International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 4; October 2017; Page No. 127-136

Histopathological studies and changes in phosphatase activities in tissues of *Spiralothelphusa hydrodroma*, following exposure to quinalphos

[*] J Manju Bashini, P Senthilkumaar
PG 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 acid phosphatase (ACP) and alkaline phosphatase (ALP), 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) [61]. 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) [53]. Environmental pollution found to be undesirable side effect of industrialization and an important aspect of environmental degradation (Jothinarendiran, 2012) [28]. 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) [55, 5, 35]. Aquatic ecosystems are more sensitive to the release of industrial wastewater (Pálaez-Cid *et al*., 2013) [43]. 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) [44]. 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) [36]. Thus, it is important that toxic effects be determined and interpreted in biochemical terms (Sneha Verma and Anurag Rawat *et al*., 2017) [54].

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 organo thiophosphate 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) [7]. 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) [34].

Biochemical analysis

The effect of pesticides on acid and alkaline phosphatases was assayed following the procedure adopted by Tenniswood *et al.* (1976) [59].

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

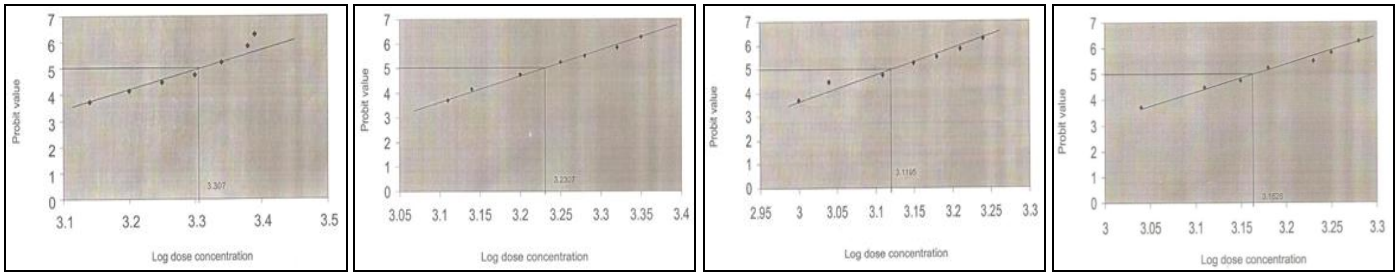
In the present investigation, an attempt was made to identify the staining reactions of the cytoplasmic contents of the tissues (muscle, gills, hepatopancreas, spermatheca and ovary) between the control and the experimental groups.

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 (Graph: 1).

Table 1: The LC50 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

Graph 1: LC50 values of Quinalphos in Spiralothelphusa hydrodroma

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.

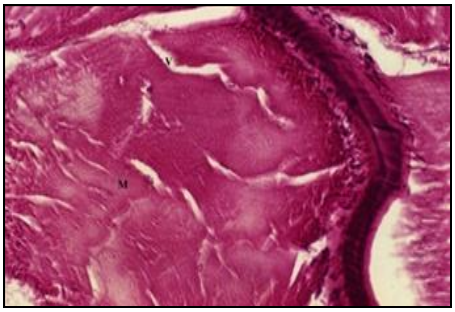


Fig 1: *S. hydrodroma* – Muscle control (45 X) M – Muscle fibres.

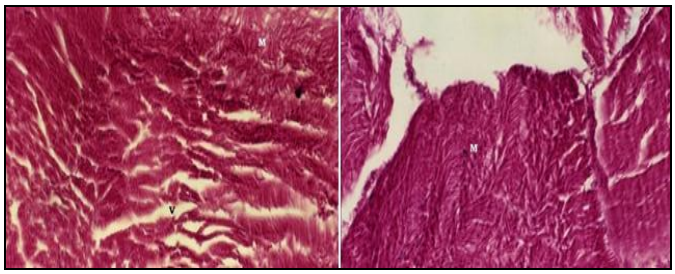


Fig 2: *S. hydrodroma* - muscles treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). M – Muscle fibres, V – Vacuole.

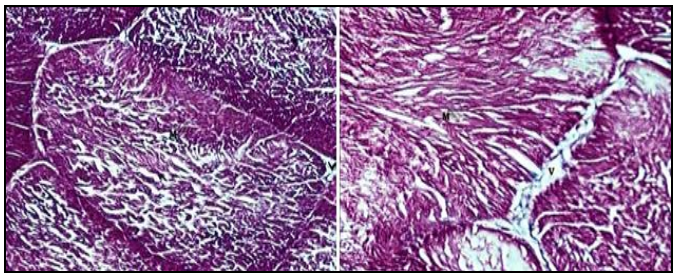


Fig 3: *S. hydrodroma* - muscles treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). M – Muscle fibres. V – Vacuole.

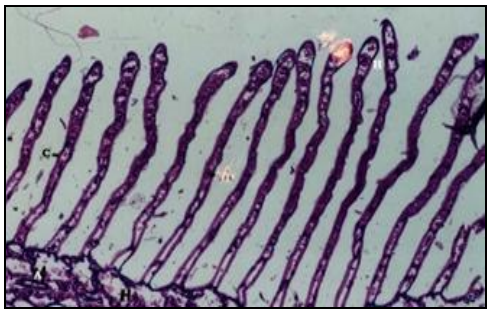


Fig 4: *S. hydrodroma* – Gills control (45 X). C – Cuticle, A–Axis, H– Haemocytes.

