Chapter IV

RESULTS

4.1. LEAD CONCENTRATION

The results of the estimation of lead concentration as total sediment load and weakly held or easily exchangeable portion of the total lead is given in the form of tables. The sampling locations are listed as spatially located in the study area from a North South direction. The sampling locations are divided into two coastal zones namely near shore water and brackish waters zone of the coast that include estuaries and back waters. In this study Muthupet, Manora are brackish water zones. The objective of the investigation is to study the behavior of lead in the coastal sediments.

The measurement of only the total sediment load of trace metals is a poor method of study because part of the metal load is loosely bounded to the sediment and particulate matter. Selective chemical methods using acetic acid or sequential extractions are developed to partition the total metal concentration load into their loosely bound are further sub divisions of total load. All these methods of extraction are strictly operational in definition.

In this study, therefore, the acetic acid method was chosen because it is one of the weakest chemical treatment that remove effectively the weakly bound part of the total metal concentrations in sediments. The acetic acid extraction removes all weakly bound metals held in ion
exchange sites, amorphous compounds if iron and manganese, carbonates and those weakly held in organic matter.

The fractionation scheme allows the definition of fractions of lead as non detrital (acid soluble) and detrital (acid in solubale) forms of total sediment load of the trace metal lead in the coastal area of Cauvery deltaic Zone of Tamilnadu.

The present study was made by monthly samplings mentioned in the above locations and the investigations were divided into four major seasons in the coastal deltaic zone. They are as follows

- Premonsoon : July, Aug, Sept.
- Postmonsoon : Jan, Feb, March
- Summer : April, May, June.

The sampling were started from June 2004 to March 2010 that includes Pretsunamic (July-Dec.2004) and Post-tsunamic (Jan-Dec.2005) studies of Lead concentrations in the deltaic area.

The Table 1 presents total lead and loosely bounded lead analysis from July to December of 2004 (ppm) which represents Pre-Tsunamic Data. In the present study the findings were as follows for the year 2004 July to December the season is classified as premonsoon. This will show signs of rainfall, as this may be the beginning for rain there are poor
concentrations of lead. There is a hike in the total lead concentration lead 0.3 ppm at Velankanni in the month of September 2004 because of the flag hoisting festival (heavy rush of tourist vehicle), similar at Nagai also. Next is Kodikarai this is also a very important tourist place shows higher concentration of total lead and more during monsoon time, here the total lead concentration level went upto 0.5 ppm in the month of November and December because of heavy monsoon and tourist vehicle movement. Muthupet estuary shows even amount of lead level. Adirampattinam is a fish landing area so there were no signs of lead pollution for the period of 2004. Karaikal and Manora were also tourist places but its seasonal so this is unseason time for tourists and moreover the parking of tourist vehicle lies about few kilometer away from beach and this is the reason for less pollution. There were no signs of presence of Loosely bound lead in the this area except kodikarai reason behind it is not only a tourist area but also a boat construction industry fish processing industries and due to the movement of mechanized fishing vehicle pollution and presence of abundant tourist vehicles (Table 1; Fig.4).

The Table 2 presents total and loosely bounded lead analysis from January to December of 2005 (ppm) which represents Post-Tsunamic Data. There were slight improvement in the concentrations of Lead in the month of May and monsoon seasons at karaikal this is because of the tourist vehicles and industrial wastes and automobile emissions liberated out and washed away towards sea. There were no signs of loosely bound lead deposits in this area. Kandhoori is one of the most important festivals
of this port-city being celebrated in the month of December each year at Nagapattinam. The Nagai and Velankanni register a high concentration because of the important festivals and arrival of a lot of tourist vehicles. Excluding this Manora and Velankanni show slightly higher concentrations during the month of May this is because of the summer holiday and tourist. The impact of Kandhoori festival and Chirstmas New Year time because of the arrival of new tourist vehicles the month of December shows lead concentrations. The above discussed total lead analysis seen in table 2. Loosely bound lead found only at Kodikarai. The results are presented (Table 2; Fig.5).

The Table 3 presents total lead and loosely bounded lead analysis from January to December of 2006 (ppm). The results of Nagai, Manora and Velankanni have registered a higher Lead concentration during festival seasons, Tourist vehicle’s movements and in Muthupet beck waters because of monsoon effects. Kodikarai recorded a high concentration 0.5 ppm of lead in April, May and December, this is because of the tourist and vehicle movements. Most of the places show not traceable for loose lead presence but found at Kodikarai at the month of May, June and November, December 0.2 ppm and 0.1 ppm. Other places like Velankanni and Nagai show a very minimal level of lead presence of September and December months, and not traceable (N.T.) (Table 3; Fig.6).
The Table 4 presents total lead and loosely bounded lead analysis from January to December of 2007 (ppm). There is an increase of concentration of lead at Kodikarai and maintains same concentration in the marine sediments. The total lead concentration touched a high upto 0.7 ppm at Kodikarai in the month of May and June. Velankanni and Nagai reported a same level of 0.5 ppm and 0.3 ppm during September and December months. The sediments of Manora, Muthupet show lead on monsoon season because of flushed rain waters because they are estuaries end. Kodikarai record high during May because of tourist vehicle flow and mechanized boats and Velankanni and Nagai recorded high during the festival seasons. Loosely bound lead found only at Kodikarai that too at Peak tourist season during festival time at Velankanni and Nagai (Table 4; Fig.7).

