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Histological alterations and changes in dehydrogenase activities in tissues of *Spiralothelphusa hydrodroma*, exposed to quinalphos

*** J Manju Bashini, P Senthilkumaar**
P.G. and Research Department of Zoology, Sir Theagaraya College, Chennai, Tamil Nadu, India

Abstract
The extensive use of pesticides to control agricultural pests poses a serious threat to organisms of the aquatic environment. 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. In the present study the impact of pesticide quinalphos on tissues (muscle, gills, hepatopancreas, spermatheca and ovary) of *Spiralothelphusa hydrodroma* was determined. Histological alterations and biochemical changes such as succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH), activities in tissues had been carried out. Overall work concluded that histological biomarkers provide reliable data to discriminate the usage of pesticides which had direct influence on loss of aquatic animals.

Keywords: quinalphos, tissues, *Spiralothelphusa hydrodroma*

1. Introduction
Pesticides have different modes of action to aquatic inhabitants. As a result release of pollutants from industrial areas, and agricultural runoff into the environment severely mixes into water bodies (Tyagi, 2000) [45]. Histological changes not only give an early indication of pollution hazard, but also provide useful data on nature and degree of damage to cells and tissues (Shaikh *et al.*, 2010) [38]. Environmental pollution found to be undesirable side effect of industrialization and an important aspect of environmental degradation (Jothinarendiran, 2012) [21]. Histological studies have a way for understanding the pathological conditions of the animal by helping in diagnosing the abnormalities or damages of the tissues exposed to toxic stress of heavy metals (Sprague, 1971; Andhale *et al.*, 2011 and Maryam, *et al.*, 2013) [40, 26, 4]. Aquatic ecosystems are more sensitive to the release of industrial wastewater (Pálaez-Cid *et al*., 2013) [31]. Stress exerted by exposure of freshwater crabs to pesticide drained into water bodies had altered activity of enzyme constituents, which indicated significant influence of toxic nature of this insecticide to crab as an important species of aquatic ecosystem (Patil *et al.*, 2014) [32]. Freshwater crabs are often exposed to biopesticides in their aquatic habitats through the agricultural runoff; generally most of the pest organisms belong to the lower trophic level of the food chain in an ecosystem. However, no attention has been paid to small invertebrates such as crabs, prawns, gastropods, bivalves, etc, which are also used as food. Hence, further study is warranted to understand the extent of such undesirable effects of the biopesticides on various economically and ecologically important fauna of the aquatic ecosystem (Mintu Deyashi *et al*., 2016) [27]. Thus, it is important that toxic effects be determined and interpreted in biochemical terms (Sneha Verma and Anurag Rawat *et al*., 2017) [39].

2. Materials and Methods
The freshwater field crab, *Spiralothelphusa hydrodroma* was collected from Neithavoyal village, Thiruvallur District, Tamil Nadu. The freshwater field crab, *Spiralothelphusa hydrodroma* was chosen for the present study because of its presence in the rice fields in the study area. The crabs were collected from the rice fields in early morning hours or late evening hours by hand picking and stored in plastic containers and brought alive to the laboratory. The crabs were immediately transferred into experimental containers. Quinalphos is an organothiophosphate chemical chiefly used as a pesticide. Ranked 'moderately hazardous' in World Health Organization's (WHO) acute hazard ranking, use of quinalphos is either banned or restricted in most nations. Quinalphos, which is classified as a yellow label (highly toxic) pesticide in India, is widely used in the following crops: wheat, rice, coffee, sugarcane, and cotton.
The acute toxicity tests were conducted in duplicates using 5L experimental containers. The duration of the test was 96h and during the study the experimental crabs were fed. A minimum of 1L water was added for 10 crabs, so that the crabs were half immersed. The experiment was carried out for finding the range of concentrations for confirmatory evaluation. The mortality was recorded for *Spiralothelphusa hydrodroma* at 24, 48 72 and 96h exposure to pesticides were corrected for natural response by Abbott's formula (Abbott, 1925). The LC_{50} values were obtained by probit regression line, taking test concentration and corresponding percent mortalities on log value and probit scales respectively. Straight line (regression line) was drawn between the points which represent the survival percentage verses concentration (APHA, 1989) [6]. Sublethal studies are helpful to assess the response of the test organism to stress caused by pesticides. Chronic time course study on the effects of pesticide on

Spiralothelphusa hydrodroma were conducted by exposing to sublethal safe concentrations for 24 hours. At the end of the treatment period the control and treated crabs were dissected and tissues namely, muscle, gills, hepatopancreas, spermatheca and ovary were collected for biochemical studies.

Histological and Histopathological Studies

To study the effect of pesticide on the histology of the test organism, the control and experimental crabs treated with Quinalphos were dissected at the end of the experimental period (24 hours) and the tissues viz., muscle, gills, hepatopancreas, spermatheca and ovary were fixed in Bouin's fluid, processed and embedded in paraffin wax. Section of 4-6 µm thickness were cut and stained in haematoxylin and eosin. The tissues were stained in chrome-alum-haematoxylin phloxine (CHP) and haematoxylin and eosin. The slides were observed under the light microscope for histological details and subsequently photomicrographs were taken using a Nikon micro photographic unit. The slides were observed under the light microscope and photomicrographs were taken using a Nikon micro photographic unit (Maharajan *et al.*, 2015) [25].

