The application of histo-cytopathological biomarkers in the mud crab *Scylla serrata* (Forskal) to assess heavy metal toxicity in Pulicat Lake, Chennai

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Abstract

The concentrations of heavy metals and their associated structural deformities in the gills, muscles and hepatopancreas of *Scylla serrata* from Pulicat Lake were determined and compared with crabs live along Kovalam coast. The concentrations of metals were high in the hepatopancreas and gills of crab from Pulicat Lake, whereas, low in crab from the Kovalam coast. Data were visualized using a principal component analysis (PCA). Significant differences were found for all variables at the plot scale; however, the overall variation was relatively low for muscle tissues in both stations. The structural deformities observed in the gills, hepatopancreas and muscle was due to metal toxicity, and the degree of damage was correlated with the elevated metal concentration. The results showed significant metal accumulation and histocytopathological lesions in the crabs from Pulicat Lake. The results suggest that these biomarkers are useful for assessing the impact of metal pollution in the coastal environments.

1. Introduction

Pulicat Lake, which is located in the North Chennai coastal region, is a typical brackish water ecosystem of great importance with regard to its biodiversity and aesthetic value. Due to its morphological and brackish water characteristics, it is the most suitable breeding and nursing ground habitat for fishes in the North Chennai coastal region. Over exploitation, mismanagement and improperly treated industrial effluents from more than 25 industries are continuously released into the North Chennai Coastal region, which make it difficult for the ecosystem to regulate itself (Kamala Kannan et al., 2008). Aquatic organisms are often subjected to multiple stressors, including aquatic diseases, anthropogenic wastes and trace metals, that are caused by man-made, industrial and urban wastes, mining and dam constructions and climatic changes (Ruddiman, 2005). Benthic invertebrates show considerable potential as sentinel marker species for ecosystem health monitoring programs because they are small, common and relatively sessile and because they tend to accumulate toxicants present in the environment. In addition, the biochemical, physiological, and histological characteristics of several common species are sufficiently well known to distinguish exposed individuals (Viarengo, 1993). Crustaceans can accumulate metals in their systems by via absorption from the surrounding water/sediment or through the ingestion of food (Bryan, 1971, 1979).

Previous studies in Pulicat Lake recorded an elevated level of heavy metal concentrations, especially iron, cadmium and mercury (Padma and Periakali, 1998; Periakali and Padma, 1998; Kamala Kannan and Krishnamoorthy, 2006). The need for monitoring environmental conditions in relation to the trace metal contamination of aquatic systems has resulted in the development of bio-indicators for this complex task (Phillips, 1977; Luoma, 1983). The interest in obtaining an accurate and easy analysis of the trace elements from marine crustacean tissues arises from nutritional, toxicological and environmental perspective. Elements such as Mg, Al, Ca, Fe, Co, Cu and Zn are necessary to maintain of optimum health and are thus nutritionally important, whereas other metals such as Pb, Cd,
As and Hg are detrimental to optimum health and have toxicological effects (Mohapatra et al., 2007). The biological impact of environmental contaminants was evaluated using sentinel organisms and biomarkers that were analyzed at the cell, tissue and organ levels (Donnini et al., 2007). Cells have evolved different networks of cellular stress responses to adapt and survive environmental changes by combating a wide variety of stress (Padmini and Usha Rani, 2010). Electron microscopic studies have revealed marked variability in the morphology of the apical surface membrane of chloride cells in different teleost species, which all share a distinctive appearance that distinguishes them from adjacent pavement cells (Carmona et al., 2004; Arockia Vasanthi et al., 2013).

