



**EFFECT OF HERBICIDE ON ENZYME ACTIVITY OF FRESH WATER FISH,  
CIRRHINUS MRIGALA**

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**ABSTRACT**

Toxicants contaminate freshwater bodies and affect non-target organisms. Various researchers have reported the effects of chemicals on aquatic organisms. Environmental factors such as pH, turbidity, alkalinity, dissolved oxygen, temperature and conductivity influence the rate of reaction of the pollutants entering the water or the lethal effects on the aquatic organisms. Pollutants in water significantly affect the ability of fish to detect and respond to chemical stimuli, feeding, growth, and reproductive performances could also be seriously affected by such polluted habitat. Pollution of aquatic habitat may result in mass fish mortality or their failure to breed in the polluted environment.

**KEYWORDS:** Herbicide, Cirrhinus mrigala, Enzyme parameters.

**INTRODUCTION**

Pesticide is one such group which consists of lactogenic compounds leading to mutations in exposed organisms. Carcinogenicity checking is becoming important aspect as people are getting exposed to different chemicals directly and indirectly in day to day life. There has been lot of concern about the cellular and molecular changes leading to carcinogenesis. Studies regarding these chemicals need screening for their properties that do not cause mutation but still are having carcinogenic potential (Hori et al., 2006).

Indiscriminate use of pesticides and their untreated effluents affects fish and other aquatic animal if concentration of pesticides reaches above certain limits in exposed organism; it leads to harms like change in metabolism, reproductive disorders, disruption of endocrine system. Death of the organisms occurs when pesticide is more toxic in nature. Several physiological and biochemical functions can get impaired due to exposure to pesticides.

Herbicides and pesticides when applied in restricted areas are washed and carried away by rains and floods to nearby aquatic system, thereby affecting aquatic biota, especially fish, which serves as a rich protein supplement for man. The herbicides affect not only the physiology and survival of aquatic organisms but also interact with their genetic make-up leading to mutations and/or carcinogenesis (NWANI et al., 2010).

Enzymatic evaluation in the diagnosis of effects of herbicides in different tissues and organs of aquatic organisms is widely used as an emerging tool in early warning of herbicidal contamination and its associated remedial measures. Alteration in the chemical composition of aquatic environment usually induces changes in the biochemical aspects of the inhabitants particularly fishes.

**MATERIALS AND METHODS**

Cirrhinus mrigala is also known as the mrigal and the white carp. It is a species of ray finned fish in the carp family. Native to the streams and rivers in India, the only surviving wild population is in the Cauvery River, leading to its IUCN rating as vulnerable. Mrigal is popular as a food and an important aquaculture freshwater species throughout South Asia.

1 ml of Glyphosate is mixed with 1000 ml of water for preparing stock solution (0.1 %). Appropriate narrow range of concentration 2-10 ml was used to find the medium lethal concentration using a minimum of 10 fishes for every 24 hours upto 96 hours. It was found as 0.62 ppm for 72 hours using probit analysis method (Finney 1961). Four groups of fishes were exposed to 0.62 ppm (1/10<sup>th</sup> of 72 hours LC<sub>50</sub> value) concentration of the herbicide 'Glyphosate' for 24, 48, 72 and 96 hours respectively. Another group was maintained as control for each experimental study. Blood samples were

collected from fish for the analysis of enzyme activity parameters like GOT, GPT and LDH.

## RESULTS AND DISCUSSION

Enzyme enticement or depression in fish or other organisms have been proposed for pollution monitoring studies. Fish respond to toxicants by altering their enzyme activities and the inhibition or induction of these enzyme activities has been used to indicate tissue damage.

The gill tissues were found to contain 27.30IU/L of GOT in the control fish. The fishes which were maintained in 0.62 ppm herbicide concentration for 24, 48, 72 and 96 hours period were found to contain 27.88IU/L, 28.30IU/L, 29.40IU/L and 29.92IU/L of GOT in their gill tissues. The control fish was found to show about 15.10IU/L of GOT activity in liver tissue. But, when subjected to 24,48,72, and 96 hours exposure in 0.62 ppm herbicide concentration they GOT levels more recorded as 16.82IU/L, 17.90IU/L, 18.80IU/L and 19.43 IU/L respectively. The mean continued value of GOT activity level in the fishes, which were maintain in short term exposure, was found to contain about 34.92IU/L in kidney tissues. The fishes which were subjected to 24, 48, 72 and 96 hours in 0.62 ppm herbicide concentration were found to have 35.65IU/L, 37.88IU/L, 38.20IU/L

and 39.20IU/L respectively. The muscle tissues of the fish, *Cirrhinus mrigala* in 0.62 ppm detergent concentration for shorter duration of for 24, 48, 72 and 96 hours contained 26.73IU/L, 27.23IU/L, 27.85IU/L and 28.20IU/L respectively. The mean control value was 25.30IU/L.

