CHAPTER IV

DISCUSSION AND CONCLUSIONS

DISCUSSION

Out of the 15 types of toxicity tests considered today, the highest rated tests include acute mortality. Acute tests were considered ecologically significant, most scientifically and legally detensible, modest in predective capability, most simple and simple and cost effective and were considered to have the greatest utility (Buikema et.al. 1982).

The present investigation aims at the understanding of the effect of distillery effluent on fish body organs and mortality by using acute toxicity test. This chapter deals with the discussion on the results obtained during the investigation and the existing literature on general toxicity. LC 50 values and histopathological alterations occured due to various pollutants. The present dissertation deals with LC 50 values for T.mossambica and R.daniconius and the histopathological alterations in gill, liver and kidney due to the distillery effluent poisoning. As it is brought to the notice, the paucity of literature on fishes selected in this area of investigation, it was hoped that such a study will throw light on toxic effects of distillery effluent on vital organ of fish which might be considered as pollution indicators.

1) Physico-chemical properties and LC 50 values :

The physicochemical properties of the distillery effluent used in this investigation are in agreement with the studies made by earlier investigators. The properties of the distillery effluent are due to different chemicals present therein. These chemicals mainly alter the pH, DO and hardness of normal water and leads to water pollution.

According to Handerson and Tarzwell (1957) the industrial wastes are chemically very complex and very difficult to define which particular factor is actually working for fish mortality. Ellis (1937) and Verma and Dalela (1976) have noticed that pollutants affect the fish life through three different ways. (i) By causing respiratory and circulatory failure through interference with the excretory functions of the gills. (ii) By specific toxic action and absorption through gills or lining of mouth or other external structures. (iii) By toxic action after absorption through the gastrointestinal tract. According to David and Ray (1966) discharge of spent wash results in the precipitation of ferric ions, High sulphide contents affect the respiratory activity of fish in choking their gills also suspended solids affect the fish adversely by causing mechanical injuries to the gills (Verma and Dalela, 1976). The presence of suspended solids change the physico-chemical properties of water.

In the present investigation the fish mortality of I. mossambica and R. daniconius at different concentrations of the pollutant was observed. The mortality could be because of fluctuations in the values of various parameters, such as pH, DO, and Hardness. The main changes in quality of water are decrease in pH from 7.5 to 4, decrease in DO from 5 mg/l to 3.3 mg/l and an increase in hardness from 53.82 mg $CaCO_3$/l to 378.48 mg $CaCO_3$/l. The pH and DO values from the experimental sets of aquaria at different concentrations of the effluent show direct correlation to each other and the values decreased with the increase in the concentration of the effluent (Fig.1,2). Where as the hardness ($CaCO_3$) values increased with the increase in the concentration of spent wash (Fig.3). All these changes causing deterioration of the water media in which the fish lives. Appearantly the decrease in the DO values below the required minimum oxygen content in the water caused death of the experimental fish due to suffocation. This process might have been enhanced by the suspended solids at higher concentration of pollutant, causing mechanical injury to gills as reported by Verma and Dalela (1976).

It is a common practice to use the LC 50, observed for short time period of exposure to determine the 'safe' concentration of a toxicant. Although this is of little practical significance, where exposure is likely to be prolonged. For any given period of exposure a harmful dose, as far as physiological, biological and behavioural aspects are concerned is much lower than the LC 50 (Fujiya, 1961). Our observations further support this, LC 50 values for Cd for 216 hrs. - was estimated to be 15% mg/l by Chung (1983), whereas LC 50 for malathion for 48 hrs was 5,592 ppm. in T.mossambica by Sailatha et al. (1981). Similarly LC 50 values of pentachlorophenol in R.daniconius are given by Gupta (1983) whereas LC 50 values for Zn in R.daniconius for 96 hrs. and 240 hrs. were 38.1 and 33.3 ppm respectively.