The Table 5 presents total and loosely bounded lead analysis from January to December of 2008 (ppm). The total lead concentration recorded high at Kodikarai during peak tourist season during this year it recorded 0.7 ppm during the month of May i.e., summer holiday time. Similarly, Manora and Velankanni during festival time and tourist seasons. Velankanni recorded a high concentration of lead during the month of September 0.5 ppm and 0.3 ppm in the month of May. Muthupet recorded low levels and some during monsoon due to back waters. Loosely bound lead found only at Kodikarai that too at Peak tourist season during festival time at Velankanni and Nagai as similar to previous month (Table 5; Fig.8).
The Table 6 presents total lead and loosely bounded lead analysis from January to December of 2009 (ppm). Here Kodikarai recorded a very high concentration of 1.2 ppm of all the places in the month of May, Manora recorded 0.5 ppm total lead a high during tourist season (Summer), May, Velankanni, Nagai recorded 0.5 and 0.3 a high during season time and festival time. Loosely bound lead found only at Kodikarai that too at Peak tourist season and monsoon time and during festival time at Velankanni and Nagai as similar to previous month (Table 6; Fig.9).

The Table 7 presents total and loosely bounded lead from January to April of 2010 (ppm). Except Kodikarai there is no vast deposition of lead in any of the area as this is a dry season and beginning of the year. Kodikarai recorded 0.5 ppm of total lead. There is no loosely bounded lead except Kodikarai i.e., 0.2 ppm (Table 7; Fig.10).

The table 7a presents pre and posttsunami study of total lead from July 2004 to June 2005. During pretsunami period from July 2004 these was considerable amount of lead deposits observed. Kodikarai recorded a highest of 0.5 ppm and all other stations with medium 0.3 to 0.1 ppm lead deposits observed. The observation after 26th December 2004 i.e., Tsunami wave made a heavy dilution in the coastal area leading to dilution in the coastal sediments as a result, there was no evidence of lead presence in the sediment in the month of January and February of 2005. But Kodikarai maintained a minimum of 0.3 ppm because of its
geographical location due to this there was a minimal deposits of lead only at Kodikarai.

The table 7 presents loosely bounded lead there was no evidence of lead presence in pre and posttsunamic period except at Kodikarai. Because of heavy tourist vehicle arrival and geographical location.

The Table 8 presents physico-chemical parameters for experimental water – *Mystus gulio* (estuarine fish). In this study an average of 36°C temperature was maintained. The parameters found in the control was pH 7.70, MOA (mg/l), 137.56, PPA (mg/l) 62.0, TA (mg/l) 199.25, Salinity (%) 0.444, TH (mg/l) 141.25 (Table 8).

The Table 9 presents physico-chemical parameters of experimental water – *Penaeus monodon* (shrimp). The physico-chemical parameters of water include pH 8.3, salinity 40 ppt, T.Alk 146 ppm, Dissolved CO₃ 12 ppm, HCO₃ 134 ppm, Hardness 8200 (Table 9).

### 4.2. ACUTE TOXICITY STUDIES OF *Mystus gulio*

The LC₅₀ values determined for *Mystus gulio* using heavy metal lead nitrate recorded 86.70 mg/l. Sublethal concentration studies on estuarine fish *Mystus gulio* using lead nitrate. Experiments are conducted at sublethal concentration-I 9.60 mg/l, Sublethal concentration-II 15.50 mg/l and Sublethal concentration-III 26.50 mg/l and results are observed (Table 10).
4.3. ACUTE TOXICITY STUDIES OF *Penaeus monodon*

The LC$_{50}$ determined for *Penaeus monodon* using heavy metal lead nitrate recorded 13.33 mg/l. Sublethal concentration studies on estuarine fish *Penaeus monodon* using lead nitrate. Experiments are conducted at sublethal concentration-I 1.66 mg/l, Sublethal concentration-II 3.33 mg/l and Sublethal concentration-III 6.60 mg/l and results are observed (Table 11).

4.4. HAEMATOLOGICAL PARAMETERS OF ESTUARINE FISH *Mystus gulio* EXPOSED TO LEAD NITRATE

A detailed observation on the Hematological study in control fish reveals the presence of total erythrocyte count is $1.49 \times 10^6/ \text{mm}^3$, total leucocyte count $10^3/ \text{mm}^3$ 17.40 Haemoglobin 7.5 g/dl, Lymphocytes 36 per cent, Monocytes 12.0 per cent, Eosinophils 2.0 per cent, Neutrophils 49.0 per cent, Basophils 1.0 per cent and Thrombocytes 2.0 per cent.