Biochemical Analysis

The effect of pesticides on mitochondrial enzymes such as LDH, SDH was analyzed by following King (1965) [22] and Nachlas *et al.* (1960) [28] Protocols.

Statistical Analysis

The data collected was statistical analyzed using SPSS software (Version 15.0). Regression and Analysis of variance (ANOVA) were used to determine the significance of difference among the pesticides. The data was entered in 15.0 SPSS software for statistical analysis.

3. Results

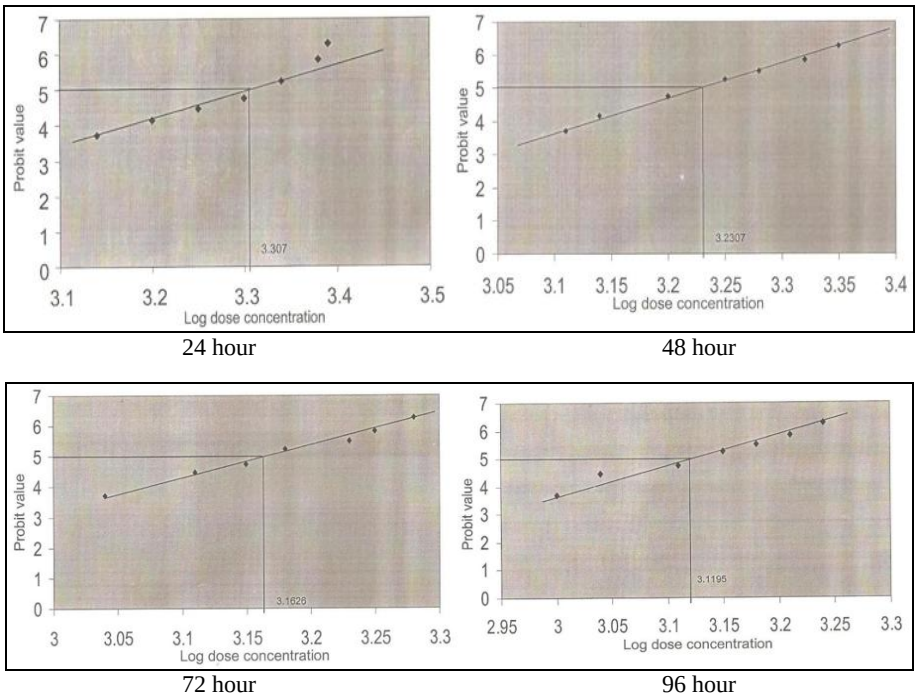
In the present investigation, an attempt was made to identify the staining reactions of the cytoplasmic contents of the tissues (muscle, gills, hepatopancreas, spermatheca and ovary) between the control and the experimental groups.

Median lethal concentration (LC_{50}) of Quinalphos

Median lethal concentration (LC_{50}) of Quinalphos for *S. hydrodroma* was observed for 96 hrs. The logarithm of 50% lethal concentration was obtained by finding the value on the abscissa for straight line which assumes the probit value 5. The concentrations resulting in 50% mortality and slope of the probit line were calculated for specific period of exposure as described by Finney (1971). The percent mortality data were subjected to probit analysis and plotted against log of dose concentrations resulting in a straight line. The values of LC_{50}, upper and lower confidence limits, slope function, correlations co-efficient square and regression results of Quinalphos on *S. hydrodroma* were given (Table: 1). The LC_{50} values for 24, 48, 72 and 96 h of exposure periods were estimated at 2.015, 1.672, 1.372 and 1.305 ppm respectively (fig: 1).

Table 1: The LC_{50} values and regression equations for *S. hydrodroma* treated with Quinalphos

Exposure periods (hours)	LC_{50} (ppm)	Upper confidence limits (UCL) (ppm)	Lower confidence limits (LCL) (ppm)	Regression results	Slope function (SF)	r^2
24	2.015	2.451	1.728	Y=-0.932X + 0.468	2.971	0.99
48	1.672	1.627	1.335	Y=-0.658X + 0.281	3.263	0.98
72	1.372	1.772	1.126	Y=-0.724X + 0.391	4.120	0.99
96	1.305	1.753	1.117	Y=-0.611X + 0.324	4.963	0.99



24 hour 48 hour

72 hour 96 hour

Fig 1: LC_{50} values of Quinalphos in *Spiralothelphusa hydrodrom*

Effect of Sublethal Concentrations of Quinalphos on *S. hydrodroma*

The experimental crabs of *S. hydrodroma* subjected to Quinalphos to two different durations of 15 days and 30 days exhibited changes in the muscle, gills, hepatopancreas, spermatheca and ovary. The variations between the control and the treated tissues were studied critically and photomicrographed.

