



IMPACT OF DISTILLERY EFFLUENT ON INDIAN MAJOR CARP, *CIRRHINUS MRIGALA*

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Abstract: Indian major carp, *Cirrhinus mrigala* was exposed to sublethal concentration (1.5%) of distillery effluent for up to 72 hours to evaluate haematological and biochemical changes in some tissues. The LC_{50} value of distillery effluent was 3.0% (v/v) for 96 hours to *Cirrhinus mrigala*. The haematological parameters like RBC counts, WBC counts and haemoglobin %, were decreased whereas lymphocytes increased in distillery effluent exposed fishes. During exposure, level of free amino acids increased in liver, kidney, muscles and gills but there was gradual decrease in protein and glycogen content distillery effluent exposed fish with reference to control levels. The significant alteration in the levels of free amino acids, protein and glycogen contents in different tissues of distillery effluent exposed fish was due to break down of glycogen and protein to fulfill additional energy requirement during stress conditions. The fish species is therefore recommended as a good bio indicator for the risk assessment of aquatic environmental pollution.

Keywords: *Cirrhinus*, Distillery effluent, Haematological parameters, LC_{50} , Organic molecules.

INTRODUCTION

Rapid industrialization in India has increased the industrial wastewater which is discharged into nearby natural water bodies without proper treatment and disturbs the ecological balance of the aquatic environment causing aquatic environmental pollution. The pollution is continuous and alarming influx to aquatic environment worldwide from both naturally occurring and anthropogenic sources (Verma and Prakash, 2019a). The disposal of industrial effluent in the aquatic environment is toxic to aquatic organisms (Prakash and Singh, 2020). The environmental pollutants or toxicants can induce physiological and biochemical changes in fishes that lead to growth inhibition (Prakash and

Verma, 2019a, 2019b & 2020a; Verma and Prakash, 2019b). Distilleries release enormous amount of waste water known as 'spent wash' which has a typical unpleasant odour of fruity smell and dark brown colour. It has large amount of heavy metals, high COD, BOD and TSS with low pH and DO, causing pollution in the receiving water (Tiwari and Prakash, 2021a). The spent wash from distilleries is not only known for the environmental problem it causes but is also known as a rich source of nutrients viz., potassium, sulphur, nitrogen, calcium, iron, magnesium, zinc and biodegradable organic matter which plays significant role in enhancing soil fertility (Bezuneh and Kebede, 2015).

Many of the toxic substances released from industries or factories are lipophilic and weren't adversely affected by water (Verma and Prakash, 2021). The toxicant present in the water are known to inhibit several biochemical and physiological mechanisms vital for fish metabolism. Due to presence of omega-3 fatty acids such as linolenic acid, decosa-hexaenoic acid and eicosapentaenoic acid, fishes are considered as a good source of animal protein. Omega fatty acid is also good for heart and helps to control diabetics by improving insulin action (Srivastava and Prakash, 2019). These substances accumulate in fish fatty tissues or become protein bound, so it is significant to know the critical concentration above which human being is affected and the commercial fish species become unsuitable food (Tiwari and Prakash, 2021a).

The toxicity tests are necessary in water pollution evolution because only chemical and physical measurements are not sufficient to assess potential effects on aquatic biota.

Haematological and biochemical parameters are the excellent health indicators of an animal and adaption to the environment because these parameters are sensitive enough to evaluate the effect of physical (*i.e.*, temperature, salinity) and chemical (*i.e.*, metallic or organic compounds) stressors (Kumar and Prakash, 2021). So a better understanding of this mechanism can help to predict the harmful effects of various toxicants on environment (Verma and Prakash, 2020). Fishes are much sensitive to changing aquatic environment and play an important role in monitoring of water pollution so they are considered as good bioindicator (Prakash and Verma, 2020b). Thus the main objective of this study was to analyze the impact of distillery effluents and their impact on mortality, haematology and serum biochemistry of Indian major carps, *Cirrhinus mrigala*.

