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Sublethal Effect of Copper Toxicity Against Histopathological Changes in the Spiny Lobster, *Panulirus homarus* (Linnaeus, 1758)

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Abstract The tissue damage induced by various organic pollutants in aquatic animals is well documented, but there is a dearth of information relating to the histological alterations induced by copper in the spiny lobster. In the present study, intermoult juveniles of the spiny lobster *Panulirus homarus* (average weight 150–200 g) were exposed to two sublethal concentrations of the copper (9.55 and 19.1 μg/l) for a period of 28 days. The muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart of the lobsters were then dissected out and processed for light microscopic studies. Exposure to copper was found to result in several alterations in the histoarchitecture of the muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart of *P. homarus*. The alterations included disruption and congestion of muscle bundle in muscle tissue; blackened haemocytes; distended lumen and F cell; necrosis of the tubules of the hepatopancreas; disarrangement of circular muscle of the midgut; accumulation of haemocytes in the haemocoelic space; swelling and fusion of lamellae; abnormal gill tips; hyperplastic, necrotic, and blackened secondary gill lamellae of the gills; damaged neurosecretory cell and sensory and motor fibre; necrotic of the thoracic ganglion; dispersedly arranged muscle bands; clumped satellite cells and nucleus of the heart. The results obtained suggest that the muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart of lobsters exposed to copper were structurally altered. Such alterations could affect vital physiological functions, such as absorption, storage and secretion of the hepatopancreas, digestion of gut and respiration, osmotic and ionic regulations of the gills, which in turn could ultimately affect the survival and growth of *P. homarus*. Thus, all possible remedial measures should be adopted to prevent the occurrence of copper contamination in the aquatic environment.

Keywords *Panulirus homarus* · Copper · Histology · Muscle · Hepatopancreas · Midgut · Gills · Thoracic ganglion · Heart

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Introduction

Cellular assay techniques are employed to study pollutant-induced injuries on the internal organ system of organisms. Toxic responses begin once metal accumulation at these sites reaches a threshold concentration that results in disruption of their physiological function, thus inducing acute toxicity. Such injuries serve as reliable biological indicators of pollution and are effectively used in assessing stress effects. Waterborne metal, copper (Cu), exerts initial toxic effects at physiologically active sites on the gill surface, interfering directly with the branchial ion transporting function. Although Cu is essential for metabolic processes, it can be extremely toxic to fish. Copper is

known to affect Na+ homeostasis Amazonian teleost tambaqui (*Colossoma macropomum*) in soft water [1].

In crustaceans, muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart display considerable cytological, cytochemical and structural alterations at chronic exposure to low levels of copper. The hepatopancreas followed by gills has been identified as the target organs of interest in toxicity investigations. Histological studies aid in locating the specific cell types in different organs which constitute the targets.

Histological studies on aquatic organisms revealed that the various pollutants produced pathological lesion changes such as necrobiotic changes caused by tubular damage in the kidney and gill lamellae and toxicoterrataxological abnormalities [2–4]. In the shore crab, *Carcinus maenas*, the acute and sublethal toxicity of waterborne copper appears directed mainly towards the gill epithelium, leading to important cytological damage of gills [5, 6]. The gill epithelium is not only the first target of the toxic action of metals but also the main site of uptake of exogenous copper in shore crab, *C. maenas* [7].

Several studies have shown that animals exposed to both acute and sublethal levels of waterborne copper can recover in the long term from harmful metal injury, even in the ambient water. In the shore crab, *C. maenas*, the recovery processes involved the cytological damage of the gill epithelium [5]. A variety of acclamatory effects have also been documented in fish chronically exposed to sublethal levels of copper [8–10]. Copper toxicity of spiny lobster, *Panulirus homarus*, in chromosomal aberrations, conductivity and bioaccumulation also has been reported [11–13]. Serious damage at cellular level in the hepatopancreas of the green mussel, *Perna viridis*, exposed to copper and mercury was reported by Pillai and Menon [14]. In the present study, the histological alterations of muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart of the spiny lobster, *P. homarus*, exposed to different concentration of copper was carried out.

Materials and Methods

Collection of *P. homarus*

The spiny lobster, *P. homarus*, weighing 150–200 g collected from bottom set gill net at a depth of 5–8 m was reared in well-aerated filtered seawater in 200-l fibreglass tanks at the Field Laboratory of the Central Marine Fisheries Research Institute at Kovalam, near Chennai, India. The seawater was treated and filtered in the laboratory both chemically and biologically to nullify the detectable level of copper and microbes. Significant sign of stress or unusual behavioural criteria were not observed in the control lobsters throughout the acclimation and test period. The lobsters were fed live marine clam *Donax cuneatus* daily in the evening, and the uneaten feed was removed next day morning followed by 100% water exchange.

Experimental Animal

All experimental animals used were in intermoult stage (stage C) to avoid change in the biochemical composition associated with the moult cycle that was determined previously [15].

Preparation of Stock Solution for Copper Toxicity Test

One gram of copper sulphate pentahydrate ($CuSO_4$ 5 H_2O) (Merck, Germany) was dissolved in 1 l of double-distilled water and used as the stock solution for preparing different concentrations of copper in rearing water. It was stored in a clean standard flask at room temperature in the laboratory

Sublethal Toxicity Tests

For sublethal toxicity tests, the lobsters were grouped into three batches. Each batch had ten lobsters and three replicates were maintained. Lobsters maintained in normal seawater served as control (group I). Lobsters were exposed to concentration of 9.55 μg/l (one tenth of 96 h LC_{50}) of copper in seawater (group II). Lobsters were exposed to the sublethal concentration of 19.1 μg/l (one fifth of 96 h LC_{50}) of copper in seawater (group III). The media were renewed every alternate day. Lobsters were fed daily with live clam *D. cuneatus*. Two specimens each from the groups I, II and III were sacrificed after 0, 7 and 28 days of the experiment.

Histopathology

Lobsters were exposed to copper at 9.55 and 19.1 μg/l for 28 days. Sampling was done on the 7th and 28th day of exposure; five lobsters in each group were sacrificed. The muscle, hepatopancreas, midgut, gills, thoracic ganglion and heart of representative lobsters from each test and control group were dissected out and fixed in Davidson's fixative for 24 h. The preserved tissues were processed by a routine histological method [16], dehydrated in alcohol series and embedded in paraffin wax. They were cut into sections of 6 mm thickness by a rotary microtome (Weswox, MT1090:1090A, India). The thin sections of the tissues were stained by haematoxylin and eosin for observation by the Nikon Bright field transmission microscope with Koechler illumination, and automatic exposure unit was used.