Though various chemicals cause mortality in different fishes through the water pollution, the LC 50 values also vary for each type of pollutant and in each fish. The LC 50 values for 48 hrs. at different concentrations of 1%, 2%, 3%, 4% and 5% of distillery effluent in the present investigation also showed different values in two species of fish used. The 48 hrs. LC 50 in *T.mossambica* was 2.5% conc. whereas in *R.daniconius* for 48 hrs it was 3.1% conc. of distillery effluent. These two different LC 50 values led to conclusion that (i) the fish *R.daniconius* is more resistant than *T.mossambica*, (ii) the juvenile stages of fish are more sensetive to pollutants as *T.mossambica* here (6-7 cm) and *R.daniconius* (7-8 cm), and average weight of 5-6 gms. Though in general *T.mossambica* is known to be sturdy variety; its juveniles are more sensitive, than the same size adults of *R.daniconius*, to distillery effluent.

FISH BEHAVIOUR :- After the fishes acclimatized in normal tap water they were transferred to the aquaria containing different concentrations of distillery effluent. The changes in behaviour of these fishes are more or less similar to their response to other pollutants. These changes in behaviour is described in Chapter III on observations.

The high rate of mortality was observed when concentration of distillery effluent was higher (5%). As conentration of distillery effluent decreased in different aquaria, the death rate also decreased and survival period increased to considerable extent. In still lower concentrations i.e. (1%), the test fishes nervertheless exhibit a narcotic effect (Verma and Dalela, 1976). The fishes showed sluggish and inactive movements and became diagonal in position and finally floated on the water surface. If the treatment continued, fish dies because of suffocation and exert a synergistic effect after entering the gastrointestinal tract and fish shows asphyxia.

<u>GILLS</u> :

Because of the increase in the pollution of the fresh water bodies, the alterations in the gill structure and function have recently become a subject matter of research. The sources of pollution, as is known very well, are of various types. Apart from the function of exchange of gases, the gill has other functions. It is involved in ion regulation (Conte, 1969; Hughes and Morgan, 1973; Maetz, 1976; Hulbert <u>et</u> <u>al</u>., 1978). Production of mucus covering on themselves and on the body parts, exposed to the ambient environment (Hughes and Perry 1979; Munshi, 1980). It has been shown that under polluted conditions the gills are the first organ systems which get damaged (Metelev et al. 1971). Because of the damage there is an impairment in the gill function. It has been illustrated that the toxins present in polluted water enter into the fish body via the gill lamellae and the skin (Metelev et al. 1971). The amount of toxins entering into the body depends upon the concentrations of the polluting agents in water. If the concentrations are less, naturally little of them are passed into the fish body. Opposite is the case with regard to higher concentrations.

The spent wash is composed of both dissolved and suspended matter. It contains both organic and inorganic substances; the fact is evident from the Table No.1. All these substances, when are mixed in the water in which the fishes live, will have a combined effect on the well being of the fish. The discussion on this aspect is done in the forgoing pages of this dissertation.

Wherever possible the results obtained presently have been discussed with regard to the information available on other toxicants. The occurrence of different polluting agents, particularly the inorganic substances lead to decrease the pH of the water. The decrease in pH has been shown to depend also on the concentration of the individual components (Metelev et al 1971). The decrease in pH means increase in the acidic condition. In the present work, it has been noted that the pH of water gets decreased considerably (from 7.5 to 4.0).

The acidic condition of the medium is toxic to the fish, and to avoid it, the fish gills and skin secrete the mucus material which gets spread over on the general body surface and thus, tries to have a protection. This protective response is also seen when the fishes are exposed to other types of industrial pollutants (Metelev et al 1971). The mucus secretion increases along with spent wash concentration in the medium. The initial increase in the mucus cells is important in this regard. As the experimental fishes need protection from the toxic substances, naturally the demand for additional mucus secretion is increased. To satisfy this demand, the mucus cells either have to increase in size or in number or both. As indicated, it has been observed that the mucus cell number and/or size gets increased, both in *T. mossambica* and *R. daniconius* at least in the sublethal doses of spent wash.

