CHAPTER X
SUMMARY AND CONCLUSION

The estuaries are unique environments that play an important role in the transfer of products of continental weathering to the ocean. Because of the major changes in physico-chemical conditions between fresh and saline waters, the distribution of chemical constituents between dissolved and particulate forms may be modified by interactions in estuaries. Traditional conventional method of coconut husk retting has adverse impacts on the ecosystem, including fauna, flora and human beings. Lack of dissolved oxygen, very high biological oxygen demand, chloride, hardness, nutrients and low pH, with foul smell of hydrogen sulphide are the characteristic features of the coconut husk retting yards. The introduction of toxic pollutants like hydrogen sulphide into a cell may interfere with their cellular functions, leading to an impaired cell function or viability. Concerns about these contaminants range from possible harmful effects on the ecosystem to possible harm to humans consuming such contaminated organisms. The importance of monitoring and preserving the aquatic environment cannot be overemphasized, because water provides the life support system for aquatic life and all life forms. Hence protection of aquatic resources is essential in protecting the entire ecosystem.

The present study was conducted to assess the physio-biochemical, haematological and histopathological changes in fishes collected from the hydrogen sulphide contaminated waters. Kadinamkulam estuary is located in Thiruvananthapuram district, Kerala and is a fresh water estuary which joins the Veli Lake and connected to Arabian Sea. A detailed survey was conducted to identify the fish species found in retting and non- coir retting areas of the estuary. Fish samples (Oreochromis mossambicus) were collected from the selected coir retting areas and non-retting areas during the premosoon, monsoon and post monsoon seasons from March 2006 to February 2007. Water and sediment samples were also collected from the selected study stations for physico-chemical analysis.

The surface and bottom water temperature were higher in the polluted retting zones with respect to the non retting zone. The highest temperature of 34°C was observed in the surface and bottom water in station I during post monsoon season. In the retting zones, the temperature regime showed that the average temperature values were highest for the premonsoon and post monsoon, and lowest for the monsoon period. The pH shows clear stratification in all stations. The bottom water pH was higher than that of the surface water pH in all stations. The maximum values in pH were observed during premonsoon season. The higher pH values were detected in the mouth of the estuary. The seasonal averages of conductivity values in the surface and bottom water showed that the monsoon season indicated the highest values followed by pre monsoon and post monsoon seasons. The intensive retting activities and poor water circulation might be the reason for the fluctuation in turbidity values. The coconut pith settled at the bottom along with the ret liquor can greatly increase the turbidity of water in retting zones. The maximum turbidity was reported at station I, where the concentration of finely divided particles and decaying organic matter was very high.

Total dissolved solids (TDS) values shows seasonal fluctuations, comparatively high values were observed during monsoon season. The sand mining and massive clay mining also contribute the suspended solids in the water body especially in the pre and post monsoon periods. The maximum TDS content was observed in the bottom water samples of station IV and the minimum value was observed at station I. The salt water infringement during monsoon and high evaporation in post monsoon season enhances the TDS content of Kadinamkulam estuary. The maximum TSS content was observed during the monsoon season at station I, II and III. The intensive retting activity prevailing in this estuary enhances the TDS, TSS and TS content, the solid wastes discharged from Parvathy Puthanar is one of the other major external 9contributors of TSS into the estuarine environment which are non-degradable and may cause harmful effect to the flora and fauna in this estuary.

The coconut husk retting areas and organic rich municipal waste receiving areas of this estuary exhibits high biological oxygen demand. The organic load in the retting zones remains high resulting in greater microbial activity leading to total depletion of oxygen and consequently high biochemical oxygen demand values, eventually leading to a state of anoxia. Mixing up of estuarine water with sea water influences the decrement of BOD values in station IV. The highest biochemical oxygen demand value was at the highly polluted area i.e. Station III which is characterized by extensive retting process. The station III showed the minimum dissolved oxygen values in all seasons and the station IV, the non retting zone showed the maximum DO content. When the seasonal values were compared the monsoon period was found to be favourable for dissolved oxygen in retting areas. During the post monsoon and premonsoon periods a total depletion of dissolved oxygen was recorded in the bottom water at SI, SII and SIII. According to water quality standards for coastal waters marine outfalls (CPCB) the physico chemical parameters like pH, temperature, hydrogen sulphide, total suspended solids, hardness, BOD are above standard permissible limit and dissolved oxygen is found to be below standard permissible limit in retting areas of this estuary.