Results

Muscle

Muscle tissue of the control *P. homarus* revealed the fascicular arrangement of myofilaments with emarginated epimysium, binding to connective tissue and tendon at the extremities of the smooth muscles. The striated muscle fibres were tightly packed. The nuclei were arranged along the margins of the muscle bundles (Fig. 1a, b). After 7 days of exposure to 9.55 μg/l concentration of copper, the muscle bundles were completely disrupted with discontinuity of striations and complete disappearance of nuclei (Fig. 1c). Similarly after 28 days of exposure, large vacuolization followed by congestion of muscle bundles and formation of gap was evident (Fig. 1d). A short-term exposure of 19.1 μg/l concentration of copper (7 days) resulted in rupture of muscle bundles, gap formation and complete disappearance of nuclei (Fig. 1e). However, nucleus congregated in the vacuole formed region, and the banding patterns were completely disturbed as a consequence of long-term exposure of 28 days (Fig. 1f).

Hepatopancreas

Transverse section of the hepatopancreas of lobster showed that the digestive gland has four types of cells: embryonic cell (E cells), resorptive cells (R cells), fibrillar cells (F cells) and blister-like cells (B cells). The F and R cells were with prominent nucleus. The F cells were fibrous with nucleus. Towards the periphery were the R cells, found as non-staining vacuoles. The star-shaped lumen contained granular materials and was bordered with B cells. The pancreatic cells were surrounded primarily with haemal sinus and within the sinus were haemocytes (Fig. 2a). After 7 days of exposure to 9.55 μg/l concentration of copper, the transverse section of hepatopancreas revealed more number of haemocytes in the sinus and distended structure of lumen. Consequently, tubular lumen was found to be disturbed and disarranged (Fig. 2b). However, after 28 days of exposure, the B cell count reduced with appearance of large vacuoles. Towards the periphery, the spongy connective sheath or the 'tunica propria' and myoepithelial layer were also found to be damaged (Fig. 2c, d). The hepatopancreas of *P. homarus* after 7 days of exposure to

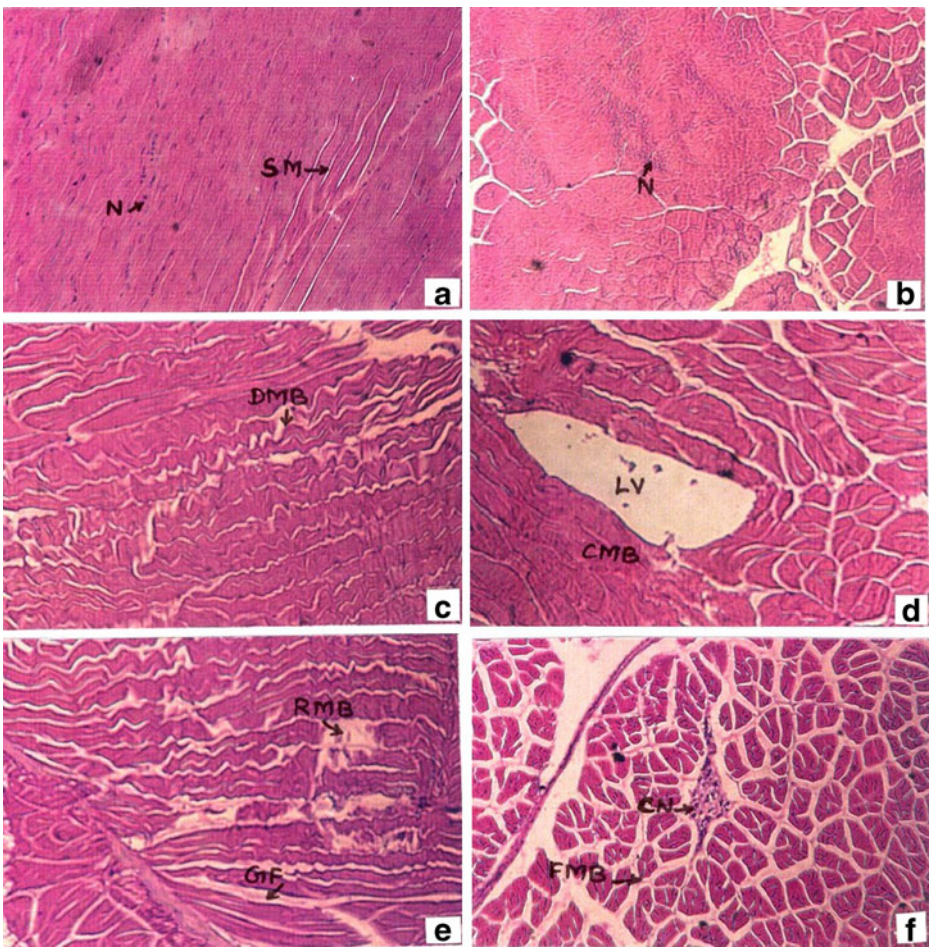

Fig. 1 Histopathological changes of muscle in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a**, **b** Control, **c** after 7 days of exposure to 9.55 μg/l concentration of copper, **d** after 28 days of exposure to 9.55 μg/l concentration of copper, **e** after 7 days of exposure to 19.1 μg/l concentration of copper, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *CMB* congestion of muscle bundle, *CN* congestion of nucleus, *DMB* disruption of muscle bundle, *FMB* fusion of muscle bundle, *GF* gap formation, *LV* large vacuole, *N* nuclei, *RMB* rupture of muscle bundle, *SM* striated muscle