The additional discharge of mucus forms a thick covering on the gill epithelia and it interferes with its basic function of respiration. No doubt mucus in very small thickness (few microns only) is helpful in the process of respiration (Ingale, 1982; Haniffa and Sundaravadhanam, 1984); But as indicated earlier the thickness of it increases tremendously and hence, the hinderance in respiration.

Loss of mucous goblet cells, has been shown to occur in certain fishes due to higher concentrations of sea bloom (Shimada et al., 1983) and due to hydrocarbons (Lopez, 1981). Here we report for the first time that the mucous cells, both in I. mossambica and R. daniconius are lost because of acute toxic effect of the spent wash. The acidic medium has a corrosive action on the gill lamellae and this increases along with the increase in acidity. To prevent it, the mucous gland cells secrete additional mucus. Metelev et al. (1971) has shown that the mucus secretion also increases to protect the gill lamellae from the solid suspensions present in the sewage water. Likewise, the spent wash also contains lot of suspended particles and to prevent damage from these, additional mucus material gets secreted.

It has been postulated by Metelev et al. (1971) that in acid media the gill epithelia absorb the acidic solution. By doing so the blood picture of the fish gets altered. This leads to the impairment of many blood functions. The combined effects, as indicated above induce coagulation of fibrin, haemolysis, clumping of blood cells, etc. In concert with these changes, the pH of the blood gets altered (Metelev et al. 1971). When such impure blood goes to the brain, its functions get severely affected. These effects are more pronounced in the respiratory and cardiac centres, which ultimately lead to the slowing up of the above functions. It has been observed in the present investigation, that after the initial excitation, the fish became sluggish. Further, the sluggishness increases along with the concentration of spentwash and time. This must have been the result of the reduction in respiratory and cardiac activities. In extreme cases, both of these activities stop completely. Such total stoppage of respiration and cardiac functions have been ampty illustrated by a number of investigators in different fishes exposed to various types of polluting agents (Metelev, et al. 1971, Van Der Putte, 1981).

The degenerative changes occurred in the gill of the two fishes studied presently could be attributed to the following factors :

Inorganic ions like Na^+, K^+ and their salts are reported to be inducing degenerative changes in the respiratory epithelium of the fish and the extent of this damage depends on their concentrations (Metelev et al. 1971; Hingorani et al. 1979; Wong, et al., 1977). These workers have shown that the damaging effects due to Na^+, K^+ and their salts have certain characteristics, e.g. disintegration of the gill epithelia and separation of it from the underlying tissue. This observation has also been made presently in both, *T. mossambica* and *R. daniconius*. The separation of gill epithelia from the underlying tissue results in disruption of the respiratory process, which leads to the death of the fish, such reports are also available in other fishes exposed to different industrial effluents containing higher concentrations of inorganic ions and their salts (Metelev et al. 1971).

The effect of heavy metal pollutants on the fish biology, particularly on their gill epithelia has been worked out by a number of workers in the field (Establier and Gutierrez, 1980; Wong and Lau, 1982; Kumar and Pant, 1981). It is well known that the heavy metals (Mn, Cr, Zn, As, Cd, Pb, Cu, Fe, Se, etc.) and their salts form important components of certain industrial wastes. These induce specific toxic effects on the fish (Skidmore and Tovell, 1972; Khangarot, 1982; Crespo et. al, 1982; Wagh et al., 1985).

Acute toxicity due to Zn and Cu leads to excessive secretion of mucus (Skidmore and Tovell, 1972; Labat et al., 1974). These workers have further shown that the secondary lamellae get inflamed and when the Cu is absorbed, it goes into the blood. It changes the chemistry of the blood. Effects of acute toxicity of $HgCl_2$ on the rainbow trout have been studied by Wobester (1975) and Lack and Van Overbeeke (1981) and they claim that $HgCl_2$ induces extensive histopathological alterations. Likewise accumulation of heavy metals from the water has been shown to cause severe damage to the gill structure (Benoit, 1975; Pequignot, 1975; Einaga, 1977; Khangarot and Somani, 1980; Establier and Gutierrez, 1980; Khangarot, 1982; Lack and Van Overbeeke, 1981). The pathological alterations in the gill structure lead to suffocation and asphyxiation. Because there is a retardation in the transport of oxygen (Hughes ~~et al.~~ Perry, 1979; Wong and Lau, 1982; Wagh et al., 1985).