MATERIAL AND METHODS

The fingerlings of *Cirrhinus mrigala* (8.2 ± 1.0 cm and 7.2 ± 1 g) were collected from local fish form, washed with 0.1% solution of KMnO_4 for five minutes and then transferred to the plastic jar containing 50L dechlorinated tap water for acclimatization. The collected fishes were

acclimated to laboratory conditions for 15 days at room temperature. After acclimation, only healthy fingerlings were used for experiments. Feeding was stopped 24 hours prior to the toxicity test, to minimize the contamination from metabolic wastes.

Feeding was stopped 24 hours prior to the toxicity test in order to minimize the contamination from metabolic wastes. To find out LC_{50} and sublethal concentration, a group of 10 acclimatized fishes were exposed to the treated distillery effluent for 24hours, 48hours, 72hours and 96 hours at different concentrations along with a control. The water was changed every 24 hours and during that time aeration was given to aquarium.

The percent concentration of test solution has been calculated by using the formula:

$$\text{Volume \%} = V_E / (V_E + V_{DW}) \times 100$$

Where, V_E = volume of effluent, V_{DW} = volume of dilution water.

For studying the impact of sublethal concentration of distillery effluent (1.5v/v), fishes from each set were sacrificed for the collection of blood. The blood was collected after severing the caudal peduncle of the living fish by a scissor and haematological parameters were determined by following standard methods. The blood was collected after severing the caudal peduncle of the living fish by a scissor. Total number of RBCs, Hb%, WBCs and DLC were estimated by following standard methods.

The fishes of both groups were sacrificed and the desired tissues were dissected out, homogenized and mixed with 5ml of deionized water then centrifuged at 3000 rpm for 10 minutes to obtain supernatant. The supernatants were filtered and the filtrates were used for analysis of the glycogen, protein and free amino acids content by standard methods of Carroll *et al.* (1956), Lowry *et al.* (1951) and Rosen (1957) respectively.

RESULTS AND DISCUSSION

Determination of LC_{50}

Toxicity is a function of concentration and duration of exposure of an organism to a toxicant

(Prakash and Verma, 2018). In the present study, during toxicity, test fishes were exposed to various concentrations such as 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0% and

5.5% of treated distillery effluent collected from distillery unit of Balrampur Chini mill, Balrampur and studied the mortality rate of the fishes.

Table 1: 96 h. LC₅₀ of fish exposed to various concentration of distillery effluents.

Distillery effluent Conc. (%)	No. of fishes Exposed	24hrs	48hrs	72hrs	96hrs	Mortality % age
0.5	10	-	-	-	-	00
1.0	10	-	-	-	1	10
1.5	10	-	-	1	1	20
2.0	10	-	-	1	2	30
2.5	10	-	-	1	3	40
3.0	10	-	1	1	3	50
3.5	10	-	1	2	3	60
4.0	10	1	2	2	2	70
4.5	10	1	2	2	3	80
5.0	10	1	2	2	4	90
5.5	10	2	2	3	3	100

The fish, *Cirrhinus mrigala* was exposed to various concentrations from 24 to 96 hours and mortality rate of distillery effluent obtained, is shown in table 1. The toxicity study of mortality of *Cirrhinus mrigala* due to distillery effluent shows that the mortality rates increase with increase in concentration and time of exposure. For treated distillery effluent, 96h LC₅₀ for *Cirrhinus mrigala* was observed at 3.0% concentration. However, reports regarding lethal toxicity in fishes exposed to distillery effluent are very scanty. LC₅₀ values at 24, 48, 72 and 96 hours for *Cyprinus carpio* exposed to untreated distillery effluent were 1.2%, 1.1%, 1.0% and 0.8, respectively (Ramakrishnan *et al.*, 2005) whereas LC₅₀ values at 24, 48, 72 and 96 hours for *Cyprinus carpio* exposed to treated distillery effluent were 2.8, 2.5, 2.3 and 2.0, respectively (Prakash and Singh, 2020) in *Cyprinus carpio* (2.0% v/v) exposed to distillery effluent. LC₅₀ for 96 hours of distillery effluent for *Mystus vittatus* was determined as 3.38 % (v/v) (Tiwari and Prakash, 2021b). At 5% concentration of treated effluent, survival of 90% fishes are not seen which is the

demand of WHO for final discharge of industrial wastewater (Prakash and Verma, 2020c). So, it can be concluded that the treated distillery effluent is also not safe to aquatic fishes.