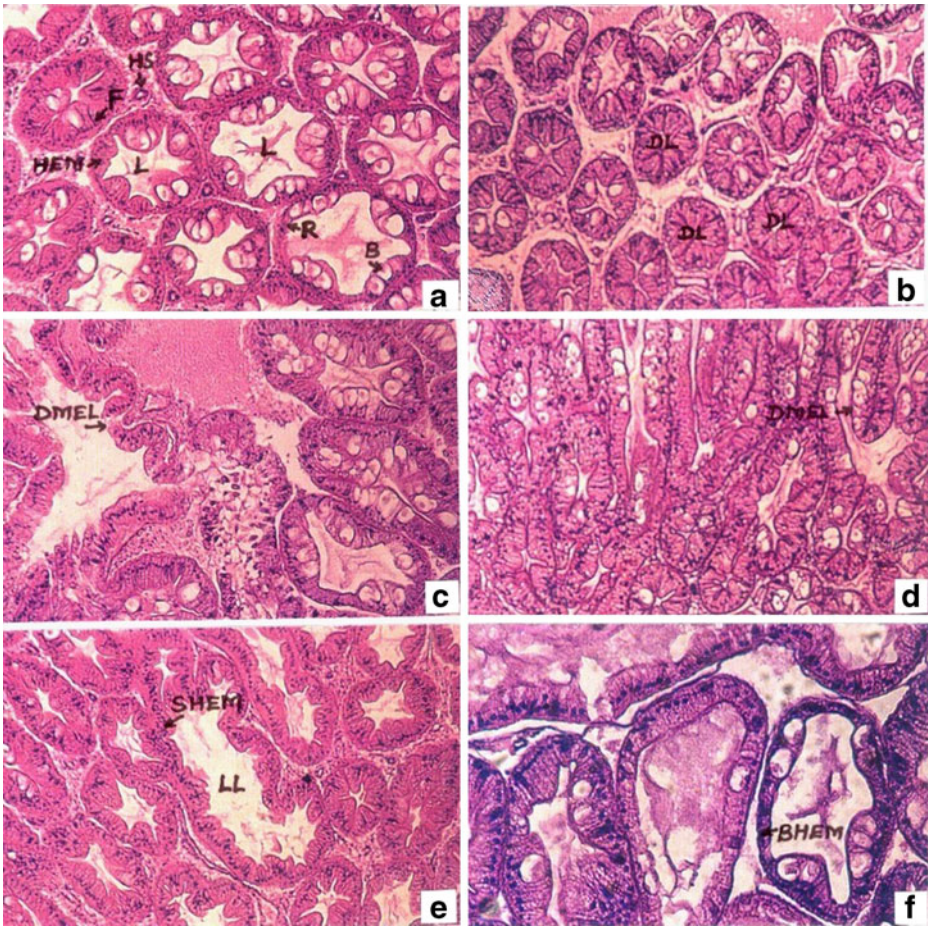

Fig. 2 Histopathological changes of hepatopancreas in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a** Control, **b** after 7 days of exposure to 9.55 μg/l concentration of copper, **c**, **d** after 28 days of exposure to 9.55 μg/l concentration of copper, **e** after 7 days of exposure to 19.1 μg/l concentration of copper, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *B* blister-like cell, *BHEM* blackened haemocytes, *DF* distended F cell, *Dl* distended lumen, *DMEL* damaged myoepithelial layer, *F* fibrillar cell, *HEM* haemocytes, *HS* haemal sinus, *L* lumen, *LL* large lumen, *R* resorptive cell, *SHEM* scatted haemocytes

19.1 μg/l concentration of copper revealed large lumen and reduced the number of B cells followed by damaged myoepithelial layer. Similarly, the haemocytes were found scattered (Fig. 2e). After 28 days of exposure, the microvillus brush border cells were found to be detached and were irregular in shape. Blackened haemocytes, damaged myoepithelial layer and distended F cell were also found (Fig. 2f).

Midgut

The transverse section of midgut revealed the epithelial cells consisting of brush border of microvilli which are joined apically by separate junctions. The lumen appeared as multichambered due to the extensive folding. Small bundle of longitudinal and circular muscles with large amount of basophilic granulocyte were observed in the control (Fig. 3a). After 7 days of exposure to 9.55 μg/l concentration of copper, cuticle detached from the epithelial layer and a gap formed in between the epithelial layer and the cuticle. Consequently, the basophilic granulocytes were absent (Fig. 3b). Prolonged exposure (28 days) revealed broken and disarranged circular muscles, vacuole formation and damage of cuticle (Fig. 3c, d). After 7 days of exposure to 19.1 μg/l concentration of copper, the cuticle detached from the epithelial layer. Absence of basophilic granulocytes, tegumental gland, basement membrane disarrangement of circular muscle, damage of epithelial layer and forming of translucent space between muscles were observed (Fig. 3e). Consequently, after 28 days of exposure, disappearance of cuticle, formation of large number of vacuoles and detachment of nucleus from the epithelial infiltration of haemocytes were also observed. Similarly, disappearance of circular muscle and damage of epithelial layer were also apparent due to the effect of copper toxicity (Fig. 3f).

Gills

The transverse section of gill tissue of normal lobster shows branching form the central axis called the primary gill lamellae. Each of the primary lamella further divides into secondary gill lamellae or filaments. Within each division of the gills are the adjacent efferent vessels and afferent vessels with haemocytes. The primary and secondary gill filaments are separated by a thin septum. The secondary non-branching filament lamella possesses epithelial pillar

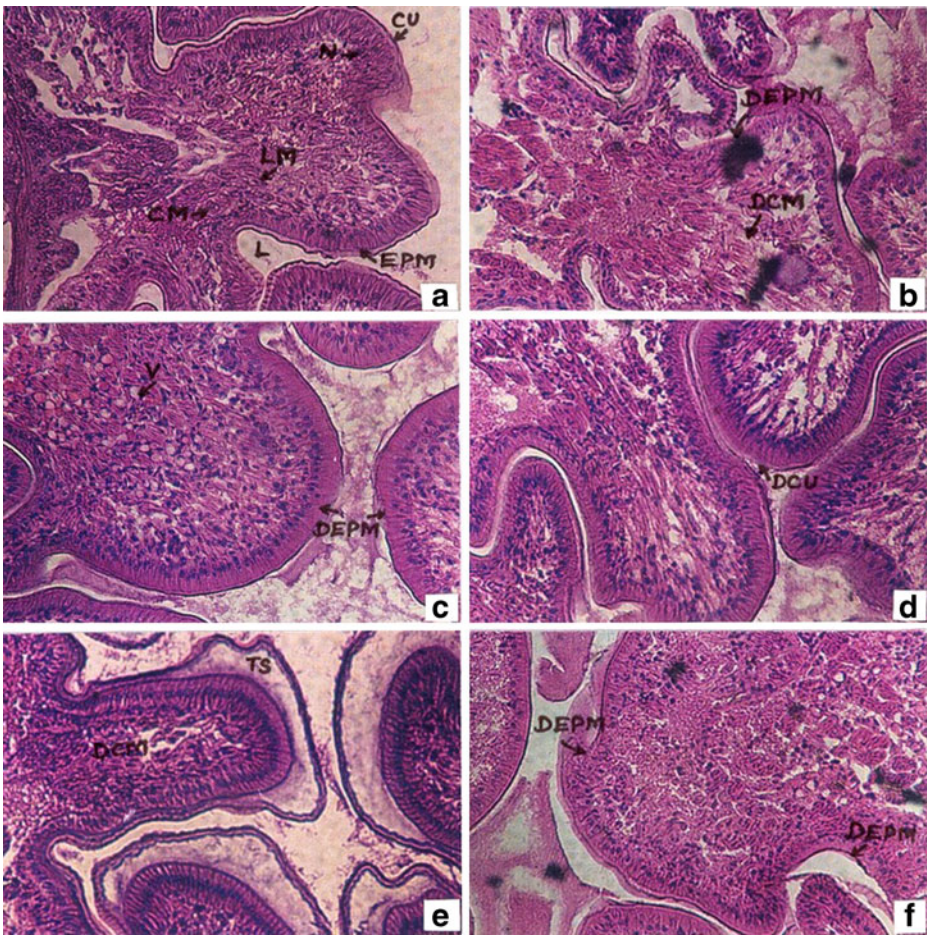

Fig. 3 Histopathological changes of midgut in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a** Control, **b** after 7 days of exposure to 9.55 μg/l concentration of copper, **c**, **d** after 28 days of exposure to 9.55 μg/l concentration of copper, **e** after 7 days of exposure to 19.1 μg/l concentration of copper, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *CU* cuticle, *CM* circular muscle, *DCM* disarrangement of circular muscle, *DCU* damaged cuticle, *DEPM* damaged epithelium, *EPM* epithelium, *L* lumen, *LM* longitudinal muscle, *N* nuclei, *TS* translucent space, *V* vacuole

