6. SUMMARY

Distilleries use waste by-product molasses obtained from the sugar mills that are available in the market. Distillery effluent is organic. It is obtained from sugarcane so it does not contain any toxic material or harmful substances. Though any thinly in excess causes hardness or ill effect.

The evaluation of nature and degree of harmful effects produced by toxic substances in the aquatic organisms are evaluated by toxicity tests. The 96 hour LC50 values have generally been found to be satisfactory for the measurement of acute toxicity. The differences in 96 hrLC50 of the same toxicant in different fishes may be attributed to individual traits including those of behaviour and additional structures such as accessory respiratory organs. The individual characteristics such as size and weight, sex and biological behaviour are important determinants for variation in LC50 values. The physico chemical factors such as temperature, alkalinity and hardness also influence the toxicity.

The present study was carried out to know the harmful effect of distillery waste. The sample was collected from Trichy distilleries, Trichy and Mystus gulio was chosen as experimental animal. Survival of fishes when exposed to different concentrations of effluent within 96 h, Lc100, Lc 50 and Lc 0 levels for M. gulio were 260, 200 and 140 respectively. The safe and sublethal levels was 80ppt of distillery waste.

The present study is an attempt to study the toxicity of the distillery waste with respect to the biochemical parameters and respiratory contents of the fish Mystus gulio. The distillery waste, which enters the body tissues of the fish, affects the physiological activities. When the fish was exposed to different concentrations
of the distillery waste there was a clear and steady increase in the rate of opercular movements.

The rate of oxygen consumption is one of the most important and major pathways for energy of all living organisms. The oxygen consumption of tested fishes was reduced with increasing sublethal concentration of distillery waste as well as exposure periods. The behaviour responses were also useful tools for identifying the polluted condition of the medium in which they were present. The dissolved toxicant stressed the fish *Mystus gulio* to escape from the medium but due to the presence of the wire mesh the fish was prevented from escaping, the fish was restless and behaved abnormally with fast and twisting movements, increased frequency of surfacing, gulping of air, vertical swimming and it swam rapidly to escape from the medium by jumping again and again.

The haematological parameters in the present study shows the reduction of RBC count and Hb content with the increasing concentration of the distillery waste. However, the WBC count was increased with increasing concentration of the distillery waste. In general all the parameters decreased with increasing concentration of the industry waste. However, the opercular beats and WBC count alone increased with the increasing concentration. The constant increase in the differential count clearly indicates that the distillery waste stress certainly stimulate the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

The biochemical contents were tested when the fish reared in different sublethal concentrations. The protein content of fish was decreased with increasing the sublethal concentrations of distillery waste. The glycogen content of fishes reared in distillery waste medium also followed the same trend of total protein
content. The glycogen content of muscle and liver tissues of *M. gulio* decreased with increasing concentrations of distillery waste.

The amino acid content and lipid content of fish also decreased with increasing concentrations of distillery waste and it was inversely proportional to water content.

The LDH activity of muscle and liver tissues of tested fishes was gradually and significantly enhanced. The activation of enzyme was more in liver than in muscle tissues of tested fishes. Unlike LDH, the SDH activity of different tissues of tested fishes was decreased with increasing sublethal concentrations of distillery waste as well as periods of exposure.

In the experimental fishes, the GOT and GPT activity level was decreased for 7, 14 and 21 days of exposure periods. The maximum decrease in the enzyme activity was found in the 21st day of treatment period.

The increase in ALP activity in the muscle and liver was statistically significant in 7, 14 and 21 days of exposure periods. In the *Mystus gulio*, the ACP activity and phosphatase activity were decreased with increasing concentrations of distillery waste.

The maximum decrease was at 80 ppt at all the exposure periods. The protease activity raised when reared in distillery waste medium at the end of the 7, 14 and 21st day exposure.

