this process paddy will be soaked in water until it reaches a moisture level of 30–35 percent by weight. The moistened paddy is then heated with steam at 100°C until it gelatinizes (Kik, M.C.and Williams, R.R.1945). After that, they are dried and milled.

Carbohydrates, proteins, and lipids all play important roles as energy precursors in the fishes under stress conditions (Idler and Clemens, 1959). The major focus on clinical diagnosis of fish physiology was utilized to identify the impacts of

environmental stressors and harmful chemicals, biochemical measures were frequently used. Biochemical profiles in stressed fish and other aquatic species serve as key bio indicators in the monitoring of aquatic environment.

Materials and Methods

Collection and acclimatization of fishes

The Indian major carp, *Labeo rohita* is a fish of family cyprinidae, found commonly in fresh water ponds, lakes and rivers in India. It is an herbivorous fish.The stock of fish was procured from a local fish form in Mangalpally, Nalgonda district, Telangana state, India. Fingerlings of *Labeo rohita* (Hamilton) weighing 8.67±0.52 gm and measuring mean body length 8.23±0.41 cm were selected for the experiment. The fish were transported to the wet laboratory and acclimatized for 15 days before being used for the experiments. The fingerlings were fed @ 4 percent of their body weight with commercially available food pellets every day. During the acclimation period, water (70 percent) was replaced on alternate days to remove any remaining feed and faecal or metabolic waste. Each tank was provided continuous aeration. Throughout the research, dechlorinated tap water was utilized for control and parboiled rice mill effluent was used for treated fishes. The physico-chemical parameters of water and parboiled rice mill effluent were determined according to American Public Health Association guidelines (APHA 2017). The following are the values: pH 5.7±0.4, Electric conductivity 1550.83±15.48, Biological oxygen demand 2537.17±81 mg/l, Chemical oxygen demand 6623.75±90.2 mg/l, Total Hardness 532.25±10.15 mg/l, Total alkalinity 693.33±13.31 mg/l, Calcium 186.5±17.52 mg/l, Magnesium 186.5±17.52 mg/l, Chlorides 783.75±22.27 mg/l, Total Dissolved solids (TDS) 1618.58±89.95 mg/l, Phosphates 29.42±1.56 mg/l.

Acute Toxicity tests

To estimate the percent mortality, two batches of twelve healthy *Labeo* fingerlings (n=12) were subjected to dechlorinated tap water (Control group) and various concentrations of untreated parboiled rice mill effluents (12.5%, 25%, 50%, 75 percent, and 100 percent) for a period of 24,48,72, and 96 hours. The fish were not fed the day before the experiment began. Following the guidelines of the Organization for Economic Cooperation and Development (OECD 2019), mortality was recorded every 24 hours after 24, 48, 72, and 96 hours of exposure, and the dead fishes were counted and removed from the test container immediately. In the control group, no fingerlings were discovered dead; however, mortality was reported in fingerlings exposed to various concentrations of untreated parboiled rice mill effluents. The percentage mortality was calculated by combining the values into a probit scale. The LC_{50} value was calculated and analyzed using the Finney probit method for various exposure times.

Chronic study

The present investigation, eighty acclimatized *Labeo rohita* fingerlings were selected and separated into different experimental groups, each containing 20 fingerlings (n=20). To evaluate the biochemical alterations in the fish *L.rohita*, the

first, second, and third groups were subjected to sublethal doses of $1/5^{th}$,$1/10^{th}$, and $1/15^{th}$ of 96 hrs LC_{50} value for long term (Chronic) exposure (30 days). Sub lethal concentrations of parboiled rice mill effluents were chosen as 17.6 % ($1/5^{th}$ of 96 hrs LC_{50}), 8.8 % ($1/10^{th}$ of 96 hrs LC_{50}), and 5.9 % ($1/15^{th}$ of 96 hrs LC_{50}) during the experiment. A fourth group was maintained simultaneously as a control group using tap water.

Biochemical analysis

The control group and three treated groups of sub lethal concentrations, three fishes from each group were sacrificed and dissected immediately on the 10, 20 and 30^{th} day. Fresh wet tissues of vital organs viz., gill, liver and muscle were isolated from the control group and treated groups after the each exposure period and preserved in 5% to 10% formalin for the estimation of biochemical constituents by the standard recommended protocols. The tissues were homogenized with 80% methanol, centrifuged at 3000 rpm for 12 minutes and clear supernatant was selected for the estimation of different biochemical constituents. The glycogen content was estimated by Kemp et al. (1954) method, Total Protein content was determined spectrometrically by using Lowry's method (1951). Lipids were estimated by the Barnes and Blackstock (1973) method.

Statistical analysis

The level of significance was calculated using one way analysis of variance and reported as the mean value ± standard deviation. For both the control and test groups, the 'n' numbers were the same. The data were statistically examined at a significance level of $p<0.05$ and $p < 0.01$. T values were calculated using the standard 't' test to determine their significance. MS Excel 2007 was used to create the graphs. A standard methodology was used to run a probit analysis of log dosage against response (mortality). The regression equations were calculated, and 96h LC_{50} value was derived from the equation.

