IV. RESULTS

4.1 Physico-chemical parameters

Monthly variations in meteorological and physico-chemical parameters viz., rainfall, air and surface water temperature, pH, salinity, dissolved oxygen, phosphate, nitrate and silicate contents in Arasalar estuarine waters were recorded for a period of one year from July 2010 to June 2011 (Table 1 and Figs.1-9).

4.1.1 Rainfall

The north east monsoon in Karaikal brings very heavy rain during October, November and December months. The pattern of rainfall facilitates the divisions of the year into post monsoon (January – March), summer (April – June), Pre monsoon (July – September) and monsoon (October – December).

Total rainfall of 1632.36 mm was recorded from July 2010 to June 2011 and the monthly rainfall (mm) varied from 0.33 to 551.8 during the study period. The maximum rainfall (551.8 mm) was recorded during the north-east monsoon (November 2010) and minimum (0.33 mm) during the month of April, 2011. (Table 1 and Fig.1).

4.1.2 Temperature

During the study period air temperature varied from 26.1 to 34.3°C. The minimum was recorded during monsoon season (November, 2010) and maximum during the summer season (June 2011). The atmospheric temperature showed a positive correlation with water temperature ($r=0.989$) of Arasalar estuary. The surface water temperature ranged from 25.1°C to 31.8°C. The minimum surface water temperature (25.1°C) was recorded during monsoon season (December, 2010) and maximum (31.8°C) was recorded during the summer season (June, 2011). (Table 1 and Fig.2). Water temperature of the Arasalar estuary showed a positive correlation with salinity ($r=0.7787$) and pH ($r=0.7175$) and a negative correlation with dissolved oxygen ($r=0.7362$). (Table 1 and Fig.3).
4.1.3 pH

The monthly mean values of hydrogen ion concentration of water varied from 7.5 to 8.4. Maximum values of pH were observed in the summer season (May, 2011) and minimum values were recorded in the monsoon seasons (December 2010). Statistical analysis showed that the pH had positive correlation with water temperature ($r=0.7175$) and salinity ($r=0.8648$) whereas dissolved oxygen had an inverse relationship ($r=-0.8905$). (Table.1 and Fig.4).

4.1.4 Salinity

The seasonal variation of salinity in Arasalar estuary are graphically represented in fig. A marked seasonal changes in salinity was observed throughout the study period. Minimum salinity (20.5‰) was recorded during monsoon (December 2010) and was slowly increased during post monsoon and attained maximum (32.1%) during summer seasons (May 2011). Salinity of the Arasalar estuary showed positive correlation between temperature ($r=0.7787$) and pH ($r=0.8648$) while it showed negative correlation with dissolved oxygen ($r = -0.8905$) (Table.1 and Fig.6).

4.1.5 Dissolved oxygen

Dissolved oxygen(DO) in Arasalar estuary was varied between 2.8 and 5.3 ml/l. Minimum D0 was recorded during the month of May, 2011 and maximum in November, 2010 (Table.1 and Fig.6). Statistical analysis showed that dissolved oxygen had a negative correlation with water temperature ($r=-0.8063$), salinity ($r=0.91205$) and pH ($r=0.8905$) (Table.6).

4.1.6 Silicate

The monthly variations of silicate of the water observed in Arasalar estuary during the study period (July 2010- June 2011) are graphically represented in Fig.7. The silicate content showed a minimum value of 14.2µg/l (April 2011) and a maximum value of 85.1µg/l (December 2010). Throughout the study period, mean seasonal temperature, pH, Salinity, Do, phosphorus, nitrate, and silicate contents were not uniform in Arasalar estuary.
4.1.7 Total phosphorus

The monthly variations of dissolved phosphate recorded in Arasalar estuary are shown in the Fig.8. The total phosphorus was minimum (0.32 µg/l) in the month of April, 2011 and maximum (1.21 µg/l) in the month of November 2010. Total phosphorus showed positive correlation with dissolved oxygen (r=0.6631) and negative correlation with pH (r=-0.7614) and salinity (r=0.7427). (Table.6)

4.1.8 Nitrate

The nitrate was varied from 2.47 to 8.69 µg/l. Minimum was recorded during the month of April, 2011 whereas maximum during the month of November, 2010 (Table.1 and Fig.9). Statistical analysis showed that the Nitrate had positive correlation with DO (r=0.7535) and negative correlation with pH (r=-0.7908) and salinity (r=-0.7908) (Table.6).

