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Acute Toxicity of Chlorpyrifos on Histological Alterations in the Anomuran Crab, *Emerita asiatica* (H. Milne Edwards, 1837)

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Abstract **- Acute toxicity of Chlorpyrifos on histological alterations in the different organs like gills, hepatopancreas and ovary of anomuran crab, Emerita asiatica was studied. Though Emerita asiatica is not a commercially viable crab, but it plays a vital role in the environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to conserve these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction. Thus, it can be concluded that the use of Chlorpyrifos which has been legally banned in India is justified. It has been proved by several workers and has been conformed in present investigation that use of this Chlorpyrifos causes serious damage to the vital organ of sand crabs gill, hepatopancreas and ovary. The application of Chlorpyrifos could be reduced in the agricultural field to nearby coastal regions.**
Keywords**: *Emerita asiatica*, Chlorpyrifos, Food Chain, Marine Ecosystem, Toxicity**

I. INTRODUCTION

Aquaculture is the cultivation of aquatic organisms in water (such as shellfish, finfish, or plants) (freshwater or marine). Food fish, sport fish, bait fish, ornamental fish, crustaceans, mollusks, algae, sea vegetables, research animals, and fish eggs are all produced by aquaculture. Aquaculture also includes the breeding of ornamental fish for the aquarium trade, as well as the cultivation of plant species for use in a variety of food, pharmaceutical, nutritional, and biotechnology products. Stock restoration, also known as "enhancement," is a type of aquaculture in which hatchery fish and shellfish are released into the wild in order to rebuild wild populations or coastal habitats such as oyster reefs.

Aquaculture is the world's fastest growing food production sector, growing 6.2% per year between 2000 and 2012 (9.5% between 1990 and 2000) [1]. Aquaculture is used in all types of water environments for animal and plant breeding, rearing, and harvesting. It is used to rebuild threatened and endangered species populations.

Aquaculture is a method of producing food and other commercial products, restoring habits, and replenishing wild stocks; it involves rearing of plant and animal for providing food to human, entertaining fishing augmentation of commercially expensive stocks, endangered species revival, and the production of bait and ornamental species. The concept of sustainable aquaculture is spreading throughout the world.

When compared to livestock, fish and aquatic species in general are a much healthier source of protein. The raise was primarily determined by marine capture fisheries, which increased production from 81.2 million tonnes in 2017 to 84.4 million tonnes in 2018. The top 20 producing countries accounted for roughly 74% of total catch fisheries production. [2]. Aquaculture is required to help feed the world. According to the UN, there will be 9.7 billion people on the planet by 2050, putting enormous pressure on food production in general, and fish in particular. Because of destructive fishing practices and overfishing, 33% of wild fish stocks have already reached their biological limit.

Over the years, there have been significant pattern transfers in terms of rising assistance from the domestic sector and, to a lesser extent, aquaculture. Coastal aquaculture, marine fisheries, inland fisheries, freshwater aquaculture, and cold-water fisheries are all taking part to the country's food basket, wellbeing, financial system, exports, service, and recreation [3]. Aquaculture is the fastest-growing sector of global food production, with marine aquaculture accounting for 38% of total supply. To the tune of 80%, the majority of aquaculture production comes from Asian countries.

India currently accounts for nearly 5% of global fish production and approximately 7% of global aquaculture production. India has enormous growth potential, and the country is on the verge of massive development in fisheries and aquaculture. Seafood exports account for approximately 3.32% of India's total exports and are the country's fourth largest contributor of net foreign exchange.

Indian aquaculture is mainly through the freshwater Indian major carps. The coastal aquaculture is mainly by the shrimps to the extent of around 2,50,000 tones. Indian

coastal aquaculture, though traditional in some parts of India in the low-lying brackish water areas of Kerala, Karnataka, West Bengal, Odisha and Goa, the farming has emerged as an important activity in improved manner in recent years.

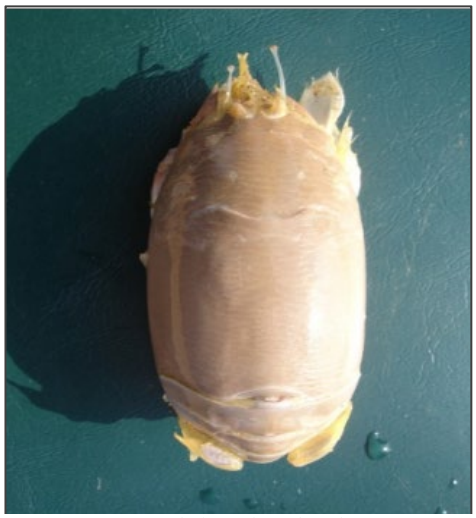
Various technological advancements in the feed and seed production and on other inputs have prompted the farmers to take up the aquaculture in improved manner and new areas have been brought into coastal farming and phenomenal growth was witnessed till the mid of 1990s. In India freshwater aquaculture production consists of approximately ponds and tanks are in 2.36 million ha, accounting for nearly 55% of total fish production in India [4].

Emerita asiatica is a small decapod crustacean genus that includes sand crabs, sand fleas, mole crabs and sand bugs (Fig. 1). These miniature animals' tunnel in the seashore sand in the surf zone and filter feed with their antennae. Emerita asiatica is the only sand crab found on the Madras coast's sandy beaches. Specimens are typically discovered buried in loose sand on the beaches [5]. Provided a brief account of the life history, along with a brief note on the occurrence of males. [6] reported on this anomuran crab's continuous breeding activity. However, we know very little about its sexual biology.

Emerita asiatica is an expert burrower, able of hiding itself entirely in 1.5 seconds. Emerita holes tail-first into the seashores and, scraping the sand from beneath its body with its pereiopods. During this stroke, the carapace is pushed into the sand as anchorage for the excavating limbs, according to Kenneth (2003). To be safe from predators, the sand must be fluidized by tidal action prior to digging, and *Emerita* must hide itself in the accurate direction previous to the wave passes[7].

Emerita has an oval-shaped cylindrical body. It has a robust exoskeleton that enables it to rotate in tidal currents and waves by keeping its thoracic attachments close to the body. Its feathery antennae filter plankton and detritus into the swash [8]. Males are typically smaller than females, and males in some species, such as *Emerita rathbune*, live attached to the female's legs. Males have carapace lengths ranging from similar to females in *E. austroafricana* to 2.5mm (0.0098) in E. rathbunae and E. talpoida. Females have carapace lengths ranging from similar to females in E. austroafricana to 2.5mm (0.0098) in *E. talpoida* and *E. rathbunae*[9].