The percentage change in the exposed fish's biochemical composition was calculated as follows:

$$\% \text{ Change} = \frac{Exposed\ value - Control\ value}{Control\ \ value} x100$$

Results

Acute toxicity test

The fingerlings were exposed to control and various concentrations of untreated parboiled rice mill effluents (12.5 %, 25 %, 50 %, %, and 100 %) for 96 hours in this study. At 24, 48, 72, and 96 hours, the percent mortality rate in fingerlings was measured. The results of acute toxicity tests are showed in Table-1.There was no mortality in fingerlings exposed for 24 hours to control, 12.5%, and 25% concentrations of untreated parboiled rice mill effluents. Three fingerlings were found dead after 96 hours of exposure at a 12.5 % and 25 % UT concentration and five fingerlings showed mortality after 96 hours of exposure at a 50% UT concentration. Three fingerlings died after 72 hours of exposure to a 75

% UT concentration, and five fingerlings died after 96 hours of exposure. Seven fingerlings died at 100% concentration of UT PRM effluent after 96 hrs of exposure. In the control and various concentrations of PRM effluents viz.12.5 %, 25%, 50%, 75%, and 100%, the percent mortality was observed to be 0, 25%, 25%.42 %, 42 %, and 58 %, respectively (Table 1). The lethal concentration (LC_{50}) of UT PBRM effluent was computed using the regression equation (y=ax+b) after the mortality rate in fingerlings was measured. After 96 hours, the LC_{50} of UT PRM effluent was calculated to be 88 percent.

Table 1: Mortality rate of *Labeo rohita* fingerlings exposed to control, different concentrations of untreated (UT) parboiled rice mill effluents at different time intervals

Sl.no	Concentration of effluent	No. of test fishes	No of dead fishes				After 96 hrs.			
			24 hr	48 hr	72 hr	96 hr	Total dead fishes	Percentage (%) Mortality	log 10	Probit analysis
1	Control	12	0	0	0	0	0	0%	-	-
2	12.50%	12	0	1	1	1	3	25%	-0.903090	4.33
3	25%	12	0	1	1	2	3	25%	-0.602060	4.33
4	50%	12	1	1	1	2	5	42%	-0.301030	4.8
5	75%	12	1	1	1	2	5	42%	-0.124939	4.8
6	100%	12	1	2	2	2	7	58%	0	5.2

Biochemical analysis

Biochemical tests are useful tools for determining the impact of aquatic pollution on the biochemical composition of fish tissues. The content of Glycogen, Protein, and Lipid in all three tissues of the fish (Gill, Liver, and Muscle) were showed to be significant decrease in the study. The effects of pharmaceuticals on fish biochemical markers may aid in the understanding of drug mechanism and mode of action (Li et al., 2011). Under chromium stress, Vutukuru (2005) found a significant decrease in several biochemical constituents in various tissues in freshwater fish, *Labeo rohita.* Mystus vittatus was exposed to mercuric chloride; Kannan et al. (2010) reported that found a decrease in protein concentration in the gills, brain, and muscle.

Table-2. Changes in the glycogen content in the tissues of Labeo rohita on long term exposure with parboiled rice mill effluent during 2021-22

Sample mg/g wet tissue	X±SD t test % Change	Control	1/15 of 96 hrs LC_{50} value			1/10 of 96 hrs LC_{50} value			1/5 of 96 hrs LC_{50} value		
			10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Gill	Mean ±SD	4.52±0.16	4.41±0.16	4.22±0.17	3.91±0.21	4.31±0.16	4.05±0.10	3.84±0.12	4.20±0.17	3.97±0.11	3.78±0.09
	t test		0.0005**	0.0005**	0.0037**	0.0001**	0.0027**	0.0024**	0.0015**	0.0025**	0.0031**
	% Change		-2%	-6%	-13%	-5%	-10%	-15%	-7%	-12%	-16%
Liver	Mean ±SD	8.23±0.11	7.95±0.14	7.69±0.12	7.20±0.04	7.72±0.10	7.30±0.07	6.87±0.14	7.51±0.14	7.11±0.09	6.59±0.05
	t test		0.003**	0.001**	0.003**	0.001**	0.001**	0.0003**	0.001**	0.001**	0.001**
	% Change		-3%	-7%	-13%	-6%	-11%	-17%	-9%	-14%	-20%
Muscle	Mean ±SD	6.21±0.14	6.09±0.10	5.79±0.19	5.40±0.25	5.93±0.16	5.62±0.07	5.36±0.02	5.76±0.23	5.50±0.30	5.13±0.03
	t test		0.021*	0.002**	0.003**	0.001**	0.004**	0.005**	0.006**	0.009**	0.002**
	% Change		-2%	-7%	-13%	-5%	-9%	-14%	-7%	-12%	-17%

Values were mean ± SD of three replicates.% change with control taken as 100%.Student 't' test shows **= Highly Significant($p < 0.01$);
* =Significant ($p < 0.05$); # =Not significant ($p > 0.05$) when treated groups were compared with controls.

Table-3.Changes in the Protein content in the tissues of *Labeo rohita* on long term exposure with parboiled rice mill effluent during 2021-22

Sample mg/g wet tissue	X±SD t test % Change	Control	1/15 of 96 hrs LC_{50} value			1/10 of 96 hrs LC_{50} value			1/5 of 96 hrs LC_{50} value		
			10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Gill	X±SD	7.72±0.56	7.21±0.51	6.89±0.49	6.51±0.37	7.06±0.32	6.68±0.41	6.29±0.39	7.08±0.49	6.57±0.48	6.14±0.36
	t test		0.013*	0.003**	0.005**	0.022*	0.003**	0.004**	0.009**	0.007**	0.009**
	% Change		-7%	-11%	-16%	-9%	-13%	-18%	-8%	-15%	-20%
Liver	X±SD	28.50±0.73	27.36±0.27	25.97±0.61	24.38±0.09	26.78±0.60	24.93±0.61	23.43±0.92	26.20±1.09	24.05±1.18	22.50±0.49
	t test		0.025*	0.009**	0.004**	0.004**	0.002**	0.001**	0.007**	0.021*	0.004**
	% Change		-4%	-9%	-14%	-6%	-13%	-18%	-8%	-16%	-21%
Muscle	X±SD	35.71±0.65	34.50±1.10	32.95±1.45	31.36±1.18	33.82±1.16	31.85±0.50	30.54±0.61	33.49±0.55	31.38±0.15	29.03±0.49
	t test		0.041*	0.017*	0.003**	0.019*	0.001**	0.001**	0.004**	0.005**	0.002**
	% Change		-3%	-8%	-12%	-5%	-11%	-14%	-6%	-12%	-19%

Values were mean ± SD of three replicates.% change with control taken as 100%.Student 't' test shows **= Highly Significant($p < 0.01$);
* =Significant ($p < 0.05$); # =Not significant ($p > 0.05$) when treated groups were compared with controls.

