CHAPTER 4
MATERIALS AND METHODS

Macroinvertebrates sampling was carried out by using two types of quantitative sampling methods.

1. Artificial substrate samplers (ASS)
2. Conventional samplers

The types of ASS and conventional samplers used in this study and relevant codes used in the text are given in table 3.

Table 3 : Types of sampling methods used in study and their relevant codes used in text.

Sampling Method	Types	Code
1. Artificial substrate Samplers	1.Wire mesh cage filled with stones	WMC
	2.Wooden box filled with dried grass	WB
	3. Multi-plate sampler	MP
2Conventional samplers	1. Berge-Ekman grab	EK
	2. Surber sampler	SS

The structure and operation of each ASS and conventional sampler are discussed below.

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4.1 Artificial Substrates Samplers

i. Wire mesh cage filled up with Stones

Cylindrical cages were made of 12.4 cm height and 13.2 cm diameter, using 24 mm diagonal mesh size wiremesh. One end of the cylinder was permanently sealed and the other end could be opened and closed (Figure 6).

Stones of three sizes with surface areas approximately 45, 65 and 85 cm^2 were collected from Mae Sa stream and Montharan stream. Stones were of approximately uniform size within each size of group. An approximate method was used to measure the surface area of the stones. Papers with surface areas 45, 65 and 85 cm^2 were cut and stones were wrapped with those papers to cover the surface area. A wiremesh cage was filled up with different sized stones and the total surface area of the stones determined by adding the surface areas of individual stones. This method was repeated for 10 wiremesh cages in order to determine the mean surface area and standard deviation for one wiremesh cage. Remaining sixty wiremesh cages were filled up with different sized stones assuming the calculated mean surface area as their surface area.

ii. Wooden box filled up with dried grass (WB)

Boxes of wooden planks were made up of size 12 × 12 × 12 cm. The top of the box was covered with 12 mm diagonal mesh size wire mesh and could be opened. Four holes each 1.2 cm diameter were made on the two opposite walls of the box.

These holes were made to facilitate the movements of the macroinvertebrates as well as to minimize the movement of the box due to water current (Figure 6).

Grass were collected from the bank of the Mae Sa stream and initially dried in sunlight, then kept in an oven until a constant dry weight was achieved. Each box was filled with about 25 g of dried grass.

To obtain the surface area for the colonizing of macroinvertebrates, the surface area of the eight holes were deducted from the total surface area of the box which was calculated from length and width measurements. Since the grass layer in the box settled to the bottom when wet, this area occupied by the grass was ignored.

3. Multi plate sampler made of baked clay tiles (MP).

In present study the original multiplate sampler of Hester and Dendy (1962) was modified by reducing the number of plates, varying the spacing between the plates and using a different type of material for the plates (Figure 6).

Oven baked clay tiles with a center hole and four nails at the four corners of each clay tile of 10 × 10 × 1 cm length, width and height were used. Five of the clay tiles were arranged one on top of the other each separated by about 1 cm space by PVC pipe spacers. To make one set of multi-plate sampler, five clay tiles and four spacers were placed alternately, on a 0.6 cm diameter eyebolt and held in place by a

nut at the top. A metal ring was fastened to the nut to facilitate hanging the multiplate samplers.

The surface area available for colonization of the macroinvertebrates was obtained by calculating the areas of the four side walls, top and bottom walls of one clay tile and multiplying by five.

4.1.1 Setting of Artificial Substrate Samplers

WMC, WB and MP artificial substrate samplers were set in all ten sites mentioned above in group of three. Each group comprising WMC, WB and MP samplers was tied together and placed in different areas in the site (Figure 7).

Figure 6: ASS used in the research. From left to right: Multi-plate sampler, Wooden box and Wire mesh cage filled with stones

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Figure 7: ASS tied up together as one unit before put in site.

Samplers were set during both wet and dry seasons. Each set of ASS were retrieved after eight weeks of colonization period. The dry season artificial substrates were set on 28th March and retrieved on 29th May and the wet season substrates were set on August 25th and retrieved on October 26th.

