MATERIALS AND METHODS
CHAPTER 2

MATERIALS AND METHODS

The study involves the estimation of the concentration of heavy metals (Hg, Cu, Fe, Mn and Zn), in water, sediment and fish.

2.1 LOCATIONS OF INVESTIGATION

The water courses chosen for this study are the following (vide Figs.2.1 and 2.2 and Plates 1, 2, and 3):

The Cauvery River

The Cauvery River flowing through Salem District, Tamilnadu is one of the major perennial rivers in South India providing water for paddy cultivation in Thanjavur District which is known as the ‘rice bowl of Tamilnadu’. The water supply is being regulated by a dam reservoir constructed at Mettur across the river. Many factories located on the bank of the river discharge their effluents below the dam site causing pollution. As a result of this, the fish population in the river gets affected very much [93]. The heavy metal pollution in this river has not been studied systematically and hence an attempt has been made to study heavy metals concentration in water, sediment and fish from this river at three locations (vide Fig.1, Lat.11°21' to 11°48 N; Long. 77°51'E).

The Cooum Rivulet

The Cooum Rivulet is flowing through the heart of Madras City and was once used for bathing and washing. In due course, as a consequence of increased
population this has become a sewage carrying canal. In addition to the sewage, this rivulet gets contaminated with effluents from Ambattur and Padi Industrial complex. The water in this rivulet is not free flowing and only during rainy seasons the floods will wash off the pollutants into the sea. It is noticed that this rivulet is so polluted that fish population is practically nil [94]. Due to the high concentration of heavy metals, the possibility of these metals getting accumulated in ground water by seepage is strong, which will ultimately lead to heavy metal pollution in the neighbouring wells causing health hazards to the users of these wells. An attempt has been made to study heavy metal concentration in water and sediment collected at 5 different locations (vide Fig.2, Lat.13°N’ Long.80°15’E).

The Chetput Pond

This pond (stagnant water) is located behind the Government Kilpauk Medical College Hospital in the heart of Madras City. The pond receives pollutants through hospital waste, sewage and highway road wash during rainy season. An attempt has been made to study heavy metals pollution in water, sediment and fish from this pond in three different locations (vide Fig.2, Lat.13°, Long. 80°15’E).

The details of possible source of pollution in the study area of the three water courses are furnished in Tables 2.1, 2.2 and 2.3 respectively.

2.2 PROGRAMME OF WORK DONE

Monthly samples of water and sediments were collected from three different points across the water course in each location and mixed.

In the Cauvery river every month the following three species of fish were caught from three different points across the water course in each location. For each variety five numbers of the samples were caught and pooled.
(i) Tilapia \((Tilapia mossambica)\) - Species 1.
(ii) Mrigal \((Cirrhinus mrigala)\) - Species 2.
(iii) Cauvery Carp \((Labeo kontius)\) - Species 3.

In the Chetput pond every month the following three species of fish were caught from three different points across the water course in each location. For each variety five numbers of the samples were caught and pooled.

(i) Tilapia \((Tilapia mossambica)\) - Species 1.
(ii) Roghu \((Labeo rohita)\) - Species 2.
(iii) Mrigal \((Cirrhinus mrigala)\) - Species 3.

The collection of the above samples of water, sediment and fish was done over a period of one year (January to December, 1986) comprising of four seasons. In South India the classification of seasons are as follows [95].

<table>
<thead>
<tr>
<th>Season</th>
<th>Month</th>
<th>Mean Temp. (^{\circ}C) in Tamilnadu</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Max.</td>
</tr>
<tr>
<td>Cold weather</td>
<td>Jan. to Feb.</td>
<td>29</td>
</tr>
<tr>
<td>Hot weather</td>
<td>Mar. to May</td>
<td>37</td>
</tr>
<tr>
<td>South West Monsoon</td>
<td>Jun. to Sep.</td>
<td>34</td>
</tr>
<tr>
<td>North East Moonson</td>
<td>Oct. to Dec.</td>
<td>30</td>
</tr>
</tbody>
</table>