In the present study, however, no heavy metals occur in the spent wash, except the iron and its salts (see table no.1)

Therefore, the histopathological changes taken place in the gill of I. mossambica and R. daniconius might have ~~accrued~~ occured because of iron and its salts.

It has been shown that the iron concentration in the natural waters of river and lake varies between 0.01 to 1.0 mg/l. Occasionally it might be slightly higher (Metelev, et al. 1971). The iron may present as ferrous or ferric sulphate, the high concentrations of iron salts, particularly the ferric salts induce acute histopathological changes in the fish gill. Such changes have been shown to be similar to the histopathological changes due to acids (Metelev, et al. 1971; Daye and Garside, 1976; Falk & Dunson ~~et al.~~, 1977). It has been shown by Metelev et al. (1971) that the pH of water gets reduced along with the increase in the iron sulfate. In the present study, the distillery effluent contained iron salt to the magnitude of 60 mg/l. After dilution the values of iron content get reduced proportionately. The minimum values range from 1 mg/l to 4.5 mg/l. Depending on the iron content variations are seen in the histopathology of the gills in both the fishes. There exists a linear correlation between the extent of histopathological change and the concentration of the iron sulfate in the spent wash. This aspect when compared between the two experimental fishes, it is seen that the histopathological alterations are more acute in I. mossambica. Metelev et al. (1971), is further of the view that the low contents of iron salts also induce toxic effects on the gills of fish. Because according to him the ferric hydroxide is formed. It forms a coating on the gill surface and thus leads to suffocation (Metelev, et al. 1971).

The organic substances used in agriculture and produced as waste products by certain industries get mixed with the water of lakes, rivers, etc. Many of them have been shown to have toxic effects on fish biology in general and on gills in particular (Mitrovic et.al. 1968; Skidmore and Tovell, 1972; Engelhardt, 1981; Leino and McCormic, 1984). Mukhopadhyay and Dehadri (1980) have shown the decrease in the enzyme activity occurs due to Malathion. This, they have shown in different fish tissue, including gills. Lindane produces a disarray in the secondary lamellae structure. In acute cases the gill epithelium breaks away (Abel, 1976; Drewett and Abel, 1983). Alkylbenzene sulphonate causes hyperplasia to the secondary lamellae (Fukuda, 1983). It results in the formation of an acute edema which causes bleeding (Fukuda, 1983). Identical results have been obtained by Studhicka *et al*. (1983) using DDT, Lindane, BHC etc. Natarajan (1982) obtained interesting results by employing different organic substances. According to him there is an extensive damage to the gill epithelia, particularly the epithelia of the secondary lamellae. He further observed that the damage is also caused to the chloride cells of *Channa striatus*. Severe gill lesions are caused by Lebaycid insecticide in cichlids including *Tilapia* (Jauch, 1981). He further claims that there occurs hyperplasia, separation of gill epithelia etc. According to him in acute toxicity the haemorrhagic condition is induced and because of this the blood gets accumulated in the gill tissues. Similar studies using various organic chemicals have been reported on different fishes (Eller, 1971; Konar, 1975; Gupta and Singh, 1982; Lakota et.al. 1983).

It is apparent that the spent wash contains certain organic substances in acute concentration and in combinations with the inorganic substances they induce histopathological changes in the gill structure of the two fishes worked out presently.