Hydrogen sulphide contamination in the retting areas in the estuary exhibited clear stratification between the surface and bottom waters, in that surface water H_2S concentration was always higher than the bottom water at all the three selected stations (SI, SII and SIII) in three seasons. The H_2S was almost absent in the water samples collected from station IV. This is may be due to the absence of retting process in this study station. Also the retting zones of this estuary showed comparatively high concentration of dissolved hydrogen sulphide in the surface and bottom waters during different seasons compared to non-retting zone. The maximum H_2S concentration was reported at station III, where extensive husk retting is being done. Moreover effective flushing is also absent in this region.

Soaking of coconut husk around the year enhances the production of large quantities of organic compounds in the medium, change the estuary into a polluted, hydrogen sulphide emanating, filthy and foul smelling water tract.

The retting process influences the salinity of Kadinamkulam estuary. In all the four stations studied the alkalinity was higher during premonsoon, followed by post monsoon and monsoon seasons. The highest alkalinity value was observed in station I during pre monsoon and the minimum values were observed in station IV. The end products of coconut husk retting make the surrounding water into stagnant and the substances like tannin, lignin and other biological materials enhance the alkalinity in retting areas. The sewage intrusion from the Parvathy Puttan Ar may also contribute the substances that will improve the alkaline condition of the Kadinamkulam estuary. The total hardness values show that the water is in the very hard category. Due to coconut husk retting activity, H_2S is massively produced in retting yards and retain it for a long time. Here the H_2S produced is converted into sulphate and sulphide. This sulphate may cause the permanent hardness to water in the retting areas and its long term effect is that it will contribute the permanent hardness of the water body. The bottom water salinity was higher than that of the surface water through out the period of study. The elevated level of salinity recorded during pre monsoon, monsoon and post monsoon seasons is due to conversion of this seasonal estuary into perennial one (opened bar mouth) in connection with the Mudalapozhi harbour construction.

Comparatively higher concentration of nitrite, nitrate and total nitrogen content was observed during the monsoon season at station II that receive sewage and organic pollution through the Parvathy Puthan Ar. The bottom water phosphorous concentrations were always higher than that of the surface water at stations II and III. The premonsoon season showed the highest phosphorous concentration followed by the post monsoon and the monsoon periods both in the surface and bottom waters at all the four stations. The acidic conditions in the retting zones and the presence of micro-organisms are the factors that favor the production of phosphate- phosphorous and this may be the reason for the relatively high values of phosphorous observed at S II and S III.

Comparatively higher sulphate values are noticed in all seasons in retting areas with respect to the non retting area. H_2S produced during retting process may retain their in the converted form as sulphate. The continuous retting activity in this estuary gradually increased the sulphate concentration in sediments and water. The silicate-silicon concentration in different stations showed significant fluctuation. The concentration of silicate showed an increasing trend from riverine region to estuarine region. The higher values of sodium were recorded during monsoon and lowest values were observed during premonsoon seasons. The potassium values were comparatively lower than the sodium concentrations. Here the minimum potassium concentration was reported at station II in bottom water samples. A comparatively greater value was obtained at the bar mouth region and this due to the influx of water from the sea through high tidal effect. The estuarine water with calcium values above 25 mg/L are classified as "calcium rich". In the present study the observed values of the estuarine water were in and around this value except station IV the bar mouth region. When the magnesium values exceed 50 mg/L, it imparts an unpleasant taste to water. The Mg values observed in the surface and bottom waters of all the stations and in all the seasons are within 50mg/L. The maximum phenol content was observed at station III during post monsoon season. The total phenol content in coir retting areas were found to be high compared to that of non retting areas through out the period of study.