2.3 COLLECTION OF SAMPLES

Water

The polythene sample bottle of 2 lit. capacity was thoroughly washed with detergent and tap water. It was then rinsed successively with 1:1 conc. \(\text{HNO}_3\) tap water, 1:1 conc. \(\text{HCl}\), tap water and finally with deionized glass distilled water. The
water samples were collected following the method of Taylor [96]. The bottle was plunged neck downwards to a distance of about one foot below the surface of water, the mouth being directed towards the current and was gently rotated. When the bottle was completely full, it was raised rapidly above the surface and the stopper was replaced immediately. The water samples were preserved by adding 3 ml of redistilled HNO₃ (1:1) per litre and were brought to a pH 2.0. The holding time for metal analysis of these samples was six months [97].

**Sediment**

Sediment samples were collected using a hand grab. The samples were placed in a plastic bag and transported to the laboratory in ice box. At the laboratory the samples were preserved in a deep freeze.

**Fish**

Fish samples were caught using a net. After catching they are separated species wise, kept in ice, brought to the laboratory and refrigerated.

2.4 PRETREATMENT OF SAMPLES

**Pretreatment of water sample for analysis of heavy metals (Cu, Fe, Mn and Zn)**

100 ml of the sample was taken in a beaker and 3 ml of Analar conc. HNO₃ was added. The beaker was kept on a hot plate and evaporated continuously to dryness without allowing the liquid to boil. The beaker was cooled and 3 ml of analar conc. HNO₃ was added to the residue. It was covered with a watch glass and kept on the hot plate. The temperature was increased slowly until a clear solution resulted. The walls of the beaker and watch glass were washed with glass distilled water and the insoluble material were removed by filtration. The volume was made upto 100 ml, labelled and used for analysis [97].
Pretreatment of water sample for analysis of Hg

100 ml of water was taken in a conical flask to which was added 5.0 ml of dil. H₂SO₄, 2.5 ml of conc. HNO₃, 15 ml of KMnO₄ and 8 ml of 5% KHSO₄ and heated at 95°C for 2 hours. The contents were cooled and treated with a 1:1 solution of sodium chloride-hydroxylamine hydrochloride in order to reduce the excess of KMnO₄. This solution was used for analysis [97].

Pretreatment of sediment for Cu, Fe, Mn and Zn

The sediment sample was wet seived through nylon screen to remove the coarse particles. Then the sample was homogenized in a blender and stored at 4°C in a sealed polythene bag. Before analysis, the sample was dried at 105°C, pulverised with mortar and pestle and thoroughly mixed.

The pulverised sediment (0.5 g) was weighed, transferred into a Kjeldahl’s flash, treated with 8 ml of conc. HCl and 2 ml conc. HNO₃ and kept overnight at room temperature. The digestion was continued next day at 105°C for 1 hour and then at 140°C until the sample was dry. After cooling, 12.5 ml of 20% (by volume) HCl were added and the mixture rewarmed at 80°C for 20 minutes. After cooling, the solution was filtered and made up to 50 ml with glass distilled water [98] and used for analysis.

Pretreatment of sediment for analysis of Hg

The sediment sample was wet seived through nylon screen to remove the coarse particles. Then the sample was homogenized in a blender and stored at 4°C in a sealed polythene bag. Before analysis, the sample was dried at 60°C and pulverised using pestle and mortar and thoroughly mixed. 0.1 g of this dry powder was accurately weighed, to which 5.0 ml of glass distilled water, 2.0 ml of aqua regia and 15 ml of 6% KMnO₄ were added and heated in a water bath at 95°C for 30 minutes. After cooling the contents to room temperature, it was treated with 1:1 solution of
sodium chloride-hydroxylamine hydrochloride to destroy the excess of KMnO₄ and filtered. The filtered solution was made upto 25 ml in a volumetric flask and the solution thus obtained was used for the analysis of mercury [96,98].

**Pretreatment of fish organs for analysis of heavy metals (Hg, Cu, Fe, Mn and Zn)**

Each variety of fish was cut open and the different organs viz. muscle, gill, liver and viscera separated. For each organ the pretreatment was done as follows.