The structural alterations were observed under light microscope in the sections of gill, kidney, intestine and liver tissues of fish from distillery waste treated group when compared to those from control group.
Liver, the first organ to face any foreign molecule through portal circulation is subjected to more damage. The parenchymatous hepatic tissue in teleosts, has many important physiological functions and also detoxification of endogenous waste products as well as externally derived toxins, drugs, heavy metals and pesticides.

The structural organization of the liver from the control group fish and the treated livers from the experimental group fish possessed a hexagonal shaped lobule with the hepatocytes displaying arrangements around the central vein and, the glycogen being distributed in a homogeneous way along the hepatic tissue. The sublethal concentration of distillery treatment groups of parenchymal vacuolation and focal coagulative necrosis were observed in the liver treated with distillery waste after 21st day.

A long-term exposure to distillery waste resulted in necrosis of the hepatic tissue. Completely vacuolated areas were observed with fat deposition. Biliary hyperplasia was observed at certain regions of the hepatic tissue. This might be indicating the regenerating hepatic cells to withstand the toxic stress condition.

Fish have five pairs of gill arches. The front four pairs, slender gill filaments form two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharengeal bone and does not play a role in respiration.

Diffusion of a xenobiotic across the gill epithelium will depend largely on lipid solubility. Generally, the fish gill arch is a curved osseous structure from which radiate the bony supports of the primary lamellae of which the surface area is increased further by the formation of regular semilunar folds called the secondary lamellae.
On short-term exposure of distillery waste, the changes observed in gills were hyperemia, clubbing and edema. After 7 days of distillery waste exposure, gills became edematous with prominent clubbing. Separation of primary gill lamellae and hemorrhage in the blood vessels outside the secondary gill lamellae were observed. Hyperemia of the gill filaments that engorged with blood vessels appeared. Hyperplasia was observed in secondary gill lamellae, which led to fusion of adjacent primary and secondary gill filaments.

The posterior kidney of freshwater fishes is adaptated to produce diluted urine and it has little participation in ion or acid-base balance. It receives the vast majority of post bronchial blood and because of that we expect renal lesions when the fish are exposed to pollutants. Therefore a study of these possible kidney changes could be a good response of environmental pollution. The body fluids of freshwater fish have a higher ionic concentration compared to surrounding water, a condition referred as hyperosmotic. To maintain the concentration gradient, the removal and conservation of ions prior to the excretion of purified water is required. This aim is accomplished in the kidney by filtration of water through glomerular nephrons comprising of a renal corpuscle and renal tubule.

Fish exposed to distillery waste were also administered along with food. Effluent was found to be suppressed, as the degeneration was seen only in a few columnar cells. Broadening of the villi and shrinkage in the submucosa were not evident. In this group fish were first exposed to effluent and then transferred to free water, and were administered distillery waste with food for 21 days. The intestine of this group did not show any severe structural damage. However, distortion of the basement membrane and slight shrinkage of the submucosa at the tips of the villi as a result of effluent poisoning were still evident. Histology of the intestine showed hyperactivity of the cells in the villi (elongated), which were found to be very well developed and healthy, and resulted in a reduction of the lumen.
The major findings of this study are that distillery waste is a toxic substance in fish, with severe histopathological alterations of fish in different exposure period. Because of the widespread, large-volume, high-frequency use of distillery waste in semiconductor manufacturing, they must be aware of its toxicity in aquatic environments. This awareness must include knowledge of its effect on fishes, more specifically of its acute toxicity, histopathology, and influence on biochemical parameters. Thus, in general, it can be inferred that respiratory mechanism of an animal under distillery waste stress is such affected and results in a shift or emphasis towards anaerobiosos at the tissue level during sub lethal intoxication.

This distillery waste has variable composition whose values are far exceed the permissible limits there by posing great danger to the aquatic biota. The dilution requirements are needed before discharge in the distillery waste safely to the ecosystem. Therefore, it is needs that the authorities concerned to ensure that treated effluent discharge comply with acceptable standard to save our environment from destruction.