Emerita has a short duration of life, possibly two to three years, and can sexually mature in its first year. The eggs are a brilliant orange colour, and when they hatch, they become larvae that can survive as plankton for more than four months and travel extended distances to ocean currents. Depending on the species, there are six to eleven zoeal stages.



Dorsal view of Sand Crab

Lateral view of Sand Crab

Fig. 1 Photograph showing the Dorsal and Lateral view of Sand Crab *Emerita asiatica*

The anomuran crab *Emerita asiatica* is the single species of the genus known from the sandy beaches of the Indian peninsula's east coast. The first Emerita species studies concentrated on distribution patterns and their relationships to physical and biological factors. Males in this genus exhibit neoteny, and it should be noted that males are generally smaller than females. The Pacific Sand Crab is a small crustacean that can reach 35 mm (1.4 in) in length and 25 mm (1.0 in) in width. The female is nearly twice the size of the male and is easily distinguished by the orange egg mass carried beneath the telson. The adult is sand-colored and well-hidden, with no claws or spines.

It has three pairs of pleopods and five pairs of legs. Sand crabs moult on a regular basis, so their exoskeletons can be found washed up on the beach (Sivakumar, 2014). The sand crab is well adapted to life in sand, which is an unstable substrate, and its shape is an elongated dome designed for rapid burrowing. The eyes are on long stalks, and the antennules are also elongated to protrude above the sand surface. These form a tube that allows water to flow downward through the gills. The retractable antennae are much longer [10]. They project above the sand surface to collect food practices when there is water overhead.

Hairy margins on the legs and uropod's aid in digging and collecting food for transfer into the mouth [5] Chlorpyrifos is a common organophosphate lipophilic insecticide used in soy crops [11]. During feeding and breathing, the skin can act as a barrier between the water and the exposed organism. Crabs are a common component of the biota of freshwater ecosystems, and those that live near agricultural areas are regularly exposed to varying pesticide concentrations.

The effects of all toxins begin with interactions with biomolecules. The consequences of their actions then cascade through the biochemical, subcellular, cellular, and tissue levels, eventually reaching individuals, populations, communities, and ecosystems.

Chlorpyrifos is widely used due to restrictions on the production and use of highly toxic organophosphate pesticides, and low concentrations of Chlorpyrifos pollution in the environment have become a common phenomenon. There are potential risks to human health, and Chlorpyrifos has been linked to human genital deformities [12].

Histological effects are the result of biochemical mechanics, which provide individuals with predictive power about effects by integrating damage done at the molecular level [11]. The gills of aquatic animals are commonly used to assess pollution. Because they are the first organ to come into contact with aquatic pollution, they have the greatest impact. Furthermore, they are extremely vulnerable to toxic chemicals, owing to their large surface area, which facilitates interaction. Toxic substances are rapidly absorbed due to the rapid absorption of gills. The gills respond quickly as well. [13] Pesticide histopathological effects are generally assessed in constant exposure settings with relatively constant biocide concentrations [14].

However, there are few records of histopathological effects observed after pesticide concentrations are reduced that we are aware of [15]. On August 18, 2021, the United States Environmental Protection Agency announced that it will phase out the use of Chlorpyrifos, a pesticide linked to neurodevelopmental problems and impaired brain function in children, on all food products across the country. Chlorpyrifos is now commonly utilized in agriculture in the United States. It is commonly sprayed on crops to kill a range of agricultural pests. Chlorpyrifos is highly toxic and has been linked to neuro developmental problems in children [16].

From the foregoing accounts there is no study on the toxicity effect of pesticides on this species past two decades. Even though the sand crabs not an economic important one, but it plays a significant role in the marine ecosystem. It also otherwise called as marine bio indicators. Hence the present investigation has been taken for toxicity effect of Chlorpyrifos on this crab.

II. MATERIALS AND METHODS

A. Collection and Maintenance of Control and Experimental Sand Crabs

Emerita asiatica is a Sand Crab. The animals were collected from the VGP Golden beach, Injambakkam, Chennai-600115, Tamil Nādu, India (Fig. 2). The sand crabs were collected from the intertidal surf zone during the early morning. The animals in the collection were both male and female by handpicking method. More than fifty sand crabs were collected in a polythene bag containing wet sand and transported to the laboratory. The intermediate sizes were discovered in the swash zone. The specimens were acclimatized to the laboratory condition in a plastic tray with adequate aeration and water for five days. The water and sand were added on daily basic for their survival days (Fig. 3).

B. Toxicity Assay

To determine the toxicity of Chlorpyrifos (Fig. 4) on sand crab, *Emerita asiatica*, the lethal experiments was conducted using 10 sand crabs in each tray. The sand crabs were acclimatized for 5 days.

Each tray had an aeration system, and the water physicochemical conditions were the same in all aquariums. With the authenticated letter, the pesticide Chlorpyrifos was purchased from a local pesticide store. Experiments were carried out in accordance with the standard method of the Organization for Economic Cooperation and Development to determine the 24-hour LD50 of these species.

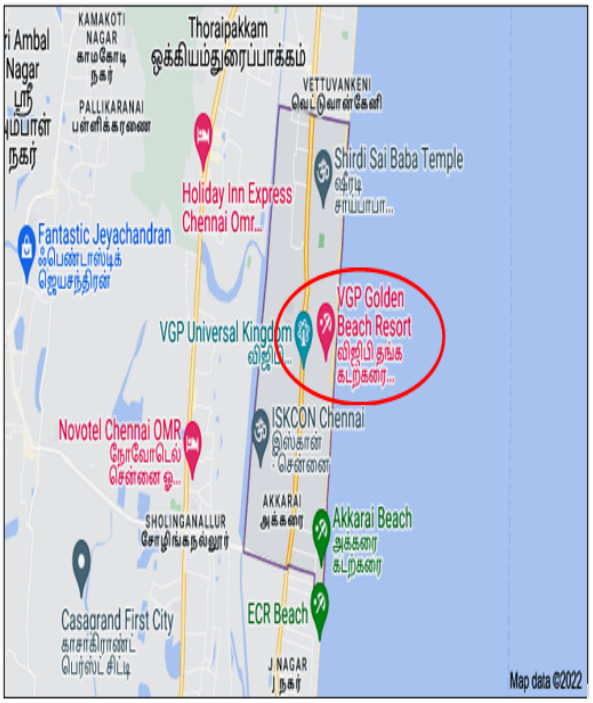
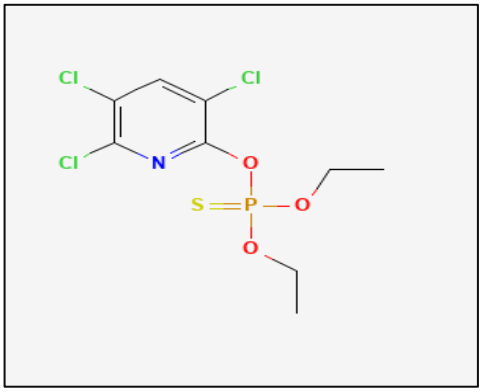