The gill tissues were found to contain 20.72IU/L of GPT in the control fish. The level of activity in the groups, which were maintained in 0.62 ppm herbicide concentration for shorter duration. The fishes which were exposed to shorter periods of 24, 48, 72 and 96 hours, the activity levels of GPT were 21.85IU/L, 22.43IU/L, 23.89IU/L and 25.27IU/L respectively. The levels of activity of the enzyme GPT in liver were 46.32IU/L, 47.88IU/L, 49.40IU/L and 50.20IU/L after short exposures of 24, 48, 72 and 96 hours periods. The mean control value in the liver tissue was about 44.23IU/L. The kidney tissue was found to record 34.44IU/L, 35.67IU/L, 37.87IU/L and 38.92IU/L of GPT activity levels shorter periods of exposure. The mean control value was found as 34.00IU/L respectively. The levels of activity of the enzymes in the muscle tissue after short term exposure were 27.82IU/L, 28.20IU/L, 28.92IU/L and 29.14IU/L however the mean value of the control group was 26.34IU/L.

**Table 1: GOT Level In Tissues Of *Cirrhinus Mrigala* Exposed To The Herbicide (Glyphosate).**

Sample Enzymes Activity	Exposure Period				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gills	27.30 ± 0.90	27.88 ± 0.54	28.30 ± 1.15	29.40 ± 1.02	29.92 ± 1.28
't' Value	52.53	-0.95	-1.18	-0.72	-2.90
% Change		2.12	3.66	7.69	9.59
Liver	15.10 ± 0.78	16.82 ± 0.78	17.90 ± 0.68	18.80 ± 0.72	19.43 ± 0.82
't' Value	35.53	-2.70	-4.68	-6.03	-6.62
% Change		1.29	18.5	24.50	28.67
Kidney	34.92 ± 0.98	35.65 ± 1.24	37.88 ± 1.29	38.20 ± 1.30	39.20 ± 1.32
't' Value	61.71	-0.79	-2.94	-3.48	-4.50
% Change		2.09	8.47	9.39	12.25
Muscles	25.30 ± 1.10	26.73 ± 1.32	27.85 ± 1.16	27.23 ± 0.59	28.20 ± 0.78
't' Value	39.83	-1.44	-2.76	-2.67	-3.72
% Change		5.6	10.07	7.6	11.4

Values are mean ± SD, n=5, figures in parenthesis are percentage Increase over control.

\*\* = Significant at one percent level, \* = Significant at five percent level, NS= Non Significant

**Table 2: GPT Level In Tissues Of *Cirrhinus Mrigala* Exposed To The Herbicide (Glyphosate).**

Sample Enzymes Activity	Exposure Period				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gills	20.72 ± 1.14	21.85 ± 0.98	22.43 ± 0.72	23.89 ± 1.06	25.27 ± 0.68
't' Value	31.48	-1.30	-2.19	-3.52	-5.93
% Change		5.45	8.2	15.29	21.95
Liver	44.23 ± 1.80	46.32 ± 1.00	47.88 ± 0.63	49.40 ± 0.72	50.20 ± 0.88
't' Value	42.56	-1.75	-3.31	-4.61	-5.16
% Change		4.72	8.2	11.68	13.4
Kidney	34.00 ± 1.06	34.44 ± 1	35.67 ± 0.92	36.87 ± 0.82	38.92 ± 0.68
't' Value	56.09	-0.51	-2.03	-3.72	-6.80
% Change		1.29	4.9	8.44	14.47
Muscles	26.34 ± 1.55	27.82 ± 0.94	28.20 ± 1.06	28.92 ± 0.63	29.14 ± 1.67
't' Value	29.43	-1.41	-1.71	-2.67	-2.12
% Change		5.61	7.06	9.7	10.63

Values are mean  $\pm$  SD, n=5, figures in parenthesis are percentage Increase over control

\*\* = Significant at one percent level, \* = Significant at five percent level, NS= Non Significant