cells separated by large lacunae (Fig. 4a). After 7 days of exposure to 9.55 μg/l concentration of copper, the gill tissues revealed large inter-lamellar space, necrosis, lamellar fusions, blackened and enlarged secondary gill lamellae and also the presence of large number of haemocytes resulting in distension of the lamellae (Fig. 4b). Similarly, after 28 days of exposure, large number of haemocytes accumulated in secondary gill lamellae and also enlargement and disarrangement of secondary gill lamellae were apparent. Followed by disappearance of epithelial layer from the primary gill lamellae, the lamellar fusion and tissue hyperplasia were predominant in secondary gill lamellae (Fig. 4c). After 7 days of exposure to 19.1 μg/l concentration of copper, a large number of haemocytes accumulated in secondary gill lamellae and resulted in enlargement of gill lamellae. Consequently, the separation of epithelial cells from the basement membrane and fusion of secondary gill lamellae were also observed (Fig. 4d). Similarly, after 28 days of exposure, blackening and enlargement of secondary gill lamellae, necrosis of primary gill lamellae and formation of large vacuoles followed by increased number of granulocytes were noticed. Furthermore, the epithelial layer detached completely from the central portion of each lamella. The secondary gill lamellae were heavily congested with large number of necrotic and blackened haemocytes (Fig. 4e, f).

Thoracic Ganglion

The thoracic ganglion revealed a network of giant nerve fibres associated with globuli cells and giant cells in the normal lobster. The neurosecretory cells were identified with large nuclei (Fig. 5a, b). After 7 days of exposure to 9.55 μg/l concentration of copper, blackening of giant cells and muscle bands followed by more number of globuli cells with large vacuoles were noticed (Fig. 5c). Similarly, after 28 days of exposure, muscle bands were damaged with enlargement of giant cells. Blackening of giant cells, necrosis and gap formation between the striated muscles were noticed (Fig. 5d). A short-term exposure (7 days) to 19.1 μg/1 concentration of copper revealed damaged neurosecretory cells followed by vascularization, enlargement of giant cells and absence of nucleus in giant cells. Condensed morphology of globuli cells also was observed (Fig. 5e). After 28 days of exposure, enlarged giant cells with blackening were

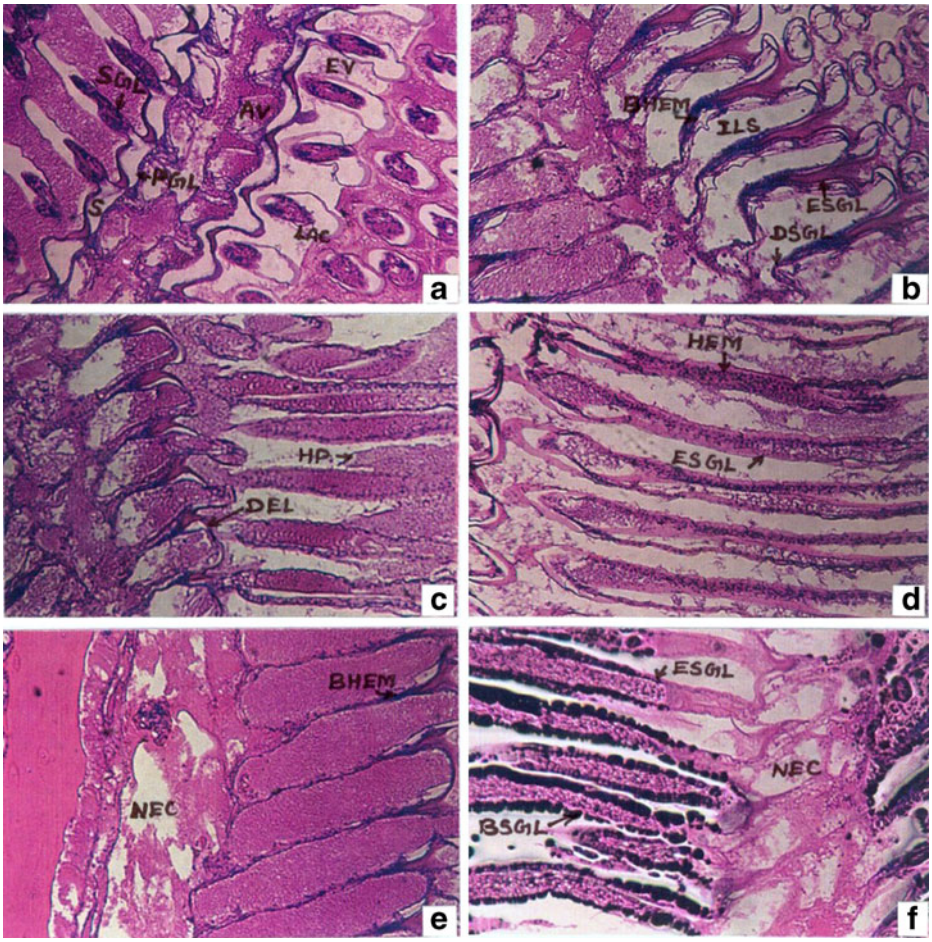

Fig. 4 Histopathological changes of gills in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a** Control, **b** after 7 days of exposure to 9.55 μg/l concentration of copper, **c** after 28 days of exposure to 9.55 μg/l concentration of copper, **d** after 7 days of exposure to 19.1 μg/l concentration of copper, **e**, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *AV* afferent vessel, *BHEM* blackened haemocytes, *BSGL* blackened secondary gill lamellae, *DEL* disappearance of epithelial lamellae, *ESGL* enlargement of secondary gill lamellae, *EV* efferent vessel, *HEM* haemocytes, *HP* hyperplasia, *ILS* inter-lamellar space, *LAC* lacunae, *PC* Piller cell, *PGL* primary gill lamellae, *S* septum, *SGL* secondary gill lamellae, *NEC* necrosis

evident. Consequently, vascularisation, condensed morphology of globuli cells with damaged sensory and motor fibres were also observed (Fig. 5f).