The Table 12 presents hematological parameters changes in TEC, TLC, thrombocytes in estuarine fish *Mystus gulio* expose to lead nitrate for 24 hrs, 48 hrs, 72 hrs and 96 hrs in three sublethal concentrations. The impact of lead nitrate on the TEC, TLC and on Thromobocytes are in decreasing order. The TEC decreases from 1.48 per cent to 1.42 per cent the TLC shows a decreasing trend from 17.38 to 17.30. The Thrombocytes shows a decreasing trend from 2.0 to Nd (Not detectable). The above findings are perused from the (Table 12).
The Table 13 presents hematological parameters changes in differential count of estuarine fish *Mystus gulio* expose to lead nitrate for 24 hrs, 48 hrs, 72 hrs and 96 hrs in three sublethal concentrations. According to the data of table 11 the lymphocytes show a decreasing path from 35 to 27 per cent, Neutrophils show a downward in number from 48.0 to 39.0, Monocytes show decrease from 11.0 to 7.0 per cent, Eosinophils also shows a decreasing attitude in number from 2.0 per cent to Nd (Not detectable limit). Similar the basophil exhibits the same as the above parameters with a decreasing trend from 1.0 per cent to Nd (Not detectable limit) (Table 13).

The Table 14 presents hematological parameters changes in haemoglobin of estuarine fish *Mystus gulio* exposed to lead nitrate from 24, 48, 72 and 98 hrs in three sublethal concentrations. The studies on haemoglobin shows a decreasing trend. The observations are 7.3 to 6.8 g/dl (Table 14).

4.5. BIOCHEMICAL ANALYSES

Biochemical analysis of *Mystus gulio*

Biochemical changes in the tissues of *Mystus gulio* due to the impact of heavy metal Lead nitrate are observed. The biochemical analysis were carried out in both control fish and on Lead nitrate treated fish. The tests were carried out in three different sublethal concentrations of 09.60 mg/L as SLC – 1, 15.50 mg/L as SLC – II, 26.50 mg/L as SLC –
III and L.C. 50 Conc. as 86.70 with 24, 48, 72 and 96 hrs the results are recorded in four different tables (Tables 15-18).

The analysis were carried out on muscle, liver and gonads, of both male and female fish.

Control values of *Mystus gulio* fish muscle, liver and gonad tissues of *M. gulio*

The protein in control muscle, liver and gonad tissue of male and female show 64.10 and 64.55, 50.15 and 52.00, 61.14 and 56.57% respectively. Carbohydrate with 16.52 and 16.82, 25.15 and 25.44, 14.50 and 15.92% respectively. Gonad concentration of muscle with 17.70 and 18.21, 21.78 and 20.06, 21.22 and 24.52% in male and female respectively.

The table 15 presents biochemical parameters of protein, carbohydrate and lipid into the muscle, liver and gonad of experimental fish *Mystus gulio* at three sublethal levels concentrations for 24 hrs. Muscle protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 63.66 in male and 63.80 per cent in female to 59.10 and 59.74 respectively this shows a decrease. Similarly the Carbohydrate level in male 16.66 and female 16.72 per cent and gradually decreases to 15.18 and 15.48 this shows depletion of carbohydrate. Similarly Lipids declines from 17.18 and 18.00 to 14.55 and 16.62 per cent respectively. The liver protein concentration in lead nitrate treated fishes SLC-I, SLC-II and
SLC-III are 49.55 in male and 50.80 per cent in female to 45.68 and 45.90 per cent respectively this shows a decrease. Similarly the Carbohydrates level in male 24.94 and female 24.20 and gradually decreases to 22.66 and 22.82 this shows depletion of carbohydrates. Similarly lipid level declines from 21.90 and 20.89 to 19.86 and 18.45% respectively. Gonad protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 59.07 in male and 55.69% in female to 56.15 and 52.80 respectively this shows a decrease. Similarly the carbohydrate level in male 13.68 and female 15.76 and gradually decreases to 12.15 and 15.00 this shows depletion of carbohydrate. Similarly lipid level decline from 19.00 and 23.15 to 17.60 and 20.10% respectively (Table 15).

The table 16 presents biochemical parameters of protein, carbohydrate and lipid into the muscle, liver and gonad of experimental fish *Mystus gulio* at three sublethal levels concentrations for 48 hrs. The muscle protein concentraton in lead nitrate treated fish SLC-I, SLC-II and SLC-III are 62.50 in male and 62.68% in female to 58.00 and 58.80 respectively this shows a decrease. Similarly the carbohydrate level in male 16.66 and female 16.72 and gradually decreases to 15.18 and 15.48 and 15.80% respectively. The liver protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 49.05 in male and 49.75% in female to 45.10 and 45.62 respectively this shows a decrease. Similarly the carbohydrate level in male 23.47 and female 23.65 and gradually decreases to 21.05 and 21.00 this shows depletion of carbohydrate. Similarly lipid level declines from 20.90 and 19.65 to 19.1 and 18.00%
respectively. Gonad protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 58.35 in male and 54.90% in female to 55.86 and 52.12 respectively this shows a decrease. Similarly the carbohydrate level in male 12.10 and female 15.58 and gradually decrease to 11.92 and 14.75 this shows depletion of carbohydrate. Similarly lipid level declines from 18.72 and 22.75 to 17.39 and 19.00% respectively (Table 16).