Table-4. Changes in the lipid content in the tissues of *Labeo rohita* on long term exposure with parboiled rice mill effluent during 2021-22

Sample mg/g wet tissue	X±SD t test % Change	Control	1/15 of 96 hrs LC_{50} value			1/10 of 96 hrs LC_{50} value			1/5 of 96 hrs LC_{50} value		
			10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Gill	X±SD	31.66±0.38	30.60±0.89	29.62±0.65	28.03±2.10	30.17±0.54	28.62±0.75	27.62±1.15	29.26±0.88	27.80±0.54	26.41±0.30
	t test		0.091#	0.029*	0.053*	0.037*	0.008**	0.012*	0.023*	0.008**	0.002**
	% Change		-3%	-6%	-11%	-5%	-10%	-13%	-8%	-12%	-17%
Liver	X±SD	57.87±0.30	56.37±0.39	54.07±0.33	51.53±0.76	54.28±0.33	52.28±0.86	50.05±0.41	52.69±0.55	50.11±0.80	47.61±0.53
	t test		0.031*	0.004**	0.004**	0.004**	0.005**	0.001**	0.003**	0.003**	0.001**
	% Change		-3%	-7%	-11%	-6%	-10%	-14%	-9%	-13%	-18%
Muscle	X±SD	68.15±0.25	65.14±1.49	62.73±1.70	59.57±1.62	63.19±1.22	59.70±1.33	56.14±1.21	60.95±0.61	57.50±1.06	53.13±1.10
	t test		0.039*	0.015*	0.006**	0.011*	0.004**	0.001**	0.002**	0.002**	0.001**
	% Change		-4%	-8%	-13%	-7%	-12%	-18%	-11%	-16%	-22%

Values were mean ±SD of three replicates.% change with control taken as 100%.Student 't' test shows **= Highly Significant($p < 0.01$);
* =Significant ($p < 0.05$); # =Not significant ($p > 0.05$) when treated groups were compared with controls.

Glycogen content

The amount of Glycogen in the tissues (gill, liver and muscle) estimated after subjecting the fishes to long term exposure periods on the Parboiled rice mill effluents are presented in Table 2. The mean Glycogen level in gill, liver and muscle tissues was found to be decreased significantly ($P< 0.05$) in test fishes treated with $1/5^{th}$,$1/10^{th}$ and $1/15^{th}$ LC_{50} value of parboiled rice mill effluents. Results revealed that the minimum level of glycogen was observed with $1/5^{th}$ of LC_{50} concentration followed by $1/10^{th}$ LC_{50} concentration and maximum level was observed at Control and $1/15^{th}$of LC_{50} concentration (Figure-1).

The gill of fishes exposed to $1/15^{th}$ of 96hrs LC_{50} Parboiled rice mill effluents for 10 days, 20 days and 30 days was found to contain a mean of 4.41±0.16, 4.22±0.17 and 3.91±0.21 mg/g of glycogen respectively. The fishes maintained as control were found to contain a mean of 4.52±0.16mg/g in their gill tissue. Liver tissue was found to contain 7.95±0.14, 7.69±0.12 and 7.20±0.04 mg/g of glycogen respectively in 10 days, 20 days and 30 days exposures in $1/15^{th}$of 96 hrs LC_{50} concentration of Parboiled rice mill effluent. Under treatment of $1/15^{th}$ of 96hrs LC_{50} concentration of Parboiled rice mill effluent for 10, 20 and 30 days exposures, the mean values of glycogen in muscle were 6.09±0.10, 5.79±0.19 and 5.40±0.25mg/g respectively (Table-2).

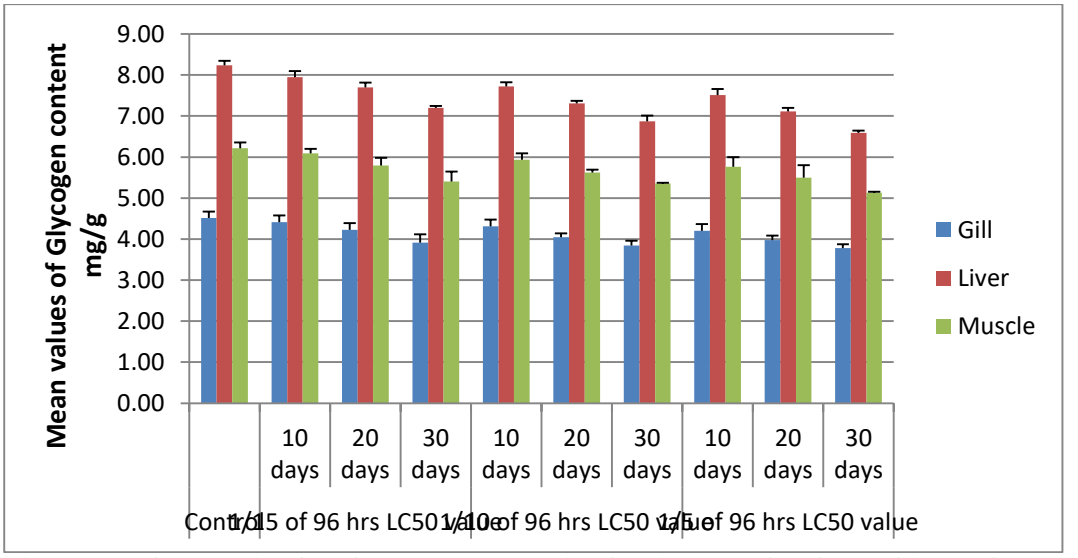

Figure-1. Changes in the glycogen content in the tissues of *Labeo rohita* on long term exposure with parboiled rice mill effluent during 2021-22. The data is presented as mean ±SD.

