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## International Journal of Biological & Medical Research

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### Original Article

# Cypermethrin Induced Toxicity to Phosphatases and Dehydrogenases in Gills and Hemolymph of Fresh Water Crab, *Spiralothelphusa hydrodroma* (Herbst)

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#### ARTICLE INFO

##### Keywords:

Gills,  
hemolymph,  
cypermethrin,  
LC<sub>50</sub>  
*Spiralothelphusa hydrodroma*

#### ABSTRACT

The fresh water field crab, *Spiralothelphusa hydrodroma* is an important food source in parts of South India. The impact of cypermethrin on the variations in the enzyme activity of gills and hemolymph of the experimental female crab *S. hydrodroma* was carried out. The toxicity of the cypermethrin on the crab was estimated using LC<sub>50</sub>. Quantitative enzymatic studies of lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) in the gills and hemolymph was undertaken.

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### 1. Introduction

Pesticides are toxic substances, non-biodegradable and accumulate in the food chain. Mostly they affect the nervous system causing tumors in living organisms. They are not only neurotoxic but also affect other systems and have shown a high degree of impact on metabolism by inhibiting enzymes like acetyl cholinesterase[1, 2]. In biological systems, the biocatalysts play a vital role in the metabolic pathways. Animal exposed to stress conditions alter their physiological status with the help of enzymes. Toxicants like pesticides are known to secrete hyper or hypo level of enzymes. The trace metal concentrations in Queensland Estuarine crabs, *Australoplax tridentate* have been observed[3]. Various chemicals entering the aquatic ecosystem through human activities, either accidentally or by design may cause adverse effects on the aquatic biota, including deleterious changes which disrupt metabolic activity at the biochemical levels[4]. The pesticide derivatives are known to alter the physico-chemical characteristics of water; these in turn interfere and interact with various physiological activities of organisms. Changes in metabolic rate among organisms exposed to pollution stress have been used as indicator of stress condition. When any

aquatic animal is exposed to polluted medium, a sudden stress is developed for which the animals should meet more energy demand to overcome the toxic stress. [5]reported on the toxic effects of sublethal concentration of copper sulphate, on certain biologically important enzymes in *Saccobranchus fossilis*. The present work was that the effect of cypermethrin on the gills and hemolymph of *Spiralothelphusa hydrodroma*.

### 2. Materials and Methods

*Spiralothelphusa hydrodroma* were collected from fresh water ponds and paddy fields on the outskirts of Walajapet, Vellore District, Tamil Nadu. The crabs were brought to the laboratory in large plastic trough and maintained in normal daylight illumination in the laboratory thereby providing normal acclimatization. The crabs were fed with uncooked oats. For all experiments, the crabs were used with carapace length ranging from 3 to 4.5cm and breadth ranging from 5 to 6.5cm. The water level was maintained carefully so that the crabs were partially immersed. Acute toxicity study was carried out to determine the potency of cypermethrin for static but renewal type of bioassay was adopted in the present investigation to estimate the LC<sub>50</sub> values. The cypermethrin was used as commercial preparation. The experiment was carried out to find the range of concentrations for confirmatory evaluation. The mortality was recorded for the crab at 24, 48, 72 and 96 hr exposure to cypermethrin; the LC<sub>50</sub> values were estimated as 2.027, 1.698, 1.452 and 1.315ppm respectively; were corrected for natural response by Abbott's formula[6].

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**Table 1: The LC<sub>50</sub> Values and Regression Equations for *Spiralothelphusa hydrodroma* treated with Cypermethrin**

Exposure periods (hours)	LC <sub>50</sub> (ppm)	Upper confidence limits (ppm)	Lower confidence limits (ppm)	Regression results	Slope function	r <sup>2</sup>
24	2.027	2.561	1.739	Y = - 0.932 X + 0.468	2.973	0.99
48	1.698	1.938	1.345	Y = - 0.658 X + 0.281	3.265	0.98
72	1.452	1.883	1.136	Y = - 0.724 X + 0.391	4.121	0.99
96	1.315	1.763	1.118	Y = - 0.611 X + 0.324	4.973	0.99

Chronic study on the effect of cypermethrin on the crab was conducted by exposing to two sublethal safe concentrations for 20 and 40 days. According to [7], 1/10th and 1/3rd of the 96 hr LC<sub>50</sub> value represent lower and higher sublethal concentrations respectively. Hence lower (0.1315ppm) and higher (0.4383ppm) sublethal concentrations of the insecticide were arbitrarily used. At the end of the treatment period, the control and treated crabs were dissected and the gills and hemolymph were collected for enzymatic assays. Lactate dehydrogenase, succinate dehydrogenase, acid phosphatase and alkaline phosphatase were estimated following the techniques adopted [8, 9]. One way analysis of variance (ANOVA) was performed based on the methods of [10].