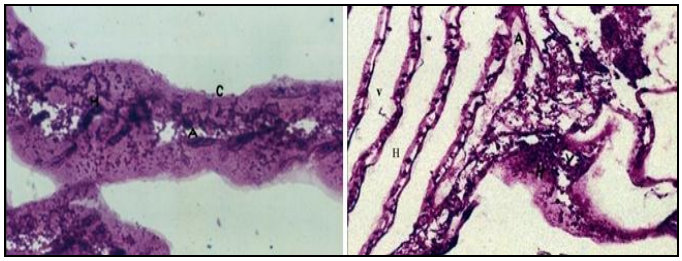


Fig 5: *S. hydrodroma* - Gills treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). C – Cuticle, A – Axis, H – Haemocytes.

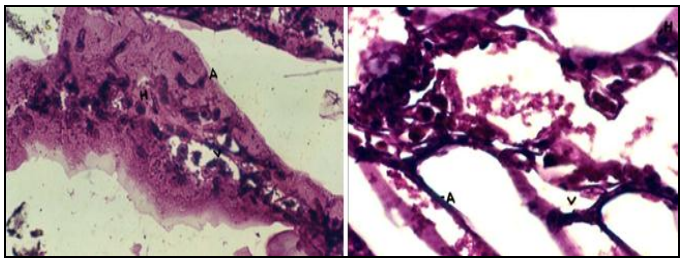


Fig 6: *S. hydrodroma* - Gills treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). A–Axis, H–Haemocytes, V–Vacuole

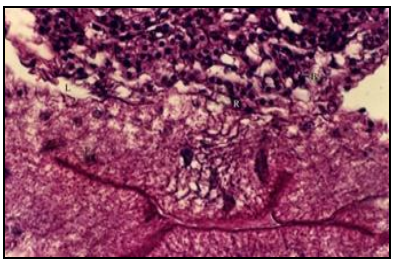


Fig 7: *S. hydrodroma* – Hepatopancreas (45 X) L–Lumen, B–'B' cell, R–'R' cell, F – 'F' cell

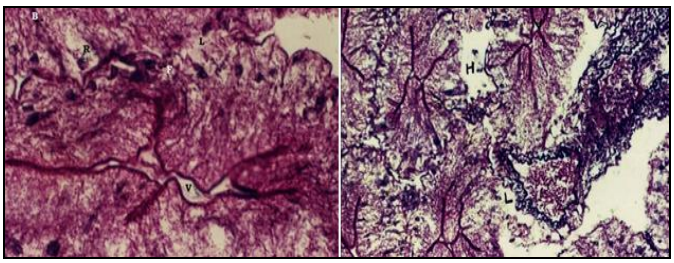


Fig 8: *S. hydrodroma* - hepatopancreas treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). H – Hamel space, L – Lumen. L – Lumen, B – 'B' cell, R – 'R' cell, F – 'F' cell, V – Vacuole.

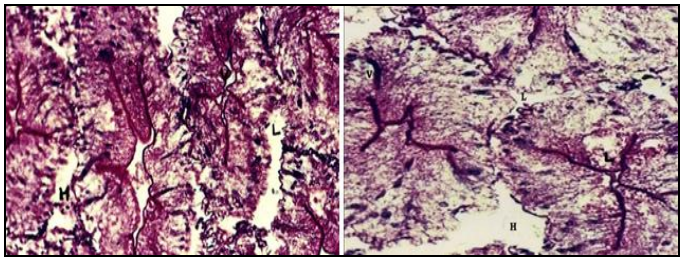


Fig 9: *S. hydrodroma* - hepatopancreas treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X). H – Hamel space, L – Lumen, V – Vacuole. L – Lumen, V– Vacuole.

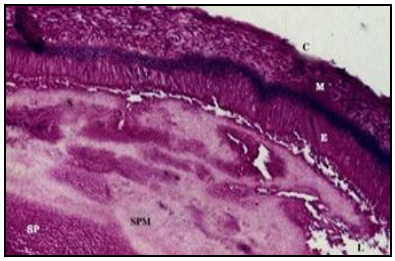


Fig 10: *S. hydrodroma* – Spermatheca control (45 X) in C – Cuticular layer, M – Muscular layer, E- Epithelial layer, L – Lumen, R – Spermathecal fluid substance.

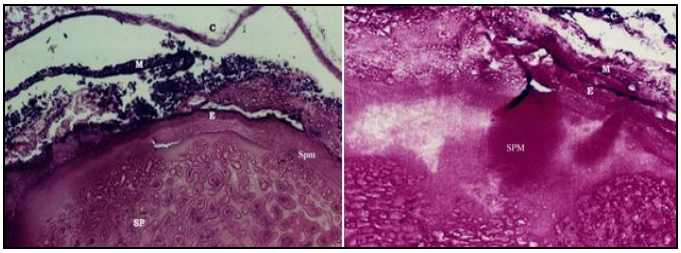


Fig 11: *S. hydrodroma* – Spermatheca treated with lower sublethal concentrations of Quinalphos for 15 and 30 days (45 X). C–Cuticular layer, M–Muscular layer, E Epithelial layer, Sp – Spermatophore, C – Cuticular layer, M – Muscular layer, E – Epithelial layer, Sp– Spermatophore, S – Free sperm. (Note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

