Fig. 2

The test fish *Labeo rohita* (Hamilton)
CHAPTER 2

COLLECTION OF TEST FISH AND ESTIMATION OF SAFE CONCENTRATIONS

INTRODUCTION

For every experiment it is essential to select the suitable test animal. The test animal should be of healthy, economically important easily available and fit for the experiment. In the present study the cultivable cyprinid fresh water fish *Labeo rohita* (Hamilton) has been selected, as it is economically important and having digestibility co-efficient of 88.60 and biological value of 78.90 at 10% level of protein intake (Anon 1962). The aim of selecting this species is to imply the result in the field, for fish culture.

Environmental scientists all over the world have been tolling the warning bells against the dangers in polluting the natural water resources without regard to the consequences. Rapid industrialisation, unchecked urban growth and unplanned developmental activities destroy the man’s natural environment causing major hazard to his health and happiness in addition to revaging the nature beyond repair. The industrial waste
waters discharged into the near by water bodies pose a serious problems and their toxic properties directly or indirectly react on the aquatic biota. So it is essential to find out the sub lethal concentrations of these effluents to safe guard the aquatic life from the disastrous effect. This in general will be done by Bio-assay tests.

"Bio-assay" is a test in which organisms are used to detect the presence or the effects of any physical factor, chemical factor or other perturbations in the environment. Sprague (1973) defined bio-assay as a test in which the quality or the strength of a material is determined by the reactions of the living organism to it. The test involves exposing the organisms to the toxicants for a definite period in the laboratory environment and observing mortality or other effects during this period. The period of exposure can be a short term of 24,48,72 and 96 hours or up to 10,000 minutes, (7 continuous days) or long term up to four months or up to the life cycle of the organism. The results of bio-assay are generally reported in terms of median lethal concentration (LC 50) or Sub-lethal concentration. This is a concentration at which 50% of
the test animal survive or it is an interpolated value based on the percentage of fish surviving at two or more concentrations at which less than half and more than half of the test fishes survive. According to the standard methods of American Public Health Association (1981), in bio-assay, the experimental organisms are subjected to a series of concentrations of a known or suspected toxicant water under adequately controlled condition.

The bio-assay test is useful in the determination of the toxicity, the degree of toxicity variability and for treatment methods. The other important use of this study is to find out the suitable concentration of the effluents for discharge or reuse. Many works has been done for standardizing the toxicity bio-assay techniques used in the water pollution studies and maximum work was done on the effect of industrial effluents on fish. Litchfield and Wilcoxon (1949) described a simplified method for evaluating dose effect experiments. Doudroff et al (1951) advocated fish as test animal for direct evaluation of toxicity of industrial effluents for toxicity tests and proposed appropriate standardisation of methodology. A number of workers like Henderson and
Torz Well (1957), Kertz (1969), Sarkar and Krishnamurthi (1977), Wilde and Parrot (1984) conducted bio-assay tests for the control of the pollution effect of the industrial effluents. Fujiya (1961) published the effects of Kraft pulp mill wastes on fish. Durve and Jain (1980) performed the toxicity tests with distillery effluent to the cyprinid weed fish Rasbora daniconius and studied the behavioral changes. Even though many bio-assay studies have been carried out by using various fishes in industrial effluents, the study on the estimation of safe concentration of the distillery effluent on cultivable fish is lacking.

So the present study is an attempt to determine the toxicity level of the distillery effluent and to find out the suitable concentration for fish culture. As there is no literature on the effect of distillery effluent on the economically important fish Labeo rohita, investigation was made to determine the safe concentration of the effluent in this fish by using long term bio-assay tests following all the standard methods described by American Public Health Association (1981).
MATERIAL AND METHODS

The test fish selected for the present study is the teleost cyprinid fish *Labeo rohita* (Hamilton). It is a commercially important fresh water fish commonly available throughout the year. It is one of the fast growing Indian major carp considered to be a best combination for composite fish culture. Being economically important, easily available, maintainable, cultivatable sensitive fish, the *Labeo rohita* has been selected for this study.

The test fish *Labeo rohita* of size ranging from 5.4 to 6.9 cm in total length, 2.1 to 3.7 g in weight were brought to the laboratory in oxygen pack from Fish Seed Farm, Medak. The fishes without any mechanical injury were selected and acclimatized to the laboratory conditions in glass aquaria for a period of four weeks prior to the experimentation. During this period the fishes were fed with conventional pelleted feed, prepared in the laboratory daily at the rate of 3% of their body weight. The feeding was done only once in the early morning, the leftover food was removed in the evening. Feeding was stopped twenty-four hours before starting the Bio-assay experiments.
The raw distillery effluent was collected from the Government distillery, Bodhan (District Nizamabad, Andhra Pradesh) and brought to the laboratory. The test concentrations were selected by conducting some pilot tests. The mortality range was from zero to hundred, and tests were conducted separately for smaller fishes with an average length of 5.6 cm and weight of 2.3 g and big sized fish of average length 6.5 cm and weight 3.2 g.