4.2 Distribution of heavy metal in water:

4.2.1 Copper

The concentration of Cu in was ranged between 1.31 and 2.64 µg/l with the annual mean of 1.97µg/l. Maximum concentration (2.64 µg/l) of Cu in the water was noted during monsoon (November 2010) and minimum (1.31 µg/l) during in summer (May 2011) (Table.2 and Fig.10).

4.2.2 Zinc

The concentration of Zn in estuarine water was ranging between 4.12 and 8.4 µg/l with the annual average of 5.92 µg/l. The concentration of Zn in the water showed the highest value (8.4 µg/l) during monsoon (November 2010) and lowest value (4.13 µg/l) in summer (April 2011) (Table.3 & Fig.11). The pre monsoon and post monsoon showed moderate level of Zn concentration.

4.2.3 Cadmium

The concentration of Cd was found varying between 0.05 and 0.13 µg/l with the mean of 0.09µg/l. Cadmium concentration in estuarine water showed its peak value (0.13 µg/l) during monsoon and low value (0.05 µg/l) during summer (April 2011) (Table.4 and Fig.12).
4.2.4 Chromium

The concentration of Cr was fluctuating between 0.54 and 1.26 µg/l with mean value of 0.89 µg/l. The highest concentration (1.26 µg/l) of Cr in the water was observed during monsoon (November 2010) and lowest concentration (0.54 µg/l) during summer (April 2011) (Table.5 and Fig.13).

4.3 Distribution of heavy metal in Sediment:

4.3.1 Copper

The concentration of Cu in sediment was found varying from 4.6 to 14.4 µg/g with a mean of 9.0µg/g. During the study period, the concentration of Cu in sediment was found to be high (14.4 µg/g) in monsoonal months and low (4.6 µg/g) summer months, whereas post monsoon and pre monsoon showed moderate values (Table.2 and Fig.10).

4.3.2 Zinc

The Zn concentration was found between 12.1 and 20.61 µg/g with the mean 0f 15.73 µg/g. The maximum concentration (20.61 µg/g) of Zn was recorded during monsoon (December 2010) and minimum (12.1 µg/g) during summer (April 2011) (Table. 3 and Fig.11).

4.3.3 Cadmium

The Cd concentration fluctuated with a mean of 2.46 µg/g. The highest (3.7 µg/g) concentration of Cd was found in November (2010) and then the values were decreasing to the minimum of 1.1µg/g in April (2011). (Table.4 and Fig.12)

4.3.4 Chromium:

The concentration of Cr was ranging from 3.2 to 8.37 µg/g with a mean value of 5.4 µg/g. The maximum Cr (8.37 µg/g) concentration was recorded in monsoon (November 2010) and minimum (3.2 µg/g) was noted during summer (April 2011) (Table.5 and Fig.13).
4.4 Bio accumulation of Heavy metals

4.4.1 Hermit crab *Clibanarius infraspinatus*

4.4.1.1 Copper

The Cu concentration in *C. infraspinatus* varied from season to season and also between the organs. Among the organs the annual mean concentration of Cu was more in the hepatopancreas (19.6 µg/g). The organ next to the hepatopancreas which accumulated Cu was gill (12.45 µg/g). The minimum levels of Cu was measured in the muscle (8.53 µg/g). The accumulation was minimum (5.3 µg/g) during summer (May 2011) season and the maximum (30.4 µg/g) during monsoon period (November 2010) (Table.7 and Fig.14).

4.4.1.2 Zinc

In *C.infraspinatus*, the annual mean concentration of zinc was higher in hepatopancreas (26.11 µg/g). The gill showed (18.27 µg/g) of Cu concentration. The minimum was observed in muscle (9.98µg/g). The accumulation was minimum (6.3 µg/g) during summer (April 2011) season and the maximum (39.7 µg/g) during monsoon (November 2011). (Table.8 and Fig.15).