Glycogen content estimation in the fishes exposed to long term periods 10, 20 and 30 days in $1/10^{th}$ of 96 hrs LC_{50} concentration of Parboiled rice mill effluent were resulted 4.31±0.16,4.05±0.10 and 3.84±0.12 mg/g in the gill tissue. The mean control value was 4.52±0.16. In Liver tissue 7.72±0.10, 7.30±0.07 and 6.87±0.14 mg/g of glycogen found respectively in 10 days, 20 days and 30 days of exposures in $1/10^{th}$ of 96 hrs LC_{50} concentration of Parboiled rice mill effluent. Under treatment of $1/10^{th}$ of 96 hrs LC_{50} concentration of Parboiled rice mill effluent for 10, 20 and 30 days exposures muscle tissue found 5.93±0.16, 5.62±0.07 and 5.36±0.02 mg/g of glycogen respectively. The mean value of glycogen content in the muscle of the control was 6.21±0.14 mg/g (Table-2).

The fishes were exposed to long term duration of $1/5^{th}$ of 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found a mean value of 4.20±0.17,3.97±0.11and 3.78±0.09 mg/g while the control fish contained 4.52±0.16 mg/g of glycogen in the gill. 7.51±0.14, 7.11±0.09 and 6.59±0.05of glycogen were present in the liver tissue respectively after 10 days,20 days and 30 days of long-term exposure of the fishes with $1/5^{th}$ of 96 hrs LC_{50} Parboiled rice mill effluents. The control mean value was8.23±0.11.The Glycogen content in the muscle of the fishes that were subjected to long term exposure of $1/5^{th}$ of 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found to contain a mean of 5.76±0.23,5.50±0.30 and 5.13±0.03 mg/g respectively. The mean control value was 6.21±0.14 recorded (Table-2).

Total protein content

The amount of protein estimated in different tissues of *Labeo rohita* with long term exposures were depicted in Table 3. The mean values of protein content in gill, liver and muscle tissues were found decreased significantly ($P< 0.05$) in test fishes treated with $1/5^{th}$,$1/10^{th}$ and $1/15^{th}$ LC_{50} value of parboiled rice mill effluents. Results were indicated that the minimum level of Proteins were observed in $1/5^{th}$ of LC_{50} concentration followed by $1/10^{th}$ LC_{50} concentration and maximum levels were observed at Control and $1/15^{th}$ of LC_{50} concentration (Table-3).

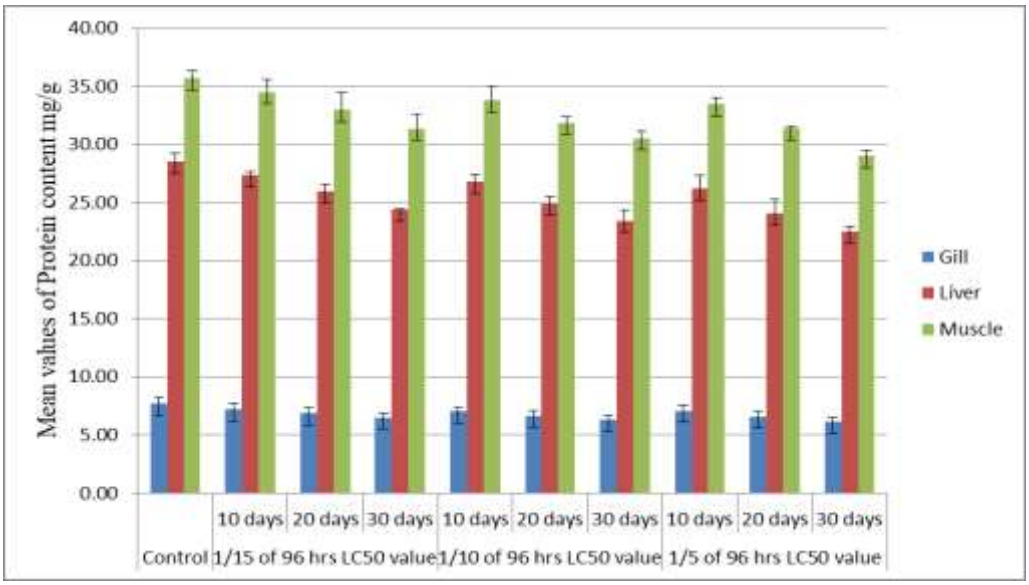

Figure-2. Changes in the protein content in the tissues of *Labeo rohita* on long term exposure with parboiled rice mill effluent during 2021-22.The data is presented as mean ±SD.

The fishes were exposed to long term duration with 1/15th of 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found mean values of 7.21±0.51, 6.89±0.49, 6.51±0.37 mg/gm, in the control fish 7.72±0.56 mg/g of protein found in the gill. In liver tissue the total protein content found 27.36 ±0.27, 25.97±0.61and 24.38±0.09 of respectively after 10 days, 20 days and 30 days of long term exposure of the fishes with 1/15th of 96 hrs LC_{50} Parboiled rice mill effluents. The control mean value was 28.50±0.73.The protein content in the muscle of the fishes that were subjected to long term exposure of 1/15th of 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found mean values of 34.50±1.10, 32.95±1.45 and 31.36±1.18 mg/gm respectively. The mean control value was 35.71±0.65 (Figure-2).