Fig. 2 Map showing the collection site-Injambakkam (ECR beach)

Fig. 3 Sand crabs maintained in the separate trays for toxicological studies



Structure of Chlorpyrifos

Chlorpyrifos Container

Fig. 4 Photograph showing the pesticide Chlorpyrifos

Distilled water was used to dilute 10 l of chlorpyrifos. 1 litre of distilled water was diluted with 20 l of chlorpyrifos. In 1 litre of distilled water, 30 l of chlorpyrifos was diluted. First, preliminary experiments were carried out on a small scale to determine a lethal concentration of this pesticide on this species, as data on Chlorpyrifos toxicity of these species had been collected. Based on this information, three concentrations of Chlorpyrifos (10, 20, and 30 l) were pipetted along the edges of each tray using a micropipette. To avoid the effects of metabolites and waste organic matter of sand crabs, all water in the trays was exchanged during the experiment, containing the same concentration of Chlorpyrifos, according to the method used (static-renewal test condition). At time 0, 30, 60, 90, 120, 150, and 180 minutes, dead crabs were removed from the tray, and mortality rates were recorded. Acute toxicity tests were conducted, and the nominal concentration of Chlorpyrifos was estimated to result in 100% mortality of sand crabs within 5 hours (5-h LD50) using statistical software SPSS version 17.

C. Histological Investigation of Sand Crab, Emerita asiatica

For histological investigation, organs such as Gills, Hepatopancreas and Ovaries were dissected out from control as well as experimental sand crabs. The histology of these organs in normal crabs was also studied. Davidson's alcohol formalin acetic acid fixativewas used to fix the organs. Later, the organs such as the gills, hepatopancreas, and ovaries were cut and stored in screw-capped bottles containing the fixative before being transferred using standard protocols. The sand crab was anaesthetized or chilled and immediately fixed with appropriate Davidson's alcohol formalin acetic acid fixative. The Sand Crab was sacrificed and the target organs (gills, hepatopancreas, and ovaries) were dissected out. It was immediately placed into the fixative and carefully labeled, and it was recommended to take at least ten volumes of fixative for each volume of tissue samples (ratio of 1part tissue to at least 9 parts formalin). Sample was allowed to fix for at least 24-48 hours before processing.

D. Processing of Tissue and Staining

Before cutting paraffin slices of various tissues, the tissues were dried and cleaned at room temperature. The tissues were washed in two changes of 70% alcohol for one hour each, dehydrated in two changes of 70% alcohol for one hour each, graded twice in 95 percent alcohol and absolute alcohol, cleaned in a mixture of absolute alcohol and chloroform (1:1 v/v), and then passed twice I. Chloroform was chosen over xylene because it does not produce tissue stiffening and brittleness. Following washing, the tissues were immersed overnight at room temperature in a 1:1 combination of chloroform and paraffin wax. The tissues were impregnated in three changes of paraffin wax with ceresin with melting temperatures ranging from 58 to 60ºC for one hour each prior to embedding. Using a manual rotatory microtome, the transverse slices were cut at a thickness of 5 to 7 m. Following xylene deparaffinization, the sections were hydrated with a graded sequence of alcohol up to 70%, stained with Harris alum hematoxylin, and counterstained with 1% alcoholic eosin. Staining slices were dehydrated in a graded series of alcohol before being mounted in DPX through xylene with a glass cover slip.

E. Light Microscopy and Photomicrography

A Carl Zeiss binocular compound microscope was used to examine the histological sections. Cellular measurements were conducted using a Carl Zeiss microscope equipped with a calibrated ocular micrometer scale with a 10 m resolution. Photographs were shot using a digital camera (Nikon) connected to a Carl Zeiss microscope equipped

with a projection eyepiece of 10 X and objectives of 10, 20, 40, and 100 X. The enlarged prints' magnification was estimated using an ocular and a stage micrometer.

III. RESULTS

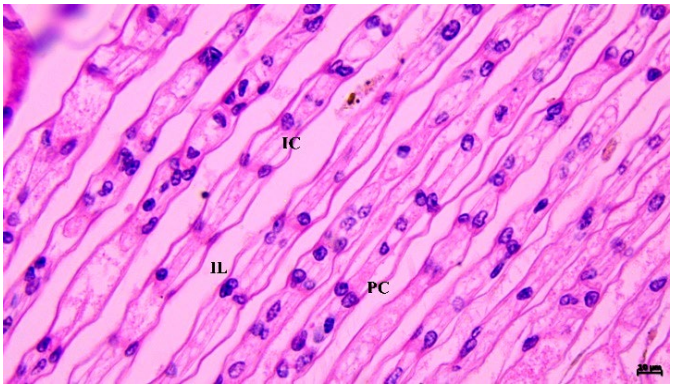
The present investigation revealed some interesting facts. After introducing the pesticide Chlorpyrifos in different concentrations on the different groups of sand crabs it was reacted immediately. The crabs were started to swim restlessly and inverted its carapace towards the sand grains. At one stage the original color of the carapace has been changed into blue color. The crabs were started to secrete the foams in the anterior region to survive in the experimental trays. Among the three categories the 30 µl concentration, the crabs were started to die quickly. In the case of 10µl concentration the crabs were survived for a period of more than four hours, whereas in 20 µl concentration the crabs were moderately survived. Toxicity effects severely damaged the gills, hepatopancreas and ovaries.

A. Histological Changes in Gills

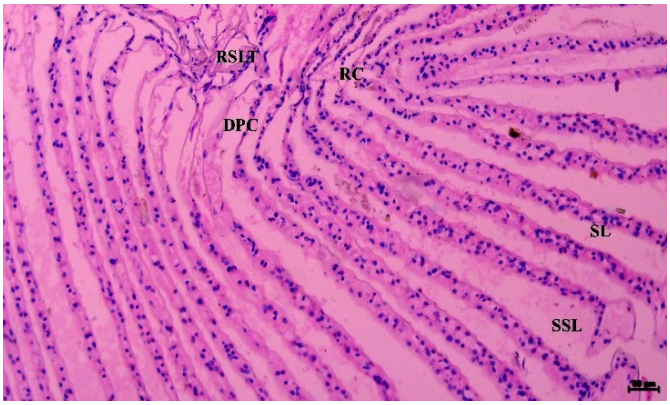
Detail microscopic examination has revealed the structure of gill as the main gill lamellae with the appropriate behind axis with gill lamellae on each side of it. Normal structure of gill is seen. The surface is covered with squamous epithelium normal cells are observed separated by the mucous cells. In the experimental samples that is after the exposure of sand crab with Chlorpyrifos that is organopesticide has revealed an abnormal structure.