4.4.1.3 Cadmium

The annual mean concentration of Cd was observed in hepatopancreas, gill and muscle of *C. infraspinatus*. Among the body components, hepatopancreas showed higher concentration of Cd (4.75 µg/g) than that of gill (2.97 µg/g). Muscle showed minimum amount of Cd (2.07µg/g). The accumulation was minimum (0.7 µg/g) in summer (April 2011) season and the maximum (6.7µg/g) during monsoon (November 2010) season. (Table.9 and Fig.16).

4.4.1.4 Chromium

In *C. infraspinatus*, the highest annual mean concentration of chromium was (8.81 µg/g) in hepatopancreas and low level of Chromium was observed in the muscle (2.9 µg/g). The annual mean accumulation of chromium was minimum (2.4 µg/g) during summer (June 2011) and the maximum (11.9 µg/g) during monsoon (November 2010) (Table.10 and Fig.17).
4.4.2 Estuarine clam *Meretrix casta*

4.4.2.1 Copper

The uptake of copper was 5.2 µg/g in foot and the maximum (43.1 µg/g) in digestive gland. The accumulation was minimum (5.2 µg/g) during summer (April 2011) and the maximum (5.2 µg/g) during monsoon (November 2010) (Table.11 and Fig.18).

4.4.2.2 Zinc

The accumulation of Zinc was 8.4 µg/g in foot and the maximum (56.2 µg/g) in digestive gland. The accumulation was minimum (8.4 µg/g) during summer (April 2011) and the maximum (56.2 µg/g) during monsoon (November 2010) (Table.12 and Fig.19).

4.4.2.3 Cadmium

The uptake of cadmium was maximum (0.6 µg/g) in foot and the maximum (7.3 µg/g) in digestive gland. The accumulation was minimum (0.6 µg/g) during summer (April 2011) season and the maximum (7.3 µg/g) during monsoon (November 2010) season (Table.13 and Fig.20).

4.4.2.4 Chromium

The accumulation of Chromium was maximum (3.1 µg/g) in foot and the maximum (7.3 µg/g) in digestive gland. The uptake was minimum (3.1 µg/g) during summer (June 2011) and the maximum (19.7 µg/g) during monsoon (November 2010) (Table.14 and Fig.21).

4.4.3 Estuarine fish *Mugil cephalus*

4.4.3.1 Copper

In *M. cephalus*, the highest annual mean concentration of Cu was observed in liver (14.07 µg/g) whereas the mean concentration of Cu in the gill was 9.1 µg/g. The minimum was observed in muscle (5.01 µg/g). The accumulation of copper was minimum (2.1 µg/g) during summer (April 2011) and the maximum (21.5 µg/g) in monsoon (November 2010) seasons (Table.15 and Fig.22).
4.4.3.2 Zinc

In mullet, the annual mean concentration of zinc was observed higher in liver (15.01 µg/g) than the gill (9.56 µg/g). The minimum was observed in the muscle (7.2 µg/g). The accumulation on Zn was minimum (2.1 µg/g) during summer (April 2011) and the maximum (25.8 µg/g) in monsoon (November 2010) seasons (Table.16 and Fig.23).

4.4.3.3 Cadmium

The annual mean higher concentration of cadmium was observed in liver (3.64 µg/g) of *M. cephalus* than that of gills (2.57 µg/g) and next decreasing trend in liver, muscle showed minimum amount of cadmium (1.44 µg/g). The accumulation of Cd was minimum (0.8 µg/g) during summer (April 2011) and the maximum (5.6 µg/g) in monsoon (November 2010) seasons (Table.17 and Fig.24).

4.4.3.4 Chromium

In *M. cephalus*, the maximum mean value of chromium was accumulated in the liver (6.76 µg/g) and the minimum concentration was observed in the muscle (4.48 µg/g). The gill showed 2.9 µg/g of Cr concentration. The accumulation of chromium was minimum (2.1 µg/g) during summer (May 2011) and the maximum (8.7 µg/g) in monsoon (November 2010) seasons (Table.18 and Fig.25).