4.1.2 Retrieving of ASS and processing of samples collected from Artificial substrates.

Before retrieval a large hand net was held under each set of ASS in order to prevent the loss of colonist animals during retrieval. Each artificial substrate was disassembled, placed in a separate bucket and washed thoroughly. The stones in the WMC and clay tiles in MP samplers were washed using a fine brush (Figure 8). The

contents of the bucket was concentrated using a small mesh hand net and placed in separate labeled plastic bottles with 4 % formalin.

Any content present in the hand net held under the ASS during retrieval were sieved through a small net and put in another plastic bottle with 4 % formalin. In the laboratory the sample was thoroughly washed with water and the number of macroinvertebrates in each family counted. They were equally divided between the three types of artificial substrate. If animals are in even number rest of the animals was put in WMC vials assuming high chance to lose the animals from the WMC when retrieval.

Figure 8: Processing of retrieved ASS. Dissembled and wash the stones in the WMC sampler

4.2 Conventional Samplers

i. Berge-Ekman grab

The grab sampler with an aperture area 15 × 15 cm (225 cm^2) was suddenly dropped with force from the bridge or near the banks in order to penetrate the water column vertically. When it was felt that the two jaws of the grab sampler hit the bottom of the ground a sample was retrieved after releasing the messenger. The Ekman grab was operated at the deeper sites such as R1, R2, polluted sites such as SC1, SC2 and irrigation canal sites IC 1 and IC 2 in both wet and dry seasons. Two replicates were done for each site and each replicate consisted of two pooled samples.

The contents of the retrieved sampler were placed in a bucket with water and swirled as much as possible. Large items like twigs, stones and leaves were washed inside the bucket and discarded after confirming that no animals were present. The contents of the bucket were concentrated using a small net and sieving and placed in labeled plastic bottle with 4% formalin.

ii. Surber sampler

A Surber sampler consists of a frame enclosing a sample area of 506 cm^2 , attached to which is 0.4 mm mesh cone shape net for sample collection, and a plastic bottle at the cod end for sample storage (Appendix A 1).

A Surber sampler was operated in all stream sites i.e. ST1, ST2, ST3 and ST4 in both wet and dry seasons. For the stream sites, samples were collected separately from three different substrate types if available i.e. sand, stones and leaf litter in order to investigate the substrate preferences of macroinvertebrates. Sampling according to different substrate types was carried out for three seasons viz. wet, dry and cool seasons.

The Surber sampler was set in particular substrate with its mouth facing the stream flow. The Substrate enclosed by the bottom frame was disturbed as much as possible allowing disturbed materials to flow into the cone-shaped mesh bag. The sample in the cod end (= plastic bottle) was put in a bucket with water and treated in the same way as the Ekman grab samples.

For substrates like stones and leaf litter, after washing these within the frame of the Surber sampler, they were removed from the enclosed area and put in a bucket with water. Each stone and leaf was brushed using a fine brush. The remains in the bucket were concentrated using small net and put in particular bottle with 4 % formalin.

4.3 Sorting and Identification

Samples were sorted and identified at the laboratory. Each sample was put in a hand net and washed thoroughly with tap water in order to remove excess formalin prior to analysis. Care was be taken to not wash under high water pressure, which would have damaged organisms in the sample making identification difficulty.

Since samples collected from artificial substrates did not have much debris, it was not necessary to have swirled the sample in bucket of water again at the laboratory. However, the sample collected from conventional methods did contain many debris yet, the same procedure was repeated to wash out excess formalin. Then the contents in the net was placed in bucket with water and swirled. Floating and suspended large materials were removed after inspection for animals.

Macroinvertebrates from each sample were sorted in a white tray using hand lens or stereo microscope, transferred to 70 % alcohol and identified to family level using the following references and keys.