Emerita asiatica's gills are made up of a series of broad flattened plates or lamellae arranged in sequence in pairs all along to a control gill stem. The primary gill lamellae are the central axis of gill tissue, which divides additional into derived gill lamellae or filaments. A lean film of cuticle covers the whole outer surface of the control gill. A continuous layer of epithelial cells lies beneath the cuticle. Pillar cells join the lamellae at irregular intervals (Fig.5). At lower concentrations of 10l, there was a noticeable swelling of interlamellar gap tightly packed out with granular material, as well as a loss of gill structure (Figs. 6 and 7). Secondary gill lamellae rupture has been observed in a medium concentration of 20 l. (Figs. 8 and 9).

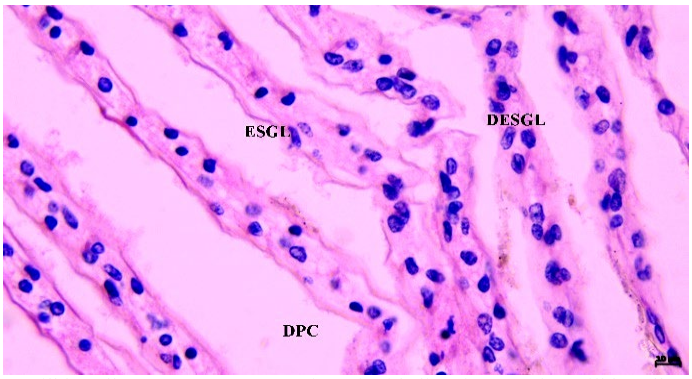
The obstruction of the pillar cells causes the gill lamellae in exposed crab to collapse. After 4 hours of exposure to a higher concentration of 30l, the alterations are hemocoel packed with coarse amorphous to leathery materials, thickened gill lamellae, and enormoushemocytic permeation (Figs. 10 and 11).



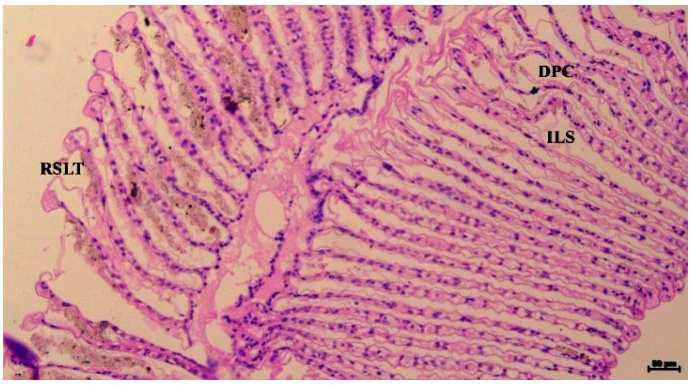
IC-Ionocytes; IL-Interlamellar space; PC-Pillar cells
Fig. 5 Photomicrograph showing the gills of control sand crab at 40X magnification



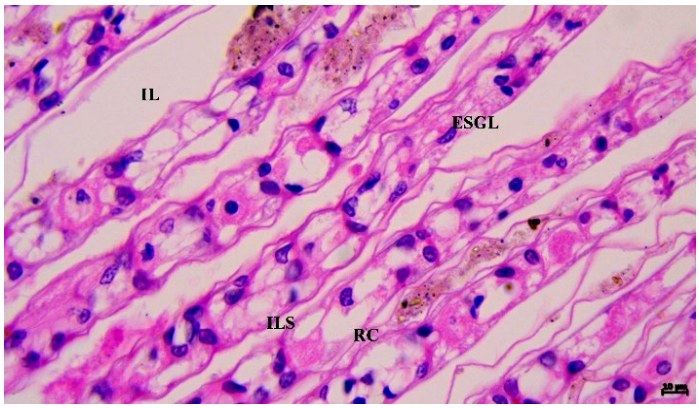
RSLT-Rupture of secondary lamellar tip; DPC-Disruption of pillar cells;
RC-Rupture of capillaries; SL-Secondary lamellae; SSL-Swelling of secondary lamellae
Fig. 6 Photomicrograph showing the gills of sand crab treated with 10 µl Chlorpyrifos at 10X magnification



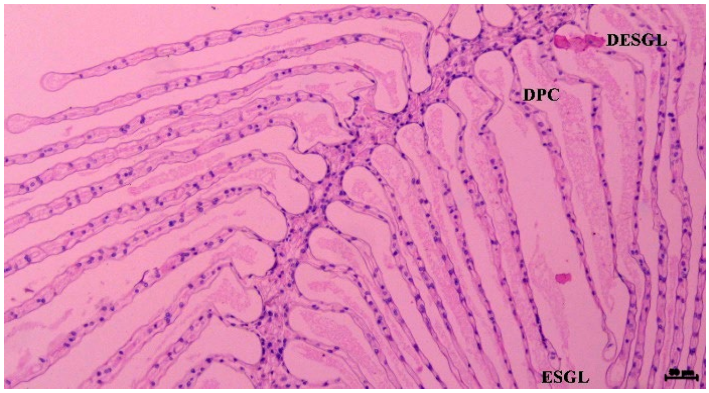
ESGL-Enlargement of secondary gill lamellae; DESGL-Degeneration of epithelium in secondary gill lamellae; DPC-Disruption of pillar cells
Fig. 7 Photomicrograph showing the gills of sand crab treated with 10 µl Chlorpyrifos at 40X magnification



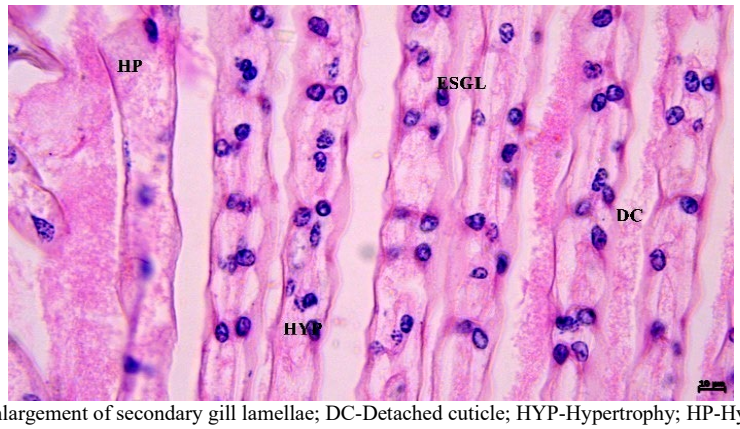
DPC-Disruption of pillar cells; ILS-Inter lamellar space; RSLT-Rupture of secondary lamellar tip
Fig. 8 Photomicrograph showing the gills of sand crab treated with 20 µl Chlorpyrifos at 10X magnification