4.5 Lethal Concentration (LC50)

4.5.1 Lethal Concentration 50 for Hermit crab

Copper caused 50% mortality of *C. infraspinatus* (96 hours) at 8.54 ppm, for zinc it was 12.21 ppm, for chromium 7.24 ppm and for cadmium, the sublethal effect was at 5.34 ppm. The LC50 values obtained at 24, 48, 72 and 96 hours exposures and the 95% confidence limits for the four heavy metals revealed that cadmium showed highest toxicity than other heavy metals. The LC50 values of copper for 24, 48, 72 and 96 hours were 8.89, 8.73, 8.6 and 8.54 ppm respectively (Table.19), the LC50 value of zinc for 24, 48, 72 and 96 hours were 13.9, 13.1, 12.6 and 12.2 ppm respectively (Table.20), the LC50 value of chromium for 24, 48, 72 and 96 hours were 11.2, 9.2, 8.06 and 7.24 ppm respectively (Table.22) whereas the LC50 value of cadmium for 24, 48, 72 and 96 hours were 5.6, 5.5, 5.4 and 5.3 ppm, respectively (Table.21)
4.5.2 Lethal Concentration 50 for Clam

Copper caused 50% mortality of *M. casta* (96 hours) at 10.4 ppm, for zinc it was 14.5 ppm, for chromium 9.1 ppm and cadmium, the sublethal effect was at 7.4 ppm. The LC$_{50}$ values obtained at 24, 48, 72 and 96 hours exposures and the 95% confidence limits for the four heavy metals revealed that cadmium showed higher toxicity than other heavy metals. The LC$_{50}$ values of copper for 24, 48, 72 and 96 hours were 10.7, 10.6, 10.5 and 10.4 ppm respectively (Table.23), the LC$_{50}$ value of zinc for 24, 48, 72 and 96 hours were 14.8, 14.7, 14.6 and 14.5 ppm, respectively (Table.24), the LC$_{50}$ value of chromium for 24, 48, 72 and 96 hours were 9.4, 9.3, 9.2 and 9.1 ppm, respectively (Table.26) whereas the LC$_{50}$ value of cadmium for 24, 48, 72 and 96 hours were 7.8, 7.6, 7.5 and 7.4 ppm respectively (Table.25).

4.5.3 Lethal Concentration 50 for Fish

A concentration of 9.6 ppm copper caused 50% mortality in *M. cephalus* during 96 hours for zinc it was 13.4 ppm, chromium 8.24 ppm and cadmium, the sublethal effect was at 6.1 ppm. The LC$_{50}$ values obtained at 24, 48, 72 and 96 hours exposures and the 95% confidence limits for the four heavy metals revealed that cadmium showed higher toxicity than other heavy metals. The LC$_{50}$ values of copper for 24, 48, 72 and 96 hours were 9.9, 9.8, 9.7 and 9.6 ppm, respectively (Table.27), the LC$_{50}$ value of zinc for 24, 48, 72 and 96 hours were 13.7, 13.6, 13.5 and 13.4 ppm respectively (Table.28), the LC$_{50}$ value of chromium for 24, 48, 72 and 96 hours were 8.5, 8.4, 8.3 and 8.3 ppm, respectively (Table.30) whereas the LC$_{50}$ value of cadmium for 24, 48, 72 and 96 hours were 6.4, 6.3, 6.2 and 6.1 ppm, respectively (Table.29).

4.6 DNA and RNA Content in Test Organisms

4.6.1 Nucleic acids composition in *Clibanarius infraspinatus*

4.6.1.1 DNA

The DNA content was the higher in hepatopancreas (2.52 mg/g) of the hermit crab reared as control than muscle (1.97 mg/g) and low in gill (1.38 mg/g). The heavy metals, copper, zinc, chromium and cadmium caused individually, pronounced dose dependent decrease in the DNA content of all tested tissues (Table.31 and Figs.26-28).
The maximum decrease of DNA was observed in the tissues of hermit crab exposed to 30% sublethal concentration of cadmium for a period of 30 days. The values were 65.21% in gill, 35.71% in hepatopancreas and 38.1% of muscle.

The minimum decline in DNA content for the same exposure period was observed in hermit crab exposed to lowest sublethal concentrations of zinc was 13.76% in gill, 6.34% in hepatopancreas and 6.59% in muscle. The percentage depletion was higher on hermit crab exposed to the sublethal concentrations of cadmium than other exposed concentrations zinc, copper and chromium.

