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CHAPTER TWO

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MATERIAL AND METHODS

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In the present investigation following twelve local fish species from river Panchganga near Kolhapur city were used. The table no. 1 gives the list of these fishes with their Scientific, Common English and Marathi names alongwith the average length and weight of the individual species. These fishes were selected for the experimental purpose because of their easy availability and <sup>small</sup> size which was found suitable for the experimental purpose. Also the fishes belonged to different niche and had varied feeding habits and behaviour. The fishes after collection were put in large sized glass aquaria (100 X 24 X 25 cms) for acclimatization.

The fishes were acclimatized for a period of 10 to 15 days and chlorine free tapwater was used for this purpose. Few drops of commercial 'Blue-O-liquid' was added in the aquaria during acclimatization period to keep fishes free from fungal attack. During this period fishes were mostly, fed on commercially available 'fish food'. The glassfish was fed upon mosquito larvae.

During acclimatization fishes showed mortality in the first 2 to 3 days. The mortality gradually decreased and ceased after 4 to 5 days.

Laboratory acclimatization could not be achieved for the fish species Chela phulo. Thrice about 100 fishes were tried but, the mortality which began on the first day never stopped and all the fishes died within 8 to 10 days.

The two effluents viz; Sugar & Distillery Effluent-Spentwash (untreated) and Paper Mill Effluent (treated) were collected

Table No.1 : The Scientific, Common, English and Local Marathi names along with the average length and weight of the fishes studied during the investigations.

| Sr. No. | Scientific name                           | Common names                                       | Vernacular names    | Average       |                |
|---------|---|--|---------------------|---------------|----------------|
|         |   |  |                     | Length in cm. | Weight in gms. |
| 0       | <u>Chela phulo</u> (Gunther)              | -  | Bhat masa, Alkut    |               |                |
| 1       | <u>Danio aequipinnatus</u> (McCletland)   | Giant Danio  | Baluki              | 7.0           | 5.0            |
| 2       | <u>Rasbora daniconius</u> (Ham.)          | Common Rasbora                                     | Dandai              | 8.0           | 6.0            |
| 3       | <u>Cirrhina fulungee</u> (Sykes)          | Carp   | Mulicha ganna       | 7.5           | 5.0            |
| 4       | <u>Garra gotyle</u> (Gray)                | hill stream fish                                   | Mallya              | 6.0           | 4.0            |
| 5       | <u>Puntius sophore</u> (Ham.)             | the Bitter carp                                    | Gerrya              | 5.5           | 3.0            |
| 6       | <u>Lepidocephalichthyes guntea</u> (Ham.) | Loach  | Wulwulya, Chikhalya | 3.0           | 1.0            |
| 7       | <u>Botia striata</u> (Rao)                | Loach, Botias                                      | Wagh masa           | 5.0           | 2.5            |
| 8       | <u>Mystus malabaricus</u> (Jordan)        | Cat-fish   | Shingati            | 8.0           | 7.0            |
| 9       | <u>Lebistes reticulatus</u> (Peters)      | Guppy, Belleyfish, Rainbow-fish, Barbados Millions | Guppy               | 2.3           | 0.52           |
|         |   |  |                     | ♀             |                |
|         |   |  |                     | ♂             |                |
| 10      | <u>Chanda nama</u> (Ham.)                 | Perch, Glass-fish                                  | Kachaki, Chambhari  | 3.5           | 3.0            |
| 11      | <u>Tilapia mossambica</u> (Peters)        | Tilapia  | Tilap               | 7.5           | 7.0            |

fresh in 5 lit. plastic cans at the site of discharge of the effluents from Kolhapur Sugar Mills, Kolhapur and Warana Paper Complex, Warananagar respectively.

The following six Physico-chemical parameters namely Temperature, Turbidity, Hydrogen-ion-concentration (pH), Hardness, Dissolved Oxygen (DO) and Free Carbon-di-oxide (CO<sub>2</sub>) were studied in the different concentrations of both the effluents and <sup>in</sup> control aquaria. The standard updated methods, recommended for water analysis by Lind (1974) and APHA (1976), were used.

1. The temperature of the effluent in test aquaria was recorded with a thermometer having 0.1°C graduations.
2. Turbidity was estimated with the help of Spectronic-20 and the results were expressed in Jackson's Turbidity Units (JTU).
3. Hydrogen-ion-concentration (pH) value was ~~estimated~~ <sup>recorded</sup> with the help of digital pH meter.
4. Dissolved oxygen (DO) was estimated on oxygen analyzer (EM-76). The results were expressed in mg/L.
5. Hardness was estimated by standard EDTA titrant method and results were expressed in mg CaCO<sub>3</sub>/L.
6. Free Carbon-di-oxide (CO<sub>2</sub>) was estimated titrimetrically and results were expressed in mg/L.