### 3. Results

In the present study, quantitative changes in lactate dehydrogenase, succinate dehydrogenase, acid phosphatase and alkaline phosphatase were observed in gills and hemolymph. Each experiment was repeated three times with different individuals and the mean value was taken.

**Table 2: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Lactate Dehydrogenase**

Exposure periods (hours)	Tissues	Control Mean ± SD	LSCMean ± SD	HSCMean ± SD	F-Value	P-Value
20 days	Gills	4.47 ± 0.51	5.44 ± 0.54	6.62 ± 1.01	10.03 **	<0.01
	Hemolymph	8.84 ± 0.72	9.52 ± 0.73	10.47 ± 0.62	5.11 *	<0.05
40 days	Gills	4.74 ± 0.44	5.80 ± 0.61	6.38 ± 0.21	14.56 **	<0.01
	Hemolymph	8.75 ± 0.81	9.86 ± 0.53	10.96 ± 0.61	10.23 **	<0.01

Mean ± SD of six individual observations  
 Values are expressed µg / 100 mg wet tissue  
 \*\* indicates significance at 0.01 level  
 \* indicates significance at 0.05 level

**Table 3: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Succinate Dehydrogenase**

Exposure periods (hours)	Tissues	Control Mean ± SD	LSCMean ± SD	HSCMean ± SD	F-Value	P-Value
20 days	Gills	7.89 ± 0.71	7.67 ± 0.46	6.93 ± 1.27	1.02*	<0.05
	Hemolymph	14.03 ± 1.08	13.79 ± 1.05	12.88 ± 0.84	1.72*	<0.05
40 days	Gills	7.58 ± 0.43	6.25 ± 0.51	5.43 ± 0.68	10.62**	<0.01
	Hemolymph	14.15 ± 0.83	13.18 ± 0.66	12.15 ± 0.62	12.25**	<0.01

Mean ± SD of six individual observations.  
 Values are expressed MIU/min/mg protein  
 \*\* indicates significance at 0.01 level  
 \* indicates significance at 0.05 level

**Table 4: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Acid Phosphatase**

Exposure periods (hours)	Tissues	Control Mean $\pm$ SD	LSC Mean $\pm$ SD	HSC Mean $\pm$ SD	F-Value	P-Value
20 days	Gills	3.67 $\pm$ 0.21	3.87 $\pm$ 0.58	4.06 $\pm$ 0.46	0.88 *	<0.05
	Hemolymph	4.03 $\pm$ 0.32	4.37 $\pm$ 0.51	4.65 $\pm$ 0.44	0.46 *	<0.05
40 days	Gills	3.58 $\pm$ 0.22	4.33 $\pm$ 1.26	5.09 $\pm$ 1.08	2.28 *	<0.05
	Hemolymph	4.08 $\pm$ 0.34	4.96 $\pm$ 0.42	5.54 $\pm$ 0.98	3.76 *	<0.05

Mean  $\pm$  SD of six individual observations.

Values are expressed mg PNPP to PNP/100 mg wet tissue

\*\* indicates significance at 0.01 level

\* indicates significance at 0.05 level

**Table 5: Effect of Lower and Higher Sublethal Concentrations of Cypermethrin on Alkaline Phosphatase**

Exposure periods (hours)	Tissues	Control Mean $\pm$ SD	LSC Mean $\pm$ SD	HSC Mean $\pm$ SD	F-Value	P-Value
20 days	Gills	4.58 $\pm$ 0.48	4.23 $\pm$ 0.68	3.92 $\pm$ 0.34	0.97*	<0.05
	Hemolymph	7.86 $\pm$ 0.52	7.36 $\pm$ 0.56	6.98 $\pm$ 0.65	0.78*	<0.05
40 days	Gills	4.55 $\pm$ 0.47	4.10 $\pm$ 0.53	3.48 $\pm$ 0.40	4.89**	<0.01
	Hemolymph	7.59 $\pm$ 0.43	7.02 $\pm$ 0.26	6.66 $\pm$ 0.62	2.87*	<0.05

Mean  $\pm$  SD of six individual observations.