In Gill proteins found a mean value of 7.06±0.32, 6.68±0.41 and 6.29±0.39 mg/g of exposed to long term duration of 1/10th of 96 hrs LC_{50} Parboiled rice mill effluents for 10 days, 20 days and 30 days respectively. The mean control value was 7.72±0.56.The protein content in the liver of the fishes that were subjected to long term exposure of 1/10th of 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found to contain a mean of 26.78±0.60, 24.93±0.61and 23.43±0.92 mg/g respectively. The mean control value was 28.50±0.73.The mean amount of protein in muscle tissue was 33.82±1.16, 31.85±0.50 and 30.54±0.61 mg/g in the fishes exposed to long term duration of 1/10th of 96 hrs LC_{50} Parboiled rice mill effluents after 24, 48, 72 and 96 hours respectively. The mean control value was 35.71±0.65.

Gill recorded a mean of 7.08±0.49, 6.57±0.48 and 6.14±0.36 mg/g of protein in fishes exposed to long term duration of 1/5th of 96 hrs LC_{50} Parboiled rice mill effluents for 10 days, 20 days and 30 days respectively. The mean control value was 7.72±0.56.The protein content in the liver of the fishes in long term exposure

of 1/5thof 96 hrs LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found mean values of 26.20±1.09, 24.05±1.18and 22.50±0.49 mg/g respectively. The mean control value was 28.50±0.73.The amount of protein in muscle tissue was 33.49±0.55, 31.38±0.15 and 29.03±0.49 mg/g in the fishes exposed to long term duration of1/5th of 96 hrs LC_{50} Parboiled rice mill effluents after 10 days, 20 days and 30 days respectively. The mean control value was 35.71±0.65 (Table-3).

Lipid content

The amount of lipid in the tissues estimated by exposing the *Labeo rohita* to long term periods of the Parboiled rice mill effluents are presented in Table 4. The mean lipid level in gill,liver and muscle tissues was found to be decreased significantly ($P< 0.05$) in test fishes treated with 1/5th,1/10th and 1/15th LC_{50} value of parboiled rice mill effluents. Results revealed that the minimum level of lipid was observed with 1/5th of LC_{50} concentration followed by 1/10th LC_{50} concentration and maximum level was observed at Control and 1/15th of LC_{50} concentration (Table-4).

The lipid content in the gill tissue of fishes exposed to long term periods of 1/15th of 96 hrs LC_{50} Parboiled rice mill effluents in term of 10 days, 20 days and 30 days were 30.60±0.89, 29.62±0.65 and 28.03±2.10 mg/g respectively. The fishes exposed to long term periods of 10, 20 and 30 days in 1/15thof 96 hrs LC_{50} Parboiled rice mill effluents contained 56.37±0.39,54.07±0.33 and 51.53±0.76 mg/g of lipid in their liver respectively against an average of 57.87±0.30 mg/g in the control. Muscle tissue was found to contain 65.14±1.49, 62.73±1.70 and 59.57±1.62mg/g of lipid in long term exposure periods of 1/15thof 96 hrs LC_{50} Parboiled rice mill effluents in terms of 10 days, 20 days and 30 days respectively (Table-4).

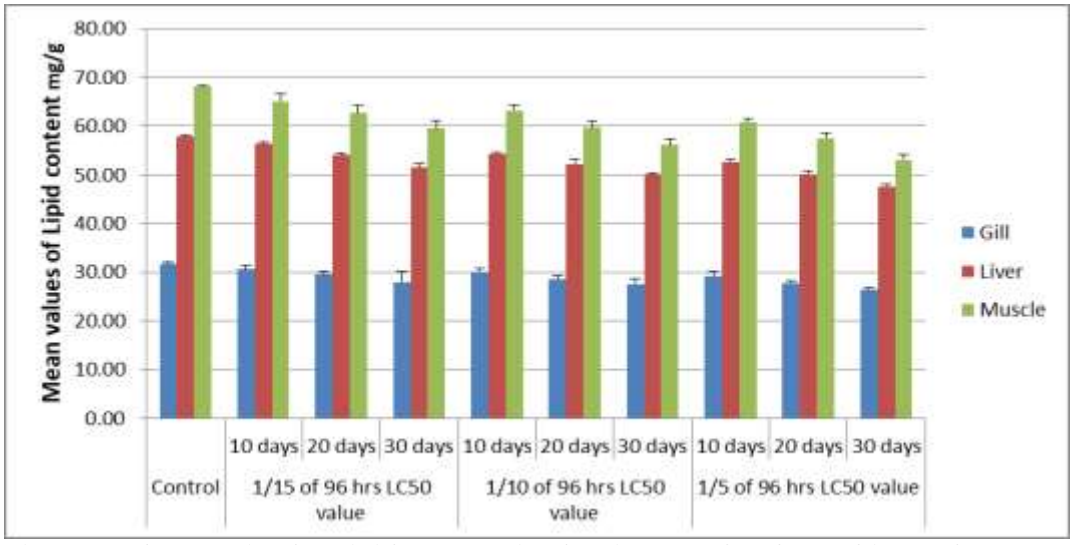

Figure-3.Changes in the Lipid content in the tissues of *Labeo rohita* on long term exposure with Parboiled rice mill effluent during 2021-22.The data is presented as mean ±SD.

The fishes subjected to long term periods of 1/10th of 96 hrs LC_{50} Parboiled rice mill effluent were found to contain 30.17±0.54 (10 days), 28.62± 0.75(20 days) and 27.62±1.15 (30 days) mg/g of lipid in the gill tissue. The control mean value in the gill tissue was 31.66±0.38 mg/g. The fishes exposed for long term periods of 1/10thof 96 hrs LC_{50} Parboiled rice mill effluent were found to contain 54.28±0.33, 52.28±0.86 and 50.05±0.41mg/g of lipid in the liver. Liver recorded 57.87±0.30mg/g of lipid in the control fishes. The lipid content in the muscle tissue of fishes exposed to long term periods of 1/10th of 96 hrs LC_{50} Parboiled rice mill effluents in term of 10 days, 20 days and 30 days were 63.19±1.22,59.70±1.33 and 56.14±1.21 mg/g respectively (Figure-3)

The lipid content in the gill of the fishes that were subjected to long term exposure of 1/5thof 96 hrs. LC_{50} Parboiled rice mill effluents for 10, 20 and 30 days were found to contain 29.26±0.88, 27.80±0.54 and 26.41±0.30 mg/g respectively (Table-4). The mean control value was 31.66±0.38. The amount of protein in liver was 52.69±0.55, 50.11±0.80 and 47.61±0.53 mg/g in the fishes exposed to long term duration of 1/5th of 96 hrs. LC_{50} Parboiled rice mill effluents after 10 days, 20 days and 30 days respectively. The mean control value was 57.87±0.30. Muscle tissue was found to contain 60.95±0.61, 57.50±1.06 and 53.13±1.10 mg/g of lipid in long term exposure periods of 1/5th of 96 hrs. LC50 Parboiled rice mill effluents in terms of 10 days, 20 days and 30 days respectively.