An important aspect of the spent wash toxicity is the alterations in the number and size of the chloride cells. The occurrence of chloride cells (also referred to as the acid cells) has been established amply (Hughes, 1979; Munshi, 1980). They are known to be involved in the osmoregulatory functions. Because of the toxic effect of the spent wash, they either get damaged or get reduced in number. Hence, the very function of them gets impaired. Similar observations using different fishes and employing other effluents have been obtained by Leino and McCormic (1984). They are further of the view that the alterations in chloride cells of Fathead minnows and Pimephales promelas are due to the change in the pH of the medium. In the present work also the various organic substances might be contributing towards the change in pH, particularly towards acidic side.

Another important histological entity of the secondary lamellae are the pillar cells. Their initial function is to lend support to the gill epithelia (Munshi, 1980). These are also adversely affected because of the toxic effects of spent wash. Because of their damage, their basic function of supporting the gill epithelia and the vascular tissue in the secondary lamella gets impaired (Haniffa and Sundarvadhanam 1984). This change in them also lead to the curling up of the secondary lamellae. It has been noted that the curling up of the secondary lamellae is in linear proportion with the concentration of the spent wash.

From the point of the curling phenomenon of the secondary lamellae, the work of Skidmore and Tovell (1972) is important. They have shown that the pillar cells contract tremendously when exposed to polluting agents. Because of this, the hydrostatic pressure in them is reduced. It leads ultimately to the curling up of the secondary lamellae, as observed in the present investigation.

LIVER :

The liver is one of the most important organ systems in the vertebrates. Many functions have been attributed to it, Basically it produces the bile needed in the digestive tract, stores the excess sugars in the form of glycogen, synthesizes the storing fats, and breakdowns the aminoacids (deamination), (Prosser and Brown, 1965). Along with the above mentioned functions it does another important function and that is detoxication. Various toxic substances, exogenous or endogenous in orgin, are broken down in the liver (Lagler *et al.*, 1977).

Under normal conditions the liver cells (hepatocytes) are not overburdened with the functions. But when the environment becomes polluted then it is functioning under stress. This is because, the toxins enter in the fish body in two ways : 1) Some part enters via food material, 2) remaining part is absorbed through the general body surface, including gills

(Metelev et al., 1971). The toxins entering via food ultimately are directed to the liver along with the digested food material (via blood) and the toxins absorbed firstly go into the blood, and then it is passed to the liver. In short the poisons or toxins are directed or passed to the liver to take due care of them.

Many reports are available to show the changes occurring in the liver (in general and hepatocytes in particular) due to the toxins. Two types of alterations occur in the liver. They are : gross level changes and changes occurring at tissue and cell level (Eller, 1971; Baktavathsalam et al., 1982; Dubale and Shah, 1979). In the present investigation also two types of effects have been observed on the liver. The liver exposed to sublethal and lethal concentrations of spent wash shows enlargement. This enlargement has a linear correlation with the concentration. Such alteration in the development of edemic condition has been also observed by many workers (Verma et al., 1980; Shaffi, 1980; 1981; Haniffa, 1984). It appears that the liver first reacts violently to the toxins (irrespective of their nature and type). To fight out the toxic effect almost all hepotocytes are mobilized and all of them get slightly enlarged, thus giving an edemic appearance to the liver. Under acute conditions the liver cirrhosis takes place. This type of damage has been observed by others in the field (Eller, 1971; Sastri and Gupta, 1978; Bhattacharya, et al., 1975).

The changes in the liver at cellular level have been described by a large number of workers (Andrews et al., 1966; Van Valin et al., 1968; Goel and Garg, 1980; Haensley et al., 1982; Murty and Devi, 1982). They have studied the changes with reference to the different pollutants. Their results are helpful in interpreting the present results. As mentioned previously the spent wash contains three major components at varying concentrations. These components have a combined effect.