Histology of Control and Treated Groups

Histological observation in control crabs were made in brain, thoracic ganglia and eyestalk. The tissues (muscle, gills, hepatopancreas, spermatheca and ovary) were less stained with fewer amounts of neurosecretory materials in the control crabs (Fig: 1, 4, 7, 10 and 13). When the exposure period was increased from 15 d to 30 d in lower sublethal concentration (0.1315 ppm), the tissues muscle, gills, hepatopancreas, spermatheca and ovary changes were more prominent (Fig: 2, 5, 8, 11 and 14). The tissues were intensely stained, whereas the neurosecretory materials were more. When the concentration of the Quinalphos was increased to higher sublethal level (0.4383 ppm) the changes were more prominent in the tissues muscle, gills, hepatopancreas, spermatheca and ovary (Fig: 3, 6, 9, 12 and 15). The tissues were more intensely stained. The neurosecretory materials were also seen in large numbers.

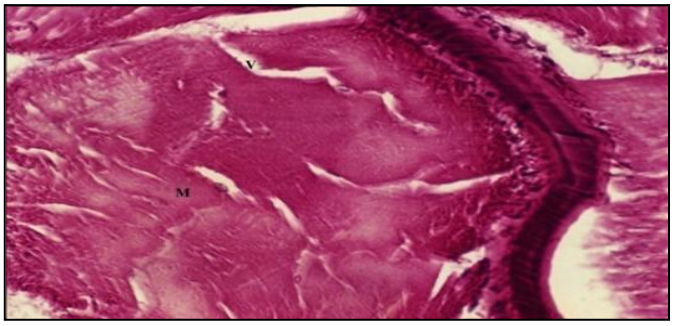


Fig 1: *S. hydrodroma* – Muscle control (45 X) M – Muscle fibres

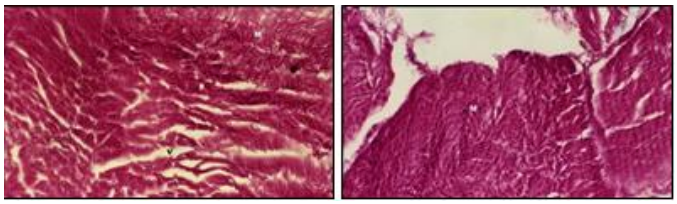


Fig 2: *S. hydrodroma* - muscles treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). M – Muscle fibres, V – Vacuole

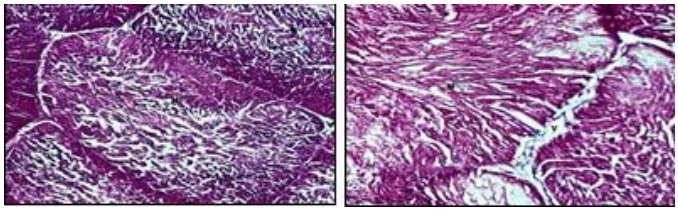


Fig 3: *S. hydrodroma* - muscles treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). M – Muscle fibres. V – Vacuole

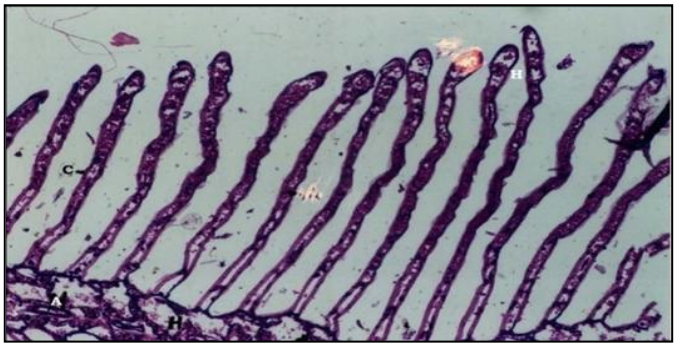


Fig 4: *S. hydrodroma* – Gills control (45 X). C – Cuticle, A–Axis, H– Haemocytes

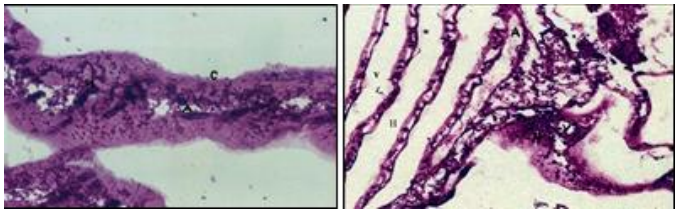


Fig 5: *S. hydrodroma* - Gills treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). C – Cuticle, A – Axis, H – Haemocytes

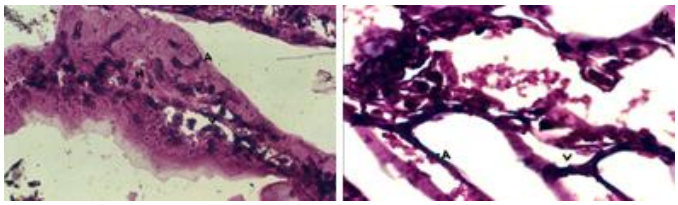


Fig 6: *S. hydrodroma* - Gills treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). A–Axis, H–Haemocytes, V–Vacuole