Values are expressed mg PNPP to PNP/100 mg wet tissue

\*\* indicates significance at 0.01 level

\* indicates significance at 0.05 level

### 3.1. Lactate Dehydrogenase

The lactate dehydrogenase (LDH) activity in the gills of the control crab was 3.87 and 4.74  $\mu$ g / 100 mg wet tissue for 20 and 40 days respectively. In the experimental crabs, the LDH activity in the lower sublethal concentration was 5.44 and 5.80  $\mu$ g / 100 mg wet tissue and for higher sublethal level, it was 6.62 and 6.38 mg / 100  $\mu$ g wet tissue for 20 and 40 days exposure periods. The increase in LDH activity of the gills calculated was found to be statistically insignificant.

In the hemolymph of the control crab, the LDH activity was found to be 8.84 and 8.75  $\mu$ g / 100 mg wet tissue for 20 and 40 days of exposure periods. In the experimental crabs, the LDH activity of the lower sublethal concentration was 9.52 and 9.86  $\mu$ g / 100 mg wet tissue and in the crabs treated with higher sublethal concentration, it was 10.47 and 10.96  $\mu$ g / 100 mg wet tissue for 20 and 40 days of treatment. In the 40 days of exposure, maximum LDH activity in the hemolymph was observed in both the sublethal concentrations. The readings were found to be statistically insignificant in 20 days and statistically significant for 40 days treatment of cypermethrin.

### 3.2. Succinate Dehydrogenase

The succinate dehydrogenase (SDH) activity in the gills of the control crab was 7.89 and 7.58 MIU/min/mg protein for 20 and 40 days of treatment respectively. In the experimental crabs, the SDH activity was decreased for both the sublethal concentrations. The succinate dehydrogenase activity for lower sublethal concentration was found to be 7.69 and 6.25 MIU/min/mg protein and for higher sublethal concentration, it was 6.93 and 5.43 MIU/min/mg protein for 20 and 40 days of exposure periods. Maximum decrease in SDH activity of the gills was observed in 40 days of exposure period, and the decrease in SDH activity of the gills was statistically significant and 20 days of treatment was insignificant.

The SDH activity in the hemolymph of the control crab was 14.03 and 14.15 MIU/min/mg protein for 20 and 40 days of treatment periods. In the experimental crabs, the SDH activity in the lower sublethal concentration was found to be 13.79 and 13.18 MIU/min/mg protein, and in the crabs treated with higher sublethal concentration was 12.88 and 12.15 MIU/min/mg protein for 20 and 40 days of experimental periods respectively. The decline in SDH activity of the hemolymph was found to be statistically insignificant in 20 days and statistically significant in 40 days of exposure period.

### 3.3. Acid Phosphatase

The acid phosphatase (ACP) activity in the gills of the control crab was 2.68 and 2.77 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment respectively. In the experimental crabs, the ACP activity increased for both the sublethal concentrations of cypermethrin. The ACP activity in the lower sublethal concentration was 2.99 and 3.24 mg PNPP to PNP/100mg wet tissue and for higher sublethal level, it was found to be 3.56 and 3.91 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure. The increase in enzyme activity of the gills was statistically insignificant in 20 days treatment and statistically significant in 40 days treatment.

The ACP activity in the hemolymph of the control crabs was analyzed as 7.07 and 7.26 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. In the experimental crabs, the ACP activity at the lower sublethal concentration was 7.39 and 7.81 mg PNPP to PNP/100 mg wet tissue and in higher sublethal concentration, it was found to be 7.48 and 7.99 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of experimental periods. The ACP activity was found to be statistically insignificant in both 20 and 40 days of treatment periods in both lower and higher sublethal concentrations.

### 3.4. Alkaline Phosphatase

The alkaline phosphatase (ALP) activity in the gills of the control crab was 4.58 and 4.55 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days respectively. The ALP of gills in the lower sublethal concentration was 4.23 and 4.10 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods and in the higher sublethal concentration was 3.92 and 3.48 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. The decrease ALP activity in the gills was statistically insignificant at 20 day and statistically significant at 40 day of exposure periods.

The hemolymph of crabs exposed to lower sublethal concentration expressed 7.36 and 7.02 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of treatment periods. When the crabs treated with higher sublethal concentration, it was 6.98 and 6.66 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. In the control crabs, the enzyme activity was found to be 7.86 and 7.59 mg PNPP to PNP/100 mg wet tissue for 20 and 40 days of exposure periods. The values were found to be statistically insignificant on 20 and 40 days of exposure period in both the lower and higher sublethal concentrations of cypermethrin.