5 g of the sample was digested with 20 ml of a 10:1:2 mixture of conc. HNO₃, H₂SO₄ and HClO₄ [99] at 60°C for analysis of mercury and at 105°C for analysis of other metals till a clear solution was got. The digested solution was filtered, the filtrate made upto 25 ml in a volumetric flask and used for analysis.

### 2.5 ESTIMATION OF HEAVY METALS

**Estimation of Cu, Fe, Mn and Zn by using atomic absorption spectrophotometer (Varian Techron AA 120)**

Preparation of reagents and calibration was done as per Varian Techron Cook Book.

**Preparation of standard solutions of strength 1000 mg/l**

**Cu Solution**

1 g of Cu metal was dissolved in 1:1 HNO₃ and made upto 1 litre.

**Fe Solution**

1 g of Fe was dissolved in 20 ml of 1:1 HCl acid, and made upto 1 litre.
**Mn Solution**

1 g of Mn metal was dissolved in a minimum volume of 1:1 HNO₃ and made upto 1 litre.

**Zn solution**

1 g of Zn metal was dissolved in 40 ml of 1:1 HCl and made upto 1 litre.

**Calibration and estimation**

The required halo-cathode lamp was inserted in the AAS and the required wavelength for various metals adjusted. Air-acetylene flame was used for atomising the metal. Initially the blank solution (the respective solutions used for digesting water, sediment and fish organs) was aspirated and the instrument was adjusted for zero absorbance. Subsequently the metal solution of different concentrations, prepared from the standard solution were atomized one after the other and the readings noted. A standard graph was plotted between concentration and absorbance. Similar procedure was followed for the samples to be analysed. From the absorbance, the concentration was worked out using the standard plot. The working conditions of the AAS instrument for different metals are furnished in Table 2.4.

**Estimation of Hg by cold vapour technique using Mercury Analyser (ECIL, 5800 MA)**

Mercury analyser works on the principle of cold vapour technique. The mercury present in the pretreated sample was reduced to elemental state by using stannous salt in an acid solution. The working wavelength was 253.7 nm and the sensitivity 0.001 μg. Hg solution of concentration 100 mg/l was prepared by dissolving 0.1354 g of HgCl₂ in 2% HNO₃ and made upto 100 ml using 2% HNO₃ and calibration was done as indicated in ECIL cook book.
**Calibration and estimation**

The apparatus was set up and connections checked for leakage. The required aliquot (2-4 ml) of the blank solution (respective solutions used for digesting water, sediment and fish organs) was taken in the reaction vessel. 8 ml of 10% HNO₃ and 2 ml of SnCl₂ were added and the stopper replaced immediately. Magnetic stirrer was switched on and the contents stirred vigorously for about 5 minutes. The pump was started to allow the air to purge through the reaction vessel. Absorbance was recorded as quickly as possible. The above procedure was followed for various concentrations of standard Hg solutions and the respective absorbance recorded. A standard graph was plotted between concentration and absorbance. The above procedure was followed for the various sample solutions and the corresponding absorbance noted (after deducting the absorbance value for the respective blank solution). The Hg concentration was calculated using the standard plot.

**2.6 STATISTICAL ANALYSIS OF RESULTS**

The collected data were tabulated and statistical analysis carried out [100, 101, 102], using the scientific subroutine package of IBM 360 computer. The following derivations were arrived at:

Correlation matrix was worked out by taking the combinations of any two metals at a time. Multiple correlation of one metal with others taken together were examined. Multiple regression analysis was done with a view to develop a system of equations keeping one metal as a dependent variable. Multiple regression coefficients were determined to develop linear equations using which, the level of any one metal can be forecast, if the concentrations of the others are known.

The analysis of variance was done using Magnum II Multiprocessor System.
Quality Assurance of Analytical Data

The sensitivity of the equipment (AAS) with respect to the estimation of Cu, Fe, Mn and Zn was checked by repeated estimation of replicate samples of known strength of the metal. It was further cross-checked by spiking these standard solutions with known concentration of the respective metal and estimating the percentage recovery.