4.6.1.2 RNA

In hermit crab kept as control RNA content of hepatopancreas was the highest in (3.14 mg/g), followed by muscle (2.65 mg/g) and moderate values were observed in gill (2.18 mg/g). Decrease in RNA levels was noted in all the tissues of fish exposed to the heavy metals individually (Table.32 and Figs.29-31). The maximum decrease of RNA content was observed in the tissues of hermit crab exposed to 30% sublethal concentration of cadmium reared for 30 days: 40.82% in gill, 37.57% in liver and 39.24% in muscle. The minimum decrease in RNA content for the same exposure period was observed in hermit crab exposed to lowest sublethal concentrations of zinc: 9.63% in gill, 5.73% in hepatopancreas and 6.41% in muscle. Though the decrease in tissue RNA of the Zn, Cu and Cr exposed hermit crab showed the same trend, the change was low magnitude compared to Cd exposure.

4.6.2 Nucleic acids Composition in *Meretrix casta*

4.6.2.1 DNA

The DNA content was the higher in digestive gland of in the clam reared as control (2.76 mg/g), than foot (2.14 mg/g), and low in gill (1.64 mg/g). The selected heavy metals, to caused pronounced dose dependent decrease individually in the DNA content of all tested tissues (Table.33 and Figs.32-34).

Decrease in DNA levels was noted in all the tissues of clam exposed to the heavy metals individually. The maximum decrease in the concentrations of DNA was observed in the tissues of clam exposed to 30% sublethal concentration of cadmium for 30 days: 56.7% in gill, 41.3% in digestive gland and 50% in foot.
The minimum decrease in DNA content for the same exposure period was observed in clam exposed to lowest sublethal concentrations of zinc: 7.92% in gill, 6.15% in digestive gland and 8.41% in foot. The percentage depletion was higher on clam exposed to the sublethal concentration of cadmium than those exposed to zinc, copper and chromium.

4.6.2.2 RNA

In the clam kept as control of M.casta RNA content was the highest in digestive gland (3.35 mg/g), followed by foot (2.96 mg/g) and moderate values were observed in gill (2.27 mg/g). Decrease in RNA levels was noted in all the tissues of clam exposed to the heavy metals individually (Table.34 and Figs.35-37). The maximum decrease of RNA content was observed in the tissues of clam exposed to 30% sublethal concentration of cadmium reared for 30 days: 48.01% in gill, 44.47% in hepatopancreas and 31.75 % in foot. The minimum decline in RNA content for the same exposure period was observed in clam exposed to lowest sublethal concentrations of zinc 7.92% in gill, 10.14% in digestive gland and 7.43 % in foot. Though the decrease in tissue RNA of the Zn, Cu and Cr exposed clam showed the same trend, the change was lower magnitude than for Cd exposure.

4.6.3 Nucleic acids Composition in Mugil cephalus

4.6.3.1 DNA

The DNA content of the fish reared as controlwas the higher in liver (3.55 mg/g), than muscle (2.62 mg/g), and less in gill (1.95 mg/g). The selected heavy metals, caused individually, pronounced dose dependent decrease in the DNA content of all tested tissues (Table.35 and Figs.38-40).

Decrease in DNA levels was noted in all the tissues of fish exposed to the heavy metals individually. The maximum decrease in concentrations of DNA was observed in the tissues of M. cephalus exposed to 30% sublethal concentration of cadmium for 30 days: 43.07% in gill, 52.95% in liver and 53.81% in muscle.

The minimum decline in DNA content for the same exposure period was observed in fish exposed to lowest sublethal concentrations of zinc 12.3% in gill, 10.98% in liver and 7.25% in muscle. The percentage depletion was higher on fish exposed to the sublethal concentration of cadmium than those exposed to zinc, copper and chromium.
4.6.3.2 RNA

RNA content was the highest in the liver (3.67 mg/g) of *M. cephalus* kept as control followed by liver (3.12 mg/g) and moderate values were observed in the gill (2.64 mg/g). Decrease in RNA levels was noted in all the tissues of fish exposed to the heavy metals individually (Table.36 and Figs.41-43). The maximum decrease of RNA content was observed in the tissues of fish exposed to 30% sublethal concentration of cadmium reared for 30 days: 28.40% in gill, 28.33% in liver and 29.16% in muscle. The minimum decline in RNA content for the same exposure period was observed in fish exposed to the lowest sublethal concentrations of zinc: 4.92% in gill, 5.72% in liver and 5.12% in muscle. Though the decrease in tissue RNA of the Zn, Cu and Cr exposed fish showed the same trend, the change was lower magnitude than Cd exposure.