The table 17 presents biochemical parameters of protein, carbohydrate and lipid into the muscle, liver and gonad of experimental fish Mystus gulio at three sublethal levels concentrations for 72 hrs. The muscle protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 60.39 in male and 60.62% in female to 56.65 and 56.82 respectively this shows a decrease. Similarly the carbohydrate level in male 16.16 and female 16.12 and gradually decreases to 14.05 and 14.30 this shows depletion of carbohydrate. Similarly lipids declines from 16.04 and 17.1 to 12.18 and 14.28% respectively. The same table 13 liver protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 48.30 in male and 50.00% in female to 44.86 and 45.48 respectively this shows a decrease. Similarly the carbohydrate level in male 23.10 and female 23.27 and gradually decreases to 21.00 and 20.22 this shows depletion of carbohydrate. Similarly lipid level declines from 20.10 and 18.75 to 18.84 and 17.90% respectively. Gonad protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 57.36 in male and 53.05% in female to 55.00 and 51.92 respectively this
shows a decrease. Similarly the carbohydrate level in male 11.42 and female 15.23 and gradually decreases to 11.78 and 14.52 this shows depletion of carbohydrate. Similarly lipid level declines from 18.46 and 22.50 to 17.00 and 18.86% respectively (Table 17).

The table 18 presents biochemical parameters of protein, carbohydrate and lipid into the muscle, liver and gonad of experimental fish *Mystus gulio* at three sublethal levels concentrations for 96 hrs. The muscle protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 59.60 in male and 60.00% in female to 56.48 and 55.60 respectively this shows a decrease. Similarly the carbohydrate level in male 16.05 and female 16.00 and gradually decreases to 13.65 and 13.34 this shows depletion of carbohydrate. Similarly lipids declines from 15.48 and 16.40 to 12.00 and 13.50% respectively. The same table 14 liver protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 47.60 in male and 48.10% in female to 41.12 and 43.05 respectively this shows a decrease. Similarly the carbohydrate level in male 22.80 and female 22.95 and gradually decreases to 20.48 and 19.60 this shows depletion of carbohydrate. Similarly lipid level declines from 19.30 and 18.20 to 17.86 and 17.00% respectively. As per the data to table 14, gonad protein concentration in lead nitrate treated fishes SLC-I, SLC-II and SLC-III are 56.80 in male and 52.55% in female to 54.48 and 51.58 respectively this shows a decrease to 11.05 and 14.30 this shows depletion of carbohydrate. Similarly lipid level declines from 18.00 and 22.00 to 16.64 and 17.89% respectively (Table 18).
4.6. BIOCHEMICAL ANALYSIS OF SHRIMP *Penaeus monodon*

The biochemical analysis were carried out in both control animals and on lead nitrate treated animals. The tests were carried out in 3 different sublethal concentrations of 1.66 mg/L as SLC – I, 3.33 mg/L as SLC – II, 6.60 mg/L as SLC – III with 24 hrs, 48 hrs, 72 hrs and 96 hrs, the results were mentioned in four different tables (Table 19-22).

The analyses were carried out on muscle and hepatopancreas of adult shrimp *Penaeus monodon*.

**Control values of muscle and hepatopancreas tissues of shrimp *Penaeus monodon***

The control value for the shrimp *Penaeus monodon* protein in muscle tissue 66.95%, carbohydrate in muscle 15.05% and lipid muscle 9.87. Similarly the protein in hepatopancreas is 58.56%, carbohydrate in hepatopancreas is 18.66% and lipid in hepatopancreas is 20.45% respectively.

The table 19 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 24 hrs. The results of table 15, the concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC-I, SLC-II and SLC-III are 65.22 to 62.25% this shows a decline and depletion of protein from the treated tissues similarly carbohydrates in muscle tissue shows a decline from 15.1 to 14.2%. The lipid concentration shows a decrease from 09.00 to 08.32%. The hepatopancreas tissue shows
a decreasing trend in protein from 57.48 to 53.7, carbohydrate from 18.57% to 15.82% and Lipid 19.20 to 17.22, these observations are found (Table 19).

The table 20 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 48 hrs. The results of table 16 shows a decrease in muscle protein the concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC – I, SLC – II and SLC –III are 65.0 to 61.56 for muscle protein 14.96 to 13.60 for muscle carbohydrate and 8.76 to 7.88 for muscle lipids all the three show a decrease in post exposure to lead nitrate similarly hepatopancreas protein shows a decreasing trend from 56.15 to 52.50, carbohydrate 18.10 to 15.22 and lipid 18.60 to 16.52 respectively (Table 20).

The table 21 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 72 hrs. The concentration of lead nitrate present in muscle tissue in three sublethal concentrations SLC – I, SLC – II and SLC – III are 64.9 to 61.1% this shows a decline and depletion of protein from the treated tissues. Similarly carbohydrates in muscle tissue shows a decline from 14.22 to 13.16%. The lipid concentration shows a decrease from 08.45 to 06.90%. The hepatopancreas tissue shows a decreasing
trend in protein from 55.9 to 51.22, carbohydrate from 17.98 to 15.05 and lipid 18.25 to 16.22, these observations are found (Table 21).