Study on the heavy metal content showed minimum values (0.001 mg/L to 0.005 mg/L.) for lead in Kadinamkulam estuary was observed. Lead toxicity of fish and other aquatic organisms is significantly influenced by the water solubility and depends on the solubility of lead compounds and on the concentration of calcium and magnesium in water. The Mn content in different stations varied from 0.06 mg/L to 0.16 mg/L. The Zn concentration varied from 0.011 mg/L to 0.025 mg/L (SI) in post monsoon season respectively. The iron content of four different stations of surface water samples varied in accordance with the influence of surface water runoff during monsoon season. According to water quality standards for coastal waters marine outfalls (CPCB, WHO and BIS), Kadinamkulam estuarine water heavy metals are under standard permissible limit. Fish may be harmed by iron compounds in poorly oxygenated waters with low pH where iron is present mainly in the form of soluble compounds. Because the gill surface of the fish tends to be alkaline, soluble ferrous iron can be oxidised to in soluble ferric compounds which they cover the gill lamellae and inhibit respiration.

The textural characteristics of Kadinamkulam estuary reveals that the sediment shows muddy in retting associated stations and sandy nature in the non retting area Because of biological degradation the debris of coconut husk influences the muddy nature in station I, II and III. The allochthonous material, intensive husk retting in these stations were subject to biodegradation, which resulted to the high percentage of silt apart from the death and decay of vegetation in Kadinamkulam backwater system. The retting activity at stations I, II and III resulted in low pH values for sediments and higher pH in non retting area. The maximum conductivity was reported at station IV and minimum at station I and II. The organic carbon concentration showed at maximum value at station I and minimum at station IV. Coconut leaf wetting, husk retting and organic wastes from Parvathy Puthanar increase in percentage of organic carbon content in Kadinamkulam estuary.

Sodium concentrations in sediments show significant variations. The higher values are observed in station IV which is the bar mouth of the estuary. The Mudalapozhi harbor construction in Kadinamkulam estuary results the salt water intrusion into the estuary, this ultimately influence the salinity of the water body result in increase in concentration of sodium content in sediments. The highest concentration of potassium content was observed at station IV. The Vamanapuram river and the Parvathy Puthanar are two major external sources of potassium concentration in this estuary. The phosphate values showed its maximum at station

III and minimum at station IV in all three seasons. The major anthropogenic activity that polluting the backwater system is husk retting, especially at station I, II and III influence the phosphate concentration. The presence of organic waste from the coir retting process and organic rich pollutants from Parvathy Puthan Ar increases the nitrogen content at station I, II and III.

The peak values of iron content were observed in monsoon season at all stations was obvious as the metals were precipitated and settled in bottom sediments, brought as a surface run-off. The maximum concentration Mn was observed in station III and the minimum was observed in non retting area the barmouth region of the estuary were the sediment is sandy. The highest zinc concentration was observed in station III and minimum values were reported in station IV in all three seasons. The anthropogenic actions in the form of sewage disposal through Parvathy puthen Ar and coconut husk retting may increases the zinc concentration in Kadinamkulam estuary. Comparatively higher concentration of lead was observed in station II. The minimum concentration was observed in station IV during post monsoon. The urban waste and water transports through mechanized boat service contribute oil pollution and lead pollution in this estuary.

From the fish diversity survey it can be seen that the fish diversity in Kadinamkulam estuary was under threat. Earlier studies showed 37 fin fish species, five species of prawns, and two species of crabs and bivalves in the Kadinamkulam estuary, of these twenty species of fin fish species were identified in the retting zones. No shell fishes were observed in the retting zones in the former study. The present study showed the fish diversity is reduced to a total of 28 species, and among the 28 species only eighteen species were found in retting zones, no shell fishes were observed in the retting zones. The fish like *Sardinella* was not found in the retting zones but it can be seen in monsoon season near Pozhi, the non retting area. Ten fish species merely associated with non retting zones. Ten species rarely found in retting zones and seven species commonly found both in retting and non- retting zones. Among the 28 species of fishes identified, the fish

Oreochromis mossambicus was the abundant species in this estuary during all three seasons. On the other hand there is a plodding diminution in fishery resource during the study period. The non retting areas of Kadinamkulam estuary supports of shell fish diversity, but the abundance of this resource in retting zones was considerably reduced.

A clear disparity can be seen in Fulton's Condition Factor (FCF) of fishes in retting areas and the fishes found in non retting areas of Kadinamkulam estuary in all three seasons of the study period. The FCF of fishes in retting areas were less than that of the fishes in non-retting areas. A decrease in condition factor can be considered as a reflection of depletion of energy reserves. The maximum values for FCF of fishes were noticed in non retting areas of Kadinamkulam estuary.