4.7 HISTOLOGY

4.7.1 Light microscopic study of structure of gill of hermit crabs under control

Phyllobranchiate type of gills was observed in *C. infraspinatus*. Histological organisation of the gill consists of a double rows of closely spaced lamellae extending both anteriorly and posteriorly from the gill shaft. The lamellae were surrounded by a thin layer of epithelial cells enclosing the central haemocoelic sinus, within the haemocoelic space of the gill, haemocytes and haemolymph were observed (Plate.1a).

4.7.2. Histological changes induced by selected heavy metals in the gill of hermit crabs

Histological lesions of gills were observed in the hermit crabs, *C. infraspinatus* exposed to the sublethal concentrations of Cu, Zn, Cd and Cr. Swellings and fusion of the gill lamellae, disintegration of epithelium, accumulation of haemocytes in the haemocoelic spaces, malformation of the tips of the gill lamellae, necrotic and hyperplastic lamellae of gills were observed (Plates.1-4)

4.7.3 Structure of hepatopancreas in control hermit crabs

The hepatopancreas of *C. infraspinatus* consist of two big lobes, each of them is divided into three lobules which inturn include numerous tubules. The tubules remain closed at one end and opened to ducts at the other end. The tubules are lined
by a single layer of epithelium and by a peritrophic membrane. Four types of cells viz., E.cells (Embryonic cell), R-cells (Absorptive cells), F-cells (Fibrillar cells) and B-cells (Secretory cells) could be differentiated in the tubules of hepatopancreas. (Plates.5a)

4.7.4 **Histological changes observed in the cells of the hepatopancreas of hermit crabs exposed to the sublethal concentrations of selected heavy metals**

After 30 days of exposure, the structural integrity of the cells of hepatopancreas was found to be affected with increase in the concentrations of heavy metals. Disorganisation of hepatopancreatic tubules, modification of epithelial cell nuclei (which become irregular flat, pycnotic), atrophy of digestive cells, necrosis and vacuolization of the digestive tubules were also observed in the hepatopancreas of heavy metals treated hermit crabs (Plates.5-8).

The highest tubular damage and heavy vaculozation of cells were noticed in the hermit crabs exposed to 30% sublethal concentration of cadmium.

4.7.5 **Structure of muscle in contral Hermit crabs**

The photomicrograph of the muscle (Plate. 9a) depicted the presence of normal muscle fibres with equally spaced muscle bundles.

4.7.6 **Histological changes observed in the muscle of hermit crabs exposed to the sublethal concentrations of selected heavy metals**

On exposure to sublethal concentrations of heavy metals (Plates. 9, 10, 11 and 12) marked degeneration of muscle bundles, vacuole, Atrophy and splitting of muscle fibres were observed. Sublethal concentration of cadmium led to pronounced intra muscular edema and splitting of muscle fibres (Plate. 11a,b).

4.7.7 **Structure of gill of clam *Meretrix casta* under control**

The histological studies of gill, digestive gland and foot were done after exposing the clam to 10 and 30% sub lethal concentrations for a period of 30 days. The selected organs of the normal, heavy metals treated clam revealed the following observations with reference to their morphology.
The normal gill filaments of *Meretix casta* showed ciliated epithelium surrounding the water tube and blood sinus (Plate.13a). The gill filaments are joined to one another by horizontal bars called inter filamentary junction. Two lamellae of lamina are joined together by inter lamellar junctions. The inter lamellar junctions between two lamellae divide the space into distinct compartments called water tubes.

