4. MATERIAL AND METHODS

Monthly sampling for analyzing the hydrological parameters and biotic components was done during the period January 2004 to December 2005. A total of eight study sites from Tirthan stream viz. Gushaini (T1), Deori (T2), Fagu Pul (T3), and Larji (T4) and Sainj stream i.e. Ropa (S1), Shalwar (S2), Tiara (S3), and Bhihali (S4) were selected for present investigation and were visited once in a month.

4.1. PHYSICAL HABITAT ANALYSIS OF STREAM

Physical habitat characteristics were assessed during the base flow conditions along a stretch of 100 mts at each site. Variables that are typically used to characterize streams habitat were measured at each site including cross-sectional and longitudinal channel dimension, substrate characteristics, bank conditions and water current. In order to classify study sites, the methodology proposed by Rosgen (1996) was followed. Quantification of habitat was carried out as described by Armantrout (1998a). The following methodology was adopted for different parameters.

1. Longitude, latitude and altitude were measured with the help of topographic map and help from experts of Department Geography, Panjab University was taken.

2. The study site gradient (both in percentage and degree) was determined between two points in linear fashion with the help of Sunnto Clinometer.

3. Water current (cm/sec) was calculated with the help of EMCON water current meter. Observations were recorded from 3-4 points having different depths by placing the propeller of EMCON water current meter at desired points. Average of all these values was considered as water current of the study site.

4. The depth of study sites was calculated with the help of graduated iron bar. It is vertical distance from bottom to upper
Material and Methods

surface layer of water. The depth was recorded from 7-8 points having different depths and the average of all these points was considered as mean depth.

5. The stream width (mts) was calculated with the help of Laser Range finder (Yardage Pro 400). It is a horizontal distance (average 5-6 points having different widths) across the bank of the study site.

4.1.1. Substrate and Habitat Structure

The particles of substrates are measured with the help of metric ruler or Vernier caliper in centimeters because rocks can be large or narrow. They are measured twice; first the length and then width, sum of two values divided by two gives the average values of substrates material were classified after Armantrout (1998a). A brief description of the substrates and habitat type are given below:-

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large boulders</td>
<td>125 cm or above</td>
</tr>
<tr>
<td>Small boulders</td>
<td>25-124 cm</td>
</tr>
<tr>
<td>Large cobbles</td>
<td>15-24 cm</td>
</tr>
<tr>
<td>Small cobbles</td>
<td>5-14 cm</td>
</tr>
<tr>
<td>Large gravels</td>
<td>2-5 cm</td>
</tr>
<tr>
<td>Small gravels</td>
<td>0.1-2 cm</td>
</tr>
<tr>
<td>Sand</td>
<td>&lt; 0.1 cm</td>
</tr>
</tbody>
</table>

4.2. CHEMICAL ANALYSIS

Air temperature (°C) was measured with the help of streamline thermometer on the spot. Water temperature (°C), dissolved oxygen (mg/l), pH and conductivity (µS/cm) were measured in the field with the help of "Multi 340i/SET water analysis kit". Total dissolved solids were
determined by using E-Merck’s TDS Scan Meter. Rest of the parameters like total alkalinity (mg/l), total hardness (mg/l), nitrates (mg/l), phosphates (mg/l) and chlorides (mg/l) were determined according to the method described in APHA (1998) in the laboratory.

4.3. BIOLOGICAL ANALYSIS

4.3.1. Plankton

For the study of phytoplankton the water (50 liters) was sieved through bolting silk (No. 25, 0.3 mm mesh) plankton net fitted with wide mouthed 500 ml PVC bottle. The samples were preserved in lugol solution and 4% formaldehyde solution was added for preservation. The samples were bought to laboratory. The samples were kept undisturbed for around one week to allow sedimentation. The supernatant was carefully removed with the help of pipette avoiding agitation leaving behind phytoplankton. A Hensen Stempel pipette was used to take one aliquots in to four Sedgwick-Rafter counting counter chambers. Each cell was then examined under the microscope for identification and counting. Density was calculated following (APHA, 1998) and expressed as units x 10^3 /L. Identification of phytoplankton made following (Ward and Whipple, 1992) and (Anand, 1998). The diatoms were cleaned in 100 ml. of boiling 20% nitric acid containing 0.25 g. of potassium-di-chromate (K₂Cr₂O₇) and mounted in DPX for the permanent preservation.