Discussion

The parboiled rice mil effluent discharge in the aquatic environment is hazardous and toxic to fish and has resulted in mortality in some cases due to abnormal and alterations on physico-chemical properties of water quality. Glycogen, Protein, and Lipid biochemical estimations were found to be significantly lower in tissues such as Gill, liver, and muscle in the investigation. Carbohydrates are necessary components of living cells and provide energy to animals. The present research study findings revealed that, a considerable reduction in carbohydrate content in all of the three tissues examined and analyzed. The carbohydrates depletion was observed due to rapid utilization to meet the energy demand under environmental stress conditions and exposed to PRM effluents.

Carbohydrates are primarily used for structural support, protection, as a source of food and stored energy to grow and decrease depending on the needs of the organism (Yerragi et al 2000). Glycogen is stored in the tissues of the fish to supply energy, under stress condition like lack of food and hypoxic condition (Olangnathan and Patterson, 2013). Carbohydrates are considered to be the first biochemical constituents to be broken when animals are under stress. In this study, a considerable decrease noticed in carbohydrate levels in the Gill, liver, and muscle tissue, may be due to the increased energy demand for detoxifying enzymes of hepatic system (Hori et al., 2006).Valarmathi and Azariah (2002) reported a lower level of carbohydrates in toxicant-exposed animals, could be due to glycogenolysis which may increase and increase activity of glycogen phosphorylase to meet energy demands in a stressful situation or the toxicant may inhibit the carbohydrate metabolism by effecting glycogenesis. The reduction in carbohydrate levels, according to Rani et al. (2000), could be due to the use of

stored glycogen, possible by anaerobic glycogenolysis under heavy metal stress in the aquatic ecosystem.

A significant reduction was found in the protein content levels in the tissues of *Labeo rohita* gills, liver, and muscle in the present study. This could be attributed due to the stress in the test fish provoked by various chemical components and toxicants present in the Parboiled rice mill effluents of study area. In both humans and animals, under stress was showed speed up protein metabolism (Nichol and Rosen, 1963). Protein depletion could be linked to stress in fish, as protein is expected to be hydrolyzed and oxidized through the TCA cycle to meet the stress induced increase in energy demand (Somnath, 1991). The present study revealed a reduced trend in protein content in various tissues of *Labeo rohita* could be attributed to metabolic requirements and consumption of keto acids in the synthesis of glucose or for osmotic and ionic regulation mechanism. The reduction on the protein levels of liver has been observed in the fish *Labeo rohita* exposed to PRME due to intensive proteolysis during environmental stress condition

Lipids play an important role in the storage of energy and vitamins in the body. Lipid is an important body constituent used in the structure of cell membranes, bile acid synthesis, and steroid hormone synthesis. According to Remiaet al. (2008), the decline in lipids content levels could be due to the fatty deposits utilization instead of glucose molecules for the energy purpose of the fish Tilapia mossambica on being exposed to Monocrotophos. The significant decrease in lipid content was observed by Mohsen Abdel – Tawwabet et al. (2013). Total lipid depletion in tissues could be due to active mobilization of lipids towards blood and/or tissue metabolism (Murthy et al., 1994). It may be suggested that, Pollutants may have inhibited lipid synthesis and started mobilizing stored lipids by oxidation or gradual unsaturation of lipid molecules (Jha and Jha 1995). The drop in lipid levels, as Rao et al. (1985) correctly pointed out, it could be due to the consumption of lipid to meet the additional energy need under stress.

The chronic exposure of fishes to pollutants of parboiled rice mill effluents may interfere with the physiological mechanisms leading to the development of stress. The overall impact of PRME on *Labeo rohita* fishes in the present study area likely a combination of direct water quality effects and indirect effects on the habitat, particularly on vegetation and food source. However, based on limited information available on PRME, it is very difficult to determine specifically which components are contributed to the toxicity of the effluents observed in this study. The present study emphasizes on the importance of PRME toxicity tests in the management of effluent discharges.

Conclusion

The PRME induces effects on *Labeo rohita* fish at cellular, molecular level and causes biochemical alterations as evident in the present results of gill, liver and muscle biochemical estimations. This could be leads to deplete low protein value in fish and nutritional deficiencies that intern will also danger to human beings due to continuous consumption of such fish. Stress induced by parboiled rice mill effluents cause alterations in carbohydrate, protein, and lipid content in fish.