ESGL-Enlargement of secondary gill lamellae; IL-Inter lamellar; ILS-Inter lamellar space; RC-Rupture of capillaries
Fig. 9 Photomicrograph showing the gills of sand crab treated with 20 µl Chlorpyrifos at 40X magnification



DESGL-Degeneration of epithelium in secondary gill lamellae; DPC-Disruption of pillar cells; ESGL-Enlargement of secondary gill lamellae
Fig. 10 Photomicrograph showing the gills of sand crab treated with 30 µl Chlorpyrifos at 10X magnification

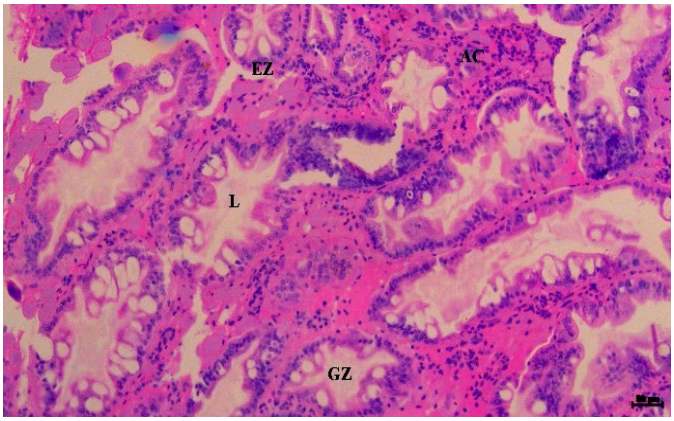


ESGL-Enlargement of secondary gill lamellae; DC-Detached cuticle; HYP-Hypertrophy; HP-Hyperplasia
Fig. 11 Photomicrograph showing the gills of sand crab treated with 30 µl Chlorpyrifos at 40X magnification

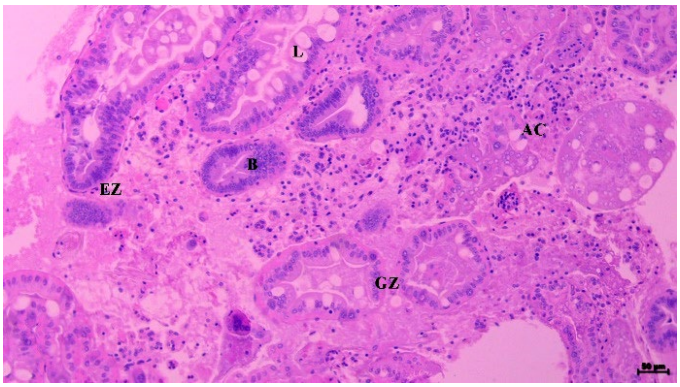
B. Histological Changes in Hepatopancreas

In the control crab the majority of cephalothoracic cavity occupied the yellowish-brown tissue called hepatopancreas. The glandular tubular structure was well-organized in the histology of the control sand crab. The tubules were consisting of composed epithelium of four type cells: B-cells (blister-like), E-cells (embryonic), F-cells (fibrillar), and R-cells (resorptive) (Fig. 12).

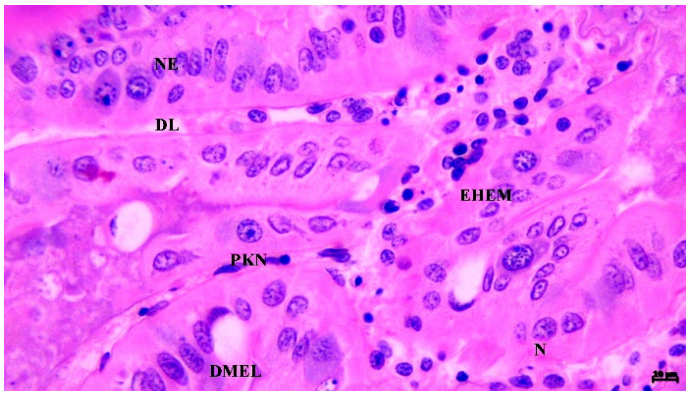
After 2 hours of exposure to a lower concentration (10l), light changes was noticed in B-secretary cells and F-Fibrillar cells (Figs. 13 and 14). After 2 hours of exposure in medium concentration (20 µl), the cells were spoiled; clumped and intercellular spaces could not be distinguished (Fig. 15 and 16). After 4 hours of exposure to a higher concentration (30l), deterioration of tubular and intertubular tissues with widespread vacuolation and total thrashing of tubular structures is observed. Great numbers of vacuoles emerged at higher concentrations, and the thickened basal lamina. There was also necrosis, bulging of cell, a decrease in cell height, and myoepithelial layer was damaged with stretched out hemocytes (Figs. 17 and 18).



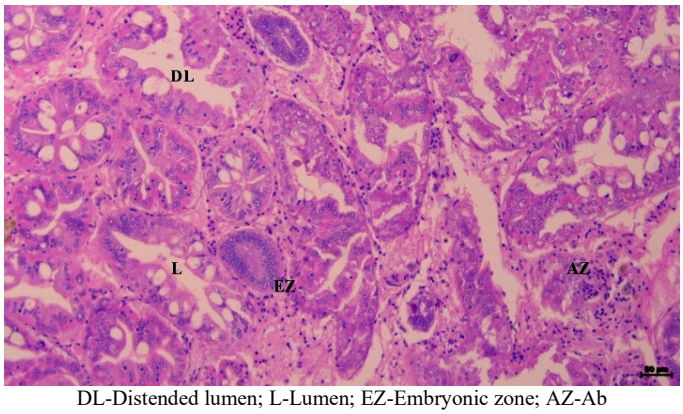
AC-Absorptive cells; L-Lumen; GZ-Germinal zone; EZ-Embryonic zone
Fig. 12 Photomicrograph showing the Hepatopancreas of control sand crab at 40X magnification