The mud crab, Scylla serrata commands a unique status by virtue of its delicacy and greater demand for consumption in both local and export markets. Due to the demand for live export and the increased price, the mud crab fishery and aquaculture have gained worldwide importance. Though, mud crabs are primarily marine dwellers, they immigrate into brackish water systems during their post-larval stages, grow fast, attain maturity and form a lucrative fishery in estuaries, backwaters and lagoons. Thus, exposure to both the marine and estuarine environments affects the metal bioavailability to the body from both natural and anthropogenic sources (Mohapatra, 2008). Three of the organ systems in crustaceans are most vulnerable to the toxic effects of contaminants: the gills, the hepatopancreas, and the antennal glands (Bryan, 1979). The gills are one of the entry points for harmful substances because they are a large adsorptive organ system. Further damage to the gills can occur when metabolized substances are excreted (Bryan, 1971). Regarding the variety of metabolic roles, the gills are comparatively unique organ within the animal kingdom (Dalla-Venezia et al., 1992). They act as a selective interface between the internal milieu and the external media, mediating exchange processes between the organism and its environment. In addition to their role in respiration, the gills play a prominent role in osmolyte, water volume and acid-base regulation (Lucu, 1990).

For field assessments, histopathology is often the easiest method of assessing both short- and long-term toxic effects (Hinton and Lauren, 1990). Histo-cytological responses in different organs of vertebrates and invertebrates have proven to be useful tools for characterizing the health status of organisms and assessing the impact of environmental contaminants on exposed organisms (Schramm et al., 2000; Gernhofer et al., 2001).

In contrast to the extensive literature on the biochemical and physiological aspects of crustacean organs, the information related to the architecture and ultrastructure of these organs is comparatively scarce (Dalla-Venezia et al., 1992). To this end, this study considers: (a) the bioavailability (accumulation) of different metals (Cu, Pb, Zn, Cd, Mn and Fe) in vital tissues and (b) the relationship underlying sub lethal effects provoked by metals in various tissues of S. serrata. While studies of this type undoubtedly provide invaluable insight into the cellular response to pollutants, they also place increasing emphasis on the effects of metals at the organ-level. Therefore, the mud crab S. serrata which lives in Pulicat Lake, where heavy metal concentrations can be increased by industrial pollution, domestic sewage and drastic changes in environmental conditions, was selected and compared with the crab collected from the less polluted Kovalam Coast.

2. Materials and methods

2.1. Study area

The study area Pulicat Lake is the second largest brackish water lagoon in India and runs parallel to the Bay of Bengal. It borders the east coast south of Andhra Pradesh and a portion of Pulicat Lake extends into the northern part of Tamil Nadu (Fig. 1). The lake is approximately 360 km² in size, and its depth (water column) varies from 1 to 6 m. The improperly treated industrial effluents from Ennore Creek and Buckingham Canal ultimately reach Pulicat Lake through its bar mouth and the Bay of Bengal coastal waters. The point sources of pollution are mainly from the North Chennai Thermal Power Plant, Ennore port activities, Manali Petrochemical Industries, other nearby industries and untreated urban wastes from the Chennai metropolitan area (Padma and Periakali, 1998; Periakali and Padma, 1998).

The reference site used for this study was the Kovalam coast (12°49′N, 80°5′E), which is located 40 km south of Chennai (Fig. 1). It runs parallel to the sea coast and extends 20 km inland. The temperature and salinity of this estuary range from 25 to 28 °C and 24 to 26 ppt respectively. The Kovalam coast was chosen as the unpolluted site for this investigation because it is surrounded by high vegetation and is free from industrial and urban pollution (Padmini and Usha Rani, 2009).

2.2. Sample collection and processing

Fifteen specimens of S. serrata were collected from professional fishermen in the study area and immediately transferred to the laboratory in insulated boxes covered with ice. In the laboratory, the crabs were placed in a refrigerator, then the sex was identified and the carapace length was measured. Tissue samples including gills, hepatopancreas and muscles were taken from each individual for heavy metal analysis. Tissue samples were dried at 100°C for 48 hours before being ground using a mortar and pestle.

In the laboratory, the gills were separated and blotted dry with filter paper. Approximately 0.5 g of sample was transferred to a 150 mL beaker. Ten milliliters of concentrated HNO₃ (Merk specialties private limited) was added and the tissue was homogenized with a pestle and mortar. The mixture was autoclaved for 30 minutes at 120°C to complete the digestion process. After cooling, the digest was transferred to a 50 mL beaker and diluted to 10 mL with DI water. The concentrations of Cu, Pb, Zn, Cd, Mn and Fe were determined using a graphite furnace atomic absorption spectrophotometer.