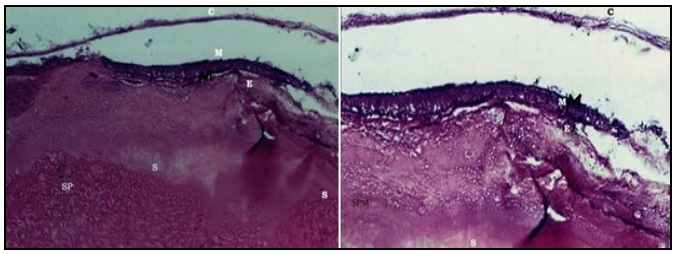


Fig 12: *S. hydrodroma* – Spermatheca treated with higher sublethal concentrations of Quinalphos for 15 and 30 days (45 X) C – Cuticular layer, M – Muscular layer, E– Epithelial layer, Sp- Spermatophore, Spm – sperm mass. (Note the elongated cells in the epithelial layer)

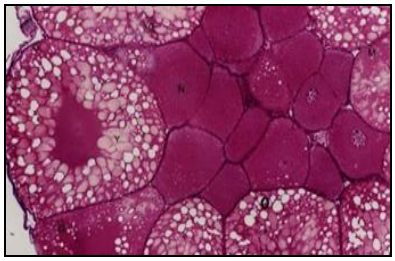


Fig 13: *S. hydrodroma* – Ovary control (45 X) M–Muscular tissue, O – Ooplasm, N – Nucleus

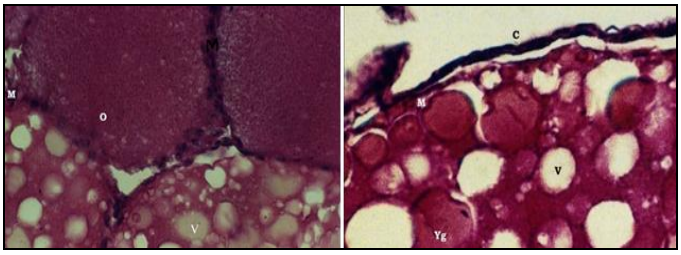


Fig 14: *S. hydrodroma* – Ovary treated with lower sublethal concentration of Quinalphos for 15 and 30 days (45 X). N – Nucleus, V- Vacuole, Y – Yolk globule, Yg – Yolk granule, C – Connective tissue, M – Muscular tissue, O- Ooplasm

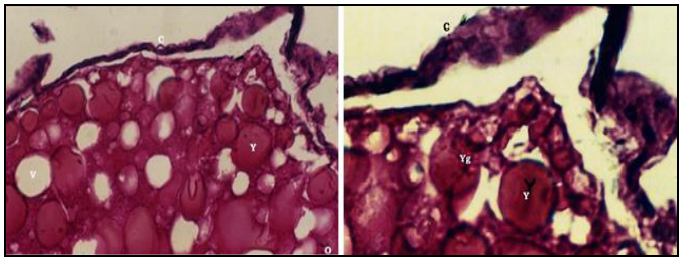


Fig 15: *S. hydrodroma* – Ovary treated with higher sublethal concentration of Quinalphos for 15 and 30 days (45 X).G– German tissue, M – Muscular tissue, Y-Yolk globule, Yg – Yolk granule, O-Ooplasm.

Acid phosphatase (ACP) activity

Muscle

In the control crabs the mean acid phosphatase activity in the muscle was 3.81 and 3.89 µg PNPP to PNP/100 mg wet tissue for the 15 d and 30 d respectively (Table: 1). When the crabs were treated with lower sublethal concentration (0.1315 ppm) of Quinalphos, the acid phosphatase activity enhanced to 4.60 and 4.39 µg PNPP to PNP/100 mg wet tissue. On the other hand, when the concentration was increased to higher sublethal level (0.4383 ppm) it was further increased to 5.10 and 4.89 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d exposure periods. The increase in acid phosphatase activity was statistically significant at $p<0.01$ for both 15 and 30 d treated crabs.

Gills

In the control crabs the acid phosphatase activity in the gill was 2.83 and 2.71 µg PNPP to PNP/100 mg wet tissue respectively for 15 d and 30 d (Table: 1). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the acid phosphatase activity was 2.91 and 3.29 µg PNPP to PNP/100 mg wet tissue, whereas in higher sublethal concentrations (0.4383 ppm) it was 3.59 and 3.99 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d exposure periods respectively. The increase in the acid phosphatase activity was significant at $p<0.05$ for 15 d and $p<0.01$ for 30 d in the experimental.

Hepatopancreas

The experimental crabs exposed to lower sublethal concentration (0.1314 ppm) of Qunialphos was analyzed for acid phosphatase activity and was found as 6.35 and 6.25 µg PNPP to PNP/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) of Quinalphos it was 6.83 and 6.84 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d of exposure periods respectively (Table: 1). However, in the control crabs it was 5.43 and 5.37 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. The decrease in enzyme activity was statistically significant $p<0.01$ at both 15 d and 30 d treated crabs.

Spermatheca

In the control crabs, the enzyme activity in the spermatheca was 5.29 and 5.38 µg PNPP to PNP/100 mg wet tissue respectively (Table: 1). In the crabs treated with lower sublethal concentration (0.1315 ppm) of Quinalphos the acid phosphatase activity was 6.37 and 6.51 µg PNPP to PNP/100

mg wet tissue, whereas in higher sublethal concentrations (0.4383 ppm) it was 6.72 and 7.19 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d exposure periods respectively. The decrease in the enzyme activity was statistically significant at $p<0.05$ for 15 d and $p<0.01$ for 30 d exposure crabs.