Of the three groups of the pollutants, the inorganic ions and salts produce deleterious changes in liver (Sastri and Gupta, 1978; Wani and Latey, 1983). Mukherjee and Bhattacharya (1975) have shown the formation of lesions on the hepatic mass of O.punctatus and C.batrachus, due to copper sulfate and sodium sulfide toxication. Mercuric chloride alteres the arrangement of the liver cord drastically (Sastri and Gupta, 1978). They have further shown that the cytoplasm becomes granulated and vacuolated and the nucleus becomes enlarged. The necrosis of the cells sets in and it leads to fat filteration and depletion of glycogen. Sastri and Gupta (1978) conclude finally that the acute toxicity finally produces liver cirrhosis in the above mentioned fishes. Wani and Latey (1983) obtained practically identical results. According to them the enzymes activity is lost and there occur alterations in the hepatic cell organelle, In the present study alterations in the hepatic histology have been observed in T.mossambica and R.daniconius. It appears that when the fishes are exposed to sublethal concentrations following changes become visible. The liver becomes edemic and hence it enlarges slightly. The inorganic substances present in the spent wash enter into the liver cells. After their entry, the very chemistry of them gets changed over and because of this it appears that the cells enlarge and give an edemic appearance to the liver. When the concentration increases, more amount the salts enter and in response to it, the cells get further enlarged. This is possible to a certain limit; after which the cells rupture and their nuclei get spread over into this distorted organisation of the liver tissue. Earlier to this the liver cord gets disturbed. The granulation of the cytoplasm continues further, and at higher concentrations the cytoplasm becomes vacuolated in both the fishes. At the end, the necrosis sets in and it leads finally to cirrhosis. Similar views have been put forward by many workers (Bhattacharya et al., 1975; Sastri and Sharma, 1978; Chatterjee et al., 1983; Gupta and Singh, 1982). In the present work, however, the effect of the spent wash has not been studied on the enzyme systems of the liver, and hence, at this juncture nothing can be said in this regard. But logically the enzyme systems must be affected because of pollutants.

Second group of spent wash is the organic chemicals. In literature there are enumerable reports on the effects of pesticides of organic origin (Mathur, 1965, 1969; Eller, 1971; Chaturvedi and Sexena, 1978; Ramlingam and Reddy, 1981; Amminikutty and Rege, 1977; Drewett and Abel, 1983; Rashtwar and Ilyas, 1984). Deformative and edemic enlargements of the liver have been observed by Mathur (1965, 1969); Eller (1971); Bhattacharya et al. (1975); Sastri and Sharma (1978); Chatterjee et al. (1983). Thus they attribute to a variety of pesticides. Most of the above researchers are of the view that the pesticides when get mixed into water, enter the fish body via general body surface and/or via gasterointestinal tract, thus, reaching the liver. As in the case of inorganic substances, the organic substances must also be altering the chemistry of the liver cell cytoplasm and based on it, the histopathological alterations take place. This view has been amply subscribed (Amminikutty and Rege, 1977; Verma et al., 1980; Shaffi, 1980, 1981; Wani and Latey, 1983, Hacking et al., 1978; Srivastava and Sriwastawa, 1984, Mukherjee and Bhattacharya, 1975; Murthy and Devi, 1982).

The remaining group of spent wash pollutants is the suspended materials. The suspended particals do not have any deleterious effect on the liver, because, these particles just cannot reach the liver and damage it.

Thus, from the above discussion, it becomes clear, that the morphological and histopathological alterations occurred in the liver of T.mossambica and R.daniconius are because of the combined effects of the inorganic and organic constituents of the spent wash. These changes seems to be dependent on the concentrations of the above substances in the spent wash. Less the concentrations, least are alterations and higher the concentration more are the deleterious effects.

KIDNEY :

The kidney in vertebrates functions in the elemination of nitrogenous waste products and also in osmoregulation (Prosser and Brown, 1965). The proportion of the above two functions varies in relation to the availability of water and habitats. Alterations in the above functions in fishes might occur because of the polluted waters. The polluting agents after detoxification are brought into the circulation and through blood these come to the kidney for their excretion. Because of this unnatural overwork there occur certain structural changes in the kidney. If the pollutants are in low concentrations, the changes taking place are less acute, while under high concentrations drastic changes take place. These may finally lead to the failure of the kidney and the death of the fish (Mathur, 1969; Holden, 1972).