2.3. Heavy metal analysis

Approximately 0.5 g of sample was transferred to a 150 mL beaker. Ten milliliters of concentrated HNO₃ (Merk specialties private limited) was added and the tissue was homogenized with a pestle and mortar. The mixture was autoclaved for 30 minutes at 120°C to complete the digestion process. After cooling, the digest was transferred to a 50 mL beaker and diluted to 10 mL with DI water. The concentrations of Cu, Pb, Zn, Cd, Mn and Fe were determined using a graphite furnace atomic absorption spectrophotometer.

Fig. 1. Location map of the study area. Pulicat Lake and Kovalam coast.
limited, Mumbai, India) and a few anti bumping granules were added to the beaker. The beaker was allowed to stand for 2 h. The volume was then reduced to approximately 3 mL by boiling on a hotplate. Ten milliliters of H$_2$O$_2$ was added, and the volume was again reduced to 3 mL. An additional 10 mL of H$_2$O$_2$ was added, and the volume was reduced to 3 mL. Finally, 10 mL of concentrated HNO$_3$ was added and the volume was reduced to 3 mL. The solution was allowed to cool and was then transferred to a 25 mL volumetric flask. In addition blanks and certified reference materials (CRM) were also analyzed. Solutions were analyzed using a Hitachi 6000 (flame) or a Hitachi 7000 (graphite furnace) atomic absorption spectrophotometer depending on the concentration of the metal. The accuracy of the analytical procedures was verified by analyzing the appropriate CRMs and analytical methods. Quantitative results were obtained for each metal in each CRM (Table 1).

2.4. Morphological characterization

2.4.1. Histology

Samples of gills, hepatopancreas and muscles of S. serrata were quickly removed from the crabs and fixed in a 5% neutral buffered formaldehyde solution (pH 7.0). After fixation, the tissues were dehydrated through a graded alcohol series and embedded in paraffin wax. Tissue sections of 6–8 µm thickness were taken and stained with hematoxylin and eosin. Photomicrographs were taken at varying magnifications using a Leica 2500 microscope (Germany).

2.4.2. Scanning electron-microscopy

The gills of S. serrata were removed, sectioned transversely into two halves and transferred to a fixative (glutaraldehyde 4% in buffered phosphate, pH 7.5) for 1 h. Subsequently, the gills were quickly rinsed with distilled water and submitted to sequential ethanol/acetone dehydration. Afterwards, they were dried repeatedly in a critical point drying apparatus (Balzers) with liquid CO$_2$, coated with gold, and finally examined with a Cambridge Stereoscan S100 scanning electron microscope.

2.4.3. Transmission electron microscopy

Specimens of gills and hepatopancreas were prefixed in a 2.5% glutaraldehyde solution, diced into 1 mm$^3$ pieces and rinsed three times for a duration of 15 min rinses with a 0.1 M phosphate buffer (pH 7.4). Post-fixation samples, were placed in cold 1% aqueous osmium tetroxide for 1 h. After another rinse with the phosphate buffer, the specimens were dehydrated in a graded ethanol series of 50–100% and then embedded in Epon 812. Ultrathin sections (approximately 92–12% of the variation in the data, and the second PC determined the variations among the studied sites and the heavy metals. The data sets were log transformed to reduce the hetero-

density, and these transformed data were used for the PCA. A vari-

max rotation was applied to the data to obtain more accurate re-

sults. A probability level below $p < 0.05$ was considered to be statistically significant.