The table 22 presents biochemical parameters of protein, carbohydrate and lipid into the muscle and hepatopancreas of experimental shrimp *Penaeus monodon* in three sublethal levels concentrations of 96 hrs. The observations found in table 18 shows a decrease in muscle protein the concentration of lead nitrate present in muscle tissue in three sub lethal concentrations SLC – I, SLC – II and SLC – III are 64.1 to 60.45 for muscle protein, 13.85 to 12.1 for muscle carbohydrate and 08.23 to 06.6 for muscle lipids all the three show a decrease in post exposure to lead nitrate similarly hepatopancreas protein shows a decreasing trend from 55.1 to 50.5 carbohydrate, 17.60 to 14.42 and lipids 18.00 to 15.86 respectively (Table 22).

4.7. BIOACCUMULATION

4.7.1. Bioaccumulation in *Mystus gulio*

Control

The lead levels were below the detectable limit for the following tissues skin, gills, muscle, liver, kidney and gonads.

The table 23 presents bioaccumulation experiments of lead nitrate accumulation in various tissues of *Penaeus monodon* in acute toxicity studies (mg/g). Various tissues were analysed for the accumulation test during toxicity studies. Gill shows the highest level of lead nitrate
absorption (3.230 mg/g) because water enters direct and makes contact with this tissue and it’s vital respiratory organ for fish. Similarly muscle shows least value (0.035 mg/g) (Table 23).

4.7.2. Bioaccumulation in Penaeus monodon

Control

The vital organ like muscle and hepatopancreas were studied and the lead nitrate was not found in the tissues and they are below the detectable limits lead nitrate treated tissues.

The table 24 presents bioaccumulation experiment of lead nitrate in various tissues of Penaeus monodon in acute toxicity studies (value in μg/g dry wt.). The muscle shows a minimal deposit of 124 μg/g for 48 hrs and 360 μg/g for 96 hrs. Hepatopancreas with 2335 μg/g for 48 hrs and 3280 μg/g for 96 hrs. Hepatopancreas accumulates more lead nitrate comparing muscle tissue (Table 24).

4.8. DEPURATION

4.8.1. Depuration of Mystus gulio

The table 25 presents metal elimination of various tissues (mg/g). The rate of depuration was high in kidney and lower in gills. This is because of detoxification process by liver and next is kidney (Table 25).
4.8.2. Depuration of *Penaeus monodon*

The table 26 presents depuration experiment of metal elimination in various tissues of shrimp *Penaeus monodon* in acute toxicity studies (value in gm %). Depuration experiment of metal elimination in various tissues of shrimp *Penaeus monodon* in acute toxicity studies (value in μg/g dry wt.). The muscle is the least that depurates 190 μg/g at 48 hrs and 92 μg/g at 96 hrs. The hepatopancreas deputation 785 μg/g at 48 hrs and 236 μg/g at 96 hrs (Table 26).

4.9. HISTOPATHOLOGY

4.9.1. Histopathological studies of *Mystus gulio*

Organ: Gill (Control) C. S.

The gills are well developed nourished and the primary gill filaments are attached to the bronchial arch and each supported by an independent gill ray. A number of secondary lamellae are seen attached on both sides of primary filament any they are the main part of gaseous exchange the C.S of gill shows secondary lamellae comes out as finger shaped form the supportive rays which acts as skeleton. Histologically, the gill shows clearly the occurrence of connective tissues, core cartilage, glandular epithelial cells and filamentous cells excluding arterioles and veinules.

Layers cuboidal cells occur on either side of the cartilage and nuclei are stained with haemotoxylin. Filaments come from rachis and traverse the cuboidal cells. The shape of the filament cells are sphericla or
oblong with a nuclei. The cytoplasm stains more with eosin than cuboidal cells. The cuboidal cells are glandular and responsible for secretion of mucous but the filament cells are essential for respiratory functions (Plate 5).

**Effect of Lead nitrate treatment (Gill) C. S.**

**Sublethal Conc. I (09.60 mg/l)**

In this concentration the damages are not that much high. The arrangement of primary gill filaments and other cells and the cellular arrangements exhibit mild damage. The inter cellular space and lamellar epithelium are with mild damage (Plate 6).

**Sublethal Conc. II (15.50 mg/l)**

In this concentration the treated gill fish shows structural changes, Necrosis in cells and erosion are seen lifting of lamellar epithelium and inter lamellar space seen. Abnormal gill cells and gill tips were damaged. The supportive rays which acts as skeleton damaged. The connective tissues, core cartilage, glandular epithelial cells and filamentous cells are wish structural damages (Plate 7).

**Sublethal Conc. III (26.50 mg/l)**

The lead nitrate treated fish gill exhibits structural changes of rachis and structural integrity is lost in most parts except staining. The cytoplasm and nuclei of glandular cells are less stained with haematoxylin and eosin due to the loss of compactness of glandular cell.
Comparing with control the filament shows necrosis at some places. Necrosis and erosion of the filaments are seen in the distal ends. Blood vessels are compact. The lead nitrate has impact over the gill cells (Plate 8).