Impact of distillery effluent on haematology of *Cirrhinus mrigala*

The impact of distillery effluent on the haematology of *Cirrhinus mrigala* was presented in table 2. The total RBC counts, WBC counts, haemoglobin content were found to be reduced with increased WBC counts in the fishes exposed to sublethal concentration of distillery effluent. In the present study, the reduction in RBC counts and haemoglobin % was found to cause anemia as noticed in fishes by Srivastava *et al.* (2007). Thus the significant decrease in haemoglobin % in effluent exposed fish was due to increased rate of destruction of haemoglobin or due to decrease rate of synthesis of haemoglobin or due to dysfunction / suppression of haemopoietic organ. The reduction in the haemoglobin content in the fish could also be attributed to the lysing of erythrocytes (Verma and Prakash, 2019b). Thus

the significant reduction in these parameters is an indication of severe anemia. In *Cirrhinus mrigala*, the reduction in total WBC count below the normal level (leucopenia) could cause loss of efficiency in the defense mechanism against stress in the species studied. Decreased number of WBC may also be related to an increased level of corticosteroid hormones, whose secretion is a non-specific response to any environmental stressor (Srivastava and Prakash, 2018).

In the differential leucocytes count, a sharp significant increase was observed in the

percentage of lymphocytes. This is an agreement with the findings of Samprath *et al.* (1993) when they exposed the Nile tilapia, *Oreochromis niloticus* to a toxic environment. They attributed to stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants. The significant decrease in eosinophils and neutrophils are in agreement with the findings of Verma and Prakash (2019b) when *Mystus vittatus* were exposed to arsenic. This was attributed to tissue damage.

Table 2: Impact of sublethal concentration of distillery effluent, 1.5% (v/v) on the haematology of *Cirrhinus mrigala*.

Parameters	After 24 hours		After 48 hours		After 72 hours	
	Control	Exposed	Control	Exposed	Control	Exposed
RBC(10^6mm^3)	2.95±0.02	1.85±0.03	3.17±0.81	1.98±0.54	3.08±0.3	1.75±0.21*
Hb (g 100ml^{-3})	9.7±0.12	7.5±0.31	10.0±0.82	8.2±0.24	11.7±0.52	8.01±0.24*
WBC (10^3mm^3)	57.9±0.20	54±0.24	59.0±0.71	55.24±0.61	55.3±0.31	45.02±0.36*
Thrombocyte	25.6±0.98	24.3±0.68	28.2±0.74	27.2±0.81	26.8±0.59	20.5±0.25*
Lymphocytes	32.5±0.25	36.62±0.51	28.4±0.32	29.4±0.33	37.2±0.09	46.60±0.05*
Monocyte	2.2±0.15	2.1±0.11	3.0±0.55	2.5±0.15	1.8±0.37	1.0±0.24
Neutrophils	13.10±0.78	12.34±0.15	14.18±0.21	13.24±0.34	11.2±0.97	9.19±0.71
Eosinophils	14.4±0.30	13.9±0.25	15.6±0.51	14±0.23	12.85±0.37	9.02±0.13*
Basophils	12.8±0.37	12.34±0.15	13.2±0.15	12.85±0.55	11.2±0.37	9.18±0.18

(each value represents the average performance of 10 individuals). *Significant at $p < 0.05$.

Impact of distillery effluent on organic molecules of *Cirrhinus mrigala*

Glycogen is immediate source of energy which gets converted into glucose by glucogenolysis to overcome the stress by pollutants (Verma and Prakash, 2019a). In the present study, there was a significant decrease in glycogen content of liver, muscles, kidney and gills of effluent exposed fishes in all the exposures from control level. The depletion trend of glycogen was Liver > Muscles > Kidney > Gills. Similar observations have been made in fishes exposed to distillery effluent (Maruti and Rao, 2001). In the present study, these alternations may be due to enhanced breakdown of glycogen into glucose through glucogenolysis in tissues to supply the energy

demand under stress condition in the form of glucose, which undergoes breakdown to produce energy rich compound ATP through glycolytic pathway.