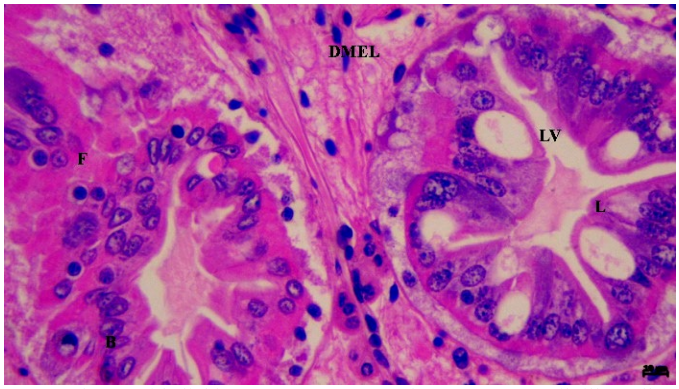
AC-Absorptive cells; GZ-Germinal zone; EZ-Embryonic zone; B-β cells; L-Lumen
Fig. 13 Photomicrograph showing the hepatopancreas of sand crab treated with 10 µl Chlorpyrifos at 10X magnification



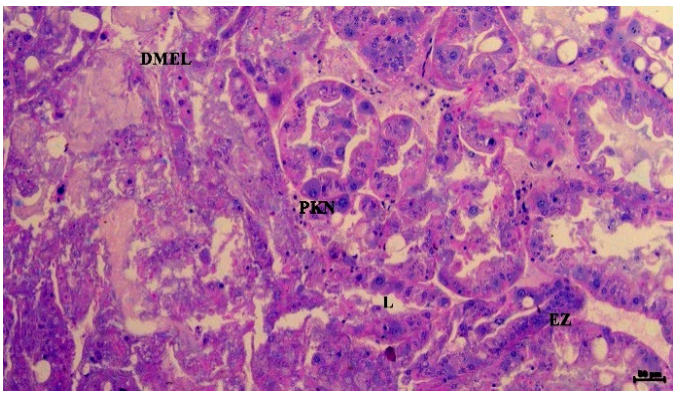
NE-Necrosis; EHEM-Elongated haematocytes; PKN-Pyknotic nucleus; DMEL-Damaged myoepithelial layer; DL-Distended lumen
Fig. 14 Photomicrograph showing the hepatopancreas of sand crab treated with 10 µl Chlorpyrifos at 40X magnification



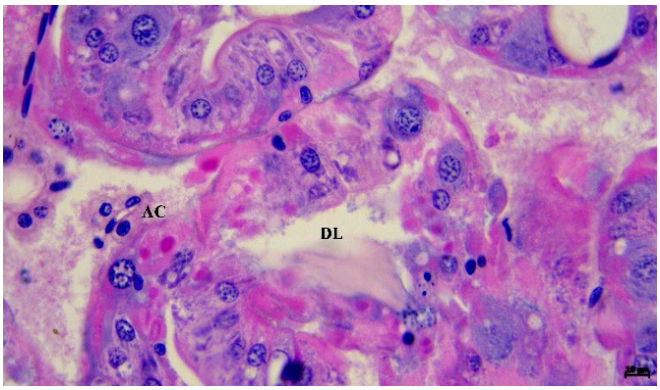
DL-Distended lumen; L-Lumen; EZ-Embryonic zone; AZ-Ab
Fig. 15 Photomicrograph showing the hepatopancreas of sand crab treated with 20 µl Chlorpyrifos at 10X magnification



DMEL-Damaged myoepithelial layer; LV-Large vacuole; L-Lumen; F-Fibrillar cells; B-βcells
Fig. 16 Photomicrograph showing the hepatopancreas of sand crab treated with 20 µl Chlorpyrifos at 40X magnification



DMEL-Damaged myoepithelial layer; PKN-Pyknotic nucleus; L-Lumen; EZ-Embryonic zone
Fig. 17 Photomicrograph showing the hepatopancreas of sand crab treated with 30 µl Chlorpyrifos at 10X magnification



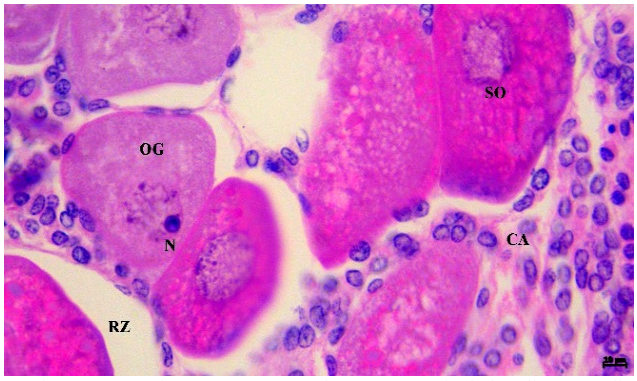
AC-Absorptive cells; DL-Distended lumen

Fig. 18 Photomicrograph showing the hepatopancreas of sand crab treated with 30 µl Chlorpyrifos at 40X magnification

C. Histological Changes in Ovaries

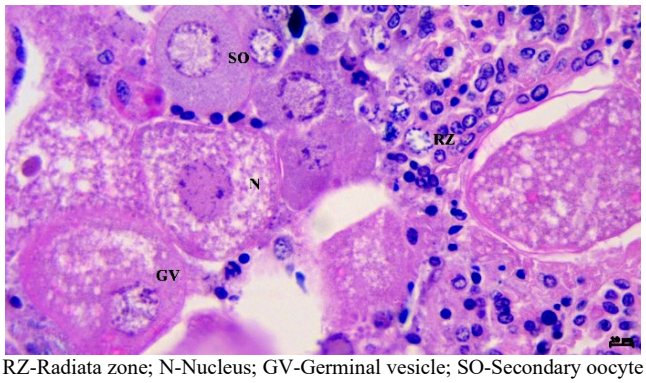
The histological findings in crabs treated with Monocrotophos in three concentrations, the ovary is distinguished by a progressive reduction in the quality (i.e., the yolk becomes more watery) and quantity of yolk in vitellogenic-sized follicles (Fig. 19). Follicles in the ovaries contain very little yolk. Cortical alveoli (yolk vesicles) are frequently fragmented or dissipated in oocytes (Fig. 20). The vitelline membrane (chorion) of oocytes is often smooth and contiguous, in contrast to oocyte atresia. However, decreased yolk formation is frequently accompanied by some degree of oocyte atresia. Somatic cells are also seen surrounding primary oocytes in the staining (Figs. 21 and 22). The staining is strongest in primary oocytes at stage 2 and previtellogenic oocytes. Primary oocytes developed at stage 2 are predominantly located in the center, whereas primary oocytes developed at stage 1 and oogonia are located in the ovary's periphery. A thick zona radiata surrounds vitellogenic primary oocytes, which are surrounded by an even layer of follicular cells. The thick zona radiata is absent in previtellogenic primary oocytes. An atretic oocyte is discovered (Figs. 23 and 24). A granulomatous inflammatory reaction caused by peritoneal cell invasion is identified.