**Effect of Lead nitrate treatment (Liver) C. S.**

**Organ Liver (Control) C.S**

This organ is enclosed by a layer of connective tissues with an inner layer of epithelium. The sections of liver narrates the presence of hepatocytes with spherical, oblong, elliptical, or trihedral in shape with different sizes centered by a nucleus. Hepatocytes present radiating from bile ductile to which their secretions are poured. The sections show lacunae which separates the hepatocytes to many lobules and bile ductile and veinule are seen. Eosinophilic cytoplasm seen. At regular gaps bile ductless are seen. Glycogen seen in scattered and stained with heamatoxylin and eosin (Plate 9).

**Lead nitrate treated liver C. S.**

**Sublethal Conc.I (09.60 mg/l)**

In this concentration the cell damages are less compared to the latter the deformities and cellular arrangements are altered. Mild structural changes observed (Plate 10).
**Sublethal Conc.II (15.50 mg/l)**

The treated fish shows changes in the staining. The internal structures get disturbed, the Hepatocytes present radiating from bile ductile (to which their secretions are poured) get damaged and dilated (Plate 11).

**Sublethal Conc.III (26.50 mg/l)**

The lead nitrate treated fish hepatocytes show difference in shape, size and staining. There are structural changes in the shape of hepatocytes with polygonal. The normal spherical nuclei are changed, the nuclei are made as polymorphic structures than the normal spherical one. Sinusoids are filled with spacy secretary materials. The bile ductules get damaged dilated. The cytoplasm of the hepatocytes less stained (Plate 12).

**Organ Kidney (Control) C. S.**

The control kidney shows the presence of Nephrons (Bowman’s capsules and glomerulus, interstitial tissues and vascular structures. The renal tubule has proximal, intermediate and distal tubules along with collecting duct. Cuboidal cells were seen in Bowman’s capsule and Glomerulus appear as vascular capillaries. Columnar cells seen in proximal and intermediate tubules with narrowing of lumen. Cells of the proximal tubule are stained high with eosin than the cells of Bowman’s capsule. The section shows vascular structures in the form of sinusoids. Interstitial tissues are compared with parenchymatous cells having nuclei haematoxylin stained (Plate 13).
Lead nitrate treated Kidney C. S.

Sublethal Conc. I (09.60 mg/l)

In this concentration the cell damages are less compared to the latter. As the concentration of the chemical is milder the cells show minor damages. Mild necrosis of the tissues observed (Plate 14).

Sublethal Conc. II (15.50 mg/l)

The lead nitrate treated kidneys exhibit abnormalities. Cellular damage observed with structural changes. Major cellular necrosis observed. Slight structural changes in the Bowman’s capsules and Glomerulars (Plate 15).

Sublethal Conc. III (26.50 mg/l)

The internal structures of the kidney show abnormalities of Bowmans’ capsules and Glomerulars. Thickening of corpuscles walls. The interstitial tissues are eroded. Tubules have no structural organization. Interstitial cells damaged found with large vacuoles and fissures. All cells exhibit necrosis due to the toxic effect of the chemical. Sinusoids dilated with change in their shape. Most of the cells stained with Haematoxylin (Plate 16).

Organ Muscle (Control) C. S.

The muscle cells present with elongated nuclei. Nerves and blood vessels seen. Deposition of glycogen seen between muscle blocks. Multinucleated striated musclecells are seen. Nuclei are elongated.
Glycogen deposits observed in between muscle blocks. Nerves and blood vessels are seen at regular intervals of the muscles (Plate 17).

**Lead nitrate treated muscle C. S.**

**Sublethal Conc. I (09.60 mg/l)**

The lead nitrate treated muscle blocks show mild degeneration and muscle blocks get mild deformation. Necrosis observed in between muscle blocks. As the concentration of the chemical is less the effect and damage of the tissue are less comparing to the latter one (Plate 18).

**Sublethal Conc. II (15.50 mg/l)**

The lead nitrate treated muscle blocks show degeneration and muscle blocks get standard deformation and vacuolation. Necrosis in between muscle blocks. Deformation of blood vessels seen (Plate 19).

**Sublethal Conc. III (26.50 mg/l)**

The lead nitrate treated muscle blocks show degeneration and muscle blocks get standard deformation and vacuolation, degeneration of sarcolemma (Plate 20).

**Organ Testis (Control) C. S.**

Epithelial and connective tissue layers seen. The testis filled with spherical or tetrahedral seminiferous tubule separated by connective tissues. The seminiferous tubule has linings of sertolicells and germinal epithelium which gives rise to spermatogonial cells which are big in size.
The spermatogonial cells divide mitotically to become primary spermatocytes later develops to secondary spermatocytes later as spermatids which gets changes and becomes as a mature sperms from the central part of lumen. Interstitial cells (Leydig cells) seen between lobules with prominent nucleus. Spermatogonial cells and sertolicells are stained more and less equal (Plate 21).