### 4. Discussion

Water is one of the basic requirements of all aquatic as well as terrestrial lives for growth and survival. Aquatic systems are contaminated by disposal of various abiotic factors. Pesticides by virtue of their design and application induce a broad spectrum biocidal effect influencing most of the organisms [11]. These pollutants also destroy the quality of aquatic ecosystems and render it unfit for various aquatic organisms, particularly fresh water crabs. In the present study, the strategy of energy production

adopted by *Spiralothelphusa hydrodroma* were assayed by tracking changes in the activities of lactate dehydrogenase, succinate dehydrogenase, acid phosphatase and alkaline phosphatase in its gills and hemolymph under stress at lower (0.1315ppm) and higher (0.4383ppm) sublethal concentrations of cypermethrin revealed highly fascinating informations.

The activity of lactate dehydrogenase, which is a cytoplasmic enzyme, shows a marked elevation in activity in the gills and hemolymph. Lactate dehydrogenase activity is generally associated with cellular metabolic activity which acts as a pivotal enzyme between the glycolysis and citric acid cycle. Thus, the elevation of lactate dehydrogenase may suggest a bias towards the anaerobic glycolytic pathway. The lactate dehydrogenase activity increased in the *U.annulipes* treated with sublethal concentrations of cadmium and mercury [12] and in *S.hydrodroma* in response to copper and zinc [13]. Increased lactate dehydrogenase activity and decreased succinate dehydrogenase activity was reported by many workers namely in *O.senex* in response to sumithion [14]. [15] studied the effect of lead on *Anabas scandens* and found that there was increase in the activity of lactate dehydrogenase and decrease in the succinate dehydrogenase activity. Lactate dehydrogenase activity increased in the hepatopancreas in the fielder crab, *U.pugilator* in response to cadmium [16]. On the contrary, lactate dehydrogenase activity decreased in the abdominal muscle in *U.pugilator* [16] and in *S.serrata* [17] when exposed to cadmium. Significantly lactate dehydrogenase activity decreased in giant fresh water prawn, *Macrobrachium rosenbergii* in accordance with increase in  $Cu^{2+}$  concentration [18]. The results of the present study are well in accordance with that of previous investigations in the increased activity of lactate dehydrogenase in cypermethrin treated crabs.

The decline in the activity of respiratory oxidative enzyme, the succinate dehydrogenase in gills and hemolymph indicates decline in enzyme synthesis, since cypermethrin disrupt the membrane bound enzyme. Mitochondrial damage leads to decreased respiration and partial coupling of oxidative phosphorylation [19]. Suppression of succinate dehydrogenase activity disrupts mitochondria in anoxic or hypoxic conditions when exposed to toxicants. Succinate dehydrogenase plays an important role in regulating osmoregulation and any change in its activity would disrupt the osmoregulatory mechanism [20]. Decrease or increase in the enzyme activity represents the metabolic stress in any organism. The results of the present study are also in conformity with those of the earlier observations.

Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme which was kept in a latent state inside the membrane of lysosomes, due to the disruption of the membrane [21]. Phosphatases play an important role in carbohydrate metabolism [22]. [23] reported increase in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzymes and carbohydrate forms the major reserve of many crustaceans accumulated in the hepatopancreas [24]. [25] were of the opinion



that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase. It was concluded that both induction and inhibition of phosphatase takes place depending on the concentration of metals. [26] concluded that sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatases. Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphate to release energy in view of impaired ATPase system during metal stress.

Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways. [27] studied the effect of pyrethroid on the fish *Clarias bairdii* and found that alkaline phosphatase decreased in response to the toxicant. [25] studied the effect of copper on oxygen consumption and phosphatase in *S. serrata* and concluded that there was decrease in alkaline phosphatase activity in muscle, hepatopancreas and haemolymph. Similar observations were noted by [28] in the same crab in response to naphthalene. In the present investigation, the activity of alkaline phosphatase was found to decrease in the experimental crabs when compared with that of the control crabs. So it was concluded that the cypermethrin, a pyrethroid compound causes a decrease in the alkaline phosphatase level in the organs which leads to the metabolic abnormalities. In the present investigation, the activity of alkaline phosphatase was found to decrease in the experimental crabs when compared with that of the control crabs.

### Acknowledgement

Authors sincerely thank Dr.R.Rangarajan, Chairman, Dr.Sakunthala Rangarajan, Vice-Chairman, VELTECH Group of Educational Institutions and Dr.K.Siddappa Naidu, Principal, VELTECH MULTITECH Dr.Rangarajan Dr.Sakunthala Engineering College, Chennai - 600 062 for their unremitting encouragement and valuable advices.

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