The zona radiata appears thinner, with some invaginations, indicating the onset of the atresia process. Atresia is also indicated by an irregular follicular layer. The graph depicts the total duration of sand crab mortality (Fig. 25).



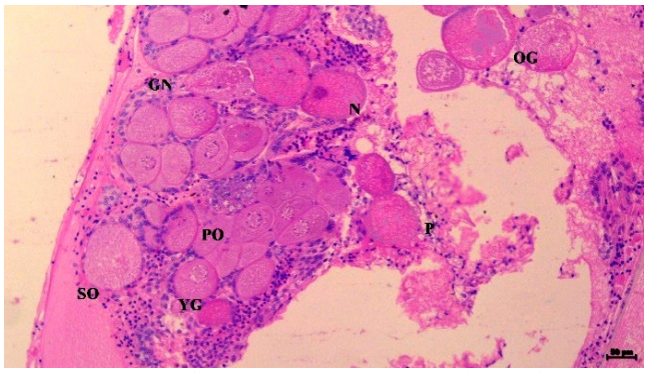
OG-Oogonia; SO-Secondary oocyte; N-Nucleus; CA-Cortical alveoli; RZ-Radiata zone

Fig. 19 Photomicrograph showing the Ovaries of control sand crab at 40X magnification

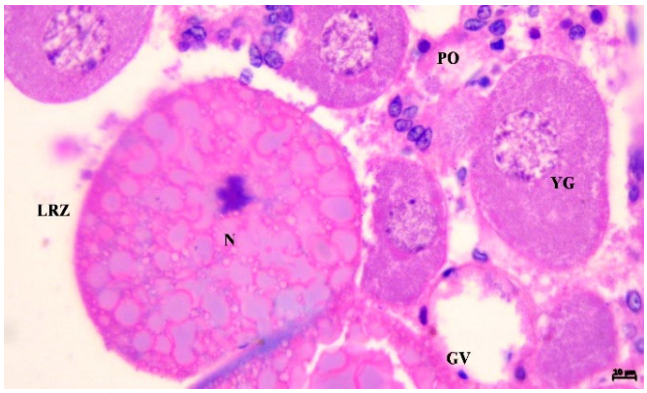


RZ-Radiata zone; N-Nucleus; GV-Germinal vesicle; SO-Secondary oocyte

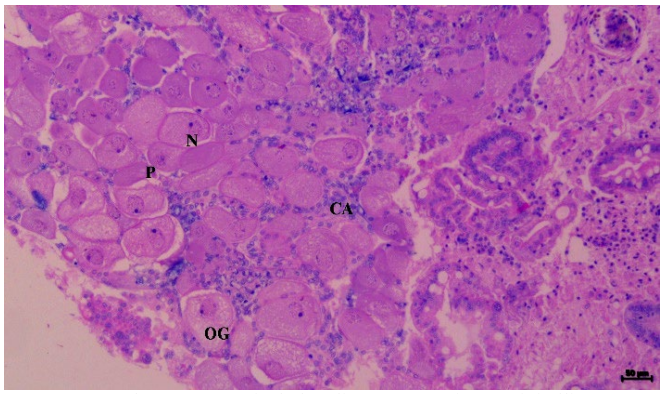
Fig. 20 Photomicrograph showing the ovary of sand crab treated with 10 µl Chlorpyrifos at 40X magnification



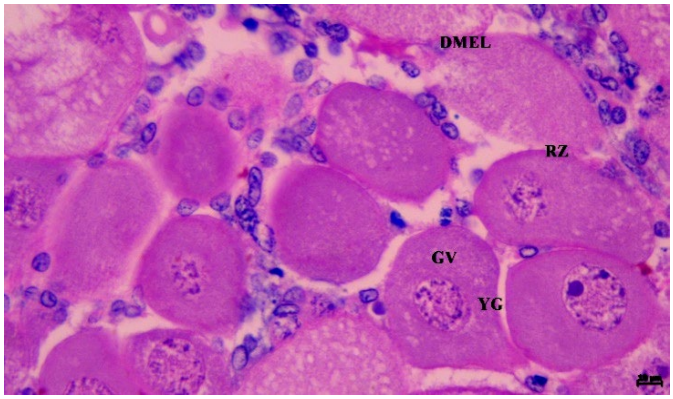
OG-Oogonia; N-Nucleus; P-Previtelin; PO-Primary oocyte; YG-Yolk granules; SO- Secondary oocyte
Fig. 21 Photomicrograph showing the ovaries of sand crab treated with 20 µl Chlorpyrifos at 10X magnification



PO-Primary oocyte; YG-Yolk granules; GV-Germinal vesicle; LRZ-Loosening of radiata zone; N-Nucleus
Fig. 22 Photomicrograph showing the ovaries of sand crab treated with 20 µl Chlorpyrifos at 40X magnification



N- Nucleus; CA-Cortical alveoli; OG-Oogonia; P-Privitelline
Fig. 23 Photomicrograph showing the ovaries of sand crab treated with 30 µl Chlorpyrifos at 10X magnification



DMEL-Damaged myoepithelial layer; RZ-Radiata zone; GV-Germinal vesicle; YG-Yolk granules
Fig. 24 Photomicrograph showing the ovaries of sand crab treated with 30 µl Chlorpyrifos at 40X magnification

IV. DISCUSSION

Gill histopathological changes can cause hypoxia, respiratory failure, and ionic and acid-base balance problems [17]. Gill surfaces changed and mucus production are increased, consistent with observed histological effects in the exposed crab, such as hyperplasia, necrosis, and lamellar aneurysms in response to sublethal concentrations of Chlorpyrifos. Changes in the architecture of the gill under Chlorpyrifos, pesticide, stress would alter the dispersing ability of the gill, resulting in hypoxic circumstances and making respiration a difficult task for E. asiatica in marine environment. Our findings recommend that the acute toxicity of Chlorpyrifos is caused by spoil togas exchange mechanisms caused by the observed gill pathologies. In the current study necrosis, edema, epithelial lifting fusion of closest secondary lamellae, and hemorrhage at primary lamellae were noticed in the crab's gills after 4 hours of higher concentration exposure. Irritating substances cause necrosis on the epithelial cells and also ruptured the gill epithelium. The animal's defense response is excessive mucus secretion as a result of stress caused by environmental change and pathologic agents that induce mucus cell abundance [18]. Lifting the epithelium, club-shaped lamellae and lamellar fusion may be protective by reducing the amount of expose gill surface area [19].