The standard deviation was worked out using a standard solution of concentration of 2 mg/l with respect to Cu, Mn and Zn. Subsequently percentage recovery was worked out by spiking the standard solutions with additional 2 mg of the respective metal. In the case of Fe the standard solution taken had a concentration of 20 mg/ml which was subsequently spiked with 20 mg of Fe. The estimations were replicated 5 times and the mean values are furnished below.

### STANDARD DEVIATION

<table>
<thead>
<tr>
<th>Metal</th>
<th>True value mg/l</th>
<th>Estimated mean value mg/l</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>2.0</td>
<td>1.97</td>
<td>0.10</td>
</tr>
<tr>
<td>Mn</td>
<td>2.0</td>
<td>2.06</td>
<td>0.18</td>
</tr>
<tr>
<td>Zn</td>
<td>2.0</td>
<td>2.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Fe</td>
<td>20.0</td>
<td>20.17</td>
<td>0.72</td>
</tr>
</tbody>
</table>

### PERCENTAGE RECOVERY

<table>
<thead>
<tr>
<th>Metal</th>
<th>Actual concentration mg/l</th>
<th>Estimated mean concentration mg/l</th>
<th>Percentage recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>4.0</td>
<td>3.97</td>
<td>99</td>
</tr>
<tr>
<td>Mn</td>
<td>4.0</td>
<td>4.04</td>
<td>101</td>
</tr>
<tr>
<td>Zn</td>
<td>4.0</td>
<td>3.78</td>
<td>95</td>
</tr>
<tr>
<td>Fe</td>
<td>40.0</td>
<td>38.50</td>
<td>96</td>
</tr>
</tbody>
</table>
Quality assessment on Hg analysis using Mercury Analyser was done on similar lines as above. The concentration of the standard solution was 20 ng/ml which was spiked with 40 ng of Hg. The estimation was replicated five times and the mean values are furnished below:

### STANDARD DEVIATION

<table>
<thead>
<tr>
<th>Metal</th>
<th>True value ng/ml</th>
<th>Estimated mean value ng/ml</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>20.00</td>
<td>20.02</td>
<td>0.78</td>
</tr>
</tbody>
</table>

### PERCENTAGE RECOVERY

<table>
<thead>
<tr>
<th>Metal</th>
<th>Actual concentration ng/ml</th>
<th>Estimated mean concentration ng/ml</th>
<th>Percentage recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hg</td>
<td>60</td>
<td>60.40</td>
<td>101</td>
</tr>
</tbody>
</table>

Based on the above data, it could be observed that the sensitivity of both the equipments were good with a very low standard deviation and high percentage recovery ranging from 95 to 101.
Metal concentration data in water

The metal concentration data for water are only 'Total', which is inclusive of dissolved and particulate. No separate analysis was done for these two categories, the reason being two of the water courses investigated (Cauvery river and Chetput pond) were almost free from particulate matter. The concentrations from other studies used for comparative purposes are also 'Total' concentrations only.
FIG. 2.1 THE COURSE OF THE CAUVERY RIVER FROM ITS SOURCE TO SEA.

SCALE: 1 inch to 16 miles

1. METTUR DAM
2. NATAMANGALAM
3. KOKKARAYANPET
FIG. 2.2 THE COURSE OF THE COOUM RIVULET AND THE CHETPUT POND IN MADRAS CITY.