3. Result

3.1. Bio accumulation

The heavy metal accumulation in the tissues of S. serrata sampled from Pulicat Lake and the Kovalam coast are presented in Figs. 2a–2f. In the gills of the crab collected from Pulicat Lake, iron showed the highest concentration level at 82.31 ± 0.894 µg g$^{-1}$, followed by manganese (13.89 ± 2.496 µg g$^{-1}$), zinc (9.894 ± 1.023 µg g$^{-1}$), copper (8.35 ± 0.894 µg g$^{-1}$), cadmium (3.251 ± 0.412 µg g$^{-1}$) and lead (1.38 ± 0.153 µg g$^{-1}$). The hepatopancreas had the highest concentrations of heavy metals, with a maximum of 85.78 ± 9.114 µg Fe g$^{-1}$, 12.978 ± 2.132 µg Mn g$^{-1}$, 11.356 ± 1.984 µg Zn g$^{-1}$, 6.59 ± 0.679 µg Cu g$^{-1}$, 2.456 ± 0.399 µg Cd g$^{-1}$ and 0.86 ± 0.103 µg Pb g$^{-1}$. Muscle tissue had the lowest levels of accumulation, with 19.45 ± 3.998 µg Fe g$^{-1}$, 8.88 ± 1.558 µg Mn g$^{-1}$, 4.596 ± 0.893 µg Zn g$^{-1}$, 2.014 ± 0.048 µg Cu g$^{-1}$, 0.276 ± 0.01 µg Cd g$^{-1}$ and 0.23 ± 0.061 µg Pb g$^{-1}$.

The tissues of crab collected from the Kovalam coast registered the highest levels of metals in the gills, except for iron and zinc. Iron and zinc recorded a maximum of 22.65 ± 2.156 µg g$^{-1}$ and 6.589 ± 0.926 µg g$^{-1}$ in the hepatopancreas, 16.37 ± 1.028 µg g$^{-1}$ and 5.468 ± 0.945 µg g$^{-1}$ in the gills and 4.89 ± 0.987 µg g$^{-1}$ and 1.423 ± 0.023 µg g$^{-1}$ in muscle, respectively. Conversely, copper (3.152 ± 0.056 µg g$^{-1}$), manganese (1.62 ± 0.358 µg g$^{-1}$), cadmium (0.241 ± 0.04 µg g$^{-1}$) and lead (0.186 ± 0.035 µg g$^{-1}$) were highest in the gills followed by the hepatopancreas and muscle. In both Pulicat Lake and the Kovalam coast, very low accumulations of the studied metals were found in muscle. Overall, the accumulation of copper, lead, zinc, cadmium, manganese and iron were high in the crab collected from Pulicat Lake. The results demonstrate that the concentrations of copper, lead, cadmium and manganese were highest in the hepatopancreas ($p < 0.05$), lower in the gills and significantly lower in muscles at both sample sites. However, the concentration of zinc and iron were highest in the gills ($p < 0.05$), followed by the hepatopancreas and were significantly lower in muscles of the crabs from the two stations.

The data yielded six principal components (PCs). Of the PCs, copper, lead, zinc, cadmium, manganese and iron were high in the crab collected from Pulicat Lake. The results demonstrate that the concentrations of copper, lead, cadmium and manganese were highest in the hepatopancreas ($p < 0.05$), lower in the gills and significantly lower in muscles at both sample sites. However, the concentration of zinc and iron were highest in the gills ($p < 0.05$), followed by the hepatopancreas and were significantly lower in muscles of the crabs from the two stations.

![Fig. 2a. Copper accumulation (µg g⁻¹) in the tissues of S. serrata collected from Pulicat Lake and Kovalam coast. X±SD of three observations. "t"-test p < 0.05.](image-url)
explained 3.99% of the variation. Combined the first and second PCs explained 96.11% of the variation in the data. Data were visualized in a biplot analysis in PCA using the Statistica package. There were significant differences in all variables at the plot scale; however, the contribution to the overall variation was relatively low for muscle tissue in the samples from both Pulicat Lake and the Kovalam coast. Among all heavy metals, Zn and Mn had the maximum loading values, Fe, Pb and Cu had moderate loading values and Cd had the lowest loading value. It was clear that muscle tissues from crab from both study areas formed an outlier compared to the other tissues. Crab muscle obtained from the Kovalam coast had a negative score, indicating that fewer pollutants were found there. Similarly, the metal concentrations in the gills and hepatopancreas of crab from the Kovalam coast were negatively correlated with each other and formed separate groups. As in the samples from the Kovalam coast, the crab muscle from Pulicat Lake had a negative score and formed an outlier in the biplot. In contrast, the concentrations of metals in the gills and hepatopancreas of crab from Pulicat Lake were positively correlated with each other. These results confirmed that there was a greater accumulation in crabs collected from Pulicat Lake than in crabs collected from Kovalam coast (Fig. 3).