Heart

The transverse section of normal heart of lobster revealed the epicardium with the majority of muscle bands associated with myocardial cells and with prominent nuclei associated with satellite cells. The epicardium was composed of cells with large distinct cytoplasm (Fig. 6a, b). After 7 days of exposure to 9.55 μg/l concentration of copper, the nuclei disintegrated and the muscle bands were dispersedly arranged (Fig. 6c). After 28 days of exposure, the muscle bands were clumped with irregular morphology which was more prominent at longer duration exposure (Fig. 6d). After 7 days of exposure to 19.1 μg/l concentration of copper, the epicardium region was damaged, satellite cells clumped followed by dislocation of muscle bands and necrosis (Fig. 6e). After 28 days of exposure, irregular morphology of muscle bands was found. Nucleus and satellite cells were clumped together in the epicardial region and many muscle bands loosened and damaged (Fig. 6f).

Discussion

The use of histopathological technique to investigate the effect of pollution is increasing, and it provides a useful tool to analyse the cellular response to various toxicants and to relate the amount of cell damage in organisms to the concentration of a pollutant or the synergistic effect of more than one toxicant, or both [17, 18]. Heavy metals at sublethal levels are known to affect the structure and functioning of cellular components, leading to impairment of vital functions of many marine organisms. It is in this context that histopathological and ultra-structural alterations are employed as effective indices of physiological and biochemical changes caused by copper induced stress. These biological indices provide insight into cellular injuries before any irreversible alterations occurs. The chronic effects of excess copper have been studied within the framework of Wilson's disease by Peisach et al. [19].

The gills serve as a route of elimination of copper. Copper may be collected from the general body circulation by haemocytes, transported to the gills and accumulated within the gill lamellae and gill processes and finally eliminated by sloughing off affected portion of the gills as

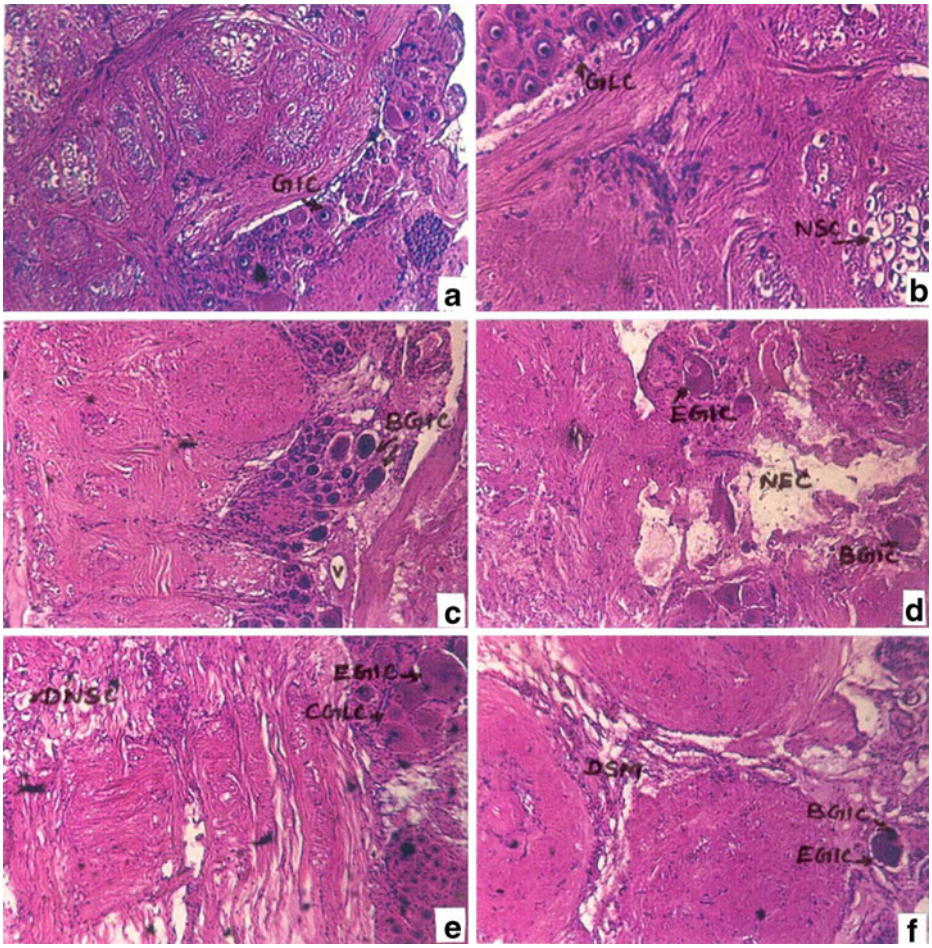

Fig. 5 Histopathological changes of thoracic ganglion in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a**, **b** Control, **c** after 7 days of exposure to 9.55 μg/l concentration of copper, **d** after 28 days of exposure to 9.55 μg/l concentration of copper, **e** after 7 days of exposure to 19.1 μg/l concentration of copper, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *BGIC* blackened of giant cell, *CGLC* condensed globuli cell, *DNSC* damaged neurosecretory cell, *DSM* damaged sensory and motor fibre, *EGIC* enlargement of giant cell, *GIC* giant cell, *GLC* globuli cell, *NEC* necrosis, *NSC* neurosecretory cell, *V* vacuole

reported in shrimps, *Penaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris* when exposed to cadmium [20]. The present study is further evidence for this hypothesis. Gill lesions were the only lesion consistently present in lobster exposed to copper and large number of haemocytes accumulated in the gill lamellae and were eliminated by sloughing off the affected portions of the gill processes. High levels of copper may be absorbed by the gill epithelium and act primarily on the cell enzymes resulting in the formation of lysosomes, vacuoles and vesicles. The apical homogenous layer of the epithelial cells may be a protected device, and the reduction of these cells observed by sectioning may enable copper to enter these cells via this route instead of, or as well as, via the haemolymph. The particulate matter normally present on the epithelial cell surface (control) occurs more extensively in animal that have been exposed to high level of copper 19.1 μg/l. Similar results have been highlighted by Sparague [21] in Atlantic salmon exposed to lethal concentration of copper and zinc. Delamination of the gill epithelium followed by the degeneration of cuticle and eventually the destruction of the gill tissues may have led to altered osmoregulatory ability of the lobster. The degeneration of gill cuticle and consequent loss of 'dumb bell' appearance was observed by Cough [22] in marine shrimp, *Penaeus aztecus*, exposed to cadmium. This complete loss of structural integrity may have contributed greatly to the dysfunction of the organisms. Similar discretion was made in the shrimps, *P. duorarum*, *P. pugio* and *P. vulgaris*, exposed to cadmium [20].

