CHAPTER 4

Results

4.1. Physico-chemical parameters of the water body in the streams

The overall results from the physico-chemical analysis of water in the two streams showed that the two streams were similar in terms of many physical and chemical parameters. From the raw data the seasonal variation was noticed quite clearly for almost all the parameters measured.

No extreme values were noticed for parameters like dissolved oxygen, pH, alkalinity, conductivity and velocity. Comparatively higher values were noticed for nutrient parameters like nitrate, ammonia and phosphorous in the month of November. (**Appendix-2**).

4.1.1. Physical parameters

4.1.1.2 <u>Water velocity</u>

The water velocity in stream A varied from 0.29 m/sec to 0.44 m/ sec and it ranged from 0.42 to 0.68 m/sec ($\bar{x}$ = 0.59 m/sec $\pm$ .09) in stream B. The highest velocity were noticed in the month of June (rainy season) in both streams.

4.1.1.2. **Depth and widths**

The depth of the streams also showed fluctuation over the year. The depth of stream A varied from 10 to 30 cm ($\bar{x}$ = 16.6 ± 7.5) and for stream B it varied from 6.0 to 28 cm ($\bar{x}$ = 8.7) as. stream B. The increase in width with increase in water volume was noticed in the month of June in rainy season and lower values were noticed in the month of March (summer) in both the streams.

4.1.1. 3 **Air and water temperature**

The air temperature ranged from 27 ^{0} C in summer to 20.5 ^{0}C in winter at the sampling sites of stream A. The air temperature range was in between 29.5 ^{0} C to 17.8 ^{0} C for stream B. The water temperature ranged from 16.8 ^{0} C to 21.4 ^{0}C for stream A and 15.8 to 23.9 ^{0} C for stream B. The water temperature was in correspondence to air temperature recorded .

4.1.2. Chemical parameters

4.1.2.1. **pH**

The pH of the water in stream A ranged from 7.1 to 8.2 ($\bar{x}$ = 7.48 ± .53). For stream B, the pH ranged from 6.8 to 7.37 ($\bar{x}$ = 7.1 ± .21). The variation of the pH from neutral to slightly alkaline in stream A was noticed in June (rainy season). The variation pattern of pH also followed the seasonal change (Fig. 5a). All the values noticed for pH were within the range of maximum allowance limit according to Thai standard for surface water (6.5-8 .5).

4.1.2.2. **Alkalinity**

The alkalinity in stream A ranged from 8 to 17 meq /l ($\bar{x}$ =14.5 $\pm$ 3.01). The recorded alkalinity for stream B ranged from 11 to 17 meq / l ($\bar{x}$ =11.6 $\pm$ 2). Alkalinity did not show much fluctuation over the year. Moreover no remarkable seasonal variation was noticed for this parameter. The values recorded from both the streams were within the range of surface water quality for running waters (20 meq/l)
20 meq/l) Fig. 5b.

4.1.2.3. **Conductivity**

The conductivity ranged from 25 to 40.4 µs/sec for stream A ($\bar{x}$ = 31.88 $\pm$7.13). For stream B it ranged from 27.5 to 39.4 9 µs/sec ($\bar{x}$ =32.54 $\pm$ 4.06). The high conductivity were recorded in rainy season during June in both the streams (Fig. 5c). The variation of conductivity also followed the seasonal change.

4.1.2.4. **Dissolved oxygen (D.O) and percent saturation**

The variation of dissolved oxygen corresponded with percent saturation recorded in each site. The dissolved oxygen recorded for stream A ranged from 6.1 to 9.2 mg/l ($\bar{x}$ = 7.5 $\pm$ 1.13). The high dissolved oxygen were recorded during the month of November (early winter) in both the streams. For stream B, dissolved oxygen ranged from 6.6 to 8.9 mg/l ($\bar{x}$ = 7.24 $\pm$ 1.88) . The variation pattern for this parameter is shown in Fig.5d.