Haematological studies in *Oreochromis mossambicus* reveals that the fishes in the stations with hydrogen sulphide pollution (retting zones) exhibit maximum decrement of red blood cells. The decrease in RBC count in fishes in hydrogen sulphide polluted coir retting areas might have resulted from the reverse anemic state or haemolysing power of toxicant particularly on the red cell membrane. The WBC count in fishes of retting areas shows relatively slight decline in all seasons with respect to the non retting station. The decrease in the number of WBC might be due to the failure of fishes to meet the pathological conditions arising from the toxicant stress. The haemoglobin content also shows decreases in the fishes of retting areas. Comparatively low levels of Hb indicated anemic condition in fish which might be due to the stress caused haemolysis. It was also observed low levels of haemocrit values in fishes of retting zones of Kadinamkulam estuary. The low Ht value indicates anaemia or oligohaemia or an alteration in the fish metabolism.

In the present investigation, liver, gills, kidney and brain of the fishes exposed to pollutants (hydrogen sulphide) in coir retting areas exhibited significant elevation MDA in accordance with the H_2S concentration in retting areas. In all three seasons studied the fishes in non retting zones have no significant difference in MDA values in their tissues. The liver tissue of fishes in retting areas recorded the highest percentage increase in MDA level when compared to that of fishes in non-retting areas. This might be due to the elevated oxidation of molecular oxygen to produce superoxide radicals indicating the importance of liver in the detoxification process. The extent of lipid peroxidation in this study may also be attributed to the fact that extent of hydrogen sulphide toxicity pressurize the life sustaining capacity of organisms in this aquatic ecosystem.

The liver, gill, kidney and brain tissue of Kadinamkulam estuary exhibited a season wise and H_2S concentration dependent decline in GSH. The non retting area of this estuary shows normal level of GSH. The GSH level was significantly reduced during post monsoon season in all tissues from the retting zones. In comparison with the tissues of fishes from non retting zone, the medium and high H_2S concentrations in retting zones of Kadinamkulam estuary persuades the superoxide dismutase activity in liver, gill, kidney and brain tissues. The fishes subjected to higher concentration of H_2S exhibits significant decline in SOD activity in premonsoon and post monsoon seasons. The accumulation of toxicants in the body of fishes might have led to the production of superoxide anions which led to the induction of SOD to convert the superoxide radical to H_2O_2. SOD catalytically scavenges superoxide radical which appears to be an important agent of toxicity of oxygen and this provides a defence against this aspect of oxygen toxicity. In comparison with non retting zone, the fishes in stations with intensive coconut husk retting process and with higher concentration of hydrogen sulphide showed significant increase in the tissue catalase activity. In Kadinamkulam estuary the order of increase in catalase activity in tissues of fishes are as follows: liver > Gill > kidney > brain.

When compare with the GPx activity from non retting zones of this estuary there is a sharp decrease in tissue GPx activity of fishes in retting areas and it is in the order live > kidney > brain > liver >gills. The low activity of GPx in different tissues of H_2S exposed fish in coir retting areas demonstrated the incapability of these organs in neutralizing the impact of peroxides. In comparison with GST activity in non retting zone, the retting zones with highest H_2S concentration in pre and post monsoon seasons, all the four tissues of the fish exhibited significantly maximum inhibition in GST activity. The reduction in GST activity level noted at the highest concentrations of H_2S indicated the impaired detoxification mechanism of the fish under long-term exposure. When compared to non retting zone the LDH activity in fish tissues exhibits hydrogen sulphide dependent increase in LDH activity was noticed in all four tissues from retting zones in all seasons. The LDH activity shows significantly maximum values in kidney and liver tissues followed by gills and brain tissues. The most pronounced increase was recorded on kidney tissues in post and pre monsoon seasons. The increase in the LDH activity of all tissues indicated the effect of H_2S stress in causing / triggering of anaerobic metabolism. The elevated LDH activity level in all the four tissues affected with the pollutants like H_2S further indicated that the fish depended mainly on anaerobic metabolism for energy requirements.