As in the earlier two organ systems, here also it will be interesting to establish a link between the type or the group of pollutant and the changes it induces in the kidney morphology. Kumar and Pant (1981) have shown that under acute toxicity of copper and zinc, the necrosis of the glomeruli and tubules sets in. Pequignot (1975) obtained identical results using lead, copper, formol and ammonia as the pollutants, in the carps. He concludes that under acute toxicity the complete failure of the kidney takes place. Biochemical and physiological alterations have been studied in a number of freshwater fishes by Shaffi (1981). His opinion is that the glycogen metabolism is hindered and it leads to depletion in glycogen of the kidney. In the present study the spent wash also contains inorganic material of which iron is the major constituent. Because of the inorganic substances alterations in the kidney have taken place. These alterations are in the form of an enlargement of the kidney and kidney tubules, distortion of the kidney tubules, vacuolation, etc. Under acute toxic condition it has been observed that the glameruli in both the fishes get severally damaged. This could logically be explained as below :

The unwanted molecules circulating in the blood might have larger size and molecular weights and these molecules might not be getting out of the capillaries of the glomeruli even after the ultrafiltration force. The condition might have become further critical because of the depletion of the glycogen as indicated by Shaffi (1981). This stress ultimately must have led in inflicting damage in the kidney. Some sort of logic could also be thought in relation to the distortion of the kidney tubules and loss of the brush border. In higher concentration these alterations must have led to the degenerative changes resulting in the complete distorted architecture of the kidney and its failure. As indicated above, this is a logical conclusion based on some guidelines. It needs, however, further confirmation.

Much information is available with regard to the effects of organic pollutants on the fish kidney (Chaturvedi and Saxena, 1978; Mitrovic, 1968; Chatterjee *et al.*, 1983; Bakthavathsalam, 1984; Dubale and Shah, 1984; Rashatwar and Ilyas, 1984). Amininkutty and Rege (1978) observed enormous swelling of the glomeruli, thickening of the endothelial wall of the capillaries and increases in the haemopoitic mass in *Widow tetra*. due to thiodan toxication and malathion produces necrosis in *C. punctatus*

(Dubale and Shah, 1984). In addition to the necrosis of kidney tissue, Chaturvedi and Saxena (1978) have shown the development of lesions in the kidney tissue due to malathion. Similar observations have been made by others in the field using different organic pollutants and fish for their study. Most of them have come to the following conclusions : Initially the protoplasm of the tubule cells is damaged. It becomes granulated and because of granulation, vacuolation sets in. These effects result in the swelling of the tubule cells and hence, in the enlargement of the kidney itself. The above mentioned alterations occur under sublethal concentrations. Under high concentrations, the effects are more drastic. And all these effects finally lead to the degerative changes.

The results obtained in the present investigation (using the kidney of *T. mossambica* and *R. daniconius* and spent wash as a pollutant) reveal more or less the same story as indicated above, i.e. the histopathological alterations become more and more drastic along with the increase in concentration. The above mentioned alterations might be occurring because of more amount of organic substances in the spent wash. These substances might also be having larger molecular weights and size and hence, the kidney tissue must be getting degenerated more drastically.

Lastly, the following postulation, especially with regard to kidney and liver may be made. In the present work organic and inorganic chemicals (constituents of spent wash) have been employed. They must be altering the chemistry of the internal environment. To cope up with these drastic changes the above organ systems might not be equipped and as in other cases, under heavy load of work they must be getting crumpelled.

CONCLUSIONS

Some of the conclusions that could be drawn from the present investigations are as follows.

The studies on the LC 50 values of fish to the distillery effluent showed that for 48 hours the values were 2.5% and 3.1% concentration for Tilapia mossambica and Rasbora daniconius respectively.

The behavioural study on test fish showed that initially fishes were restless and showed agitation but subsequently sluggishness increased with time and increase in the concentration of the toxicant.