The effects of heavy metals on the gills of fishes have been widely investigated. Philpott and Copeland [23] described the results on the winter, flounder *Pseudopleuronectes americanus* exposed to high doses of copper. A concentration of 0.18 mg/l of copper caused vacuolation of the epithelial layer and a concentration of 3.2 mg/l, detachment of the epithelial layer from the central portion of the lamellae in *P. americanus* [2]. The results of this study on *P. homarus* is in agreement with the above results in that pathology of gill increases with increase in copper concentration from 9.55 to 19.1 μg/l. In the shrimp *Penaeus japonicus*, the nuclear matrices were condensed with vacuoles around the nucleus, and the cell became extensively vacuolated on exposure to copper [24].

The *hepatopancreas* is a vital organ in crustaceans, with secretory, absorptive, digestive and excretory functions. This organ has been identified as a target organ for heavy

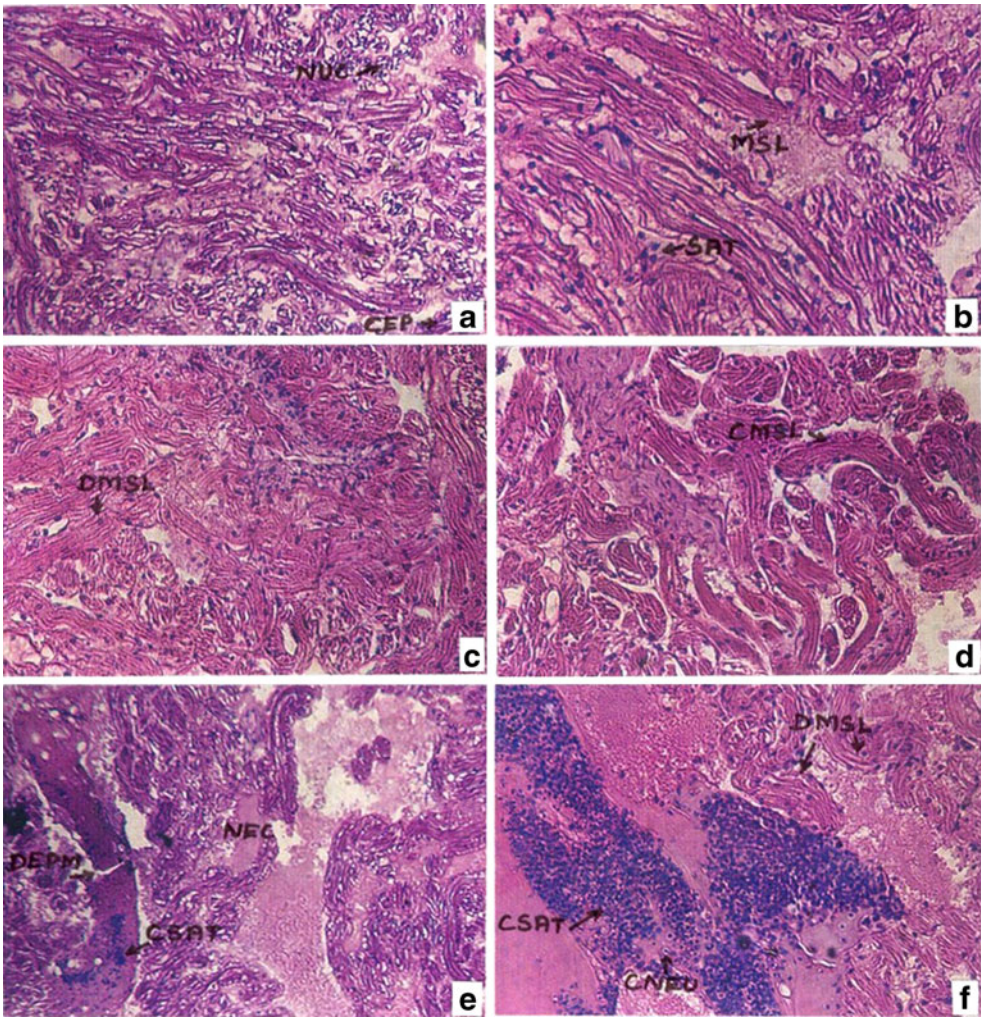

Fig. 6 Histopathological changes of heart in *P. homarus* photomicrographs of the paraffin section stained with haematoxylin and eosin (×40). **a**, **b** Control, **c** after 7 days of exposure to 9.55 μg/l concentration of copper, **d** after 28 days of exposure to 9.55 μg/l concentration of copper, **e** after 7 days of exposure to 19.1 μg/l concentration of copper, **f** after 28 days of exposure to 19.1 μg/l concentration of copper. *CEP* cuticular epidermis, *CMSL* clumped muscle bands, *CNEU* clumped nucleus, *CSAT* clumped satellite cells, *DEPM* damaged epicardium region, *DMSL* dispersedly arranged muscle bands, *MSL* muscle bands, *NEC* necrosis, *NUC* nuclei of myocardial cell, *SAT* satellite cell

metal pollution because it shows critical histopathological and ultra-structural alterations at very early stages of exposure to toxicants. The present study on copper exposed *P. homarus* elucidates the major structural alterations brought about in the vital organelles of hepatopancreas cells. Copper is primarily accumulated and deposited in the hepatopancreas which is a major storage organ in decapods [25]. In *P. homarus*, copper acquired from the water was directed into the hepatopancreas for detoxification within copper granules.

In the present study, the structural details of various cellular components of the hepatopancreas in control lobster confirmed with the descriptions given in the literature. The hepatopancreas of *P. homarus* exposed to the 9.55 and 19.1 μg/l concentration of copper had damaged myoepithelial layer, and distended lumen was also apparent. Similarly, Manisseri and Menon [26] observed distorted nuclei with 'scalloped' edges, damage of the nuclear membrane, vacuolization and overall shrinkage resulting in the nuclei losing their characteristic shape in penaeid shrimp, *Metapenaeus dobsoni*. These changes would profoundly influence the normal functioning of the nuclei.