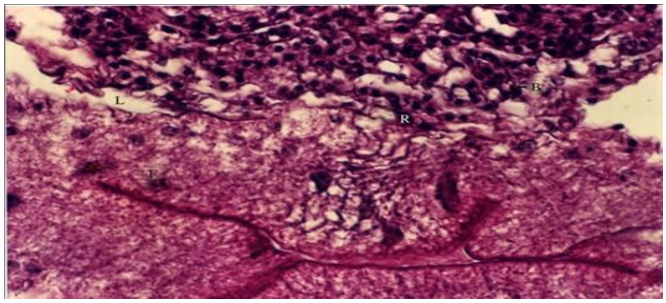


Fig 7: *S. hydrodroma* – Hepatopancreas (45 X) L–Lumen, B–'B' cell, R–'R' cell, F – 'F' cell

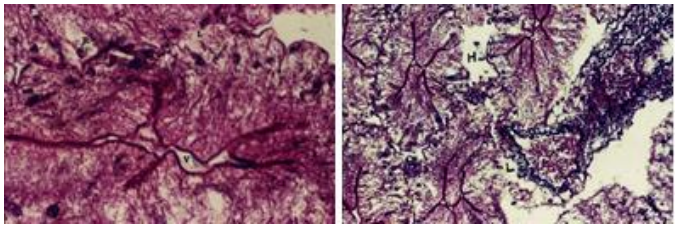


Fig 8: *S. hydrodroma* - hepatopancreas treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). H – Hamel space, L – Lumen. L – Lumen, B – 'B' cell, R – 'R' cell, F – 'F' cell, V – Vacuole

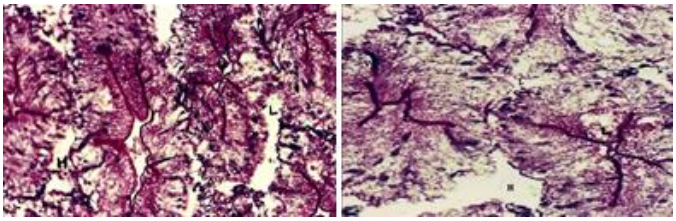


Fig 9: *S. hydrodroma* - hepatopancreas treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). H – Hamel space, L – Lumen, V – Vacuole. L – Lumen, V– Vacuole

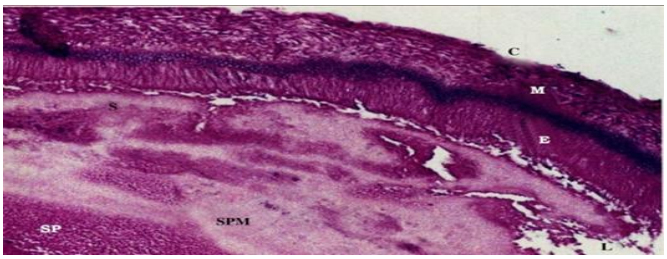


Fig 10: *S. hydrodroma* – Spermatheca control (45 X) in C – Cuticular layer, M – Muscular layer, E- Epithelial layer, L – Lumen, R – Spermathecal fluid substance

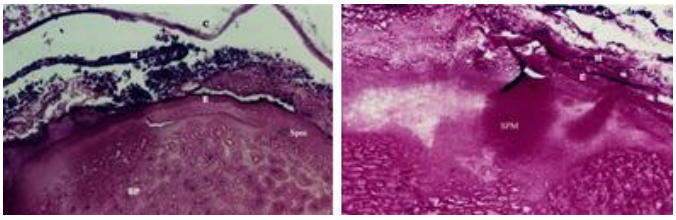


Fig 11: *S. hydrodroma* – Spermatheca treated with lower sublethal concentrations of Quinalphos for 15 and 30 days (45 X). C–Cuticular layer, M–Muscular layer, E Epithelial layer, Sp – Spermatophore, C – Cuticular layer, M – Muscular layer, E – Epithelial layer, Sp– Spermatophore, S – Free sperm. (Note the changes in the wall layers)

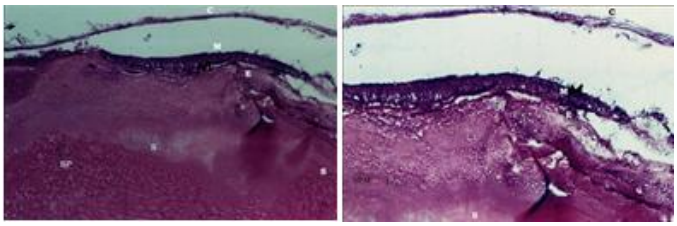


Fig 12: *S. hydrodroma* – Spermatheca treated with higher sublethal concentrations of Quinalphos for 15 and 30 days (45 X) C – Cuticular layer, M – Muscular layer, E– Epithelial layer, Sp- Spermatophore, Spm – sperm mass. (Note the elongated cells in the epithelial layer)

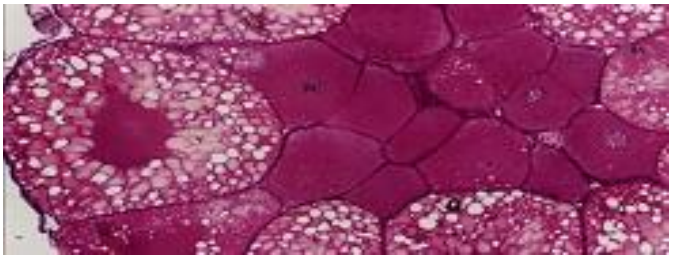


Fig 13: *S. hydrodroma* – Ovary control (45 X) M–Muscular tissue, O – Ooplasm, N – Nucleus