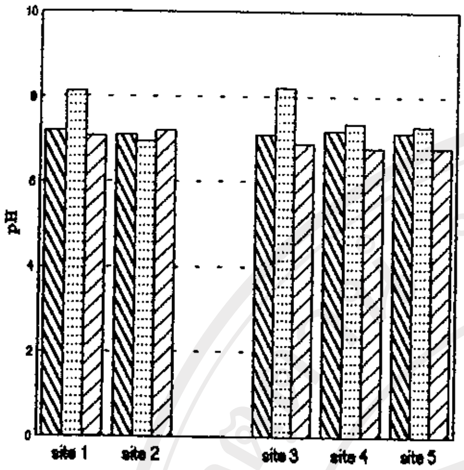


Fig 5a pH

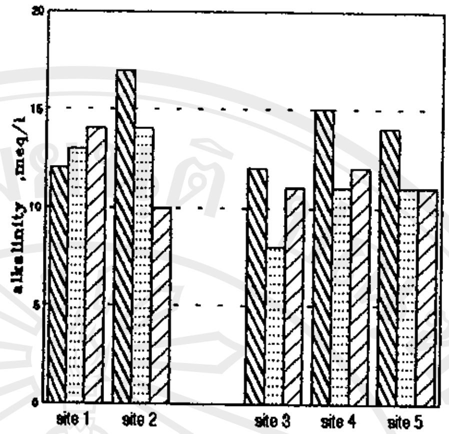
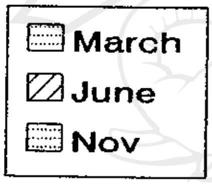


Fig. 5b Alkainity (meq/l)



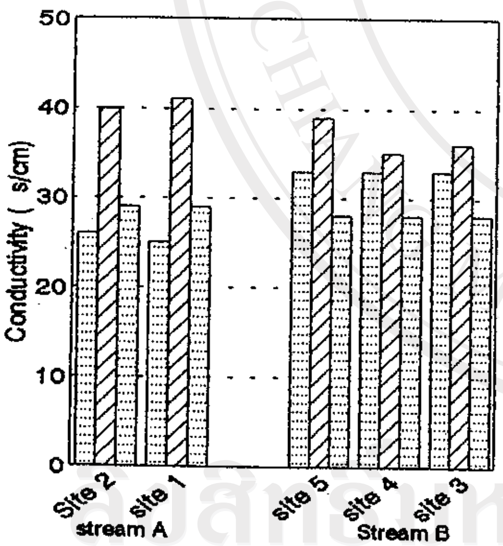


Fig 5c Conductivity

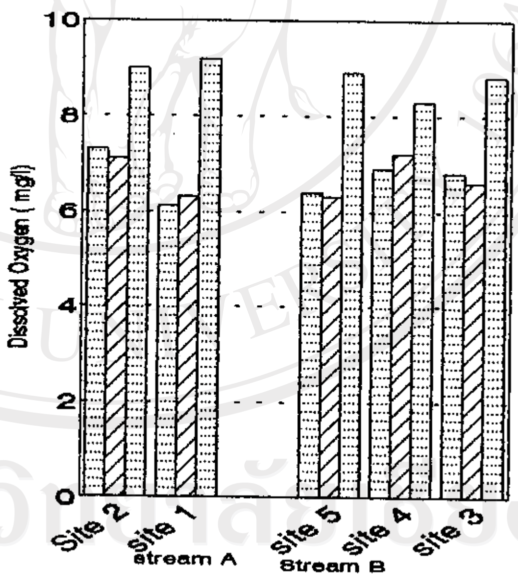


Fig 4d Dissolved oxygen

Fig 5 Graph showing the variation of different phyical and chemical parameterin the two stream

4.1.2.5 **Nitrate (NO_3-N)**

The nitrate concentration in stream A ranged from 0.1 to 0.2 mg/l and 0.1 to 2.6 mg/l in stream B. The higher concentration of nitrate in stream A was recorded in rainy season in site 1. But for stream B, all the sites showed higher values in November. Though the nitrate concentration noticed in site 1 in rainy season (1.6 mg/l and sites 4 (1.6 mg/l) and 5 (2.6 mg/l) are comparatively higher compared to other seasons and sites, the values were within the range of maximum allowance limit for surface water quality by Thai surface water standard (5 mg/l).The variation pattern for this parameter is displayed in Fig. 6a.

4.1.2.6. **Ammonia (NH_3-N)**

The NH_3-N was non detected in the month of March in site 2 and site 5. The highest values for this parameter was noticed in the month of November followed by June in both the streams. The highest values for stream A was noticed in site 1 in November (0.82 mg/l). The highest recorded ammonium concentration for stream B was 1.01 mg/l in site 3 in the month of November. The variation pattern for this parameter is shown in Fig.6b.