4.3.2. Benthos

Benthic macroinvertebrates colonizing the substratum were collected from each site. The substratum in form of small boulders, cobbles and pebbles were lifted carefully from marked area (1m²). All the stones in marked area were washed in bucket full of water by dipping it number of times to dislodge the attached fauna. The fauna which remains attached to substrate surface were removed with the help of brush and forceps.
Material and Methods

The bucket water was filtered through meshed cloth of 0.5mm sieve to retain benthic macroinvertebrates (Singh and Nautiyal, 1990). Then the contents were transferred to small bottle and were preserved in 5% formalin for further analysis. Various benthic taxa were identified with the help of Kudo (1966), Pennak (1978), Ward and Whipple (1992), and APHA (1998).

4.3.3. Collection of Ichthyofauna

The sites were sampled with the help of cast net of 1-2 meter diameter and mesh size are of 1 cm. (knot to knot). In addition to cast net, scoop net and drag net were also used. The fishes were collected and brought to laboratory for identification using standard references (Day, 1878; Johal and Tandon, 1979, 1980; Talwar and Jhingran, 1991; Johal, 1998; Jayaram, 2010). While dealing with the fish density, during each visit, ten fishing operations using cast net were made and the number of fish samples caught were counted. Here the density relates to number of fish samples irrespective of weight, size and sex.

4.4. SEM ANALYSIS

For studying adhesive apparatus of insect, the organisms were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer pH 7.2-7.4 for 24 hours at the temperature of stream. The organisms were rinsed with several washing in double distilled and dehydrated to various grades of acetone. The specimens after acetone treatment were transferred to emylacetate solution. The specimens were dried in Polaram critical point dryer and mounted on metal stubs with double adhesive tape. The specimens were coated with 100 Å thick layers of gold/ palladium in JEOL sputter ion coater. The specimens were examined with JEOL JSM 6100 SEM at 10kv and images were observed on screen. Negatives were prepared for the photography.

The attachment terminology previously used by Beutel and Gorb (2001) and cuticular outgrowth terminology of Richards and Richards (1979) have been employed.
4.5. STATISTICAL ANALYSIS

The data was statistically analyzed for calculating mean, standard deviation and coefficient of correlation by using software SPSS version 10. Species diversity indices were calculated using the Shannon and Weiner (1949) index:-

4.5.1. Shannon-Weiner diversity index ($H'$)

$$H' = \pi \log_2 (\pi_i)$$

Where

$$\pi_i = \frac{n_i}{N}$$

Where, $H =$ Shannon Wiener index of diversity; $\pi_i =$ total number of individuals of a species; $N =$ total number of individuals in sample.

4.5.2. Morisita-Horn index ($C_{mh}$)

$$C_{mh} = \frac{2\sum_{i=1}^{s} [\frac{(an_i)(bn_i)}{da + db}(aN)(bN)]}{(aN)(bN)}$$

This index is calculated from the equation:-

$aN =$ the total number of individuals of all species collected at site A

$bN =$ the total number of individuals of all species collected at site B

$an_i =$ the number of individuals of the $i^{th}$ species collected at site A

$bn_i =$ the number of individuals of the $i^{th}$ species collected at site B

and in the denominator, there are two terms summed that are defined as:

$$da = \frac{\sum_{i=1}^{s} an_i^2}{aN^2} \quad \text{and} \quad db = \frac{\sum_{i=1}^{s} bn_i^2}{bN^2}$$

It was originally developed to measure niche overlap and is Horn’s (1966) modification of an index of similarity proposed by Morisita (1959).
4.6. HYDROLOGICAL VARIABLES

4.6.1. Froude Number

Dimensionless number expressing the ratio of the internal and gravitational force in a fluid was calculated from water current.

\[ Fr = \frac{U \sqrt{gD}}{U} \]

- \( U \) = mean water current, \( ms^{-1} \)
- \( g \) = acceleration due to gravity, \( ms^{-2} \)
- \( D \) = average depth, \( m \)

Fr < subcritical flow
Fr = critical flow
Fr > supercritical flow

4.6.2. Hydraulic discharge

\[ Q = W D U \]

Where
- \( W \) = Average width
- \( D \) = Average depth
- \( U \) = water velocity respectively