A significant elevation in alkaline phosphatase activity was observed in liver, gill, and kidney tissues of the fish subjected to hydrogen sulphide pollution in retting areas, the non retting areas of Kadinamkulam estuary exhibits the normal ALP activity in all three seasons. In all three seasons the higher concentration of protein content was reported in tissues representing the unpolluted area. The fish tissues from retting zones with high hydrogen sulphide content shows decrease in protein content. Because of oxidative streets due to hydrogen sulphide and other toxic circumstances in retting zones influence the decrement of protein content in fish tissues.

Distinct histological alterations were observed in the liver, gill, and kidney tissues of the fishes subjected to hydrogen sulphide pollution in this estuary. These histological changes in the vital organs have affected their normal functioning and these were reflected in the biochemical and haematological perturbations exhibited by the fish. The liver tissue exhibits vacuolation of hepatic cells, appearance of blood streaks, visible nucleus with hyperhaematoxyllinophilia, necrosis, and degradation of hepatic cytoplasm. The other changes such as vacuolization, nuclear hypertrophy, lymphocyte infiltration and degraded cells were also detected in hepatic tissues of fishes collected from the retting and non retting area. The histological analysis of control gill tissues exhibit some structural alterations but the tissues from retting areas exhibits clubbing of secondary gill lamellae, hyperhaematoxyllinophilia, curling of secondary gill lamellae and dilation of primary gill lamellae.

The histology of control kidney shows normal characteristics but some of the abnormalities found in this station also; such as hypotrophied convoluted segments, swollen renal tubules and inflammatory cells. The other tissues from retting zones exhibits inflammatory cells, swollen renal tubules, decrease in size of epithelial cells, increased tubular lumen, hypotrophied convoluted segments, vacuolation, degradation of tubules, glomerulus, formation of vacuoles in the renal interstitial tissue, necrosis, heavy infiltration of inflammatory cells with some debris and decrease in size of the epithelial cells respectively. The analysis of the seasonal variation in the histological parameters leads to the conclusion that the changes observed in the three organs were apparently related to the pollutants in the retting zones of Kadinamkulam estuary.

The aquarium experimental study reveals the changes in biochemical, behavioural, haematological and histopathological changes in two different concentrations of hydrogen sulphide exposure. In the present experimental study the pH, temperature, sulphate concentrations of aquarium water in test groups shows marginal increase with respect to the control aquarium water. Here the marginal increase in concentration of physico-chemical parameters was in accordance with the concentration of hydrogen sulphide. But the dissolved oxygen concentration shows decreases in initial stage and recovers its concentration slightly at the end of the experimental period (96 h).

Oreochromis mossambicus exposed to hydrogen sulphide for two different concentrations (4.9 and 6.6 mg/L) exhibited noticeable changes in morphological and behavioural characteristics when compared to control fish. The skin colour of the exposed fishes becomes dark grey in comparison with normal yellowish to olive brown colour. The black spots on fins were also found to loose their intensity under H_2S exposure. Further, there was copious secretion of mucus under hydrogen sulphide exposure. The morphological changes in dead fishes were observed, and it showed change in colour of the gill lamellae from reddish to dark brown and coagulation of mucus on gill lamellae. The body weight of fishes in T_1 and T_2 groups showed no significant change compared to that of control fishes. There is a schooling behavior was observed to be disrupted and the fishes occupied twice the area than that of the control group. They were spread out and appeared to be swimming independent of one another. And they exhibited irregular, erratic and darting movements followed this with imbalanced swimming activity. The fish progressively showed signs of tiredness and lost positive rheotaxis characterized by weakness and apathy after 48 hours of experiment. The fish exhibited peculiar behaviour of trying to leap out from the H_2S exposed medium, which can be viewed as an escaping phenomenon. The frequency of surfacing phenomenon was greater in fishes in T_2 group wherein the fish frequently come to the water surface. In both test groups, respiratory disruption was observed in the normal ventilating cycle (cough, yawn) with a more repeated opening and closing of the mouth and opercular coverings. Partially extended fins and singlewide opening of the mouth and opercular coverings accompanied by hyperextension of all fins were found and the fish was in a state of excitement after 12 hours.