Metal can interact with nuclear proteins, altering the complex structure of chromatin or the catalytic activity of the enzymes involved in DNA and RNA metabolism [27]. Metal cations can also induce depolymerisation and favour hydrolysis of RNA, affecting the correct replication of transcription of DNA, and alter the fidelity of the translation of RNAs during the process of protein synthesis at the ribosomal level [28]. Extensive disruption and disintegration of the F and R cells observed in lobsters exposed to copper may indicate serious deleterious alterations associated with heavy metals. The extent of damage seemed to be directly proportional to the concentration of toxicant in the medium. According to Al Mohanna and Nott [29], the R cells in the hepatopancreas of *Penaeus semisulcatus* can take up particulate material from the haemolymph by pinocytosis at the basal cell membrane. It is also reported that copper is accumulated in large dense vacuoles. The present findings support this hypothesis. Icely and Nott [30] carried out studies on the accumulation of copper within the hepatopancreatic caeca of the amphipod *Corophium volutator*. White and Rainbow [31]

also reported the presence of copper-rich granules containing homogenous electron-dense material (with no nucleus or core) in the R and F cells of the ventral caeca of *Palaemon elegans*, inhabiting copper-polluted localities. Similar observations were made by Weeks [32] in talltrid amphipod *Orchestia gammarellus*. The presence of electron-dense granules in the epithelial cells of *P. homarus* indicates the role played by these granules in the detoxification and elimination of copper from the tissues of copper-contaminated individuals.

The results of this study provide a detailed description of the fate of hepatopancreas in *P. homarus* exposed to copper. The primary role of the hepatic arterioles is to distribute blood to the extensive network of haemal sinuses of this large organ. However, the toxicity of copper damages the arterioles. Therefore, the flow of blood through the digestive gland is important in transferring the products of digestion and stored nutrients from the digestive tubules to the other organs of the lobster. The prominent ridges on the apical and lateral surfaces of the digestive gland were damaged and boarded with lesions, thereby decreasing the surface area for the capture and uptake of foreign particles during phagocytosis [33]. The nuclear matrices were condensed and vacuoles were large and prominent with less basal in foldings and less number of B cells in mud crab *Scylla serrata* [34]. Detachment of the basal membrane was a common observation in the hepatopancreatic cells of lobster exposed to copper. The same was evident in *Penaeus monodon* postlarvae exposed to copper [35]. Likewise, loss of structural integrity was observed in the hepatopancreatic tissues of *P. homarus* exposed to 19.1 μg/l of copper. The study further demonstrates that the hepatopancreas is the central site of metabolism, and it easily and readily reacts to changes in water conditions such as presence of pollutants. Bioaccumulation of the copper from the water has occurred as in the pink shrimp, *P. duorarum*, and tiger prawn, *P. monodon*, which accumulated the metal in great amounts resulting in structural changes of the hepatopancreas and gills [36]. Even in lobster exposed to lowest concentration of copper (9.55 μg/l), the same damage in the architecture of the hepatopancreas was observed. Numerous electron-dense inclusions seen near the basal lamina of gut cells of copper exposed *P. homarus* could be the metal-rich bodies in the process of being transported from the haemolymph to the cell and to hepatopancreas for further sequestration and elimination.

In the present investigation, consistent histological changes were observed in the muscle tissue of *P. homarus* exposed to sublethal concentrations of copper. Haemocytic infiltration and accumulation of the metal increased after 28 days of 19.1 μg/l concentration of copper exposure. Lawson et al. [37] have reported that the presence of haemocytic infiltration in cell implies some degree of degeneration. Tissue necrosis was also noted in the muscle tissue of *P. homarus* that was exposed to copper, and this may be one of the reasons for the presence of large number of haemocytes. Dislocation of circular muscles with prominent deposits of copper granules, vacuole formation and disappearance of cuticle of copper-treated *P. homarus* depicted hypertrophy. These appear to be the general response of the midgut to heavy metal pollution as in Rainbow trout, *Salmo gairdneri* and *Punctius conchorius* [38, 39]. The changes observed in the gut may probably be due to the increased copper deposits as observed in *Therapon jarbua* [40].

Copper reduces the active potential of the ganglion influencing the complete detachment of the nerves and consequently the nucleus from them [27]. Similar effect of copper was reported in *Mytilus edulis* by Calabrese et al. [41]. The clumping nature of the epicardial cells depicted by an initial hypertrophy and vacuolization is general phenomena of *P. homarus* exposed to longer duration of 28 days in either concentrations (9.55 and 19.1 μg/l) of copper as observed in tunas exposed to mercury [42, 43]. The progressive necrosis, lacunae of epicardial cells and concentrated copper granules in *P. homarus* have also been noticed in *S. gairdneri* [38] and *Puntius sophore* [44] exposed to intoxication with mercury. The epicardial cells were uniformly spared in the control lobsters unlike the treated ones.

References

1. Matsuo AYO, Wood CM, Val AL (2005) Effects of copper and cadmium on ion transport and gill metal binding in the Amazonian teleost tambaqui (*Colossoma macropomum*) in extremely soft water. Aquat Toxicol 74:351–364
2. Baker JTP (1969) Histological and electron microscopical observations on copper poisoning in the winter flounder (*Pseudopleuronectes americanus*). J Fish Res Board of Canada 26:2785–2793
3. Skidmore JF (1970) Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. J Exp Biol 52:484–494
4. Ramalingam K (1980) Studies on the effects of sublethal concentration of a few toxicants on biochemistry, physiology and histology of *Tilapia mossambica* (Peters). Ph.D. thesis, University of Madras, Chennai, pp 1–118
5. Nonnotte L, Boitel F, Truchot JP (1993) Water borne copper causes gill damage and hemolymph hypoxia in the shore crab *Carcinus maenas*. Canada J Zool 71:1569–1576
6. Truchot JP, Rtal A (1998) Effects of long term sublethal exposure to copper on subsequent uptake and distribution of metals in the shore crab, *Carcinus maenas*. J Crus Biol 18(2):224–231
7. Rtal A, Truchot JP (1996) Haemolymph transport and tissue accumulation of exogenous copper in the shore crab, *Carcinus maenas*. Mar Pollu Bull 32:802–811
8. Lauren DJ, McDonald DG (1987) Acclimation to copper by rainbow trout. Physiol Canada J Fish Aquat Sci 44:99–104
9. Saucier D, Astic L (1995) Morphofunctional alternations in the olfactory system of rainbow trout (*Oncorhynchus mykiss*) and