4.1.2.7 **Phosphorous (PO_4-P)**

Soluble phosphorous ranged from 0.1 to 0.26 mg/l ($\bar{x}$ = 15 $\pm$ .06). in stream A and the highest concentration (0.2 mg/l) was recorded in rainy season in site 3. For stream B, the high PO_4 was recorded in November and it ranged from 0.1 to 0.32 mg/l ($\bar{x}$=0.6 $\pm$.08). The site 2 in summer (June) and site 5 in November (early winter) showed next higher concentrations. All the values recorded were well above the eutrophic state of lakes (0.05 mg/l). The variation pattern for this parameter is shown in the Fig.6c.

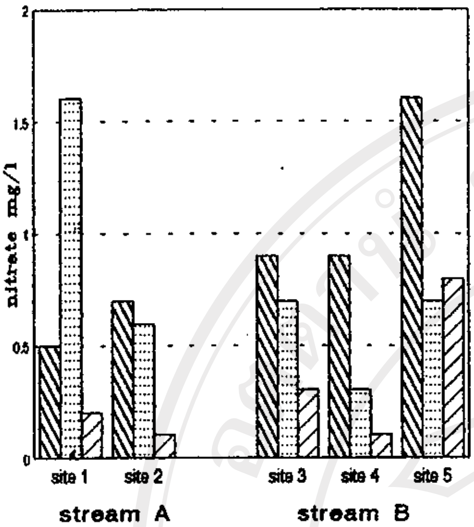


Fig.6a Nitrate (mg/l)

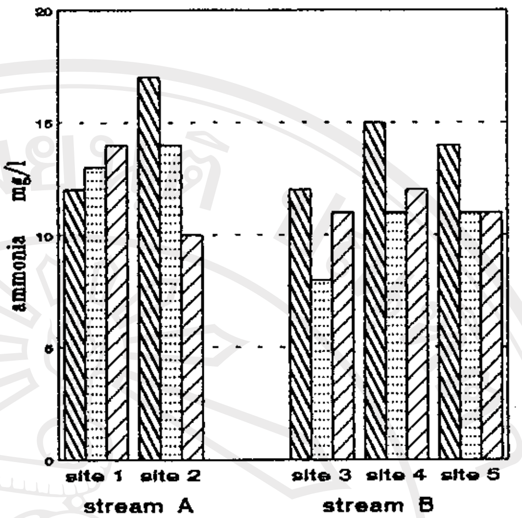


Fig. 6b Ammonia (mg/l)

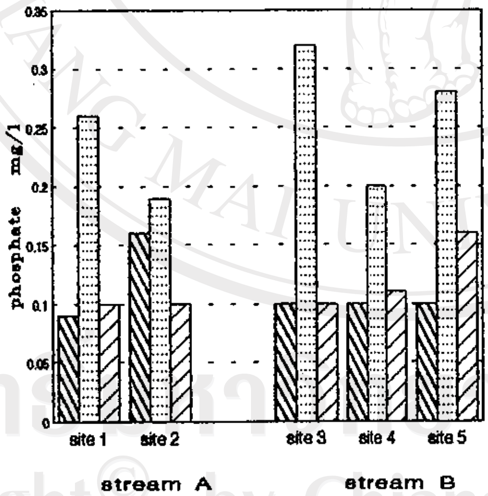


Fig. 6c Phosphorous (mg/l)

Fig.6 Figures showing the variation pattern of nutrient parameters in the two streams A and B in different seasons

4.1.3 Cluster analysis based on physico- chemical parameters of water quality

The cluster analysis was done using average linkage clustering with standardized Z score, and pearson's correlation coefficient based on the physico-chemical parameters of water quality. The resulting dendrogram showed that there is no clear distinction between the two streams based on the measured water quality parameters as no distinct cluster was formed representing the sites from each stream (site 1 and 2 representing stream A and site 3,4 and 5 representing stream B) . The clusters formed significant distinct grouping for the month of November only. Though the other groupings were formed for the month of March and June, these cluster did not represented significant level. Therefore, they were not considered as significant representative of each rainy and cool season. However, the general grouping tendency showed more seasonal variation than anthropogenic influences as shown in the **Fig 7** .