The T_1 and T_2 test group fish's exhibits dose dependent change MDA content. With respect to time the MDA content gradually increases in all four tissues during the 96 hour of the experiment. With respect to duration of exposure the maximum increase in percentage was observed in liver followed by gills, kidney and the brain tissues. There is a dose dependent variation in glutathione content was observed in T_1 and T_2 test groups. In all four tissues the glutathione concentration shows significant increases during the initial H_2S exposure and then it gradually reduces towards the 96 hour of the experiment. In two test group organisms the maximum percentage of decrement in glutathione content during the 96 hour was observed in brain followed by Kidney < Gill < Liver tissues.

The superoxide dismutase activity shows an initial significant increase with respect to hydrogen sulphide concentration in all four tissues After the initial steep increment all four tissues shows duration dependent decrement in enzyme activity towards the 96^{th} hors of the experiment. In comparison with the control fish catalase activity, the liver, gill, kidney and brain tissues of the exposed fish showed an initial elevation and inhibition in enzyme activity during the two H_2S exposure periods. In hydrogen sulphide exposed fish the highest decrease in activity was observed in liver > gill.> kidney > brain.

When compared to the control GPx activity in fish tissues, there is a dose dependent increase in glutathione peroxidase activity was observed in test group fishes during the initial stages of experiment. When the fish exposed to hydrogen sulphide for a longer period influence the reduction in enzyme activity was observed in T_1 and T_2 test groups. The low activity of GPx in different tissues of H_2S exposed fish demonstrated the incapability of these organs in neutralizing the impact of peroxides. The decreased level of GPx in the intoxicated fish might debilitate the antioxidant defence system of the fish which would eventually affect their survival. When compared to the control fish LDH activity of the test group fish tissues shows significant dose dependent increase up to the middle stages and marginal decrement in activity during the 96^{th} hours of experiment. The increase in the LDH activity during the initial stages indicated the effect of H_2S stress in causing / triggering of anaerobic metabolism in fishes.

A significant dose dependent increase in ALP activity was observed during the initial stages in liver, gill, kidney and brain tissues of T_1 and T_2 test group fishes. There is a dose and duration dependent decrement in protein content was recorded in all the four tissues of fishes in both the experimental periods. In the present investigation the maximum decrement in protein content was observed in liver followed by gill, brain and kidney tissues. The protein depletion in treated fishes was the physiological strategy played by the fishes to adopt itself to the changed metabolic systems. This leads to degradative processes like proteolysis and utilization of degraded products for increased metabolism.

Haematological parameters have been recognized as valuable tools for the monitoring of fish health. In the present study a significant dose and duration dependent decrease in RBC count, WBC count, haemoglobin content and haematocrit value was observed in the blood of *Oreochromis mossambicus* as a response to hydrogen sulphide exposure. The reduction in Hb content in H_2S exposed fish could be due to the inhibitory effect of the toxic substance on the enzyme system in the synthesis of Hb and the low Ht value would indicate anaemia or oligohaemia or an alteration in the fish metabolism.

Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The histological studies of hydrogen sulphide exposed fish tissues like liver, gills and kidney showed marked histological changes in different exposure periods. The hepatic tissues of fishes in T_1 test group exhibits appearance of blood streaks, vacuolation of hepatic cells during the 24 hour. After 96 hours, hyper haematoxyllinophilia were found. In T_2 test group fishes, appearance of blood streaks were detected in the liver tissue during the 24 hour, and in 96 hours it exhibited vacuolation of hepatic cells, appearance of blood streaks and degradation of hepatic cytoplasm. The degeneration of hepatic tissues and necrosis may be due to the cumulative effect of dissolved hydrogen sulphide and the increase in their concentrations in the fish liver.

The gills of T_1 test group fishes showed dilation of primary gill lamella and clubbing of secondary gill lamellae during the 24 hour, and in 96 hours the gills has clubbing of secondary gill lamellae and appearance of hyper haematoxyllinophilia. There is clubbing of secondary gill lamellae in 24^{th} hour, during 96 hour the gill tissues exhibits dilation of primary gill lamellae, mild hyperplasia of gill lamellae, hyper haematoxyllinophilia and clubbing of secondary gill lamellae are visible.

The histological studies in the kidney tissues of T_1 test group fishes showed swollen renal tubules, and decrease in size of epithelial cells during the 24 hour, and in 96 hours the structural alterations include swollen renal tubules and formation of vacuoles in the renal interstitial tissue. In T_2 test group fishes, the kidney tissues exhibited decrease in size of the epithelial cells, and formation of vacuoles in the renal interstitial tissue during the 24 hour. But hypotrophied convoluted segments in the tissue and swollen renal tubules are found during the 96 hours of H_2S exposure.