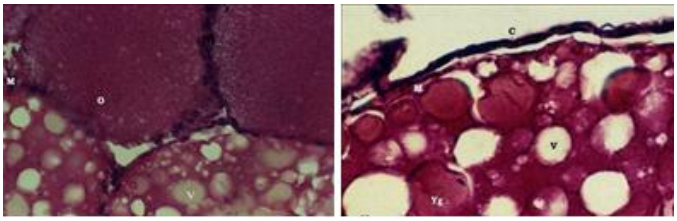


Fig 14: *S. hydrodroma* – Ovary treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). N – Nucleus, V- Vacuole, Y – Yolk globule, Yg – Yolk granule, C – Connective tissue, M – Muscular tissue, O- Ooplasm

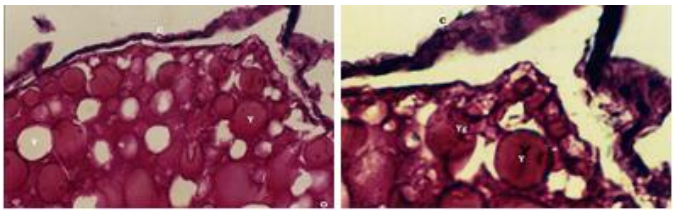


Fig 15: *S. hydrodroma* – Ovary treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X).G– German tissue, M – Muscular tissue, Y-Yolk globule, Yg – Yolk granule, O- Ooplasm

Enzyme activities

Succinate Dehydrogenase (SDH) Activity

Muscle

In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the SDH activity was 10.67 and 10.29 MIU/min/mg protein, whereas in higher sublethal concentration 0.4383 ppm) it was 9.61 and 9.29 MIU/min/mg protein for 15 d and 30 d exposure respectively. On the other hand, the enzyme activity in control revealed 11.81 and 11.99 MIU/min/mg protein for 15 d and 30 d respectively (Table: 2). The decrease in the SDH activity was statistically significant at $p<0.05$ for 15 d and $p<0.01$ for 30 d.

Gills

In the control crab the SDH activity in the gills was found to be 7.83 and 7.51 MIU/min/mg protein respectively for 15 d and 30 d (Table: 2). In the crabs treated with lower sublethal concentration (0.1315 ppm) the SDH activity was 7.63 and 6.29 MIU/min/mg protein for 15 d and 30 d of exposure periods respectively. Higher sublethal concentration revealed 6.99 and 5.49 MIU/min/mg protein for 15 d and 30 d respectively. The decrease in the values of SDH activity was statistically significant ($p<0.05$) at 15 d and ($p<0.01$) for 30 d of exposure.

Hepatopancreas

In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the SDH activity was 6.25 and 6.01 MIU/min/mg protein, whereas in higher sublethal concentration 0.4383 ppm) it was 5.58 and 5.14 MIU/min/mg protein for 15 d and 30 d exposure respectively. On the other hand, the enzyme activity in control showed 6.93 and 7.01 MIU/min/mg protein for 15 d and 30 d respectively (Table: 2). The decrease in the SDH activity was statistically significant at $p<0.05$ for 15 d and $p<0.01$ for 30 d.

Spermatheca

In the control crabs the mean SDH activity in the spermatheca was 7.69 and 7.98 MIU/min/mg protein respectively for 15 and 30 d. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos (Table: 2), the SDH activity was 6.52 and 6.94 MIU/min/mg protein, whereas in higher sublethal concentration (0.4383 ppm) it was 6.12 and 5.82 MIU/min/mg protein for 15 d and 30 d of exposure periods respectively. The decrease in the SDH activity was statistically significant ($p<0.05$) at both 15 d and 30 d experimental.

Ovary

The ovary of *S. hydrodroma* treated with sublethal concentrations of Quinalphos was tested for succinate dehydrogenase activity. As observed for the results (Table: 2) the SDH activity of the control crab was found to be 5.69 and 5.56 MIU/min/mg protein for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the SDH activity reduced to 4.39 and 4.17 MIU/min/mg proteins for 15 d and 30 d of exposure periods respectively. On further increasing the concentration of Quinalphos to higher sublethal level (0.4383 ppm) the SDH further decreased to 3.69 and 3.39 MIU/min/mg proteins for 15 d and 30 d respectively. The decrease in SDH activity was statistically significant for 15 d ($p<0.05$) and 30 d ($p<0.01$) of Quinalphos treated crab.

Lactate Dehydrogenase (LDH) Activity

Muscle

In the control crabs, the mean LDH activity in the muscle was found to be 7.71 and 7.89 µg/100 mg wet tissue for 15 d and 30 d respectively (Table: 3). When the crabs were treated with lower sublethal concentration (0.1315 ppm) of Quinalphos, the LDH activity enhanced to 8.30 and 8.79 µg/100 mg wet tissue. On the other hand, when the concentration was increased to higher sublethal level (0.4383 ppm) it further increased to 9.16 and 9.89 µg/100 mg wet tissue for 15 d and 30 d respectively. The increase in the LDH activity was significant at $p<0.01$ for both treated crabs.