- McCafferty (1981) : Pennak (1978) : Merritt and Cumnins (1988) :Williams (1991) : Sannarm (1993) : Needham (1969) : Lehmkuhl (1979)

Data sheets were prepared separately for each sampling method and different substrate types.

4.4 Determination of colonization sequence curve

Twenty set of artificial substrate, each set consisting one WMC, one WB and one MP were randomly set at site R 1. The site selected had two main characteristics. The river bank already had poles, where the artificial substrates could be hung (Figure 3). Due to closeness to a private house, the substrates also had less vandalism. After setting the substrates, two sets of each artificial substrate were retrieved each week

within the first month and processed as described above. During the second and third months two sets of samples were retrieved twice a month. Collected macroinvertebrates were identified only to family level.

Graphs were drawn separately for each type of ASS to show the number of macroinvertebrates families present and the total number of individuals on a weekly scale.

4.5 Data analyses

Water quality in study sites was assessed by biological impairment of the benthic community. The metrics used for Rapid Bioassesment Protocol II (RBP II) for macroinvertebrates (Plafkin *et al.*, 1989) and the protocol used in the GEMS/ water system (Thorne, 1993) were used.

The data analysing scheme used in RBP (II) and GEMS/Water system integrates several community, population and functional parameters into a single evaluation of biotic integrity. Since it is based on several parameters, it provides more assurance of a valid assessment. Each parameter measures a different component of community structure and has a different range of sensitivity to pollution stress (Plafkin *et al.*, 1989)

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The following metrics were used to evaluate water quality in the study sites using collected macroinvertebrates.

i. Taxa or family richness

ii. Modified Family Biotic Index

iii *A*verage *S*core *P*er *T*axa (*ASPT*) values based on *B*iological *M*onitoring *W*orking *P*arty (*BMWP*) Scores adopted for India.

iv. Number of EPT (Ephemeroptera, Plecoptera and Trichoptera) families

v. Ratio of EPT and Chironomids abundance

vi. Percent contribution of dominant family

vii. Community Similarity/ Loss Indices

a. Community loss index

b. Jaccard Coefficient of community similarity.

c. Sorensen's similarity Index

A brief interpretation of each metric is given below

i. Taxa Richness

This index measures the total number of taxa or families present and simply gives an idea of the health of the community. Generally it increases with increasing water quality, habitat diversity and habitat suitability.

ii. Modified Family Biotic Index

This index was developed to summarize the various tolerances of the benthic arthropod community with a single value (Hilsenholf, 1988). It calculates a score based

on a average tolerance per individual and therefore takes into account abundance as well as presence/absence. Tolerance score ranges from 0 to 10, indicating animals live in excellent water quality and very poor water quality with severe pollution respectively (Appendix B 1). Tolerance values will vary accordance to region and level of taxonomic identification.

The following formula was used to calculate this index.

$$\mathbf{FBI} = \Sigma\,(x_i \cdot t_i/n)$$

Where x_i - number of individuals within a taxon/family

t_i - tolerance value of a taxon (from Hilsenholf, 1988)

n - total number of organisms in the sample

iii. *A*verage *S*core *P*er *T*axa (*ASPT*) values based on *B*iological *M*onitoring *W*orking *P*arty (*BMWP*) Scores adopted for India.

Each family has a score range 1-10, reflecting its general tolerance to organic pollution. High score are registered by the least tolerant groups and low scores for highly tolerant groups (Appendix B 2). The ASPT is calculated by dividing the sum of BMWP scores by the number of corresponding scoring families.

$$\text{ASPT} = \frac{\Sigma \text{ BMWP score for family}}{\text{No: of families with Score (No: of indicator family)}}$$

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iv. Number of EPT families

The insect orders Ephemeroptera, Plecoptera and Trichoptera are considered to be pollution sensitive and therefore the total number of families of these three orders will proportionately relate to the water quality.

v. Ratio of EPT (Ephemeroptera, Plecoptera and Trichoptera) and Chironomids abundance

Uses the abundance of these four indicator taxa as a measure of community balance. It is generally considered unpolluted sites should have a fairly even distribution of these groups but the chironomids will become more dominant in a stressed environment.

vi. Percent contribution of dominant family

This metric simply measures the distribution of individuals among the families. Stressed communities typically consist of few families and have a high dominance index. Therefore environmental degradation can be measured by fluctuation of dominant family/taxon score.