Ovary

The ovary of control crabs was tested for acid phosphatase activity (Table: 1) and was found to be 6.19 and 6.06 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) the acid phosphatase activity increased to 7.39 and 7.17 µg PNPP to PNP/100 mg wet tissue. On further enhanced to 7.99 and 8.12 µg PNPP and PNP/100 mg wet tissue for 15 d and 30 d respectively. The increase in acid phosphatase activity was statistically significant at $p<0.05$ for 15 d and $P<0.01$ for 30 d in Quinalphos treated crabs.

Alkaline phosphatase (alp) activity

Muscle

In the control crabs, the mean alkaline phosphatase activity in the muscle was found to be 4.31 and 4.29 µg PNPP to PNP/100 mg wet tissue for the 15 d and 30 d respectively (Table: 2). When the crabs were treated with lower sublethal concentration (0.1315 ppm) the enzyme activity decreased to 4.10 and 3.69 µg PNPP to PNP/100 mg wet tissue and in higher sublethal level (0.4383 ppm) it further reduced to 3.36 and 3.23 µg PNPP to PNP/100 mg to wet tissue for 15 d and 30 d respectively. The decrease in enzyme activity was significant at $p<0.05$ for both 15 d and 30 d of experimental crabs.

Gills

In the control crabs, the mean alkaline phosphatase activity in the gill was 4.53 and 4.51 µg PNPP to PNP/100 mg wet tissue respectively for 15 d and 30 d (Table: 2). In the crabs treated with lower sublethal concentrations (0.1315 ppm) of Quinalphos, the alkaline phosphatase activity was 4.21 and 4.19 µg PNPP to PNP/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) it was 3.99 and 3.49 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d exposure periods respectively. The decrease in the alkaline phosphatase activity showed higher value ($p<0.01$) for 30 d and the value was $p<0.05$ for 15 d treated crabs.

Hepatopancreas

The crabs exposed to lower sublethal concentrations (0.1315 ppm) of Quinalphos was analysed for alkaline phosphatase activity and was found 6.45 and 6.52 µg PNPP to PNP/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) of Quinalphos it was 6.73 and 5.64 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d of exposure periods respectively (Table: 2). However, in the control crabs the mean alkaline phosphatase activity was 7.13 and 7.17 µg PNPP to PNP/100 mg wet weight tissue for 15 d and 30 d respectively. The significant decrease in enzyme activity was $p<0.05$ for 15 d and $p<0.01$ for 30 d exposure periods.

Spermatheca

In the control crabs, the mean alkaline phosphatase activity in the spermatheca was 2.99 and 2.93 µg PNPP to PNP/100 mg wet tissue respectively for 15 d and 30 d (Table: 2). In the crabs treated with lower sublethal concentration (0.1315 ppm) the alkaline phosphatase activity was 2.47 and 2.11 µg PNPP to PNP/100 mg wet tissue, whereas in higher sublethal concentration (0.4383 ppm) it was 2.22 and 1.99 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d of exposure periods respectively. The decrease in the enzyme activity was statistically significant at $p<0.05$ for 15 d and $p<0.01$ for 30 d treated crabs.

Ovary

As observed from the results (Table: 2), the alkaline phosphatase activity of the control crab was found to be 6.89 and 6.66 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. In the crabs treated with lower sublethal concentration (0.1315 ppm) the alkaline phosphatase activity reduced to 5.96 and 5.57 µg PNPP to PNP/100 mg wet tissue and in higher sublethal level (0.4383 ppm) if further decreased to 5.19 and 4.12 µg PNPP to PNP/100 mg wet tissue for 15 d and 30 d respectively. The decline in enzyme activity was statistically significant at $p<0.01$ for both concentrations and exposure period.

Table 1: Acid phosphatase (ACP) activities in *Spiralothelphusha hydrodroma* treated with of Quinalphos

Exposure period in days	Tissues	Control	Lower sublethal concentration	Higher sublethal concentration	F-value	P-value
15	Ovary	6.19 ± 0.49	7.39 ± 0.74	7.99 ± 1.26	2.96*	<0.05
	Spermatheca	5.29 ± 0.65	6.37 ± 0.71	6.72 ± 0.67	13.89*	<0.05
	Hepatopancreas	5.43 ± 0.52	6.35 ± 0.62	6.83 ± 0.56	5.82**	<0.01
	Muscle	3.81 ± 0.59	4.60 ± 0.59	5.10 ± 0.59	4.89**	<0.01
	Gill	2.83 ± 0.37	2.91 ± 0.69	3.59 ± 0.50	2.18*	<0.05
30	Ovary	6.06 ± 0.56	7.17 ± 0.62	8.12 ± 0.49	5.32**	<0.01
	Spermatheca	5.38 ± 0.42	6.51 ± 0.78	7.19 ± 0.64	6.49**	<0.01
	Hepatopancreas	5.37 ± 0.77	6.25 ± 0.68	6.84 ± 0.88	4.19**	<0.01
	Muscle	3.89 ± 0.57	4.39 ± 0.63	4.89 ± 0.32	4.59**	<0.01
	Gill	2.71 ± 0.37	3.29 ± 0.39	3.99 ± 0.84	4.58**	<0.01