10.1 Conclusions

Kadinamkulam backwater, being the largest in Thiruvananthapuram district, holds great environmental, economic and social significance. Because of human intervention the estuary is facing serious environmental deterioration like intensive coconut husk retting, sand mining and land reclamation. The below detectable levels of dissolved oxygen and high amount of hydrogen sulphide makes this water body as a puddle of toxic pollutants. There is a plodding diminution in fishery resource was observed in the study period. The earlier research studies in this estuary recorded 37 fin fish species, now it is reduced into 28 species. Because of uncontrolled pollution in the estuary mainly due to coconut husk retting increased the loss of fish diversity in this estuary. Aquatic ecosystems should be protected against all kinds of adverse conditions which may lead to dramatic changes in the health of its inhabitants. Fishes are more susceptible to stress than many other animals because of a greater dependence upon their surrounding environment. The pollutants like hydrogen sulphide in the retting area caused oxidative damage to the fish tissues especially to the liver, kidney, brain and gills. The aquarium study also proved that the hydrogen sulphide at sublethal and medium lethal concentrations altered the rate of lipid peroxidation and activities of antioxidant systems in various organs of the test fish, *Oreochromis mossambicus*. The lipid peroxidation product malondialdehyde in tissues are better indicators for the oxidative damage in aquatic organisms. The damage may alter cell functions through changes in intracellular calcium or intracellular pH, and eventually can lead to cell death.

Both the experimental and field study about the extent of lipid peroxidation reveals the fact that the extent of hydrogen sulphide toxicity pressurizes the life sustaining capacity of organisms in this aquatic ecosystem. The highest percentage of increase in MDA in liver reveals the elevated oxidation of molecular oxygen to produce superoxide radicals indicating the importance of liver in the detoxification process. The increased GSH level in fishes could be an adaptive mechanism to slight oxidative stress but decreased GSH level could be due to loss of adaptive mechanisms and the oxidation of GSH to GSSG. The oxidative damage caused by metabolites of the toxicant could be mediated by uncoupling of mitochondrial oxidative phosphorylation, which would generate reactive oxygen species. The detoxification of ROS and hydroperoxides implied the oxidation of reduced glutathione to glutathione by peroxidase. The superoxide radical by itself or after its transformation to H_2O_2 caused a strong oxidation of the cysteine in the enzyme, and decrease in the superoxide activity in fishes when they are exposed to H_2S. The accumulation of toxicants in the body of fishes might have led to the production of superoxide anions which led to the induction of SOD to convert the superoxide radical to H_2O_2.

The oxidative stress due to hydrogen sulphide caused an increase in catalase activity and is an adaptive response against antioxidants. The low activity of glutathione peroxidase in different tissues of H_2S exposed fishes in retting area and in test groups demonstrated the incapability of these organs in neutralising the impact of peroxides. A decline in Glutathione-S-Transferase in tissues suggests a failure in detoxification and occurrence of oxidative stress in the cells. The increase in the lactate dehydrogenase activity of all tissues indicated the effect of H_2S stress in causing/triggering of anaerobic metabolism for energy requirements and the increase in alkaline phosphatase activity might indicate increased uptake of certain metabolites and ions. The protein depletion in hydrogen sulphide exposed fish was the physiological strategy played by the fish to adopt itself to the changed metabolic system leads to degradative process like proteolysis and utilization of degraded products for increased metabolism. The Fulton's condition factor of fishes in retting areas was less than that of the fishes in non-retting areas. A decrease in condition factor can be considered as a reflection of depletion of energy reserves.

The morphological and behavioural changes articulated by the fishes imply the intensity of H_2S poisoning. The general haematological parameters of fishes in hydrogen sulphide contaminated water in estuary and in controlled experimental conditions showed slight decline in their levels. The low haematocrit value noted in the fishes of H_2S contaminated water would indicate anaemia or oligohaemia, and the lower haemoglobin levels might be due to the disruption of the iron synthesizing machinery or due to the inhibitory effect of the toxic substance on the enzyme system in the synthesis of Hb. Marginal decrease in leucocyte count of fishes in hydrogen sulphide contaminated water shows the weakening of its immune system. The diminutions in RBC count in fishes of contaminated water suggest the decrease in their erythropoietin activity due to the interference of H_2S in the normal functioning of kidney or resulted from the reverse anemic state or haemolysing power of toxicant particularly on the red cell membrane.