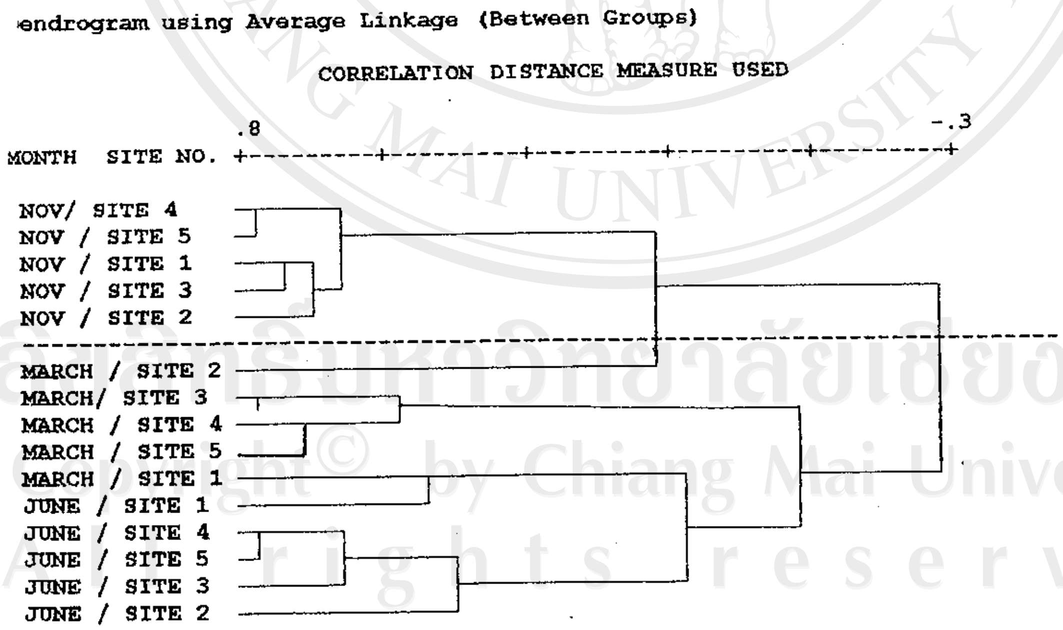


Fig.7 : Dendrogram showing the seasonal variation of physico-chemical parameters of water quality in different seasons and sites.

4.1.4 Factorial analysis for water quality parameters

The principle component analysis for water quality parameters were done using rotated factor analysis. 3 factors were extracted for stream A with eigenevalues more than 1 which explained 91.4 % for the whole physical and chemical data. Factor 1 and factor 2 alone explained 75.9 % of the water quality for stream A. The main factor loading parameters for factor 1 were phosphate, conductivity, velocity, nitrate, depth , pH and alkalinity. The values above 0.5 were considered as significant with reference to the similar approach made by Lohani (1984). For stream B, only two factors were extracted out of 10 factors as principal components. Factor 1 and factor 2 explained 86.3 % of the whole data. The most important factor loading parameters for factor 1 for stream B were phosphate, conductivity, velocity, nitrate, alkalinity, dissolved oxygen and percent saturation. The extracted factor 2 explained 24.5 and 25 % for stream A and stream B respectively. Factor 3 for stream A explained only 15.5 % of the total variation. Factor 3 was not extracted for stream B. The positive factor loading parameters for factor 2 for stream A and B and factor 3 for stream A were as follows:

	Stream A	Stream B
Factor 2	Ammonia, Dissolved Oxygen, Percent saturation and Ammonia	Ammonia, pH and depth

The eigen values, factor loading parameters, percent variance and communality for each of the streams are shown in **appendix 3 and 4** for stream A and B respectively.