4.7.8 **Histological changes of gill induced by selected heavy metals in the gill of clam**

In sublethal exposure of heavy metals, the gill of *M. casta* showed marked histological changes. Appreciable changes were noted in the histology of gill after 10% sublethal treatment including swelling lamellae with haemocytes, damaged epithelial cells and sloughing of basal epithelium. The damage was more severe and progressive after 30% sublethal exposure. Clumping and enlargement of filaments, sloughing of gill filaments and vacuolization of basal epithelia were observed. Clogging of respiratory lamellae, hypertrophy of gill filament and vacuolization were observed. However, such changes were drastic to the extent that hypertrophy and widening of ostial spaces in gill lamellae were found in the clam exposed to 30 days of 30% sublethal concentration of Cd treated (Plates.13-16).

4.7.9 **Structure of digestive gland of clam under control**

The digestive gland is made up of a number of fine digestive tubules (DT) which communicate with the lumen of stomach through partially ciliated main ducts and non ciliated secondary ducts. The ducts are circular or oval in shape.

Two types of cells namely digestive cell (DC) and secretory cells (SC) can be differentiated in the digestive tubules of digestive gland of clam (Plate.17a). The digestive cells are responsible for absorption and intracellular digestion of most of the food ingested. The secretory cells produce digestive enzymes.

4.7.10 **Histological changes observed in the cells of the digestive gland of clam exposed to the sublethal concentrations of selected heavy metals**

Marked effects were observed at structural and cellular levels in the digestive gland. In 30 days of 10% sublethal exposed clam, shrunken digestive and secretory cells and enlarged lumen were observed (Plate.17 & Fig.b,c).
After 30 days of zinc exposure, rupturing of cell membrane and degeneration of digestive tubules were also observed. At the end of 30 days of chromium treatment, the digestive gland was highly damaged. Vacuolization, necrosis and atrophy of digestive cells were also observed (Plate 18 a,b).

The tissue of digestive gland was severely injured in cadmium treated M. casta, compared to other heavy metals treated individuals. At the end of 30 days of 10% sublethal exposure, rupturing of cell membrane, necrosis and vacuolation were also observed. After 30 days of 30% sublethal concentration, digestive gland was highly damaged and vacuolization, necrosis and atrophy were also observed (Plate 19-20).

4.7.11 Structure of foot of clam under control

The photomicrograph of the foot of M.casta (Plate. 21a) showed the arrangement of muscle bundles with epithelial layer the muscle bundles consisting of elastin cells.

4.7.12 Histological changes observed in the cells of the foot of clam exposed to the sublethal concentrations of selected heavy metals

In clam exposed sublethal concentrations of heavy metals after 30 days the foot photomicrograph showed shrinkage of inter epithelium and damaged muscle bundles were observed (Plate. 21, 22, 23 & 24).

4.7.13 Structure of gill of fish Mugil cephalus under control

The histological studies on organs like gill, liver and muscle were done after exposing the fish to sub lethal concentrations for a period of 30 days. The selected organs of the control, heavy metal treated fish revealed the following observations with reference to their morphology.

Structure of gill of M. cephalus consists of highly vascular plate structures called primary and secondary lamellae. The primary gill lamellae (PGL) are laterally compressed flat leaf like structure on either side of the interbranchial septum. Each one of them bears a row of secondary gill lamellae (SGL) on both sides perpendicular to the long axis of primary gill lamellae. The secondary gill lamellae are highly vascularised and surrounded by a thin layer of epithelial cells. Between the two adjacent respiratory lamellae lie the inter lamellar region (Plate 25a).
4.7.14 Histological changes induced by selected heavy metals in the gill of fish

In sublethal exposure of heavy metals, the gill of *M. cephalus* exhibited marked histological changes. Appreciable changes were noted in the histology of gill after 30 days of Cu treatment including detachment of epithelium from the axis respiratory lamellae, fusion of secondary lamellae and vacuolization (Plate.25 b,c). In 30 days of zinc treated fish, changes like hypertrophy of gill filaments, necrosis and fusion of secondary lamellae were evident (Plate.26). The damage was more severe and progressive after 30 days of exposure of chromium. The gill epitheliums surrounding the axis of primary and secondary lamellae were damaged to a great extent (Plate.28). Clogging of respiratory lamellae, hypertrophy of gill filament and necrosis were observed. However, such changes were drastic to the extent that detachment of epithelium, hypertrophy and complete erosion of secondary gill lamellae were found in the 30 days of 30% cadmium SLC treated fish (Plate. 27a,b).