The histological changes observed in the gills, kidney and liver tissues of test and estuarine fishes indicate that the fishes were responding to the direct effects of the contaminants as much as to the secondary effects caused by stress. The field study and aquarium study on hydrogen sulphide contamination in fishes showed behavioral, biochemical and physiological changes in *Oreochromis mossambicus*. The results of behavioural observations and biochemical changes indicate that hydrogen sulfide is a significant environmental factor that influencing the ecological distribution of fishes.

Because of unscientific and uncontrolled human interference, the environmental and economic quality of Kadinamkulam estuary is getting deteriorated. The introduction of exotic species like *Oreochromis mossambicus* into the water bodies of Kerala may considerably influences the biodiversity of inland water bodies. Because of their wide tolerance about salinities and pollutants like hydrogen sulphide make easy to survive both in fresh and brackish water. The omnivorous nature of this fish species causes the deterioration of other species in these water bodies. It is too late to formulate appropriate policy to save these backwater systems because rich natural resources make the country rich. The present study on the effects of aquatic hydrogen sulphide pollution on fishes shows that hydrogen sulphide induces oxidative stress in fishes as indicated by increased lipid peroxidation and changes in antioxidant enzyme activities. Therefore the measurement of oxidative stress biomarkers in fishes plays an important role in the quality assessment of the hydrogen sulphide polluted aquatic medium in which they survive and can be used for monitoring the fish health in coir retting areas of estuaries.

10.2 Management measures for the Conservation of Kadinamkulam estuary and its biota

The 590 km coastal belt of Kerala is unique, having 30 major wetland ecosystems popularly known as back waters. Retting of coconut husk in the estuaries of Kerala is a major source of pollution, leading to deterioration in environmental quality and production potential. Coconut husk retting is covering the wetland ecosystem into a serious and complex ecosystem of micro aerobic and aerobic properties. The large scale reclamation of the estuaries due to dumping of husk, coir pith and related materials has resulted in horizontal and vertical shrinkage of estuaries. Traditional retting activity has led to acidic pH conditions with anoxia resulting in the production of high amount of hydrogen sulphide leading to drastic reduction in the occurrence and abundance of plankton, benthic fauna and fishery resources.

The following management measures are suggested:

- To keep the estuary mouth (Pozhi) permanently open, allowing continuous flushing and uninterrupted sea/estuary interaction, to avoid deterioration in water quality and the consequent damage to aquatic organisms.

- To entrust the local panchayats for licensing of the retting areas and form a local committee comprising of government representatives, local people and technical personal to advice the government on retting operations.

- Coconut husk retting, clay, sand mining, land repossession are the major apprehension about the shrinkage of biodiversity especially in fish diversity. So strictly prohibit the uncontrolled clay/ sand mining and traditional coconut husk retting in this estuary.

- To close the worst hit retting activity areas for a few years and allowing their ecological restoration.

- To allow retting of coconut husks in specially constructed tanks using the technology of 'Biomethanation coupled extraction of quality coir fibre'. The coir units should install solid waste recycling plant for minimizing the dumping of solid wastes from the retted husk into the backwater system.

- To conduct the studies on the carrying capacity of each estuaries in the context of number of husk that can be soaked, which will in turn enable for regulating the quality of husks.

- Parvathy Puthanar is one of the major external sources which add pollutants into the estuary other than coir retting. The effluents from this canal should be treated biologically and chemically from its source/ constructing water treatment plants for improving the water quality.

- Shrinkage of water body in Kadinamkulam backwater due to indiscriminate reclamation especially for the purpose of coir industry by private parties legally or illegally is a matter of concern. Indiscriminate reclamation of water body should be strictly prohibited.

- Biomonitoring is an essential element needed to assess the environmental health of an aquatic ecosystem. Improving the quality of water alone is not enough to raise the quality of aquatic ecosystem, but the banks and substratum also have to be handled with care as well.