MATERIALS AND METHODS

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In the field of industrial toxicology it is a problem to determine exact impact of a particular pollutant from the number of factors existing in the environment on a given organism. As the fishes have different habitats and ecological niche, it is difficult to assess the cummulative effect of pollutant in natural environment. It becomes further difficult because each species have individual degree of sensitivity to pollutant and their range of adaptation to the changing environment. The effects of pollutants on fish is studied by various methods like pathological. clinical, histological, anatomical, physiological, biochemical, biophysical, enzymological etc. Developing methods for determining toxicity in aquatic environment and quick diagnosis of fish poisoning is important in studying indicator organism for early warning system.

The present study deals with the impact of industrial effluents on fish, therefore an attempt has been made to study the impact of industrial effluents on the major carp *Labeo rohita.*

Survey :

Survey was made for the selection of the industrial effluents for the present investigation in and around Kolhapur. Finally three industrial units were selected for the studies.

1. Textile mill industry : Various chemicals, H_2SO_4, NaOH, sodium silicate, Na_2O_2, caustic soda, Alkaline hypochlorite,

sodium bisulfite peroxides and dyes are used during the processes like sizing, desizing, scouring, mercerizing, bleaching,. dyeing, printing and finishing.

2. Tannery industry : Chemicals used are NaCl, Lime, Na_2S, NH_4Cl, $(NH_4)_2SO_4$, chromium enzymes, trypsin, chymotrypsin, H_2SO_4, dyes etc. during the process such as beam housing, soaking, liming, deliming, tan yard processing, chrome tanning, bating, pickling, tanning, dyeing and flat liquoring.

3. Electroplating industry : Chemical salts containing heavy metals such as Ni, Cu, Zn, Ag, Au, Cd, Cr, Sn, Pb, Al, Fe, free ammonia are used in electroplating baths. Beside chemicals, such as acids, H_2SO_4, HCl, HNO_3, alkalies, sulphate, NaOH, NO_2CO_3, Na_2PO_4, Na_2SiO_3, solvents like benzene, trichloroethylene, ferrous sulphate, borate, carbonates, fluoborate, cyanide, fluoride, tortrate, phosphate, chlorides, sulfides, sulfites, sulfamate, nitrate etc. are used in electroplating industry during the processes such as surface cleaning, stripping and plating.

Collection of Samples :

The effluents from textile mill, tannery and electroplating industries at Kolhapur were collected monthly and brought to the laboratory in 5 litres plastic cans for analysis of physico-chemical

parameters (APHA,1985; Trivedi and Goel, 1986) and in 25 litres plastic cans for toxicological studies.

Physico-Chemical Parameters :

1. Temperature : Temperature was recorded at site by using ordinary standard mercury thermometer ranging from 0^0 to 100^0C. The readings were recorded at the time of sample collection of the effluents.

2. Total Solids : Total solids were determined as the residue left after evaporation of the unfiltered sample. Total dissolved solids were determined as the residue left after evaporation of the filtered sample. Total suspended solids were determined as the difference between total solid and total dissolved solids. The results were calculated according to Trivedy et.al. (1987) in mg/lit.

3. Turbidity : It was recorded on Nephelo Turbidity meter (131 systronic) and results were calculated with the help of Jackson's units Table prepared by Hash Chemical Co., USA and presented in NTU.

4. Colour : Colour of the effluent was recorded by observation.

Chemical Parameters :

During chemical investigation nine major chemical parameters namely pH, dissolved oxygen, free carbondioxide, hardness, chloride, alkalinity, acidity and important nutrients like inorganic phosphates and nitrates from different effluents were

studied. After collection the samples were brought to the laboratory for analysis.

1. pH - Hydrogen ion concentration (pH) was measured with pocket digital pH meter (Hanna Instrument) at collection site.
2. Dissolved oxygen : was estimated by modified standard Winkler's method and expressed in mg/ l.
3. Free carbondioxide : Free CO_2 was determined by titration method by using phenolpthalien indicator. The results are expressed in terms of mg/ l.
4. Hardness : Total hardness was estimated with standard EDTA method using Erichrome Black T. indicator and expressed in mg $CaCO_3$/l.
5. Chlorinity : Chlorinity was estimated by titration method using silver nitrate as titrant. The results are expressed in mg/ l.
6. Alkalinity : Total alkalinity was determined by titrimetric method by titrating against (0.1 N) Hydrochloric acid. The results are expressed in mg/ l.
7. Acidity : Total acidity was determined by titrimetric method by titrating against (0.05 N) sodium hydroxide. The results are expressed in mg/ l.
8. Phosphate : Phosphate content in the samples was determined by stanous chloride reagent method. The optical densities of the blue colour of ammonium molybdate developed in the

samples was measured using spectronic-20 at a wavelength of 690 nm as given by Murphy et.al. (1962). The results are recorded in mg/ l.