4.2 Substrate composition

The result of the substrate composition based on the mean percent area coverage showed that the two streams were completely different in terms of their substrate composition. Stream A was found mainly dominated by sand and silt forming more than 70 % of the stream bed coverage while for stream B, the major substrate composing the stream bed were stones (> 2 cm) and gravels. About 70 % of the stream B was covered with stone and gravels .The overall view of the substrate composition is shown in the **fig. 8a and 8b.**

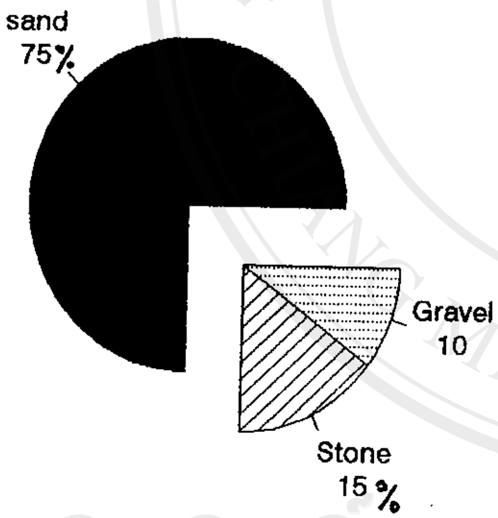


Fig. **8a** substrate composition in stream A

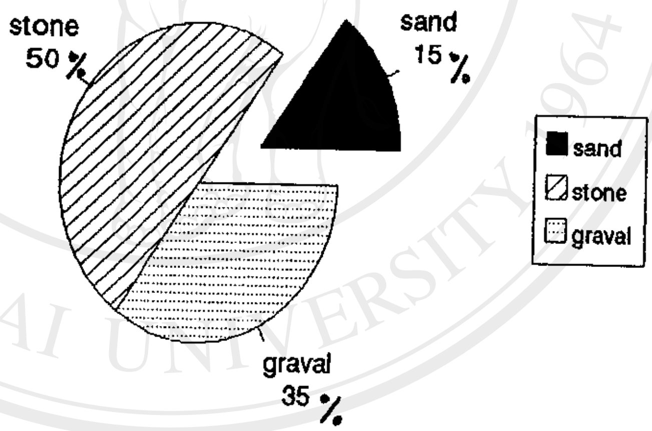


Fig. **8b** Substrate composition in stream B

Fig.8 : Pie charts showing the composition of stream bed with different substrate type. The charts clearly displays the dominance of sand /silt in stream A and dominance of stones and gravel in stream B.

4.3 Pesticide residue analysis

The result from pesticide residue analysis showed that traces of organochlorine pesticides were detected in both streams. The concentration of detected organochlorine pesticides in stream A was higher than in control stream B. The metabolites of heptachlor pesticides e.g. heptachlor epoxide was detected only in stream A (**Table 2**). This can also be supported by the findings of the fact that heptachlor pesticide in agricultural practice in the highlands of northern Thailand.

The mean percent recovery for the pesticides is shown in the **Appendix -5.** The percent recoveries of heptachlor (105 %), heptachlor epoxide (119 %), p,p'- DDE (77.6 %), o,p'-DDT (96.8 %), and p,p'-DDT (84.4%) were satisfactory while the recoveries of o,p'-DDE (165 %), dieldrin (185.5%), p,p'-DDD (157.57 %) were rather high. The concentrations shown in table 2 are not corrected according to the recovery of the respective pesticides.

The higher standard deviation of the recovery of individual pesticides shown in the appendix 5 is attributed to the difference in the textural combination of different sediment samples. The result of the textural analysis of the sediment which is shown in **Appendix 6** showed that the organic matter content is relatively higher in the sediment samples of stream B. Since, it is known that the organic matter is liable to contribute in absorbance of pesticides in higher concentration, it was expected that the sediments of stream B, having comparatively higher concentration of organic matter would absorb more of these pesticides. The higher standard deviation in pesticide analysis was attributed to difference in textural differences of the sediment samples analyzed.

Table.2 Distribution of pesticides in the sediments of stream A and B. Stream Ashows detection of some organochlorine pesticides in comparatively higher concentration than stream B

Pesticides	stream A(μg/ kg)	stream B (μg/kg)
Heptachlor	7.7	4.7
Hept. epoxide	5.8	Nd
o,p'-DDE	15.6	0.2
p,p'-DDE	2.5	3.3
Dieldrin	23.6	3.1
p,p'-DDD	5.0	3.2
o,p'- DDT	3.0	3.9
p,p'-DDT	11.4	5.8

Nd = Not detected

4.4 Benthic community analysis

4.4.1. Cluster analysis based on benthic community

The result from the hierarchical classification using average linkage with standardized Z score and pearson's correlation coefficient as a basis of clustering showed that there is relative similarity between sites 1 and 2 in stream A and among the sites 3, 4 and 5 in stream B in every season. The presence of distinct faunal groupings is confirmed by the two distinct clusters formed representing the two streams A and B as shown in the Fig 9. Distinct clusters can be noticed for sites 1 and 2 for the month of March, June and November representing stream A. Similarly, distinct cluster can be noticed for the sites 3, 4 and 5 for the month of November, June and March representing stram B.