The acclimated test fish *Labeo rohita* were exposed in a batch of 5 in glass aquaria with 10 lits of distillery effluent in different concentrations in triplicate. Different concentrations of the distillery effluent were prepared by mixing with dechlorinated tap water in the laboratory at room temperature. Simultaneously the control troughs were also maintained. As the aim of this study is to utilize the distillery effluent for fish culture the bio-assay test were carried out for a period of 168 hours and the sub-lethal concentrations were estimated. During this experimentation period the fishes were fed daily once in the morning with 3% ration of artificial pelleted feed prepared in the laboratory. In the evening the excess food has been sucked out with the help of a small hose.
pipe without much disturbance. During the test the
behaviour of the fishes were noted and when the
opercular movements ceased the fishes were considered
dead (Sprague 1973). The dead fish were removed and
their total length and weight were recorded. By
estimating the percentage of the death fishes during
the period of 168 hours the LC 50 value were calculated.
The calculations were done by graphical method (Gosh
1962). As the aim of the present study is to imply the
result into the land for intensive fish culture 100% survival value concentrations were also estimated.

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RESULTS & DISCUSSION

The industrial waste water discharge into water bodies having potential to either kill the organisms or incapacitate them. Toxicity tests involve the study of various concentrations of these chemicals on organisms and finding out the safe concentration of these substances, which will cause no or minimum hazard to aquatic ecosystem. The effect of organism include the mortality as well as other changes in behaviour, physiology and reproduction. The test determine the concentration which will not pose any harm to the environment and give the suitable conditions for discharging the effluents into fresh water bodies. In the present study the toxicity tests were made in distillery effluent by using the fish Labeo rohita.

The safe distillery effluent concentration were estimated by using smaller and bigger size fishes separately. The LC 50 values for different concentrations of the distillery effluent upto 168 hours were observed. The survival and mortality percentage for 24, 48, 72, 96, 120, 144 and 168 hours were observed and are given in table No. 3 and 4.
The fishes exposed to distillery effluent for a long term exposure showed different general, external behaviours. The behavioural pattern and the period of their suffocation varied from concentration to concentration.

In the higher concentration the movement of the fish were limited to the surface and the frequency of jumping out was more. The opercular movements increased rapidly and then the fishes lost their equilibrium and the opercular movement ceased. The colour of the fishes also showed some changes. The colour become more darker in high concentrations and pale in lower concentrations. Excess mucous deposition was observed in fishes died in higher concentrations.

Percentage survival of fishes in different distillery effluent concentrations gives the toxic level of the effluent in different periods. For smaller sized fishes the LC 50 value obtained for 168 hours and 144 hours was 1.5% concentration.

For 72 hours the LC 50 value was 1.8% concentration and for 48 hours 2% concentration. Above that concentration it was observed to be lethal for *Labeo rohita*. 
For big sized fishes LC 50 value observed for 168 hours and 144 hours was 1.9% concentration. For 72 hours and 48 hours the LC 50 value was 2% concentration and for 24 hours 2.5% concentration. Above this concentration it was fatal.

The fishes exposed to sub-lethal concentrations showed normal activities such as constant opercular movements, normal balance, behaviour and movement. They came to the surface of the water only after a constant time limit and there was no aggressive or irregular movements. The colour was yellowish when compared with the control.

It is clear from the result that the distillery effluent in higher concentration is toxic to the fishes and pumping of the effluent without dilution in any water body or fields will affect the fauna of that environment. In lower concentrations it can be utilized properly for the culture of fishes.

The safe concentration study and the Physical and Physiological changes in the body and the activities of fish in higher concentration of distillery effluent is in agreement with different studies in fishes exposed to pesticides. Toor and Kamaldeep Kumar (1974) reported
behavioural changes in *Cyprinus carpio* when exposed to different pesticides. According to Durve and Jain (1980) the *Rasbora daniconius* when exposed in distillery effluent showed various behavioural changes, which is also observed in the present work. Rashatwar and Ilyas (1984) reported the jerky movement of the fish *Nemachelius denisoni* when exposed to phosphomidon. Hingoroni et al (1980) recorded that in *Labeo rohita* the oxygen consumption decreases with increase of industrial effluent concentration. Samuel Paulraj (1988) established the fact that there was no mortality of *Labeo rohita* fingerlings when exposed to domestic sewage upto 70% concentration. Kamble and Keshavan (1986) reported the surfacing activity, spreading of pectoral fins in *Clarius batrachus* when exposed to different pesticides. Kamble (1983) studied the behavioural changes in *Lepidocephalichthys thermals* when exposed to pesticides.

The distillery effluent in general is very dense and dark in colour with low PH, high BOD and COD. Due to this characteristic features the fishes suffer with suffocation and leads to death. In the present study the effluent was diluted with dechlorinated tap water
and the PH was raised in between 6.9 to 7.4. The BOD level and COD level were also reduced by dilution. Because of the non-harmful parameters of the diluted effluent the fishes survived well. According to the present observation the diluted distillery effluent below the toxic level can be used for fish culture.

Warren and Davis (1967) pointed out that the deleterious effects of pollutants on survival, growth, behaviour and reproduction of animals under normal laboratory conditions give very little, which are very useful in determining water quality to protect aquatic resource, although experiments carried out in the laboratory are criticised as they do not reflect the condition in the field.

According to the present study the distillery effluent in low concentration can be re-used for fish culture and it is an environmentally pleasing approach and economically viable one.

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TABLE 4

TOXICITY TEST RESULT WITH DIFFERENT DISTILLERY EFFLUENT CONCENTRATION IN % FOR BIG Sized FISH.
(Under normal laboratory conditions)

Average length of the fish: 6.5 cm.
Average weight of the fish: 3.2 g.

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