The study revealed that at lower concentration of the effluent (i.e. 1%) there was no mortality recorded in R.daniconius but in T.mossambica even at this low concentration 20% mortality was reported. This clearly shows that even at such low conc., if continued, some species of fish die due to suffocation or exert a synergistic effect leading to asphyxia. At higher concentrations the death is certain due to acute toxicity.

From the experiments it is evident that with the increase in concentration of the effluent from 1% to 5% in test aquaria there is a direct correlation in the physico-chemical parameters studied. When the conc. increases there is an increase in the $CaCO_3$ hardness where as proportional decrease is witnessed in the pH and DO values leading to the acute toxic nature of the pollutant.

Histopathological studies revealed the damage in the normal structure of gill, liver and kidney caused due to the effluent. Gills being sensitive and directly exposed to the polluted water, was the first organ to get damaged. It was due to the mechanical injury and also because of the heavy secretion of mucus adversely affecting the process of respiration. At the end, the mucous cells were lost due to the acute toxicity. This leads to the seperation of the epithelia from the disruption of the respiratory process leading to the death of the fish.

There were two types of effects observed during the present investigations in the liver of both the test fishes. With the increase in the concentration of spent wash the liver shows linear correlation (i) gross level changes and (ii) changes occuring at tissue and cell level. Under the acute toxicity conditions liver cirrhosis takes place and with increase in the concentration of the effluent the enlarged cells and liver cord get ruptured.

During the toxicological studies the enlargement of the kidney, kidney tubules distortion and vacuolation observed was attributed to the inorganic substances from the pollutant, where glomeruli in both the test fishes were seen severely damaged. From the experience gained during the investigations the following comments can be made.

Sensitivity of different fish, to the given toxicant and its concentration, depends upon its species characters, its niche and the habitat it belongs to.

The age of the test fish perhaps plays an important role in the toxicity experiments. It can be seen from the experiment where the juveniles of the known sturdy fish Tilapia mossambica were found more sensitive to the pollutant than the same sized adult individuals of Rasbora daniconius. Apparently the weight of the fish has no contribution to the response to toxicity.

It is necessary to study the behaviour of the test fish when exposed to pollutant in order to know its response. The knowledge of this behavioural pattern would help in assessing the impact of pollution, in advance, in the water body and thus suitable antipollution measures could be thought of in time.

It is important to monitor the physico-chemical changes in the lotic and lentic ecosystems where the correlation of the histopathological and physiological changes in fish with reference to the fluctuations in the values of abiotic parameters needs to be studied for better understanding.

Biological assessment of water pollution has depended historically on observing effects directly in natural systems. At times individual organisms are exposed to different pollutants in laboratory to study their reactions to the toxicants.

These practices are unsatisfactory because of two main reasons (a) Best monitoring practices require prevention rather than documentation of damage and (b) direct observation of an event in one system does not necessarily permit one to make claims about events in other systems (Clairs, 1981).

The documentation of damage is a necessary part of biological monitoring, and methods for determing whether biological integrity has been impaired are still being developed although a wide variety of methods for different types of ecosystems is presently available for practical use. As a consequence, the most important of the future needs in biological assessment of pollution are (a) development of a predicture capability, and (b) means of validating the accuracy of the predictions which will in turn enable correlations to be made when the predictions are in error.

In Indian context, it has to be realised that mere study of histology and histopathology in hundreds of local fish species will not give any preventive solutions for the ever increasing pollution problems. Instead a systematic study of sensitive fish species and their response to the existing and probable pollutants needs to be undertaken immediately. This will enable the environmental scientists to assess the impact caused by the various pollutants, like spent wash, existing today on the "indicator fish species", in the given ecosystem. Therefore prevention of pollution rather than mere documentation of damage caused by pollution requires priority, and immediate attention needs to be given to the existing pollutants and commercially or ecologically dominant organisms affected by them in nature.

The recovery of the fish species exposed to different pollutants at lower concentrations needs to be studied in detail in order to recommend the management policy for the pollution control studies.