1. KOYAMBUDU BRIDGE
2. AMINJIKARI BRIDGE
3. MC NICHOLS BRIDGE
4. PERIYAR BRIDGE
5. NAPIER BRIDGE
6. CHETPUT POND
PLATE-II-THE CHETPUT POND

STATION - 1

STATION - 2

STATION - 3
PLATE-III THE COOUM RIVULET

STATION - 1

STATION - 2

STATION - 3
PLATE-III THE COOUM RIVULET

STATION - 4

STATION - 5
### TABLE 2.1: STATIONS OF SAMPLE COLLECTION AND SOURCE OF POLLUTION IN THE CAUVERY RIVER

<table>
<thead>
<tr>
<th>Station No.</th>
<th>Location</th>
<th>Distance from Mettur Dam (Km)</th>
<th>Possible source of pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Mettur Dam</td>
<td>0</td>
<td>Cluster of chemical industries.</td>
</tr>
<tr>
<td>2.</td>
<td>Nattamangalam</td>
<td>7</td>
<td>Confluence of Ellis-surplus channel carrying industrial effluents with the Cauvery</td>
</tr>
<tr>
<td>3.</td>
<td>Kokkarayanpet</td>
<td>60</td>
<td>Fishing spot, 6 Km below paper industry.</td>
</tr>
</tbody>
</table>

### TABLE 2.2: STATIONS OF SAMPLE COLLECTION AND SOURCE OF POLLUTION IN THE COOUM RIVULET

<table>
<thead>
<tr>
<th>Station No.</th>
<th>Location</th>
<th>Distance from the sea (km)</th>
<th>Major source of contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Koyambedu Bridge</td>
<td>18</td>
<td>Cattle and lorry washings</td>
</tr>
<tr>
<td>2.</td>
<td>Aminjikarai Bridge</td>
<td>13</td>
<td>Domestic wastes</td>
</tr>
<tr>
<td>3.</td>
<td>McNichols Bridge</td>
<td>9</td>
<td>Cattle and sewage wastes</td>
</tr>
<tr>
<td>4.</td>
<td>Periyar Bridge</td>
<td>5</td>
<td>Metal workshops and bus depot</td>
</tr>
<tr>
<td>5.</td>
<td>Napier Bridge</td>
<td>1</td>
<td>Chloride from sea water.</td>
</tr>
</tbody>
</table>

Foot Note: Apart from the major sources of contamination mentioned, untreated domestic wastes are let out at various points throughout the entire route within city limits of the rivulet.
### TABLE 2.3: STATIONS OF SAMPLE COLLECTION AND SOURCE OF POLLUTION IN THE CHETPUT POND

<table>
<thead>
<tr>
<th>Station No.</th>
<th>Location</th>
<th>Source of Pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Near the sewage point</td>
<td>Sewage out fall from neighbouring area, road wash during rainy seasons and seepage from hospital.</td>
</tr>
<tr>
<td>2.</td>
<td>Middle of the pond</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Behind hospital</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE 2.4  DETAILS OF WORKING CONDITIONS OF AAS

<table>
<thead>
<tr>
<th>Metal</th>
<th>Wave Length nm</th>
<th>Spectral Band Pass nm</th>
<th>Optimum Working Range mg/l</th>
<th>Typical Sensitivity mg/l</th>
<th>Lamp Current mA</th>
<th>Detectable Limit mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>324.7</td>
<td>0.2</td>
<td>2.0 - 8.0</td>
<td>0.040</td>
<td>3</td>
<td>0.003</td>
</tr>
<tr>
<td>Iron</td>
<td>248.3</td>
<td>0.2</td>
<td>2.5 - 10.0</td>
<td>0.062</td>
<td>5</td>
<td>0.005</td>
</tr>
<tr>
<td>Manganese</td>
<td>279.5</td>
<td>0.2</td>
<td>1.0 - 4.0</td>
<td>0.024</td>
<td>5</td>
<td>0.003</td>
</tr>
<tr>
<td>Zinc</td>
<td>213.9</td>
<td>0.2</td>
<td>0.4 - 1.6</td>
<td>0.009</td>
<td>5</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table 2.4a DETAILS OF WORKING CONDITIONS OF MERCURY ANALYSER

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>Measuring range</th>
<th>Sensitivity</th>
<th>Radiation source</th>
</tr>
</thead>
<tbody>
<tr>
<td>253.7 nm</td>
<td>20-200 ng/ml</td>
<td>3 ng absolute for 1% absorption</td>
<td>Mercury Lamp</td>
</tr>
</tbody>
</table>