**Lead nitrate treated testis. C. S.**

**Sublethal Conc. I (09.60 mg/l)**

As the concentration of the test chemical are comparatively lesser the tissues exhibit less damage. Mild damage and alternations of the interstitial calls and sertoli cells observed (Plate 22).

**Sublethal Conc. II (15.50 mg/l)**

Walls of the seminiferous tubule damaged and the shape changed. The interstitial and sertoli cells alterations of interstitial cells and sertoli cells are observed. The interstitial cells and lumen are deformed (Plate 23).

**Sublethal Conc. III (26.50 mg/l)**

The seminiferous tubules its shape deformed and rupture and the wall are damaged. The distribution of different stages of spermatocytes absent in the seminiferous tubule. The seminiferous tubules with layers of cells stained with hamemotoxylin seen. The seminifrous tubules are thick.
Spermatid and sperm cells are seen in the lumen but interstitial cells and lumen are deformed. The interstitial and sertoli cells are altered (Plate 24).

**Organ ovary (Control) C.S.**

The ovary is well developed and constructed by two layers one an outer connective tissues cell, an inner germinal layer. With frequent gaps the germinal bulky at ovocoel and proliferates the primordial germ cells which later becomes as oogonia cells. Oogonia cells are found along the lamellar folds. Single nucleus seen in each cells and oogonia seen in groups as nest. The C.S shows 7 stages of oocyte development.

**Stage 1**

The first stage shows oocytes came out of oogonia. Multi nucleoli present in the nucleus. A strip of basophilic cytoplasm surrounds nucleus of oocyte. The oocyte is surrounded by incomplete layer of simple squamous cells indicating the follicular layer from oocytes (Plate 25).

**Stage 2**

Nuclear (Yolk) present near nuclear membrane. Nucleoliformed in early stage 1 have moved to the periphery of nucleus and nucleus and nucleolus becomes chromophilic in this stage (Plate 26).

**Stage 3**

Nuclear contents get disappeared and released in to cytoplasm. A layer of follicular cells envelops the oocyte. Cytoplasm is basophilic but
intensity of haemotoxylin eosin staining make a difference between peripheral and central zones of cytoplasm (Plate 27).

Stage 4

The oocyte gets surrounded by follicular cells and with another layer called vitelline membrane also covers. Corticular and granular layers seen in periphery of cytoplasm in oocytes. The nuclear membrane undulated and stained with haematoxylin (Plate 28).

Stage 5

The oocytes appear bigger with yolk granules and vesicles. Vitelline membrane and zona radiate are seen in this section. Yolk granules deposited from periphery of oocyte and found distributed all over the cytoplasm (Plate 29).

Stage 6

The size of the yolk granules and oocytes bulges out thus sized up and yolk granules and globules are seen in cytoplasm. The oocyte has another covering called theca outer to follicular layer made of fibroblastic cells (Plate 30).

Stage 7

Oocyte is fully packed with yolk materials. Nucleus is not prominent and moves towards animal pole. Haematoxylin stained granules seen (Plate 31).
Stage 8

Well developed ripe oocyte, the cytoplasm is loaded with yolk material with three layers envelop 1. Vitelline membrane, 2. Zone radiate, 3. Theca (Plate 32).

Lead nitrate treated ovary

Sublethal Conc. I (09.60 mg/l)

As the concentration of the test chemical are comparatively lesser the tissues exhibit less damage. Mild damage observed. Germinal epithelium and the follicular layers show mild deformation and mild necrosis (Plate 33).

Sublethal Conc. II (15.50 mg/l)

Structural changes of germinal epithelium seen. Erosion and necrosis are seen. Follicular layer shows deformation and necrosis. Cell damage observed. Follicular layer covering of the oocyte damaged (Plate 34).

Sublethal Conc. III (26.50 mg/l)

Follicular layers shows deformation and necrosis. The germinal epithelium looks like damaged in structure. Oocytes are seen.

Without follicular layer covering. Follicular layer shows necrosis and deformation. Yolk depositions are disturbed in the later stages of oocyte. The secondary vitellogernic oocytes are absent. The ovaries show
deformation in the muscle layer of their walls. Erosion and necrosis are observed. The shape of the vitellogenic oocyte are affected. Fissures appear in the yolk cytoplasm (Plate 35).

4.9.2. Histopathological studies of *Penaeus monodon*

*Penaeus monodon* was exposed to sublethal concentrations of Lead nitrate. The experiment was conducted for 24 hrs, 42 hrs, 72 hrs and 96 hrs in 3 sublethal concentrations. SLC-I-1.66 mg/L, SCL-II-3.33 mg/L, SLC-III-6.60 mg/L this is an economically important species cultured in India. The microscope study of the tissue provides essential knowledge of living cells as the basic building blocks of all living organisms. It examines the structural organization of the cells in different tissue types allowing better understanding of morphological and physiological process of different organs.