4.7.15 Structure of Liver in normal *Mugil cephalus*

The normal liver is made up of continuous mass of hepatocytes arranged in irregular cords. The hepatic cells are polygonal in shape with distinct nuclei. Large number blood sinusoids were also seen around the hepatocytes (Plate.29a).

4.7.16 Histological changes observed in the liver of fish exposed to the sublethal concentrations of selected heavy metals

Marked toxic effects were observed at the structural and cellular level in the liver. In 30 days of copper exposed fish, disintegration of cell boundaries and slight dilation of blood sinusoids were observed (Plate.29 b,c). In fish at 30 days of zinc treatment, vacuolation, pyknotic nuclei and necrosis were evident (Plate.30). After 30 days of chromium, many hepatic cells were completely damaged and intracellular vacuolation was also apparent (Plate.32). Degeneration of hepatocytes showing distinct vacuoles and necrosis were maximum in the clam reared at 30% sub lethal concentration of cadmium (Plate.31 a,b).

4.7.17 Structure of Muscle in normal fish *Mugil cephalus*

Histological observations of the fish muscle (Plate. 33a) showed normal muscle fibres with equally spaced muscle bundles.
4.7.18 Histological changes observed in the muscle of fish exposed to the sublethal concentrations of copper, zinc, cadmium and chromium

Muscle tissues of the fish exposed to sublethal concentration of heavy metals showed irregular, ruptured and shrinkaged and vacuolization of muscle fibres (Plate. 33-36)

4.8 Scanning electron microscopic (SEM) study

4.8.1 Scanning electron microscopic (SEM) study of normal hermit crab gills

Scanning electron micrographic (SEM) examination of the photomicrograph of the gill (Plate. 37a) showed the arrangement of primary and secondary gill lamellae. The primary gill lamellae are flat like structures and secondary lamellae were equally spaced attached to their bases of primary lamellae.

4.8.2 Histological alterations of gill in heavy metals treated hermit crab under SEM observation

In hermit crab exposed to sublethal concentrations of heavy metal copper, zinc, cadmium and chromium after 30 days the gill photomicrograph (Plate. 37, 38) showed fusion and damaged gill lamellae. At sublethal concentration cadmium for the same exposure completely disrupted secondary gill lamellae was well marked (Plate. 38a,b)

4.8.3 Scanning electron microscopic (SEM) study of normal clam gills

In the gills of clam under control the filaments exhibited normal architecture and consist of two V – shaped demibranchs. Each demibranch has inner and outer lamellae connected by projections on their abofrontal surface. All the gill filaments have a inter-filamentar spaces. (Plate. 39).

4.8.4 Histological alterations of gill in heavy metals treated clam under SEM observation

The damages and fusion of secondary lamellae with mucous and hypertrophy were observed after 30 days of exposure. On exposure to heavy metals for 30 days, necrosis, fusion of secondary lamellae, mucous and vacuolization were observed (Plate. 39-40). The clam, treated upto 30 days, exhibited the changes in the gill with fusion adjacent lamellae, erosion of gill filament and necrosis. The damage was very severe and progressive in cadmium treated gills.
4.8.5 Scanning electron microscopic (SEM) study of normal fish gills

In the gills of *M. cephalus* under control, the primary gill lamellae exhibited normal architecture and mucous free and uniform branching of secondary lamellae from primary lamellae. The gill filaments bear micro ridges on the surface epithelium (Plate.41).

4.8.6 Histological alterations of gill in heavy metals treated fish under SEM observation

The damages, fusion of secondary lamellae and edema of primary lamellae, were observed after 30 days of exposure. On exposure to Cu, Zn, Cr and Cd for 30 days, epithelial hypertrophy, fusion of secondary lamellae and necrosis were observed. In fish, treated up to 30 days, the changes observed in the gill of *M. cephalus* were deformation and edema of primary and secondary lamellae, fusion adjacent lamellae and with mucous opening (Plate.41-42). The damage was more severe and progressive in cadmium treated gills.