Table 2: Alkaline phosphatase (ALP) 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	6.89 ± 0.79	5.69 ± 0.34	5.19 ± 0.26	9.26**	<0.01
	Spermatheca	2.99 ± 0.55	2.47 ± 0.11	2.22 ± 0.17	3.19*	<0.05
	Hepatopancreas	7.13 ± 0.52	6.45 ± 0.62	6.73 ± 0.56	4.82*	<0.05
	Muscle	4.31 ± 0.79	4.10 ± 0.54	3.36 ± 0.49	2.26*	<0.05
	Gill	4.53 ± 0.47	4.21 ± 0.64	3.99 ± 0.30	0.98*	<0.05
30	Ovary	6.66 ± 0.73	5.57 ± 0.42	4.12 ± 0.69	13.42**	<0.01
	Spermatheca	2.93 ± 0.57	2.11 ± 0.48	1.99 ± 0.54	4.39**	<0.01
	Hepatopancreas	7.17 ± 0.57	6.52 ± 0.78	5.64 ± 0.68	5.89**	<0.01
	Muscle	4.29 ± 0.67	3.69 ± 0.53	3.23 ± 0.56	2.19*	<0.05
	Gill	4.51 ± 0.43	4.19 ± 0.59	3.49 ± 0.44	4.88**	<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 LC50 value. The acute 96 h LC50 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). Decrease or increase in the enzyme activity represents the stress in any organism that results in metabolic burden (Hanson *et al.*, 1992) [25]. 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) [51, 52]. Ovarian histopathological changes were observed by many authors in crustaceans namely *Caridina rajadhari* in response to organophosphorus pesticides (Victor and Sarojini, 1985) [65, 67], *Macrobrachium idae* in response to cadmium toxicity (Victor *et al.*, 1985), 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*) Oziotelphusa sensex sensex* in response to cythion (Victor, 1989). Devi (1996) [17] reported similar histopathological changes in *Uca triangularis* with urea and naphthalene and Suresh (2001) [58] also observed such changes in *U. annulipes* in response to heavy metal toxicity such as cadmium and mercury. Jayakumar (2002) [27] 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) [6], Kulasekaran (1994) [31], Sujatha (1998) [56] and Suresh (2001) [58]. 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) [27]. 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) [12] 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) [17]. Anderson *et al.* (1997) [4] 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) [11]. The disorganized condition of hepatopancreas was observed in *U. annulipes* in response to cadmium and mercury toxicity (Suresh, 2001) [58]. 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) [3, 38, 60]. 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) [13, 14]. 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). Gate and Mulherkar (1979) [21] 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). Damages in the gills were observed in *Carcinus maenas* exposed to copper (Nonnotte *et al.,* 1993; Lawson *et al.,* 1995) [40, 33]. 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) [8, 46]. 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) [25]. In the present study, the phosphatase activity like 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. 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 disruption of the membrane (Deduve *et al.,* 1955). Phosphatases play an important role in carbohydrate metabolism (Goodman and Rothstein, 1957) [23]. Norseth (1967) [41] 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 (O'Connor and Gilbert, 1968) [42]. Bhatia *et al.,* (1972) [9] were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas causes release of acid phosphatase. Since hepatopancreas was an important site of intermediary metabolism in crustaceans (Kulkarni and Nagabhushanam, 1979) [32] higher acid phosphatase activity was noted in hepatopancreas.

Dutta *et al.* (1983) [20] concluded that both induction and inhibition of phosphatase take place depending on the concentration of metals. Reddy *et al.* (1984) concluded that sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatase. Increased acid phosphatase activity suggested glycogenolysis during metal toxicity and enhanced breakdown of phosphatase to release energy in view of impaired ATPase system during metal stress (Reddy *et al.,* 1994; 1996). The acid phosphatase activity increased in the copper and zinc treated crabs as reported by Jayakumar (2002) [27]. Alkaline phosphatase is a brush border enzyme that splits various phosphorus esters at an alkaline pH and mediates membrane transport (Goldfisher *et al.,* 1964). It is also involved in synthesis of certain enzymes (Sumner, 1965) [57], active transport (Denielli, 1972) [16], protein synthesis (Pilo *et al,* 1972) [45], glycogen metabolism (Gupta and Rao, 1974) [24] and secretory activity (Ibrahim *et al.,* 1974) [26]. Any alteration in the activity of alkaline phosphatase affects the organisms in a variety of ways. Bhatnagar *et al.* (1995) [10] studied the effect of pyrethroid and mortality on the fish *Clarias bactrachus* and found that alkaline phosphatase decreased in response to the toxicant. Ahmed *et al.* (1997) [2] 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. 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.

Organophosphates inhibit acid phosphatase and alkaline phosphatase activity in different tissues of fishes which may adversely affect nucleic acid synthesis (Sastry and Sharma, 1981) [51, 52]. In the present study, we noticed an increase of ACP and decrease of ALP. *S. hydrodroma* was more sensitive to quinalphos toxicity than that of other pesticides. The toxicity of quinalphos caused severe metabolic distress, which was evident from the escaping tendency of test from the aquaria and such behaviour was based on dosage of these

pesticides, which eventually leads to death of test crabs. The accumulation of quinalphos in the present study, in the tissues was in the order of hepatopancreas, haemolymph, gill, ovary, muscle. Maximum accumulation of quinalphos in the hepatopancreas in various crustaceans was reported by many workers namely in *Penaeus duorarum* in response to cadmium (Nimmo *et al.,* 1977) [39]; *Rangia cuneata* exposed to mercury (Dillon and Neff, 1978) [18]; *Potamonautes warreni* exposed to zinc and lead (du Preeze *et al.,* 1993) [19]; *M. malcolmsonii* in response to cadmium and zinc (Vijayaraman *et al.,* 1999) [68] and nickel (Kabila *et al.,* 1999) [29]; *U. annulipes* in response to cadmium and mercury (Suresh, 2001) [58]. 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.