Gills

In the control crabs, the mean LDH activity in the gills was found to be 4.43 and 4.71 µg/100 mg wet tissue for 15 d and 30 d respectively (Table: 3). When the crabs were treated with lower sublethal concentration (0.1315 ppm) of Quinalphos, the LDH activity enhanced to 5.41 and 5.79 µg/100 mg wet tissue. On the other hand, when the concentration was increased to higher sublethal level (0.4383 ppm) it further increased to 6.69 and 6.39 µg/100 mg wet tissue for 15 d and 30 d respectively. The increase in the LDH activity was significant at $p<0.01$ for both treated crabs.

Hepatopancreas

The experimental crabs, exposed to lower sublethal concentration (0.1315 ppm) of Quinalphos was analysed for LDH activity in hepatopancreas and it was 6.45 and 6.10 µg/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) of Quinalphos it was 7.35 and 7.14 µg/100 mg wet tissue for 15 d and 30 d of exposure periods respectively (Table: 3). However, in the control crabs the mean LDH activity was 5.53 and 5.60 µg/100 mg wet tissue for 15 d and 30 d respectively. The increase in enzyme activity was statistically significant at $p<0.01$ for both 15 d and 30 d treated crabs.

Spermatheca

In the control crabs, the mean LDH activity in the spermatheca was 5.49 and 5.28 µg/100 mg wet tissues respectively for 15 d and 30 d (Table: 3). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the LDH activity was 6.67 and 6.34 µg/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) it was 7.32 and 6.92 µg/100 mg wet tissue for 15 d and 30 d of exposure respectively. The increase in the LDH activity was statistically significant at $p<0.01$ for both 15 d and 30 d in treated crabs.

Ovary

The ovary of control crab was tested for LDH activity (Table: 3), and was found to 5.39 and 5.16 µg/100 mg wet weight tissue for 15 d and 30 respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the LDH activity was increased to 6.09 and 5.82 µg/100 mg wet weight tissue. On further increasing the concentration of Quinalphos to higher level (0.4383 ppm) it was further increased to 6.59 and 6.92 µg/100 mg wet weight tissue for 15 d and 30 d respectively. The increase in LDH activity was statistically significant ($p<0.05$) for 15 d and 30 d ($p<0.01$) in Quinalphos treated crabs.

Table 2: Succinate dehydrogenase (SDH) activities in *Spiralothelphusha hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Ovary	5.69 ± 0.39	4.39 ± 0.54	3.69 ± 0.61	7.06*	<0.05
	Spermatheca	7.69 ± 0.55	6.52 ± 0.58	6.12 ± 0.77	3.93*	<0.05
	Hepatopancreas	6.93 ± 0.72	6.25 ± 0.42	5.58 ± 0.69	3.82*	<0.05
	Muscle	11.81 ± 0.41	10.67 ± 0.30	9.61 ± 1.14	5.19*	<0.05
	Gill	7.83 ± 0.77	7.63 ± 0.42	6.99 ± 1.22	1.08*	<0.05
30	Ovary	5.56 ± 0.36	4.17 ± 1.12	3.39 ± 0.59	9.12**	<0.01
	Spermatheca	7.98 ± 0.22	6.94 ± 0.72	5.82 ± 0.54	6.93**	<0.05
	Hepatopancreas	70.10 ± 0.67	6.01 ± 0.58	5.14 ± 0.28	14.71*	<0.01
	Muscle	11.99 ± 0.73	10.29 ± 0.88	9.29 ± 0.73	8.39**	<0.01
	Gill	7.51 ± 0.47	6.29 ± 0.59	5.49 ± 0.61	10.68**	<0.01

Table 3: Lactate dehydrogenase (LDH) activities in *Spiralothelphusha hydrodroma* treated with Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Ovary	5.39 ± 0.49	6.09 ± 0.74	6.59 ± 0.36	3.26*	<0.05
	Spermatheca	5.49 ± 0.45	6.67 ± 0.31	7.32 ± 0.57	14.73**	<0.01
	Hepatopancreas	5.53 ± 0.42	6.45 ± 0.92	7.35 ± 0.46	10.22**	<0.01
	Muscle	7.71 ± 0.79	8.30 ± 0.69	9.10 ± 0.99	2.19*	<0.05
	Gill	4.43 ± 0.57	5.41 ± 0.59	6.69 ± 1.10	10.08**	<0.01
30	Ovary	5.16 ± 0.56	5.87 ± 0.42	6.92 ± 0.69	7.42**	<0.01
	Spermatheca	5.28 ± 0.42	6.34 ± 0.62	6.92 ± 0.54	7.49**	<0.01
	Hepatopancreas	5.60 ± 0.47	6.10 ± 0.58	7.14 ± 0.68	12.49**	<0.01
	Muscle	7.89 ± 0.73	8.79 ± 0.78	9.89 ± 0.62	7.29*	<0.05
	Gill	4.71 ± 0.47	5.79 ± 0.69	6.39 ± 0.24	14.58**	<0.01