3.2. Histological and ultrastructural observation

3.2.1. Gills

Sections of the gill lamellae of the crab collected from Kovalam coast (control) exhibited a very thin cuticle, a single layer of epithelial cells and characteristic pilaster cells. The cellular layers in each leaflet were regularly connected by pilaster cells forminghilars across the haemolymph space. The periphery of each lamella was expanded to form a marginal canal. Between the lamellae, water channels were aligned parallel to each other (Fig. 4A and B).

In contrast, the gills of crab collected from Pulicat Lake exhibited collapsed lamellae due to the disruption of pilaster cells. The disintegrated cuticle showed a thickened epithelia and a completely disorganized distal filaments. The marginal canals were broadly dilated. Epithelial necrosis and hyperplasia combined with
the absence of pilaster cells, led to a reduction in the intra-epithelial space. The disappearance of pilaster cells was more than fully compensated by the enlargement of necrosed epithelial tissue and a damaged marginal canal. The lamellae were obliterated by hemocytic infiltration, causing them to become distended and the water channels to become enlarged (Fig. 4C and D).

Under scanning electron microscopy, the gill section of crab from the Kovalam coast (control) showed thick flattened lamellae at regular arrangements along the central shaft. An afferent and an efferent blood vessel ran along the epibranchial (dorsal) and hypobranchial (ventral) edges, respectively. The vessels were connected by a shaft that separates individual lamellae into two halves. Peripherally the lamella was expanded to form a marginal canal, which may be further dilated locally to form spacing nodules (Fig. 4E and F). Dense filaments were noted in both afferent and efferent vessels. In contrast, the ultra-thin section of the gills in crab from the polluted site revealed fusion of the lamellae. The marginal canal in the lamellae became folded and showed scattered filamentary arrangements both from the afferent and efferent blood vessels (Fig. 4G and H).

Ultra-thin sections of crab gill collected from the less polluted site showing the general view of the cuticle showed distinct and multilayered areas with dark filaments on the dorsal side and clear filaments on the ventral side. The intercellular junctional complexes were well developed. The cytoplasm had a well-developed rough endoplasmic reticulum, mitochondria and Golgi apparatus. In addition, the cytoplasm possessed various mucous secreting granules and lipid droplets. The nucleus contained heterochromatin which was distributed randomly in the periphery of the nucleoplasm. No distortion was observed in the multilayered structure.
of the cuticle in crab collected from the less polluted site (Fig. 4I and J).

In contrast, the ultra-thin Gill sections of crab Gill from the polluted site showed distortions in the multilayered cuticle. The most conspicuous structural alterations included the enlargement of intercellular spaces and the appearance of vacuoles and lamellar bodies in the cytoplasm. The augmentation of lipid droplets with a decrease in cellular organelles and the presence of an extremely large membrane-bound vacuole represented the complete degeneration of the gills. The intercellular junctional complexes were poorly developed, and some large vesicles appeared to be unconnected with the folds. The majority of the inner folds were dilated into broad vesicles or bleb-like structures that gave the superficial appearance of cell vacuolization and resulted in the enlargement of the subcuticular compartment (Fig. 4K and L).

3.2.2. Hepatopancreas

In the control crabs, the hepatopancreatic tubules appeared to be densely packed, and the inter tubular spaces were joined by a thin layer of connective tissue that most likely contained blood vessels. The tubular tissue was composed of a columnar epithelium delimited at its base by a basement membrane and characterized at its distal end (next to the lumen) by a striated border. In this section, B and R cells appeared in all tubules, and the lumina were star-shaped; this is the normal condition for many decapod species (Fig. 5A and B).