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

1. Abbot WS. A method of computing the effectiveness of an insecticide. J. Econ. Entomo., 1925; 18:265-267.
2. Ahmed MR, Elumalai M, Balasubramanian SE, Balasubramanian MP. Individual and combined effect of copper and chromium on oxygen consumption and phosphatases of a marine edible crab, Scylla serrata. Biochemical Letter., 1997; 55:147-152.
3. Anderson IG, Law AT, Shariff M, Nash G. A parvo-like virus in the giant freshwater prawn Macrobrachium rosenbergii. J. Inver. Pathol., 1990; 55:447-449.
4. Anderson MB, Reddy P, Preslan JE, Fingerman M, Bollinger J, Jolibois L, Maheshwarudu G, George WJ. Metal accumulation in crayfish, Procambarus clarkii exposed to a petroleum contaminated bay in Louisiana. Ecotoxicol. Environ. Saf., 1997; 37:267-272.
5. Andhale AV, Bhosale PA, Zambare SP. Histopathological study of nickel induced alterations in the fresh water bivalve, Lammellidens marginalis. Journal of Experimental Sciences. 2011; 2(4):01-03.
6. Anilkumar G, Adiyodi KG. Spermatheca of the freshwater crab, Paratelphusa hydrodromous Herbst. Advan. Invt. Reprod. eds., Adiyodi, K.G. and R.G. Adiyodi Peralam Kennath, Kerla, India, 1977; 1:269-275.
7. APHA. Standard methods for the examination of water and waste water. American Public Health Association, Washington, D.C. 17th Ed, 1989.
8. Bansal SK. Pesticide induced alterations in the oxygen uptake rate of a freshwater major carp, Labeo rohita. Ecotoxicol. Environ. Saf., 1979; 3:374.
9. Bhatia SC, Sharma SC, Venkatasubramanian TA. In vivo subacute physiological stress induced by sumithion on the hepatopancreatic acid phosphatase activity in the freshwater crab, Oziotelphusa senex senex. Water Air Soil Pollut., 1972; 22:299-302.
10. Bhatnagar MC, Tyagi M, Tamata S. Pyrethroid induced toxicity to phosphatases in Clarias bactrachus Linn. J Environ Biol. 1995; 16(1):11-14.
11. Bhavan PS, Geraldine P. Histopathology of the hepatopancreas and gills of the prawn Macrobrachium malcolmsonii exposed of endosulfan. Aquat. Toxicol., 2000; 50:331-339.
12. Ceccaldi HJ. A synopsis of the morphology and physiology of the digestive system of some crustacean species studies in France. Rev. Fish. Sci., 1998; 6:13-39.
13. Das BK. Studies on the effect of some pesticides and commonly used chemicals on Indian major carps and their ecosystem. PhD. Thesis, submitted to Orissa University of Agriculture and Technology, Bhubaneswar, Orissa, 1998.
14. Das BK, Mukherjee SC. A histological study of carp Labeo rohita exposed to hexachlorocyclohexane. Vet. Archiv. 2000; 70(4):169-180.
15. Deduve C, Pressman BC, Gianetto R, Wattiaux R, Appelmans F. Intracellular distribution patterns of enzymes in rat-liver tissue. Biochemical Journal, 1985; 60:604-617.
16. Denielli JF. Structural factors in cell permeability and secretion. Symp. Soc. Exp. Biol., 1972; 6:1-15.
17. Devi S. Studies on the effects of urea and naphthalene in a brackish water crab Uca celuca triangularis Bengali Crane, 1975 of Pulicat lake Tamilnadu. Ph.D Thesis, University of Madras, Tamil Nadu, India, 1996.
18. Dillon TM, Neff JM. Mercury and the estuarine marsh clam Rangia cuneata Cray II. Uptake, tissue distribution and depuration. Mar. Environ. Res., 1978; 1:67-77.
19. Du Preeze HM, Steenkamp VE, Schoonbee HJ. Bioaccumulation of zinc and lead in selected tissues and organs of the freshwater crab, Potamonautes warreni. Proc. Euro. Conf. Ecotoxicol., 1993; 1&2:469-478.
20. Dutta HS, Lall B, Haghighi AZ. Mercuric chloride induced changes in serum protein of bluegill, Lepomis macrochirus. Ohio. J Sci., 1983; 83:119-120.
21. Gate HV, Mulherkar L. Histological changes in the gills of two freshwater prawn species exposed to copper sulphate. Indian J. Exptl. Biol., 1979; 17:838-840.
22. Goldfisher SE, Esser E, Novikoff AB. Use of histological and histochemical assessment in the prognosis of the effects of aquatic pollutants ed. DE. Hinton MW Kendall and BB. Silver. Sect. 528, ASTM, Philadelphia, 194-208.
23. Goodman J, Rothstein A. The active transport of phosphate into the yeast cell. J. Genet. Physiol., 1957; 40:915-925.
24. Gupta V, Rao G. Histological studies on the chloride