Contaminants can have an effect on cells, even at the molecular level, resulting in biochemical alternations. The level of biochemical constituents was found to be decreased significantly in the selected tissues of test fish exposed to $1/5^{th}$ of 96 hrs. LC_{50} value when compared to the other two concentrations ($1/10^{th}$ and $1/15^{th}$) and the control in the present investigation. The test fish's metabolic profiles have changed, indicating a hypoxic situation due to effluents of parboiled rice mills in the study area. The results of this study revealed that, at sublethal concentrations, parboiled rice mill effluents changed the biochemical composition (glycogen, protein, and lipid) of the various organs of test fish, due to the use of biochemical energy to overcome stress induced by the heavy metals present in the effluents. Therefore, it can be concluded that the untreated parboiled rice mill effluents have a potential to induce acute toxic effects in *Labeo rohita* fingerlings Furthermore, the entry of parboiled rice mill effluents into the aquatic bodies to be monitored to protect the aquatic organisms such as cultivable fish *Labeo rohita* which is major rich protein food source for many Indians

Acknowledgements

The authors of the present study are very grateful to the Prof. S. Jitender Kumar Naik, Head of the department of Zoology, Prof. M. Madhavi, Chairperson, Board of Studies, Department of Zoology, Osmania University, Hyderabad, Telangana for providing all the basic facilities to carry out this research work. We wish to take this opportunity to express our immense gratitude to Dr. Ghanshyam, Principal, Govt. Degree College for Women, Nalgonda for providing all the necessary facilities.

References

Agrawal, A.; Pandey, R.S.; Sharma, B.Water pollution with special reference to pesticide contamination in India. J. Water Resour. Protect. 2010, 2, 432–448. (CrossRef)

Ali, S.H.; De Oliveira, J.A.P. Pollution and economic development: An empirical research review. Environ.Res. Lett. 2018, 13, 123003. (CrossRef)

Authman MS, Zaki M, Khallaf E, *et al.,* Use of fish as bio-indicator of the effects of heavy metals pollution.JAquac Res Dev. 2015;6(4):328.

Barnes, H. and J. Blackstock: Estimation of lipid in marine animals, detail investigation of sulphophospho vanillin method. J. Exp. Mar. Biol. Ecol.,12, 103-118 (1973).

Behera G, Sutar P P. 2018. A comprehensive review of mathematical modeling of paddy parboiling and drying: Effects of modern techniques on process kinetics and rice quality. Trends Food SciTechnol, 75: 206-230

Cooke, S. J, Allison, E. H., Beard, T. D., Arlinghaus, R.,Arthington, A. H., Bartley, D. M., Cowx, I. G., Fuentevilla,C., Leonard, N. J., Lorenzen, K., Lynch, A. J., Nguyen,V. M., Youn, S. J., Taylor, W. W. &Welcomme, R. L. (2016). On the sustainability of inland fisheries: finding a future for the forgotten. Ambio 45, 753–764.

Duffus JH. Environmental toxicity. London, U.K: Edward Arnord Publ. Ltd; 1980.

Dwivedi, A.C, P. Mayank and A. S.Mishra (2018a). Food structure of two exotic fish species *Cyprinuscarpio*and *Oreochromisniloticus* from the Ganga River. *J the KalashSci*, 6(2): 37-39.

Ebrahimpour, M.; Mosavisefat, M.; Mohabbati, V. Influence of water hardness on acute toxicity of copper and zinc on fish. Toxicol. Ind. Health 2010, 6, 361–365. (CrossRef)(PubMed)

Edokpayi, J.N.; Odiyo, J.O.; Durowoju, O.S. Impact of wastewater on surface water quality in developing countries: A case study of South Africa. Water Qual. 2017, 401–416.

Fales, F.W. (1951).The Assimilation and Degradation of Carbohydrates of Yeast Cells. Journal of Biological Chemistry, 193(1): 113-124. http://www.jbc.org/content/193/1/113.full.pdf

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, *17*(4), 581–613. https ://doi.org/10.1007/s11160-007-9059-5

Finney D. J., (1971), Probit analysis, 3rd Ed. Cambridge University Press, Cambridge. p. 333. http://www.standardmethods.org

Ganeshwade, R.M.; Rokade, P.B.; Sonwane, S.R. Behavioral responses of Cyprinuscarpio to industrial effluents.J. Environ. Biol. 2006, 27, 159. (PubMed)

Hadi, A.A.;Shokr,A.E. and Alwan, S.F. (2009). Effects of Aluminum on the Biochemical parameters of Fresh water fish, *Tilapia zillii*. *Journal of Science and its applications*.3 (1):33-41.

haematological parameters and some biochemical profiles of the Indian major carp, *Labeorohita*. *International Journal of Environment Research and Public Health*. 2: 456-462.

Idler, D.R. and Clemens, W.A.1959. The energy expenditures of fresh water river sockage salmon during spawning migration to chikes and stuar lakes. Pacific Salmon fish comm. Prog. Jackson.Printing, New westminister B.C. 80.

Jangu S, Brraich OS. Histopathological studies of fish liver as a biomarker of heavy metal pollution in internationally important Harike wetland, India. Curr Top Toxicol. 2018; 14:67–72.

Jha, B. S and A. K. Jha, 1995 Biochemical effect of nickel chloride on the liver and gonads of the freshwater climbing perch Anabus testiduneus (Bloch). *Proc. Nat. Acad. Sci. India.*, 65: 39-46.

Juliano, B.O.; Bechtel, D.B. The Rice Grain and Its Gross Composition. In *Rice Chemistry and Technology*, 2nd ed.; Juliano, B.O., Ed.; American Association of Cereal Chemists: Eagan, MN, USA, 1985; pp. 17-57.

Kannan, K., Rajasekaran, G. and Raveen. 2010. Heavy metal mercuric chloride induced biochemical changes in the fresh water cat fish, *Mystus vittatus*. *J. Ecotoxicol. Environ. Monit.*, 20(1) : 33-38.

Kik, M.C.; Williams, R.R. (June 1945). "The Nutritional Improvement of White Rice". Bulletin of the National Research Council. 112: 61–.

Li, Z.H., Velisek, J., Zlabek, V., Grabic, R.,Machova, J., Kolarova, J., Li, P. and Randak, T. 2011. Chronic Toxicity of Verapamil on Juvenile rainbow trout (Oncorhynchusmykiss): effects on morphological indices, haematological parameters and antioxidant responses. *J. Hazard. Mater*. 185:870-880.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randel, R. J. (1951): Protein measurement with Folin-phenol reagent. The Journal of Biological Chemistry, 193, 265- 275.