The formula used in this metric is

$$D = \frac{n_{max}}{N} \times 100$$

Where D = percent dominate index

n_{max} = maximum number of individuals in the sample

N = total number of individuals in the sample

vii. Community Similarity/ Loss Indices

When a reference community exists community similarity indices can be used. In this study three types of community similarity/loss index were incorporated.

a. Community loss index

This index developed by Courtemanch and Davies (1987) is more discriminating than many similarity indices (Plafkin *et al.*, 1989). It measures the loss of benthic taxa between the reference site and a comparison site. Values range from 0 to infinity and increase with dissimilarity. The formula used is given below.

b. Jaccard Coefficient of community similarity

This measures the degree of similarity in taxonomic composition between two sites considering taxon presence or absence. Coefficient values ranging from 0-1, increase as the degree of similarity with the reference station increases. The formula for this index is given below.

c. Sorensen's similarity index

This also measure the degree of similarity between two sites in terms of taxonomic composition. Values range from 0-10 and increase as the degree of similarity with the reference site increases.

The formulae used in these three index are

$$\text{Community loss index} = \frac{D - A}{E}$$

$$\text{Jaccard Coefficient of community similarity} = \frac{A}{(A+B+C)}$$

$$\text{Sorensen's similarity index} = \frac{2A}{D + E}$$

Where A = number of families common to both samples

B = number of families present in site of comparison but not in reference site

C = number of families present in reference site but not in site of comparison

D = total number of families present in reference site

E = total number of families present in site of comparison

Using benthic data, values calculated for each metric and expressed as a percentage to the reference site except percent contribution of the dominant family in which the actual percent value itself used. Quality points were allocated for each metric according to following quality points ranges (Table 4).

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Table 4: Quality point ranges for each score

Quality points	6	4	2	0
Taxa richness	> 80 %	61-80 %	40-60 %	<40 %
ASPT	>92 %	81-92 %	68-80 %	<68 %
BMWP	>80 %	51-80 %	20-50 %	<20 %
Modified FBI	>85 %	71-85 %	50-70 %	<50 %
EPT : Chironomids abundance	>75 %	51-75 %	25-50 %	<25 %
Community loss	<0.5	0.5-1.5	1.6-4.0	>4.0
% dominant family	<30 %	30-40 %	41-50 %	>50 %
No : of EPT family	>90 %	81-90 %	70-80 %	<70 %

Source : Plafkin *et al.* (1989), Thorne (1993).

In order to combine all indices and give a single score for each site, quality points are totaled for each site. Calculated totals are then compared to the total from the reference site and expressed as a percentage of these totals. Study sites can be classified into four broad categories of water quality based on final percentage classification as shown in table 5.

Table 5: Quality points ranges for the final classification of sites.

% of reference score	Biological condition category	Attributes
>80 %	Non-impaired	Comparable to the condition at the reference site, with good community structure.
51-80 %	Slightly impaired	Loss of some sensitive taxa/families. contribution of tolerant forms increased.
20-50 %	Moderately impaired	Fewer families/taxa due to loss of most of sensitive groups especially EPT.
<20 %	Severely impaired	Few families/taxa. Community dominated by one or two groups

Source : Thorne, (1993)

In addition data were analyzed statistically using SPSS (Statistical Package for Social Sciences) program. Cluster analyses were performed using prescribed criteria. *AN*alysis *O*f *VA*riance (*ANOVA*) was used to compare the relative efficiencies of different artificial substrates and artificial substrates with conventional methods in terms of number of animals and families found, as well as differences between the animals inhabiting different substrate types.