However, the section of hepatopancreas in crab collected from the polluted site showed necrotic tubules in the hepatopancreas that contained tissue debris in the lumen. Moreover, there appeared to be a severe walling off of the tubules by hemocytes around the thickened basal laminae. The internal organization of the tubule and lumina most likely disrupted and the connective tissue appeared to be completely loose. The basement membrane became detached with an expanded tubule. Vacuolization was observed in the epithelial cells (Fig. 5C and D).

In the control crabs, the ultra-thin section of hepatopancreas showed normal hepatocytes that were lined by a simple epithelium with a variety of cells. Mitochondria were observed to be uniformly distributed around the nucleus. Many of the microvilli possessed visible filaments that penetrated the apical cytoplasm of the cell. The cytoplasm possessed subapical vacuoles, and the enclosed membrane remnants of digested cell organelles indicated their autophagic function. Golgi vesicles were observed along the microvillous border. Another prominent feature was the presence of electron light vacuoles in the gland lumen (Fig. 5E and F).

In contrast with the control, the ultra-thin section in hepatopancreas of crab from the polluted site was characterized by an irregular microvillous border. The microvilli structure seemed to be disrupted and stunted in many areas and electron-dense material had accumulated in the tips of the microvilli. Large numbers of mitochondria were closely packed between the interdigitating membranes and the electron-dense nucleus. In addition damage to the mitochondrial cristae and the disappearance of the mitochondrial outer boundary were observed. Other cell organelles, such as the rough endoplasmic reticulum, were much less abundant. Inside the cell, most of the folds were dilated into broad vesicles or bleb-like structures that gave the superficial appearance of vacuolization in the cell (Fig. 5G and H).

3.2.3. Muscle

Sectioning through the muscle tissue of crab collected from the less polluted site revealed the fascicular arrangement of myofila-
ments with emarginated muscle bundles and fibers, binding to connective tissue. The striated muscle fibers were tightly packed. The nuclei were arranged along the margins of the muscle bundles (Fig. 6A and B).

In contrast, the muscle section of crab from Pulicat Lake was completely disrupted, with discontinuous striations and the complete disappearance of nuclei. The muscle bundles showed atrophy and damage to the connective tissues. The muscle bundles became disrupted and possibly split the muscle fibers. Large vacuoles occurred due to necrosis in the muscle tissues, and the muscle bundles were congested (Fig. 6C and D).