possible acclimation in response to long-lasting exposure to low copper levels. Comp Biochem Physiol 112A:273–284
10. Grosell M, Boetius I, Hansen HJM, Rosenkilde (1996) Influence of pre exposure to sublethal levels of copper on 64 Cu uptake and distribution among tissues of the European eel (*Anguilla anguilla*). Comp Biochem Physiol 114C:229–235
11. Maharajan A, Vaseeharan B, Rajalakshmi S, Vijayakumaran M, Kumarasamy P, Chen JC (2011) Effect of copper on morphology, weight and chromosomal aberrations in the spiny lobster, *Panulirus homarus* (Linnaeus, 1758). Biol Trac Elem Rese. doi:10.1007/s12011-011-9110-1
12. Maharajan A, Rajalakshmi S, Halder S, Vijayakumaran M (2010) Sublethal effect of copper on the frequency of electrical conductivity in the muscle tissue of spiny lobster, *Panulirus homarus* (Linnaeus, 1758). J Aqua Biol 25(2):1–4
13. Maharajan A, Rajalakshmi S, Vijayakumaran M, Kumarasamy P (2010) Sub lethal effect of copper on bioaccumulation in the spiny lobster, *Panulirus homarus*, (Linnaeus, 1758). Biol Seg 1((2)BS/1528.P):1–8
14. Pillai S, Menon NR (1998) Structural alterations accompanying acute and chronic toxicity of heavy metal exposure in hepatopancreas of green mussel, *Perna viridis*, (Class-Pelecypoda). Indian J Mar Sci 27:416–420
15. Radhakrishnan EV (1989) Physiological and biochemical studies on the spiny lobster, *Panulirus homarus*. Ph.D. thesis, University of Madras, Chennai, p 168
16. Humason GL (1972) Animal tissue techniques, 3rd edn. W.H. Freeman and Company, San Francisco
17. Crandall CD, Goodnight CJ (1963) The effects of sublethal concentrations of several toxicants to the common guppy, *Lebestes reticulatus*. Trans Am Fish Soc 82:59–73
18. Brown VM, Mitrovic VV, Stark GTC (1968) Effects of chronic exposure to zinc on toxicity of a mixture of detergent and zinc. Water Res 2:255–264
19. Peisach J, Aisen P, Blumberg WE (1967) The biochemistry of copper. Academic, New York, pp 475–503
20. Nimmo DWR, Lightner DV, Bahner LH (1977) Effects of cadmium on the shrimps, *Penaeus duorarum*, *Palaemonetes pugio* and *Palaemonetes vulgaris*. In: Vernberg FJ, Calabrese A, Thurberg FP, Vernberg WB (eds) Physiological responses of marine biota to pollutants. Academic, New York, pp 131–183
21. Sparague JB (1964) Lethal concentrations of copper and zinc for young Atlantic salmon. J Fish Res Boa Can 21:17–26
22. Cough JA (1977) Ultra structural study of lesions in gills of a marine shrimp exposed to cadmium. J Invert Pathol 29:267–288
23. Philpott HCW, Copeland DE (1963) Fine structure of chloride cells from three species of *Fundulus*. J Cell Biol 18:389–401
24. Soegianto Chrmantier M, Daures JP, Trilles G, Charmantier (1999) Impact of copper on the structure of gills and epipodites of the shrimp *Penaeus japonicus* (Decapoda). J Crus Biol 19(2):209–223
25. Vogt G, Quinitio ET (1994) Accumulation and excretion of metal granules in the prawn *Penaeus monodon*, exposed to water borne copper, lead, iron and calcium. Aqua Toxic 28:233–241
26. Manisseri MK, Menon NR (1995) Copper induced damage to the hepatopancreas of the penaeid shrimp, *Metapenaeus dobsoni* an ultrastructural study. Dis Aquat Org 22:51–57
27. Viarengo A (1985) Biochemical effects of trace metals. Mar Pollu Bull 16:153–158
28. Arumugam M, Ravindranath MH (1987) Copper toxicity in the crab *Scylla serrata*, copper levels in tissues and regulation after exposure to a copper-rich medium. Bull Environ Contam Toxicol 39:708–715
29. Al Mohanna SY, Nott YA (1987) R cells and the digestive cycle in *Penaeus semisulcatus* (Crustacea: Decapoda). Mar Bio 95:129–137
30. Icely JD, Nott JA (1980) Accumulation of copper within the "hepatopancreatic" caeca of *Corophium volutator* (Crustacea: Amphipoda). Mar Biol 57:193–199
31. White SL, Rainbow PS (1986) Accumulation of cadmium by *Palaemon elegans* (Crustacea: Decapeda). Mar Ecol Prog Ser 32:17–25
32. Weeks JM (1992) Copper rich granules in the ventral caeca of talltrid amphipods (Crustacea: Amphipoda: Talltriae). Ophelia 36:119–133
33. Factor JR, Naar M (1990) The digestive system of the lobster *Homarus americanus* II. Terminal hepatic arterioles of the digestive gland. J Morph 206:283–291
34. Narayanan KR, Ajmalkhan S, Pechimuthu SM (1994) Histopathological changes due to effect of sublethal concentration of CuSo4 of the hepatopancreas of edible oyster crab, *Scylla serrata*. J Exp Biol 15(4):288–303
35. Vogt G (1987) Monitoring of environmental pollutants such as pesticides in prawn aquaculture by histological diagnosis. Aquacult 67:57–164
36. Kelly JF (1980) A description of the histological structure of normal and microsporidian infected pink shrimp, Penaeus duorarum. University Microfilms International, Ann Arbor, p 88
37. Lawson SL, Jones MB, Moate RM (1995) Effect of copper on the ultrastructure of the gill epithelium of *Carcinus maenas* (Decapoda: Brachyura). Mar Pollut Bull 31(1):63–72
38. Wobeser G (1975) Acute toxicity of methyl mercury chloride and mercuric chloride in rainbow trout (*Salmo gairdneri*) fry and fingerlings. J Fish Res Boa Cana 32:2005–2013
39. Kumar S, Pant SC (1981) Histopathologic effects of acutely toxic levels of copper and zinc on gills, liver and kidney of *Punctius conchorius* (Ham.). Indian J Exp Biol 19:91–194
40. Krishnakumari L, Varshney PK, Gajbhiye SN, Govindan K, Nair VR (1983) Toxicity of some heavy metals on the teleost, *Therapon jarbua* (Forsskal, 1775). Indian J Mar Sci 12:60–63
41. Calabrese A, Maclnnes JR, Nelson DA, Greig RA, Yevich PT (1984) Effects of long-term exposure to silver or copper on growth, bioaccumulation and histopathology in the blue mussel, *Mytilus edulis*. Mar Environ Res 11:253–274
42. Kureishy TW, George MD, Sen Gupta R (1979) Total mercury content in some marine fish from the Indian ocean. Mar Pollut Bull 10:357–360
43. Richard AG, Krzynowek J (1979) Mercury concentration in three species of tuna collected from various ocean waters. Bull Environ Contam Toxicol 22:120–127
44. Khengarot BS, Somani RC (1980) Toxic effects of mercury on the gills of a freshwater teleost, *Puntius sophore* Hamilton. Curr Sci 49:832–834