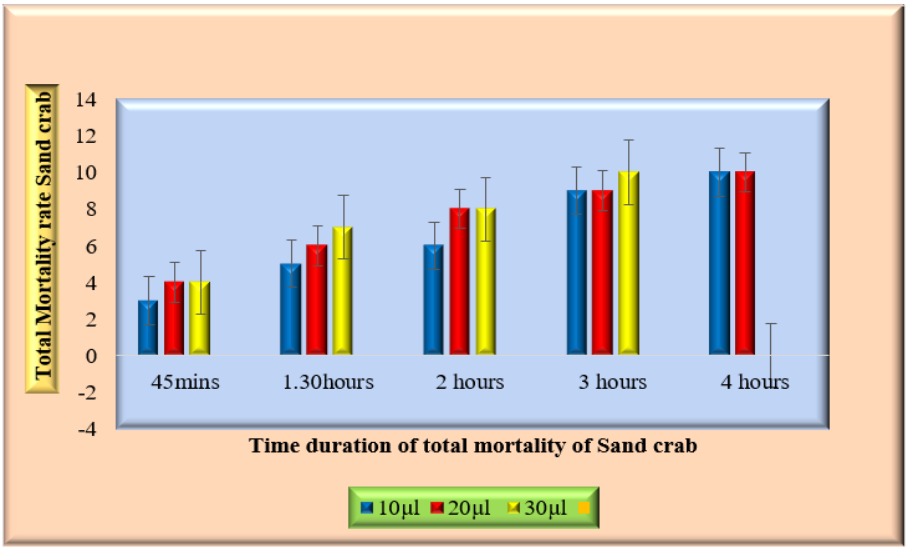


Fig. 25Graph showing the toxicity effect ofChlorpyrifos on*Emerita asiatica*

The hepatopancreas is not only doing the digestion process such as absorption, digestion, storage, and secretion abilities, but it is also a main position of biotransformation and detoxification in crustaceans. In the case of low concentration of Chlorpyrifos the hepatopancreas showed changes in B and F cells and were found clustered and intercellular vacuums indistinguishable in medium concentrations, and a general degeneration, loss of tubule structures, vacuolation, star shape of lumen, and necrosis of cells in high concentrations of Chlorpyrifos exposed *E. asiatica*. The star shape of the lumen was lost due to morphological changes in the tubular epithelial cells, as some cells shrank from a normal columnar height to a low cuboidal form. One of the most noticeable changes in the current study is the proliferation of B-cells in the dosed crabs, indicating a high rate of excretion from the hepatopancreas. A large number of F-cells converting to B-cells may affect the accumulation and elimination of the xenobiotic entering the Hepatopancreatic tubules. The hepatopancreas is involved in a variety of metabolic processes in crustaceans [20, 21]. Chlorpyrifos caused structural changes such as decreased tubular epithelial cellular height, decreased secretary and lipid vacuoles, hemocyte infiltration, atrophy, pyknotic nuclei, cytolysis, and melanized encapsulation of necrotic tissues.

[22] Found elongated and shrunken cells in of hepatopancreas in *Macrobrachiumlamerrei* was exposed to small amount of (0.0065 ppm) and high (0.0215 ppm) copper concentrations. In *Penaeus indicus* exposed to Zn at a low concentration of 100 ppb, destructive and deteriorative changes in the hepatopancreas and gills were observed [23]. Due to the accumulation of Chlorpyrifos in the hepatopancreas the histopathological changes were observed. As this organ is the center of metabolism, detoxification and storage. The rupture of basal laminae in the Hepatopancreatic channels indicates that tissue integrity was compromised in crabs due to Chlorpyrifos exposure.

Because hemocytes are the most important form of cellular defense in crustaceans, abnormal infiltration of hemocytes in the interstitial sinuses in the hepatopancreas of test animals suggests that the mechanism of cellular/host defense was in operation to neutralize the tissue damage caused by Monocrotophos [24, 25, 26]. Necrotic hepatopancreatic tubules showed in test crabs due to the formation of cell distortion disintegration and death occurred in *E. asiatica. E. asiatica* was given the highest sub-lethal dose of Chlorpyrifos. As a result, Chlorpyrifos toxicity disrupts the normal integrity of the hepatopancreas of *E. asiatica.*

In the current study, numerous histopathological modifications were also noted in the muscles of *E. asiatica* when exposed to sub lethal concentration of Chlorpyrifos. The pathological findings include degeneration of muscles, necroses of muscle fibers with hemorrhages and RBC like pigmented cells. The structural changes noticed in the muscle tissue as wavy appearance, necrosis, atrophy and granular cells in between the muscle fibers, fragmentation, loss of muscle structure, appearance of basophilic deposits of the muscle fibers was caused as a result of exposure of crabs to the sub lethal concentrations [27, 28].

During pesticide exposure, pollutants affected the muscle epidermis abruptly. Pigmented cells are an important feature of chronic inflammatory response. The current study supported a similar report by [29] in the muscle tissues of *Artemia urmiana* in response to carbamates pesticide, which resulted in degeneration, Zenkers necrosis of muscle fibre with hemorrhages and RBC like cells. The exposure of *Labeorohita* to hexachlorocyclohexane caused muscle bundle separation and intracellular edoema in the muscle tissues [30]. These findings were also made in the muscle tissues of *Channa punctatus* after exposure to Chlorpyrifos [31]. [32] Histopathological changes in the direct exposure of polluted river water on muscle tissues of Heteropneustes fossils were documented.

As a result, we can conclude that the use of chlorpyrifos in coastal ecosystem agricultural fields should be limited. In conclusion, the present investigation is the first, latest and the only report on the histological toxicity effect of Chlorpyrifos on histological alterations in the anomuran crab, *Emerita asiatica.* Though *Emerita asiatica* is not a commercially viable crab, but it plays a significant function in the environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to consume these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction.

V. CONCLUSION

The current study on Sand crab, *Emerita asiatica* is the recent study that is characterizing the histological toxicity effect of Chlorpyrifos in the different organs like gills, hepatopancreas and ovary. Though *Emerita asiatica* is not a commercially viable crab, but it plays a vital role in the environment to maintain a stable marine ecosystem. Several steps and precautions measures should be taken to conserve these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction. Thus, it can be concluded that the use of Chlorpyrifos which has been legally banned in India is justified. It has been proved by several workers and has been conformed in present investigation that use of this Chlorpyrifos causes serious damage to the vital organ of sand crabs gill, hepatopancreas and ovary. The application of Chlorpyrifos could be reduced in the agricultural field near to the coastal region.

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