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# In vivo biochemical changes in liver and gill of *Clarias batrachus* during cypermethrin exposure and following cessation of exposure

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**Abstract**

The sublethal effect of a synthetic pyrethroid, cypermethrin on total protein, amino acids, ammonia, glycogen, and enzymes like aminotransaminases (AIAT, AAT), glutamate dehydrogenase, and glycogen phosphorylases (a and ab) was studied in physiological important tissues viz; liver and gill tissues of freshwater teleost air breathing fish, *Clarias batrachus*. The study was conducted during exposure of 1/3 (33%) of $LC_{50}$ concentration and followed by cessation of exposure. Thirty-six fish were exposed to 0.07 mg/L cypermethrin for 10 days. After 10 days, 18 fish were released to freshwater and kept in the same for 10 days (recovery group). Thirty-six fish were kept in freshwater as control batch. Protein content in liver tissues decreased at the end of 1st and 5th day followed by slight increase at the end of 10th day. Gill tissue showed statistical significant decrease ($P < 0.001$) in protein content during exposure period of 10 days. Recovery in protein content was observed to a large extent in both the tissues. Total free amino acids were increased in liver and gill tissues throughout the treatment period, recovery response was seen after cessation of exposure. Ammonia level was decrease in both the tissues throughout the exposure period except in liver tissue at the end of 1st day of exposure. Recovery response was exhibited by both the tissues. A decreased in glycogen content of liver tissue was observed during exposure period, gill tissue also showed decrease in glycogen at the end of 1st and 5th day followed by increase at the end of 10th day of exposure period. When the fish were transferred to freshwater, recovery in glycogen content was noted. The activity level of alanine, aspartate aminotransaminase, glutamate dehydrogenase, and phosphorylases (a, ab) was increased in both the tissues, followed by recovery response after released of fish into freshwater. The present study showed that cypermethrin caused alterations in certain biochemical mechanisms of *C. batrachus*. This fish indicated recovery response when transferred to cypermethrin free water.


*Keywords:* Sublethal toxicity; Cypermethrin; Liver; Gill; Metabolites; Enzymes; Recovery response

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## 1. Introduction

Pesticides are used extensively in agriculture but their residues often reach aquatic ecosystem. They can be transferred through phytoplankton to fish and ultimately to humans. The low toxicity to mammals of synthetic pyrethroid has encouraged their use in intensive agriculture. In aquaculture they have been used as replacement for more toxic pesticides such as organophosphates. The hypersensitivity of fish to pyrethroid intoxication is due partly to species specific differences in pyrethroid metabolism, but principally to the increased sensitivity of the piscine nervous system to these pesticides. Moore and Waring [17] reported that even low levels of cypermethrin in the aquatic environment might have a significant long-term effect on Altantic Salmon populations through disruption of reproductive functions. α-cypermethrin is practically non-toxic to birds but is highly toxic to fish and aquatic invertebrates. This is mainly because it is metabolized and eliminated significantly more slowly by fish than mammals or birds [10,26].

In fish culture, cypermethrin is used against lice infestation [23]. Therefore, it is necessary to investigate the deleterious effect of this insecticide on fish and their ecosystem. Reports are available on the effects of cypermethrin on fish [5,20]. There is no reported literature on the toxicity of cypermethrin during exposure and following cessation of exposure in economically important food fish. Hence, the present study was undertaken to examine the effect of cypermethrin on biochemical aspects of physiologically important tissues of Indian food fish, *Clarias batrachus*, during exposure and after transferred into freshwater. The biochemical aspects like total proteins, total free amino acids, ammonia, glycogen, aminotransaminases (aspartate and alanine), glutamate dehydrogenase, and glycogen phosphorylases (a and ab) were estimated in liver and gill tissues of the fish, *C. batrachus* exposed to sublethal concentration of commercial grade cypermethrin insecticide.

## 2. Materials and methods

Healthy freshwater commercially available food fish, *C. batrachus* (Linn) in the weight range of $38 \pm 2$ g and length $20 \pm 1$ cm were purchased from a local fish market and brought to the laboratory. They were kept in glass aquaria and fed ad libitum with egg albumin, and commercial fish feed and acclimatized in the laboratory for 2 weeks at $25\,^{\circ}\mathrm{C} \pm 2$ under natural photoperiod (12 h light/12 h dark) prior to the use for experiment. Only healthy and one day starved fish were used for experimentation. Cypermethrin (*RS*) α-cyano-3 phenoxybenzyl-(I*R*,I*S*) *cis*,*trans*-3 (2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate was purchased from United Phosphorus (Bombay, India). The purity of commercial compound used in the study is 10% emulsifiable concentrate. Its common trade names are Ripcord and Cyperkill. The solution of the cypermethrin insecticide was made by dissolving in 100% ethanol and diluting with laboratory water to obtain the desired concentration (0.1 ml/L), same concentration of ethanol was used in the control group. The sublethal concentration of cypermethrin (0.07 mg/L) is 33% of the 96 h $LC_{50}$ value (0.21 mg/L) determined by the method of Finney [7]. Experimental fish were divided into two groups of 36 each and placed in separate glass aquaria. Group I fish reared in cypermethrin free water served as control. Group II exposed to 0.07 mg/L for 10 days served as exposed group. After 10 days of exposure, 18 fish from exposed group were transferred to freshwater served as recovery group. The aquaria were cleaned daily and the water was renewed in all the groups. Cypermethrin was added daily in the exposed group only in order to keep the concentration constant throughout the study period of 10 days. Six fish each from control, exposed, and recovery group were removed at the end of 1, 5, and 10 days, and sacrificed for liver and gill tissue sampling. Tissues were immediately removed, frozen, and used within an hour for the assay of metabolites and enzymes.

Proteins were estimated by the method of Lowry et al. [15], total free amino acids by Moore and Stein [16], Ammonia with Nessler's reagent as described by Bergmeyer [1], and glycogen by the method of Kemp and Kits Van Heijinger [12] using 10% homogenate (w/v). The enzymes, aminotransaminases (AIAT, AAT) were assayed by Reitman and Frankel as described by Bergmeyer [1],