9. Nitrate : Nitrate content of samples was determined by Brucine method. The resultant yellow colour developed by the reaction of nitrogen and brucine was measured using spectronic-20 at 410 nm and values are expressed in mg/ lt. referring to the standard graph (Charles, et.al. 1945).

Samples were diluted wherever it was necessary.

Heavy metals :

The samples were collected from textile mill, electroplating and tannery industries in 5 litre cans. They were brought to the laboratory for further analysis. The collected samples were well filtered and the nitric acid treatment was given in order to avoid further degradation of heavy metals. The heavy metals were detected by Atomic absorption/ emission spectrophotometer (Chemito 201).

Acute toxicity :

Acute toxicity of these effluents was studied by using static bioassay experiment. The fish, *Labeo rohita* was selected for the present investigation for the following reasons :

1. They are found in the river systems as well as reared in ponds.

2. The fish is freshwater and available throughout the year.
3. They are expected to be good indicators of aquatic pollution.

The detail classification of *Labeo rohita* (Ham.) is as follows:

Grade	-	Pisces
Class	-	Osteicthyes
Subclass	-	Actinopterygii
Order	-	Cypriniformes
Suborder	-	Cyprinoidei
Family	-	Cyprinidae (Minnows and carps)
Subfamily	-	Cyprininae
Genus	-	*Labeo*
Species	-	*rohita*

Live fingerlings of 7-8 cm in length and 10-12 gm in weight were collected from the local Government fish rearing centre. Fishes were brought to the laboratory and stocked in rectangular glass aquaria of dimension. 45 cm x 22 cm x 30 cm and capacity of 25 litres for acclimatization. Ordinary chlorine free tapwater was used during acclimatization. Fishes were acclimatized for 15 days in the laboratory condition. During this period, water was changed twice a day and fishes were fed with groundnut cake once in a day. pH, dissolved oxygen and hardness of the water used for acclimatization was recorded.

Feeding was stopped 24 hr prior to the exposure of the fish to be effluents. The well acclimatized fishes to laboratory condition

were used for experimentation. Pilot experiments were carried out to find out the toxic range (in percentage) of the industrial effluents. Group of 10 fingerlings were held separately in plastic container of 10 litre capacity containing industrial effluents of different concentrations making total volume of the five litres.

Fishes were exposed to tannery effluent in the month of October 1999 and the test concentrations used were 10%, 15%, 20%, 25% and 30%. Experiment for electroplating effluent was carried out in the month of December 1999 and the test concentrations used were 2%, 3%, 4%, 5%, 6%, 8%. Experiment for textile mill effluent was carried out in the month of February 2000. The test concentrations used were 16%, 18%, 20%, 22% and 24%.

The experiments were conducted for 96hr while studying the toxicity of the effluents. For every experiment, control group without effluent was also run simultaneously. All the experiments were conducted in the natural day light rhythm. The effluent medium i.e. test concentrations from the experimental containers were renewed after each 24 hours. The mortality rate and behaviour of fishes were recorded before each change of the water from the plastic container. The toxicity tests were repeated thrice and values for 96 h LC_0 and LC_{50} were determined.

Biochemical Studies :

After acute toxicity (96 hr) experiments for textile mill, tannery and electroplating effluents, alive fishes were immediately sacrificed (5 from each group) from control, LC_0 and LC_{50} group separately to obtain gill, liver, muscle, kidney and brain. The pooled samples of these organs were properly blotted weighed and used for biochemical estimations i.e. total glycogen (DeZwaan and D.I. Zandee, 1972), total protein (Gornall, et.al. 1949) and total lipid (Barnes and Blackstock, 1973). Level of significance at 0.05 and 0.001 was, statistically calculated by using student 't' test.