Amino acids are essential intermediates in the process of protein synthesis and their degradation products appear in the form of different nitrogenous substances (Prakash and Verma, 2020a). Amino acids and some nitrogenous compounds play important role during osmotic stress hence increase or decrease in free amino acid content provide valuable information during stress phenomenon at the tissue level (Magar and Shaikh, 2012). In the present study, there was a significant increase in

Table 3: Effects of sublethal concentrations of distillery effluent, 1.5%(v/v) on organic molecules (mg/g) in different tissues of *Cirrhinus mrigala* at different period of exposure.

Tissue	Biochemical Parameters	Period of Exposure			
		Control	24 hrs	48 hrs	72 hrs
Liver	Glycogen	17.48 ±0.12	15.88±0.11	15.09±0.15	14.88±0.14*
	Protein	108.83±0.14	99.25±0.21	82.34±0.21	76.64±0.32*
	F.A.A	28.39±0.23	31.57±0.15	33.75±0.17	35.25±0.13*
Muscles	Glycogen	16.78±0.22	15.06±0.11	13.14±0.21	11.48±0.23*
	Protein	136.34±0.11	124.43±0.12	112.11±0.34	101.16±0.11*
	F.A.A	41.12±0.21	37.13±0.11	35.25±0.12	31.15±0.23*
Kidney	Glycogen	6.45±0.22	5.86±0.21	4.54±0.32*	4.17±0.15*
	Protein	91.43±0.32	84.43±0.43	70.32±0.55*	65.36±0.35*
	F.A.A	26.54±0.12	29.43±0.14	33.24±0.16*	29.21±0.32
Gills	Glycogen	15.22±0.23	12.56±0.23	10.78±0.22	8.75±0.23*
	Protein	97.46±0.65	88.05±0.62	81.12±0.53	72.01±0.45*
	F.A.A.	38.12±0.34	40.43±0.13	42.24±0.15	34.11±0.23*

(Values are mean ± SD of six replicates). *Significant at $p < 0.05$

free amino acids level. Similar remarkable changes in free amino acid content have been made in fishes exposed to distillery effluent (Maruti and Rao, 2001). Prakash and Verma (2020d) reported that the enhanced free amino acids may be due to depletion of reserved glycogen so that the fish can try to yield metabolic energy by gluconeogenesis process. Increment in free amino acid level is the result of breakdown of protein for energy requirement and imparted incorporation of amino acids is protein synthesis (Verma and Prakash, 2021). Thus it can be concluded that increase in free amino acid level in tissues and later their sudden decline show that these are utilized in the glycogenesis to compensate the energy demand under effluent stress.

Proteins are building blocks of animal body. The protoplasm of the cell is composed of protein. They play vital role in the process of interaction of cellular medium. In the present study, there was a significant decrease in protein content of liver, muscles, kidney and gills of effluent exposed fishes in all the exposures from control level. Similar observations have been made in

fishes exposed to distillery effluent (Kumar and Gopal, 2001). Verma and Prakash (2021) reported that the depletion of protein level in the tissues of fish is due to diversification of energy, to meet the impending energy demands during stress condition. In the present study, decrease in protein content may be due to reduced protein synthesis or degradation of protein into free amino acid (proteolysis), which is used for different metabolic activity during stress condition.

CONCLUSION

Based on the results of the present study, it can be concluded that alteration in haematological and biochemical content in *Cirrhinus mrigala* indicates biochemical manifestation due to the toxic action of industrial effluent. Industrial effluent affects biologically active molecules such as carbohydrates, proteins and amino acids. The changes in biochemical composition of fishes will naturally affect the nutritive value of aquatic fauna. Thus any alteration in the biochemical parameters will affect the efficiency of the fish, in turn reducing the consumption value.

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