Maleki, A.; Mahvi, A.H.; Vaezi, F.; Nabizadeh, R. Ultrasonic degradation of phenol and determination of theoxidation by-products toxicity. Iran. J. Environ. Health Sci. Eng. 2005, 2, 201–206.

Malla Reddy P Bashamohideen MD. Alteration in protein metabolism in selected tissue of fish, Cyprinuscarpio, during sub-lethal concentration of cypermethrin. Environ. Monitoring. Asses. 1995; (36):183-190.

Miah, M. A. Kaddus; Haque, Anwarul; Douglass, M. Paul; Clarke, Brian (2002). "Parboiling of rice. Part II: Effect of hot soaking time on the degree of starch gelatinization ". International Journal of Food Science and Technology. 37 (5): 539–545. doi:10.1046/j.1365-2621.2002.00611.x

Mishra, S., Sharma, D. and Melkania, V. 1988. Physico-chemical. Characteristics of effluents from a textile mill at Ra.lnpur and their Effect on fish mortality. IXth Annual Session of and National Symp. On Fish. Assess, and Management of Acad.

Mohsen Abdel-Tawwab., Mohamed, N.M. Mousaad., Khaled M Sharafeldin and Nahla,E.M.Ismaiel. 2013. Changes in growth and biochemical status of common carp, *Cyprinuscarpio* L. exposed to water-borne zinc toxicity for different periods. *International Aquatic Research.*5:11.

Murthy, K. V., M. Bhaskar and S. Govindappa, 1994. Studies on lipid profile of fish liver on acclimation to acidic medium. *J. Environ. Biol.,* 15(4): 269-273.

Nichol, C. A. and F. Rosen, 1963. In: Advances in enzyme regulation (Ed: G. Weber) *Pergamon Press, Oxford.,*1: 341.

OECD (2019), *Test No. 203: Fish, Acute Toxicity Test*, OECD Guidelines for the Testing of Chemicals, Section 2,OECDPublishing,Paris, https://doi.org/10.1787/9789264069961-en.

Olangnathan R. and J. Patterson, 2013. Effect of anthroquinone dyes on the carbohydrates, protein and lipid content in the muscle of *Channapunctatus*and*Cyprinuscarpio.Int. J. Pharma. Appli.,*4(1): 11-18.

Omoregie E, Ofojekwu PC Amali EI. Effects of sublethal concentrations of formalin on weight gain in the Nile Tilapia Oreochromisniloticus (Trewavas). Asian Fish. Soc., 1998; (10): 323-327.

Osman, H., Suriah, A.R. and Law, E.C. 2001. Fattyacid composition and cholesterol content of selected marine fish in Malaysian waters. *Foodchemistry.*73: 55-60.

Pawar, C. D. 1988.Studies on fish and fisheries of river, Panchganga. M.Phil. Dissertation, Shivaji University, Kolhapur, India.

Prakash S. and Verma A.K. (2020b). Effect of Arsenic on Serum Biochemical parameters of a fresh water cat fish, *Mystusvittatus*. *International Journal of Biological Innovations.* 2 (1): 11-19. https://doi.org/10.46505/IJBI.2020.2102

Priestly, R.J. (1976). Studies on parboiled rice: Part I. Comparison of the characteristics of raw and parboiled rice. Food Chemistry, 1, 5-4.

Rani, A.S., R. Sudharsan, T.N. Ready, P.U.M. Ready and T.N. Raju,2000. Effects of sodium arscnite on glucose and glycogen levels in fresh water teleost fish, Tilapia mossambicus. *Poll.Res.,*19(1): 129-131.

Rao, K. S. P., K.R.S. Rao, I.K.A. Sahib and K.V.R. Rao, 1985. Combined action of carbaryl and phenthoate on tissue lipid derivative of muscle of Channapunctatus (Bloch). *Ecotoxicol. Environ Saf.,*9(1): 107- 111.

Rao, R.S.N. and Juliano, S.O. 1970. Effect of parboiling on some physico-chemical properties of rice.Journal of Agricultural and Food Chemistry, 11, 289–294.

Remia, K.M., Logaswamy, S., Logankumar, K. and Rajmohan, D. 2008. Effect of an insecticides (Monocrotophos) on some biochemical constituents of the fish, *Tilapia mossambica. Poll.Res.*, 27: 523-526.

ShaziaQuadir, Abdul Latif, Muhammad Ali and Furhan Iqbal. 2014. Effects of Imidacloprid on thehaematological and serum biochemical profile of *Labeorohita*. *Pakistan J. Zool.*, 46(4): 1085-1090.

Somnath, B., 1991. Effect of acute and sub lethal concentration of tannic acid on the protein, carbohydrate and lipid levels in the tissue of the fish *Labeorohita*. *J. Environ. Biol.,* 12(2): 107-112.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937

V. Subrahmanyan, "Recent advances in rice processing". J. Sci. Ind. Res., vol 30, pp729-731, 1971.

Valarmathi, S. and J. Azariah, 2002. Impact of two sublethal concentrations of copper chloride and chlorine on the excretory products of crab *Sesarmaquadratum*(Fabricicus). *Turk. J. Zool.,* 26: 357-361.

Vutukuru, S.S. 2005. Acute effects of Hexavalent chromium on survival, oxygen consumption,

Xia, C.; Fu, L.; Liu, Z.; Liu, H.; Chen, L.; Liu, Y. Aquatic toxic analysis by monitoring fish behavior using computer vision: A recent progress. J. Toxicol. 2018, 11, 2591924. (CrossRef)

YerragiSG., V.A. Kali and S. Yeraj, 2000. Effect of pesticides Malathion on protein metabolism of the marine crab (Ucamarionis). *J. Exotoxicol. Environ. Monit.,*10(1): 59-62.