**Control Hepatopancreas**

In the control shrimps, the hepatopancreas exhibited a well organized glandular tubular structure. The tubules were closed distally, but opened out proximally into ducts which, in turn, united to form longer ducts that were ultimately connected to the digestive tract. A single layer of epithelial cells can be found lining the tabules. The cells showed normal differentiation into E (embryonic) cells, at the narrow distal end of the tubule; young R (restzellen) cells; F (fibrillenzellen) cells, a short distance away from the distal region; and B (blasenzellen) cells, in the middle and proximal regions of the tubules. Some of the E-cells exhibited
figures. The B-cells exhibited apical secretory granules, the R-cells were found to contain high amounts of rough endoplasmic reticulum and lipid droplets, and the F-cells were found to be nonvacuolated and deeply stained. The interstitial sinuses between tubules were histologically normal x 400 (Plate 36).

**Effect of Lead nitrate treatment (Hepatopancreas) C.S.**

The histopathology, compared with those of the control group (Figure A), the number of R-cells ® in the tubular epithelium of the hepatopancreas of the group treated with 1.66 mg/L lead showed a significant increase and abnormal lumen (ALU) were observed (Figure B). In the group exposed to 3.33 mg/L lead, abnormal lumen (ALU) and hemocytic infiltration (HI) in the interstitial sinus (IS) were observed (Figure C). Exposure to 6.60 mg/L lead resulted a continuous increase in hemocytes in the interstital sinus, severe abnormal lumen (ALU), coagulation (CO) and necrotic tissue cells (N.T).

**Sublethal Conc.I (1.66 mg/L)**

Lead nitrate induced alterations in the histoarchitecture of the hepatopancreas of *Penaeus monodon*. Presence of greater number of R-cells (Restzellen cells) and significant increase of abnormal lumen (ALU) in hepatopancreas tubules x 400 (Plate 37).

**Sublethal Conc.II (3.33 mg/L)**

Lead nitrate induced alterations in the histoarchitecture of the hepatopancreas of *Penaeus monodon*. Abdominal lumen (ALU) and
hemocytic infiltration (HI) in the interstitial sinus was observed x 400 (Plate 38).

**Sublethal Conc.III (6.60 mg/L)**

Lead nitrate induced alterations in the histoarchitecture of the hepatopancreas of *Penaeus monodon*. Severe abnormal lumen (ALU) and hemocytic infiltration (HL) in the interstitial sinus was observed. Coagulation (CO) in the thickened basal laminae and Necrotic hepatopancreas tubule (NT) are observed.

The observed histopathological damages were documented as a general tubular necrosis lesion, the separation of necrotic cells of the hepatopancreas from basal laminae, and the formation of hemocytic infiltration in the interstitial sinuses (Plate 39).

**Gill Histopathology of Penaeus monodon**

**Control**

Inter lamellar space normal, No Haemocytes infiltration, Gill tips are normal Cells are normal, pillar cells are normal, Haemocytes not clear, No cell necrosisiof gill lamellae and cells, Epithelial cells are normal L, Lamellar epithelium are normal. The gills are well developed nourished and the primary gill filaments are attached to the branchial arch and each supported by an independent gill ray. A number of secondary lamellae are seen attached on both sides of primary filament any they are the main part of gaseous exchange the C.S. of gill shows secondary lamellae comes out
as finger shaped from the supportive rays. Histologically, the gill shows clearly the occurrence of connective tissues, core cartilage, glandular epithelial cells and filamentous cells excluding arterioles and veinules. The shape of the filament cells are spherical or oblong with a nuclei. The cytoplasm stains more with eosin than cuboidal cells. The cuboidal cells are glandular and responsible for secretion of mucous but the filament cells are essential for respiratory functions (Plate 40A and B).

**Sublethal conc. (1.66 mg/L)**

The impact of lead nitrate shows mile structural changes and damage on the basal attachment of gill. Deformities in the (Branchial arch) basal gill attachment area. The cells shows signs of damage and deformities (Plate 41).

**Sublethal Conc.II (3.33 mg/L)**

In this concentration the inter lamellar space seen, lifting of lamellar epithelium. Nectrosis of gill lamellae and cells. Abnormal gill tips were damaged (Plate 42A and B).

**Sublethal Conc.III (6.60 mg/L)**

Deformities in the (Branchial arch) basal gill attachment area. The cells show signs of damage and deformities. Abnormal gill cells and gill tips were damaged. Vaculation (Lacunae) seen.

Pillar cells damaged, proliferation of epithelium. Lifting of Lamellar epithelium. Necrosis and erosion of the filaments are seen in the distal ends. The lead nitrate has impact over the gill cells, primary gill
filaments are attached to the branchial arch damaged. Extensive damage of gill structure and cellular alterations are observed (Plate 43A-C).

4.10. STATISTICAL ANALYSIS
4.10.1. Relationship between the estuarine fish *Mystus gulio* Vs Biochemical parameters

The female protein and lipid content are positively correlated to the estuarine fish *Mystus gulio*. The carbohydrate and lipid (male only) and lipid content are negatively correlated to the *M. gulio*. The detailed results are given by the table 27.

4.10.2. Relationship between the biochemical parameters of marine shrimp *Penaeus monodon*

The protein content of 24, 48, 72 and 96 hrs will be positively correlated. The carbohydrate content of the various time estimation are negatively correlated, and the lipid content of the various hours are negatively correlated. The results are given in Table 28.