4. Discussion

The results obtained in the present study on the toxicity effect of Quinalphos, an organophosphorus compound on a freshwater field crab, *Spiralothelphusa hydrodroma* at two different sublethal concentrations and two different exposure periods showed interesting results. The results at lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos on the muscle, gills, hepatopancreas, spermatheca and ovary revealed various histopathological changes. The crabs treated with quinalphos at the acute toxicity level were expressed in terms of LC_{50} value. The acute 96 h LC_{50} value for quinalphos on *S. hydrodroma* was found to be 1.315 ppm concentration. Exposure of fingerlings of *L. rohita* to sublethal concentrations of quinalphos produced changes in the protein, DNA and RNA levels of muscle and the activities of ALP, ACP, AChE, LDH, SDH and ATPase in different tissues. A fall in muscle protein is indicative of reduced protein synthesis and low assimilation of food and low amino acid uptake for protein synthesis. Organophosphates are known to methylate and phosphorylate cellular proteins directly (Wild, 1975) [53]. Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et aL,* 1992) [19]. In the present study, the enzyme activity in succinate dehydrogenase, lactate dehydrogenase, acid phosphatase, alkaline phosphatase and acetyl cholinesterase were estimated in both control crabs and the crabs treated with lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos.

Gametogenesis might be hindered if pollutant stress reduced the build of sufficient energy stores (Sastry and Miller, 1981) [37]. Ovarian histopathological changes were observed by many authors in crustaceans namely *Caridina rajadhari* in response to organophosphorus pesticides (Victor and Sarojini, 1985) [46], *Macrobrachium idae* in response to cadmium toxicity (Victor *et al.,* 1985) [47], presumably that was the reason for reduced oocyte development. Histopathological changes were also observed in *P.hydrodromous* in response to urea and naphthalene (Victor and Sundarraj, l988*)* [48] *Oziotelphusa sensex sensex* in response to cythion (Victor, 1989). Devi (1996) [16] reported similar histopathological changes in *Uca triangularis* with urea and naphthalene and Suresh (2001) [42] also observed such changes in *U. annulipes* in response to heavy metal toxicity such as cadmium and mercury. Jayakumar (2002) [20] also reported the similar such histopathological changes in *S. hydrodroma* when treated with copper and zinc. They observed swelling of oocytes, vacuolization in oocytes, degeneration of oolemma, and loss of normal shape of oocyte, necrosis, and fusion of adjacent oocytes, pycnosis in ooplasm and nucleus, atresia, turgidity in ovary, disorganized ooplasm, hyperchromatic nuclei, necrotic oocytes and fibrosis of ovarian wall. There was high energy demand during toxicity stress and related tissue hypoxia. Such changes together with other changes in the internal organs resulted in mortality. Similarly, the effects of quinalphos on ovaries were well evidenced on the treated crabs. The ovaries exhibited vacuolization of oocytes, degeneration of connective tissue, loss of normal shape of oocytes, necrosis, vacuole formation in the nucleus, alteration in size and shape.

The histological sections of spermatheca of the control crab showed three different layers namely outer cuticular, middle muscular and inner epithelial layers. The lumen of the spermatheca showed spermatophores, sperm mass, free sperms lodged in pouches and the spermathecal fluid substances. The observations were similar to that of the findings of Anil Kumar and Adiyodi (1977) [5], Kulasekaran (1994) [23], Sujatha (1998) [41] and Suresh (2001) [42]. In copper and zinc treated crabs at sublethal concentrations the wall layers were highly distorted and flocculated and slits were observed in the lumen in *S. hydrodroma* (Jayakumar 2002) [20]. Similary, in the present study, spermatheca exhibited morphological and histological changes when the experimental groups were compared with the control. In the experimental crabs there was enlargement or disruption of the wall layers and highly fibrous in nature. The luminal content consisting of granular substances, sperm mass and spermatophores exhibited much difference in size and shape. The sperm mass and spermatophores exhibited non-homogeneity in distribution. The spermathecal fluid substances showed refractive condition in addition to the empty spaces. In some sections, necrosis was observed.

The tubules of the hepatopancreas were enclosed by a basal lamina and contained a central lumen. The three different types of cells namely R-cells, B-cells and F-cells were observed in the present investigation as previously reported by Ceccaldi (1998) [11] and Vogan *et al.* (2001). Victor *et al.* (1990) observed histopathological changes in the hepatopancreas of *P. hydrodromous* in response to cythion resulting in reduction in the height of tubular epithelium, enlargement of lumen, vacuolation and atrophy. The histopathological changes indicated that the animals were not able to digest and store food properly. The lack of nutrients

resulted in atrophy of hepatopancreas. Extensive vacuolation of hepatopancreas was observed in *U. triangularis* exposed to urea and naphthalene (Devi, 1996) [16]. Anderson *et al.* (1997) [3] concluded that the R-cells were responsible for the increase in vacuolation in response to chemical exposure and in *M. malcolmsonii* in response to endosulphan (Bhavan and Geraldine, 2000) [10]. The disorganized condition of hepatopancreas was observed in *U. annulipes* in response to cadmium and mercury toxicity (Suresh, 2001) [42]. In the present study, the crabs treated with lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos exhibited various changes in the hepatopancreas. The tubules were disfigured and the lumen size was enlarged, disorganization and extensive vacuolation in the cytoplasm of the cells were observed.