4. Discussion

The accumulation of tissue-specific trace metals has been observed in many marine organisms (Ng et al., 2007). Metal bioaccumulation in crabs can be affected by the chemical composition of
the sediment and interstitial seawater (Sadiq, 1992). The tissue distribution of metals can be useful for defining accumulation pathways and possible detoxification routes (Rainbow and Black, 2005; Alquezar et al., 2007). In the present study, a comparison of the bioaccumulation levels of different metals in various tissues indicated elevated concentrations in the gills and hepatopancreas but not in the muscles. The pattern of accumulation most likely varies not only with the different functions of tissues, but also with the conditions. The accumulation pattern in S. serrata similarly varied with the site. The highest concentrations of Cu, Pb, Mn and Cd were found in hepatopancreas tissues in crabs from both Pulicat Lake and the Kovalam coast. Higher concentrations of Cu, Fe, and Pb have been found in the hepatopancreas of Portunus pelagicus compared to the levels found in gills or muscles (Ghazaly, 1988). Particularly high concentrations of certain metals in specific organs may be related to the utilization of that metal. Notably, the concentration of Zn is highest in the gills, though most related studies have found the highest concentration of Zn in the hepatopancreas in other species of crabs. The result is most likely related to the chemical environment of the habitat. High concentrations of Zn and most other trace metals in the gills of S. serrata likely result from the direct accumulation of metal ions from seawater. The concentration of Zn in the gills of S. serrata from Pulicat Lake was close to the highest concentration of Zn found in Charybdis longicollis (Mohapatra et al., 2007; Firat et al., 2008). The higher concentrations of the metal levels in Pulicat Lake are most likely due to the possible input sources from the chemical industries and petrochemical parks located nearby. The concentrations of Pb found in crabs were reflective of the environment, which was expected because Pulicat Lake has a long history of contamination. The higher concentrations of Cu and Zn in the hepatopancreas were also expected because the hepatopancreas has higher levels of metallo-thioneins, which help sequester these metals. In the current study, the Fe concentration was higher in the gills than in the hepatopancreas or muscle tissues. Fe is critically involved in enzymatic and respiratory processes of crustaceans, therefore, Cu and Fe are abundant in the gills (Mohapatra et al., 2009). The higher Fe content in the gills of the mud crabs is most likely due to the presence of absorbed particulate matter on the gills rather than to the active biological uptake of the metal (Szefler et al., 1990; Paez-Osuna and Ruiz-Fernandez, 1995). High values of Fe in gill tissue have been reported previously for Cancer irroratus (Martin, 1974), S. serrata (Depledge et al., 1986) and P. vannameli (Paez-Osuna and Tron-Mayen, 1996). Several possible reasons may explain the lower accumulation of metals in muscles. The most likely reason is that muscle does not come into direct contact with the toxicant medium because it is totally covered by the skin which helps the organism to avoid the penetration of the toxicant (Arockia Vasanthi et al., 2013). Several reports in the literature examine the histopathological effects of pollutants in the gills of crustaceans and fish (Arockia Vasanthi et al., 2013). Our results suggest that the effect of heavy metals is a result of damage to gas exchange mechanisms as a consequence of the gill pathologies observed. It is evident that both acute and long-term, exposure to metal compounds produces conspicuous histopathological changes in the hepatopancreas and gills of S. serrata. Similarly, crustacean gills are important in both respiration and osmoregulation, and cellular damage resulting in the disorganization of the gill tissue would thus have serious consequences. In fact reports have indicated that heavy metals affect the osmoregulatory ability of estuarine crabs (Thurberg et al., 1973; Revathi et al., 2011) described that the effect of cadmium on the gills was pronounced with abnormalities in lamella compared with the control. These abnormalities included abnormal gill tips, lifting of the lamellar epithelium, lamella hypertrophy, cuticular layer atrophy and lamellar fusion. Similar observations have been reported in the posterior gill lamellae of various species after exposure to heavy metals such as mercury, cadmium and copper (Mazon et al., 2004; Arockia Vasanthi et al., 2012). Mallatt (1985) stated that the swelling and lifting of the lamellae might simply reflect a physiological adaptation to stress. However, the loss of cellular ions most likely exacerbates the impairment of the gill functions and leads to dysfunctional or even nonfunctional gills, eventually resulting in asphyxia (Tamse et al., 1995; Revathi et al., 2011).

The scanning electron micrograph clearly revealed the reduction in the number of lamellae in the gills of crab from Pulicat Lake. However, the lamella was organized in the gills of crab from the Kovalam coast. Peripheral swellings in the marginal canal of Gecarcinus lateralis were identified as interlamellar spacers by Copeland (1968). Similar lesions have been reported in the posterior gill lamellae of various species after exposure to several heavy metals (Bubel, 1976; Victor et al., 1990; Mazon et al., 2004). Cell degeneration has been frequently described as the negative effect of pollutants on gill epithelia, and this description generally refers to death of cells by necrosis (Mallatt, 1985). Under transmission electron microscopy, the lamellae showed disarrayed epithelial structure, condensed nuclei, mucous production, lipid droplets and vacuole formation. Mucous cells can be efficient in seizing toxic agents and thereby helping prevent the entry of these agents into the gills (Perry and Laurent, 1993). According to Takashima and Hibiya (1995), the hyper-secretion of mucous can be the result of a chronic defensive mechanism against parasitic or bacterial infections or chemical irritation. The results of the present study illustrated the excessive secretion of mucous from the surface of the secondary lamellae. Mucous is normally found in the filaments but can also be found on the respiratory epithelium when it is exposed to stress conditions, suggesting that the mucous layer protects lamellar surfaces against toxic metals.