**Table 3: LDH Level In Tissues Of *Cirrhinus Mrigala* Exposed To The Herbicide (Glyphosate).**

Sample Enzymes Activity	Exposure Period				
	Control	24 hrs	48 hrs	72 hrs	96 hrs
Gills	31.50 $\pm$ 1.08	32.10 $\pm$ 0.88	32.90 $\pm$ 0.63	33.12 $\pm$ 1.00	34.25 $\pm$ 0.23
‘t’ Value		-0.74	-1.93	-1.90	-4.31
% Change	50.51	1.90	4.44	5.14	8.73
Liver	50.20 $\pm$ 0.72	51.25 $\pm$ 1.06	52.80 $\pm$ 0.33	53.18 $\pm$ 0.88	54.00 $\pm$ 0.72
‘t’ Value		-1.41	-5.68	-4.53	-6.46
% Change	120.7	2.09	5.17	5.93	7.5
Kidney	41.20 $\pm$ 1.20	42.90 $\pm$ 1.06	43.00 $\pm$ 0.23	44.25 $\pm$ 0.98	45.20 $\pm$ 0.78
‘t’ Value		-1.83	-2.55	-3.40	-4.84
% Change	59.46	4.12	4.36	7.40	9.70
Muscles	27.80 $\pm$ 0.94	28.22 $\pm$ 0.72	29.40 $\pm$ 0.53	30.20 $\pm$ 1.08	31.87 $\pm$ 0.98
‘t’ Value		-1.22	-3.96	-3.69	-6.48
% Change	-78.40	1.51	5.7	8.63	14.64

Values are mean  $\pm$  SD, n=5, figures in parenthesis are percentage Increase over control

\*\* = Significant at one percent level, \* = Significant at five percent level, NS= Non Significant

The gill tissues were found to contain 31.50IU/L of LDH in the control fish. The fishes which were maintained in 0.62 ppm herbicide concentration for 24, 48, 72 and 96 hours period were found to contain 32.10IU/L, 32.90IU/L, 33.12IU/L and 34.25IU/L of GOT in their gill tissues. The control fish was found to show about 50.20IU/L of GOT activity in liver tissue. But, when subjected to 24, 48, 72, and 96 hours exposure in 0.62 ppm herbicide concentration the GOT levels more recorded as 51.25IU/L, 52.80IU/L, 53.18IU/L and 54.00IU/L respectively. The mean continued value of LDH activity level in the fishes, which were maintain in short term exposure, was found to contain about 42.40IU/L in kidney tissues. The fishes which were subjected to 24, 48, 72 and 96 hours in 0.62 ppm herbicide concentration were found to have 42.90IU/L, 43.00IU/L, 44.25IU/L and 45.20IU/L respectively. The muscle tissues of the fish, *Cirrhinus mrigala* in 0.62ppm detergent concentration for shorter duration of for 24, 48, 72 and 96 hours contained 28.22IU/L, 29.40IU/L, 30.20IU/L and 31.87IU/L respectively. The mean control value was 27.80IU/L.

Increased enzymatic activities of GOT and GPT measured in our study may be correlated with cell-membrane damage or changed permeability caused by herbicide leading to selective leakage to blood stream. This contention GOT and GPT are diagnostically most useful to detect cellular damage.

The increase in GOT and GPT activity may be due to decrease in metabolic activity, disruption of enzyme system by blocking active sites and tissue damage. Beyer (1996) reported the increased activity of GOT and GPT in the intestine tissues of the fish, *Catlacatlaon* exposed to Triazophos. The increase in LDH level indicates a metabolic change, which is the glycogen catabolism and glucose shift towards the formation of Lactate in stressed fish, primarily the muscle tissue (Vander Oost, 2003).

The decreased activities of ACP and ALP indicate disturbance in cell organelles like endoplasmic reticulum and membrane transport system. Similar findings decreased ACP were reported in Labeorohitaon exposure to Arsenic.

Lactate dehydrogenase (LDH) is an enzyme which takes part in anaerobic pathway of carbohydrate metabolism. Increase of LDH activity is a diagnostic index widely used to recognize increases of anaerobic metabolism resulting from depletion of energy under anaerobic and environmental stress conditions (Banaee, 2013). Sastry and Sharma, (1980) who stated that the increased enzymatic activities in the liver might be due to increased enzyme synthesis to counter the damage caused by toxicants. In contrast, the decreased activities of some enzymes may be attributed to decreased enzyme synthesis, or it may also be due to changes in permeability of hepatic cells.

The metabolic pathway in liver of fish are affected by various pollutants including organic chemicals due to the alteration of cellular enzymatic activities. The decreased activities of GOT, GPT, ACP and ALP, indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Such damage to cell organelles has been reported in various studies (Karatas and Kalay 2002).

## CONCLUSION

The present investigation indicated that the herbicide is toxic to fish and further earlier findings that enzymes su as GPT, GOT, LDH in fish could be effectively used as biomarkers of herbicide toxicity. The increase in GOT and GPT activity may be due to decrease in metabolic activity, disruption of enzyme system by blocking active sites and tissue damage. These are also key enzymes of nitrogen metabolism and are important in energy

mobilization. These are precursors in studying alterations in protein and carbohydrate metabolism. GOT and GPT levels were found to be increased after pesticide exposure. The increase in GOT and GPT activity suggests that proteins are channeled into the metabolic pathway.

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