For the bioassay investigations the available literature was studied and standard methods recommended by APHA (1976), Buikema et al. (1982) and Alabaster & Lloyd (1982) were followed.

The fishes selected for the experiments were collected

from the waters receiving the pollutants as suggested in ASTM (1970).

The glass aquaria used for the bioassay tests were of the dimensions of 45 X 20 X 30 cms. Each aquarium was filled with 20 lit of effluent concentrations to be tested. Chlorine free tapwater was used for control as well as for the preparation of the various concentrations. If more than 10 % mortality occurred in control, the fish lot for that test was discarded.

Laboratory tests for acute toxicity with Static Method were adopted. The tests were conducted for 96 hr. and fish mortality was recorded at an interval of 24 hr, 48 hr and 96 hr. duration. For range finding studies 2 fishes of each type were put in 0.1 %, 1.0 %, 10 % and 100 % effluent concentrations for 24 hr duration. After approximation, definitive tests with 10 number of each fish species in each effluent concentration, including control, were conducted for a period of 96 hr. A parallel experiment was conducted side by side for confirmation purpose.

During the present investigation eleven freshwater fish species were studied in the two effluents and two tests for each pollutant per fish species were carried out. Normally 5 to 6 different concentrations of a pollutant and a control were used in each test depending on the sensitivity of the test fish species to the pollutant. Therefore, altogether around 44 tests, <sup>i.e. 220 experiments</sup> were carried out during investigations.

The effluent concentrations for the definitive test based

on logarithmic interval (APHA, 1976) were prepared as shown in table no. 2. From the mortality data recorded, 48 hr  $LC_{50}$  value for each fish species was calculated by graphical method as a standard procedure.

The mortality data of each fish species in various effluent concentrations at 24 hr, 48 hr and 96 hr intervals was presented in tabular form. This data was used for the final analysis and the graphs were prepared to determine the  $LC_{50}$  values.

The effluent concentration in such methods is plotted on the 'absciss' or 'X-axis' and mortality on 'ordinate' or 'Y-axis'. Often the concentration axis is plotted on a log scale and mortality is plotted in an arithmetical scale, this yields a idealized 'Dose Response Curve' (Buikema et al., 1982). By transforming the dose response data to a probit scale (Sprague, 1973) the dose response curve becomes a straight line. In such cases, concentration is on a logarithmic scale and per cent mortality is on a probability or "probit" scale. The probit scale never reaches 0 or 100 per cent mortality, a line is next fitted to the points by eye, the concentration causing 50 per cent mortality is read from the fitted line and this is the  $LC_{50}$  value for that exposure time (Sprague, 1973).

The results are expressed in tabular form and also graphically, in case of all the eleven fish species, tested for both the effluents.

Table No. 2 : Guide to selection of experimental concentrations based on progressive bisection of intervals on logarithmic scale (APHA, 1976).

| Column 1 | Column 2 | Column 3 | Column 4 | Column 5 |
|----------|----------|----------|----------|----------|
| 10.0     |          |          |          | 8.7      |
|          |          |          | 7.5      |          |
|          |          | 5.6      |          | 6.5      |
|          |          |          |          | 4.9      |
|          | 3.2      |          | 4.2      | 3.7      |
|          |          | 1.8      |          | 2.8      |
|          |          |          | 2.4      | 2.1      |
|          |          |          |          | 1.55     |
|          |          |          | 1.35     | 1.55     |

The taxonomic classification of the twelve freshwater fish species used for the toxicological studies is given below. The system of classification adopted here is based on Berg (1940).

- Class : Osteichthyes (Bony fishes)
- Subclass : Actinopterygii (Modern bony fishes)
- Order : Cypriniformes
- Family : Cyprinidae (Minnows or Carps)
- Subfamily : Abramidinae
- 1) Chela phulo (Gunther)
- Subfamily : Rasborinae
- 2) Danio aequipinnatus (McClelland)
- 3) Rasbora daniconius (Hamilton)
- Subfamily : Cyprininae
- 4) Cirrhina fulungee (Sykes)
- 5) Garra gotyla gotyla (Gray)
- 6) Puntius sophore (Hamilton)
- Family : Cobitidae (Loaches)
- 7) Lepidocephalichthys guntea
- 8) Botia striata (Rao)
- Order : Siluriformes
- Family : Bagridae (Cat-fishes)
- 9) Mystus malabaricus (Jordan)
- Order : Atheriniformes
- Suborder : Cyprinodontoidei
- Family : Poeciliidae (Livebearers)
- 10) Lebistes reticulatus (Peters)



Order : Pegasiformes

Suborder : Percoidei

Family : Centropomidae (Perches)

11) Chanda nama (Hamilton)

Family : Cichlidae (Cichlids)

12) Tilapia mossambica (Peters)