The muscle tissue from the abdomen of control crabs showed lobes held together by connective tissue. The fibres of the muscle tissue were observed in the present study and were in accordance to the results in *M. rosenbergii* (Nash *et al.,* 1987; Anderson *et al.,* 1990; Tung *et al*, 1999) [29, 2]. They reported muscle fibre degeneration, fragmentation, flocculation, granulation, haemocytes infiltration and shrinkage of muscle fibres. Similar histopathological changes reported in fish *Labeo rohita* exposed to hexachlorocyclohexane (Das, 1998; Das and Mukherjee, 2000) [15]. They observed thickening and separation of muscle bundles, intramuscular oedema and dystrophy in response to the toxicant. Similarly, in the present investigation the crabs treated with quinalphos showed disintegration of lobes, vacuolation, fragmentation and muscle fibre disorganization.

Gills of *S. hydrodroma* were of phyllobranchiate type with a central axis and gill lamellae arranged in two rows on either side. The central axis contained haemocytes and fixed nephrocytes. The nephrocytes had brown materials in its vacuoles. The structure of the gills observed in the present investigation was well in accordance with earlier works where oedematous, necrosed lamellae with extensive vacuolation, ulceration and hyperplasia were observed (Victor *et al.,* 1985; 1990; Victor, 1994; Vogan *et al*., 2001) [16, 17]. Gate and Mulherkar (1979) reported infiltration of haemocytes in gills of prawn *M. kistensis* exposed to copper. Oedematous and necrosed gill lamellae lead to loss of osmotic barrier and hyperplasia that reduced the respiratory ability when *M. idae* treated with cadmium and mercury. Shrinkage of respiratory surface in the gill causes severe respiratory failure. Similar changes in *M. idae in* response to cadmium and mercury was noted by Victor *et al*., (1985; 1990) [47]. Damages in the gills were observed in *Carcinus maenas* exposed to copper (Nonnotte *et al.,* 1993; Lawson *et al.,* 1995) [30, 24]. They observed extensive structural alterations namely cellular hyperplasia, vacuolation, necrosis, thickening of gill epithelium, reduction of haemal spaces and tissue hypoxia. Depletion in the rate of oxygen consumption as a result of heavy metal toxicity might be the reason for mortality (Bansal, 1979; Rao *et al.,* 1998) [7, 33]. The present study, in the crabs treated with quinalphos also showed various histopathological changes including necrosed gill lamellae, vacuolation and damaged cuticle.

Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et al,* 1992) [19]. In the present study, the enzyme activity in succinate dehydrogenase and lactate dehydrogenase were estimated in both control crabs and the crabs treated with lower (0.1315 ppm) and higher (0.4383 ppm) sublethal concentrations of quinalphos. Present investigation, clearly showed decreased succinate dehydrogenase activity. Since the succinate dehydrogenase enzyme is an important enzyme in TCA cycle, its inhibition suggests that the metabolic pathway might have turned anaerobic to meet the increased energy demand during pollution stress. The results of the present study are also in conformity with those of the earlier observations. Decreased succinate dehydrogenase activity and increased lactate dehydrogenase activity was reported by many workers namely in *O.senex senex* in response to sumithion (Reddy *et al.,* 1983; Bhagyalakshmi *et al.,* 1984) [12, 8]. On the contrary lactate dehydrogenase activity increased in the hepatopancreas in the fiddler crab, *U. pugilator* and decrease d in the abdominal muscle when exposed to cadmium (Devi *et al.,* 1993) [17] and in *S. serrata* in response to cadmium (Reddy *et al.,* 1994) [14]. The increased lactate dehydrogenase activity in the abdominal muscle reflects anaerobic carbohydrate metabolism when exposed to heavy metal. Reduction in the enzyme activity in fishes was observed in response to heavy metals. Chandravathy and Reddy (1994; 1995) [12, 14] 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. The lactate dehydrogenase activity increased in the *U. annulipes* treated with sublethal concentrations of cadmium and mercury (Suresh, 2001) [42] and in *S. hydrodroma* in response to copper and zinc (Jayakumar, 2002) [20]. The results of the present study are well in accordance with that of previous investigations in the increased activity of lactate dehydrogenase in quinalphos treated crabs.

5. Conclusion

Hence, the present investigation clearly showed that the quinalphos caused damages to the tissues at higher sublethal concentrations. There was a marked decrease in the succinate dehydrogenase, alkaline phosphatase activities and increase in lactate dehydrogenase and acid phosphatase activities clearly indicate that the quinalphos caused metabolic stress in the experimental crabs. High levels of accumulation of quinalphos in the present investigation indicated that the intake was exponential in an environment where the quinalphos routinely used as biocides and fertilizers which is highly toxic was concluded.

6. References

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