plexes of the goat embryos II. Histological distribution of acid and alkaline phosphatase. Acta Histochem, 1974; 49:60-63.
25. Hanson JI, Mustafa T, Depledge M. Mechanism of copper toxicity in the shore crab, Carcinus maenus: Effects Na, K-ATPase activity, haemolymph electrolyte concentrations and tissue water contents. Marine. Biology. 1992; 114(2):253-257.
26. Ibrahim AM, Higazi MG, Demian ES. Histochemical localization of alkaline phosphatase activity in the alimentary tract of the snail Marisa carnvarietus L. Zool. Soc. Egypt. Bull., 1974; 26:94-105.
27. Jayakumar S. Effects of copper and zinc toxicity on a fresh water crab, Spiralothelphusa hydrodroma, Ph.D. Thesis, University of Madras, India, 2002.
28. Jothinarendiran N. Effect of dimethoate pesticide on oxygen consumption and gill histology of the fish, Channa punctatus. Current Biotica. 2012; 5(4):500-507.
29. Kabila P, Saravanabhavan P, Geraldine P. The freshwater prawn, Macrobrachium malcolmsonii an accumulator of nickel. J. Environ. Bio. 1999; 20(4):307-312.
30. King J. In: Practical clinical enzymology. Eds., D. Van Norstand Co London, 1965.
31. Kulasekaran R. Some aspects of reproduction in a freshwater crab, Spiralothelphusa hydrodroma Herbst of Salem district, Tamil Nadu, Ph.D. Thesis, University of Madras, Chennai, 1994.
32. Kulkarni GK, Nagabushanam R. Mobilization of organic reserves during the ovarian development in a marine penaeid prawn, Parapenaeopsis hardwickii Miers. Aquaculture. 1979; 18:373-377.
33. Lawson SL, Jones MB, Moate RM. Effect of copper on the ultrastructure of the gill epithelium of Carcinus maenas Decapod: Brachyura. In proceedings Trace metals in the aquatic environment. Proc. III Internatl. Conf., Aarhus. Philips D.J.H. and P.S. Rainbow eds. 1995; 31(1-3):63-72.
34. Maharajan A, Narayanasamy Y, Ganapiriya V, Shanmugavel K. Histological alterations of a combination of Chlorpyrifos and Cypermethrin Nurocombi insecticide in the fresh water crab, Paratelphusa jacquemontii Rathbun. The Journal of Basic & Applied Zoology, 2015; 72:104-112.
35. Maryam SM, Khalili V, Abbasi A, Hedayati. Sublethal effect of copper toxicity on liver lesions of Roach Rutilus rutilus caspicus Juveniles. JNASCI. 2013; 2(4):119-123.
36. Mintu Deyashi, Kamales Kumar Misra, Siddhartha Sankar Bhattachary, Suman Bhusan Chakraborty. Acute Toxicity of a Neem Seed Kernel based Biopesticide, Nimbecidine Plus on an Edible Fresh Water Crab, Varuna litterata Fabricius, 1798. Int. J. Adv. Res. Biol. Sci. 2016; 3(10):122-130.
37. Nachlas MM, Margulius SI, Selligman AM. A colorimetric method for the estimation of SDH. The Journal of Biological Chemistry, 1960; 235:499-503.
38. Nash G, Chinabut S, Limsuwan C. Idiopathic muscle necrosis in the freshwater prawn, Macrobrachium rosenbergii de man, cultured in Thailand. J. of Fish Diseases, 1987; 10:109-120.
39. Nimmo DWR, Lightner DV, Bhaner LH. Effects of cadmium on shrimps, Penaeus duorarum, Palaemonetes pugio and P. vulgaris. In: Physiological response of marine biota to pollutants, eds., F.J. Vernberg *et al.*, Academic Press, New York, 1977, 131-183.
40. Nonnotte L, Boitel F, Truchot JP. Waterborne copper causes gill damage and haemolymph hypoxia in the shore crab, Carcinus maenas. Can. J. Zool. Rev. Can. Zool. 1993; 71(8):1569-1576.
41. Norseth T. The intracellular distribution of mercury in rat liver after single injection of mercuric chloride. Biochem Pharmacol., 1967; 17:581-593.
42. O'Connor JD, Gilbert LI. Aspects of lipid metabolism in crustaceans. American Zoology, 1968; 8:529-539.
43. Pálaez-Cid AA, Velazquez-Ugalde I, Herrera- González AM, García-Serrano J. Textile dyes removal from aqueous solution using Opuntia ficus-indica fruit waste as adsorbant and its characterization. Journal of Environment Management. 2013; 130(1):90-97.
44. Patil VN, Kadam GH, Raut PD. Enzymological alterations produced by chronic malathion exposure in freshwater crab, paratelphusa barytelphusa jacquemontii. International Journal of Science, Environment and Technology. 2014; 3(1):303-313.
45. Pilo B, Ansari MV, Shah RV. Studies of wound healing and repair in pigeon liver III. Histochemical studies on acid and alkaline phosphatase activities during the process. J. Anim. Morphol. Physiol., 1972; 9:205-212.
46. Rao LM, Vani S, Ramaneswari. Metal accumulation in tissues of Macrobrachium rudae from Mehadrigedda stream, Visakhapatnam. Poll. Res. 1998; 17(2):137-140.
47. Reddy PS, Bhagyalakshmi A. Changes in oxidative metabolism in selected tissues of the crab, Scylla serrata in response to cadmium toxicity. Ecotoxicol. Environ. Saf. 1994; 29(3):255-264.
48. Reddy PS, Bhagyalakshmi A, Ramamurthi R. In vivo subacute physiological stress induced by sumithion on the hepatopancreas acid phosphatase activity in the freshwater crab. Toxic. Lett., 1984; 22:299-309.
49. Reddy PS, Devi M, Sarojini R, Nagabhushanam R, Fingerman M. Cadmium chloride induced hyperglycemia in the red swamp crayfish, Procambarus clarkia: Possible role of crustacean hyperglycemic hormone. Comp. Biochem. Physiol. 1994; 107(C):57-61.
50. Reddy PS, Katyayani RV, Fingerman M. Cadmium and naphthalene-induced hyperglycemia in fiddler crab, Uca pugilator. Differential modes of action on the neuroendocrine system. Bull. Environ. Contam. Toxicol, 1996; 56:425-431.
51. Sastry AN, Miller DC. Biological monitoring of marine pollutants. Verberg, F.J, Calabrase, A., Thurberg, F.P. and Vernberg, W.B. eds. Academic Press, London and New York, 1981.
52. Sastry KV, Sharma K. Effect of mercuric chloride on the activities of brain enzymes in Heteropneustes fossilis. Matsya, 1981; 7:66-69.
53. Shaikh FI, Ustad IR, Ansari NT. Effect of heavy metal on the ovary of freshwater crab, Barytelphusa cunicularis Westwood. Bioscan. 2010; 5(2):335-338.
54. Sneha Verma, Anurag Rawat. Effect of chlorpyrifos on protein and carbohydrate content of Heteropneustes