The hepatopancreas in crustaceans is analogous to the liver in higher organisms, which is a sensitive organ and is liable to be damaged by waterborne pollutants (Baticados et al., 1987;
Baticados and Tendencia, 1991). The hepatopancreas is essentially composed of branched tubules and four different types of epithelial cells (E-cells, R-cells, F-cells, and B-cells) that line the tubules. Revathi et al. (2011) described hepatopancreas basement membrane disruption as the presence of more vacuoles and disorganized epithelial cells when it is exposed to cadmium. Such structural damage to the hepatopancreas of Macrobrychiun rosenbergii can significantly affect its absorption, secretion and digestion functions. Similarly, the section of hepatopancreas in crab from Pulicat Lake has shown necrotic tubules, thickened basal lamina and vacuolization. Additionally, tissue necrosis was noted in the muscle tissue of Panulirus homarus that was exposed to copper, this may be one of the reasons for the presence of a large number of haemocytes (Maharajan et al., 2011). Copeland (1968) considered that the apparent vesicles at the base of the apical infoldings in Callinectes sapidus were pinocytotic. However, the presence of vesicles does not necessarily imply pinocytosis and the results described here suggest that at least some of the apparent vesicles could be infolded. Histopathology shows that the crustacean hepatopancreas is a sensitive organ and is liable to injury by waterborne pollutants (Bhavan and Geraldine, 2000). Exposure to Hg\(^{2+}\) for 40 days resulted in the hepatopancreas tubules of E. sinensis losing both structural integrity and cellular organization, and the extent of this damage increased with the Hg\(^{2+}\) concentration, which was evident by the thickening of the basal lamina, deposition of melanin-like material in cells, vacuolization of cytoplasm, and disorganization of cells. The thickening of the basal lamina observed in our study most likely represents a defensive mechanism against the toxicant that is attributable to the production of collaguenous fibers and melanin or to the coagulation and walling-off by hemocytes (Bautista et al., 1994). The hepatocytes showed oxidative stress to the organism, and the condition was mediated by the redox cycling property of the heavy metals contaminating the estuary (Padmimi and Usha Rani, 2009). The morphological perturbations are the results of defensive mechanism or adaptive changes to heavy metal contamination in the study area (Au, 2004). Our findings leave us to conclude that the structural modifications in the tissues at the contaminated sites are most likely associated with changes at the membrane level, as implied by the tissue perturbations.

5. Conclusion

In conclusion, many statistically significant differences were observed in the mean metal values obtained for crab species of Pulicat Lake. The differences in the pattern of metal occurrence in various organs of the crab, S. serrata, and the significant increase in the metal concentrations are likely associated with the contribution from the surrounding industries. These results clearly indicate the ability of decapods to accumulate metals to detectable levels. The results of this study supply valuable information about the metal contents in examined species and indirectly indicate the crustacean health status. The results clearly indicate the ability of decapods to accumulate metals to detectable levels. The results of this study supply valuable information about the metal contents in examined species and indirectly indicate the environmental contamination along the coastal areas. Though it reinforced the difficulty of establishing cause-effect relationships under field conditions, particularly with a co-occurrence of different stressors, the implemented strategy integrating environmental chemical data and crab biomarkers is promising. Moreover, these results can be used to understand the chemical quality of crab and evaluate the possible risk associated with their consumption.

Acknowledgements

The authors gratefully acknowledge the UGC Dr. D.S. Kothari Post-doctoral Scheme for funding support for this study. Special appreciation and gratitude to Mr. Gobal Pillai, Canada for his constructive comments, which helped improve the manuscript. We also express our immense gratitude to Dr. T. Sathish, Scientist, for his assistance in data analysis using the statistical package.

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