fossilis Bloch, 1794. International Journal of Fisheries and Aquatic Studies. 2017; 5(1):463-466.
55. Sprague JB. The measurement of pollutant toxicity to fish III, sub lethal effect and safe concentration. Water Res., 1971; 5:245-266.
56. Sujatha P. Studies on the reproductive aspects in a brackish water crab, Uca celuca triangularis Bengali Crane, 1975 of Pulicat Lake, Tamil Nadu., Ph.D. Thesis, University of Madras, Tamil Nadu, India, 1998.
57. Sumner AT. The cytology and the histochemistry of the digestive gland cells of Helis. Quart. J. Microsc. Sci., 1965; 106:173-192.
58. Suresh V. A study on the effects of heavy metals toxicity on a brackish water crab, Uca celuca lacteal annulipes Crane, 1975 of Pulicat Lake, Tamil Nadu, Ph.D. Thesis, University of Madras, Tamil Nadu, India, 2001.
59. Tenniswood M, Bind CE, Clark AF. Acid phosphatases androgen dependent markers of rat prostate. Canadian Journal of Biochemistry, 1976; 54:340-343.
60. Tung CW, Wang CS. Histological and electron microscopic study on Macrobrachium muscle virus MMV infection in the giant freshwater prawn, Macrobrachium rosenbergii de Man, cultured in Taiwan. J. of Fish Diseases, 1999; 22:319-361.
61. Tyagi P, Dharam B, Choudhary R, Sawhney BL. Physico chemical quality of Ground water in industrial areas of India. A review. Poll. Res. 2000; 19(3):443-445.
62. Victor B. Histology evaluation of cythion toxicity on the oocyte development of Oziotelphusa senex senex. J. Environ. Biol. 1989; 10(1):65-71.
63. Victor B. Gill tissue pathogenicity and hemocyte behaviour in the crab Paratelphusa hydrodromous exposed to lead chloride. J. Environ. Sci. Health. 1994; 29(A):1011-1034.
64. Victor B, Sundaraj A. Changes in the developmental morphology of oocytes in urea toxified crab Paratelphusa hydrodromous. In: Recent advances in invertebrate reproduction and aquaculture ed. Palanichamy, S., Palani Paramount Publication, Palani, India, 1988, 219-227.
65. Victor B, Sarojini R. Toxicity of organophosphorous on the ovaries of the caridian prawn, Caridina rajadhari Bourvier. Curr. Sci. 1985; 54(8):398-400.
66. Victor B, Jeyaraj DA, Ramakrishnan P. Effect of cythion on food utilization, tissue pathology and behavior of the freshwater crab Paratelphusa hydrodromous Herbst. Impacts of environment on animals and aquaculture eds., Manna GK and BB Jana, 1990, 265-269.
67. Victor B, Mahalingam S, Sarojini R. Cytopathological effects of cadmium on the freshwater prawn Macrobrachium idea. Indian J. Fish. 1985; 32(4):475-480.
68. Vijayaraman K, John G, Sivakumar P, Mohammed RR. Uptake and loss of heavy metals by the freshwater prawn, Macrobrachium malcolmsonii. J. Environ. Biol. 1999; 20(3):217-222.
69. Vogen CL, Coasta RC, Rowely AF. A histological study of shell disease syndrome in the edible crab Cancer Pagurus. Dis. Aquat. Org., 2001; 47:209-217.
70. Wild D. Mutagenicity studies on organophosphorus insecticides. Mutat. Res., 1975; 32:135-150.