The clustering of the families as variables with similar criterion as cases formed two significant groupings at 0.05 confidence level. The two groupings were named as group I and group II as shown in the **Appendix 7**. The group 1 comprises of families Hydraenidae, Notonectidae, Palingeniidae, Ptilodactylidae, Chloroperlidae, Taeniopterygidae. Ametropodidae, Pteronarcyidae and Athericidae. Group II comprises of families Empididae, Hydrophilidae, Ephemeridae, Psephenidae, Philopotamidae, Ephydridae, Glossomatidae, Perlidae, Lepidostomatidae, Perlodidae, Siphlonuridae, Leptophlebiidae, Helodidae, Ephemeridae, and Pleidae.

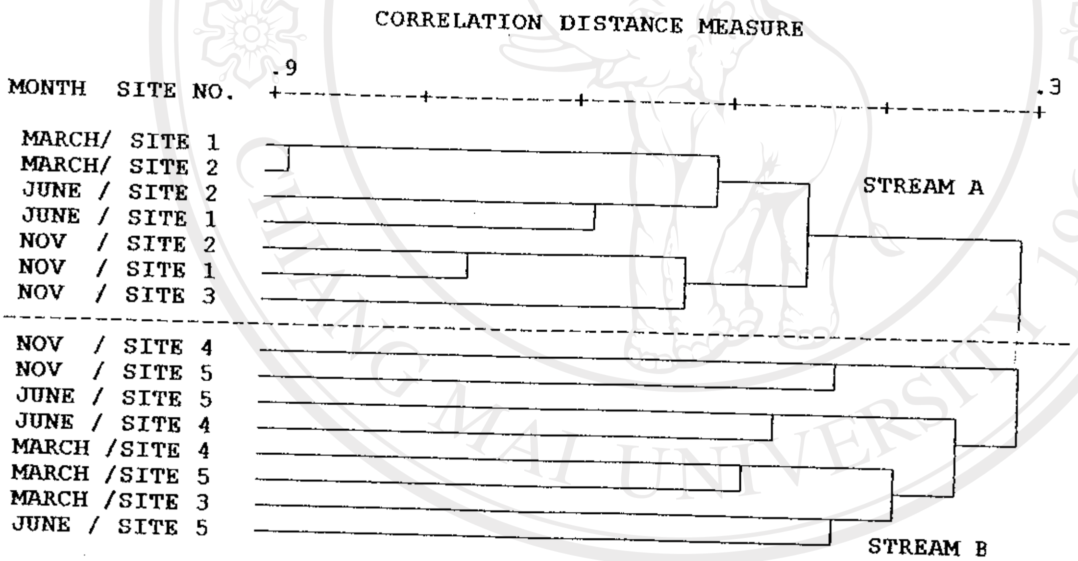


Fig. 9 : Dendrogram showing the groupings based on representative sites from each stream. Site 1 and site 2 represent stream A . Site 3, 4 and 5 represent stream B. The grouping pattern shows that the faunal groupings are diferent in each of the stream.

4.4.2 Diversity and evenness

The result from Shannon Wiener Diversity Index showed that the calculated " H" value is relatively lower for the sites 1 and 2 compared with sites 3, 4, and 5 (**Fig. 10**). The number of rare families (N_1) and number of very abundant family (N_2) values are also always lower for site 1 and 2 compared to sites 3,4, and 5. Among the sites in stream B, site 3 has lower N_1 and N_2 values. Modified Hill's ratio (E_5) value is explaining the uneven distribution of the species in site 1 and 2 mainly during summer. The E_5 values seems to be higher following rainy and winter season for site 1 and 2. For sites 3, 4 and 5, the N_1 and E_5 values are always higher in all the sampling seasons (**Appendix 8**).

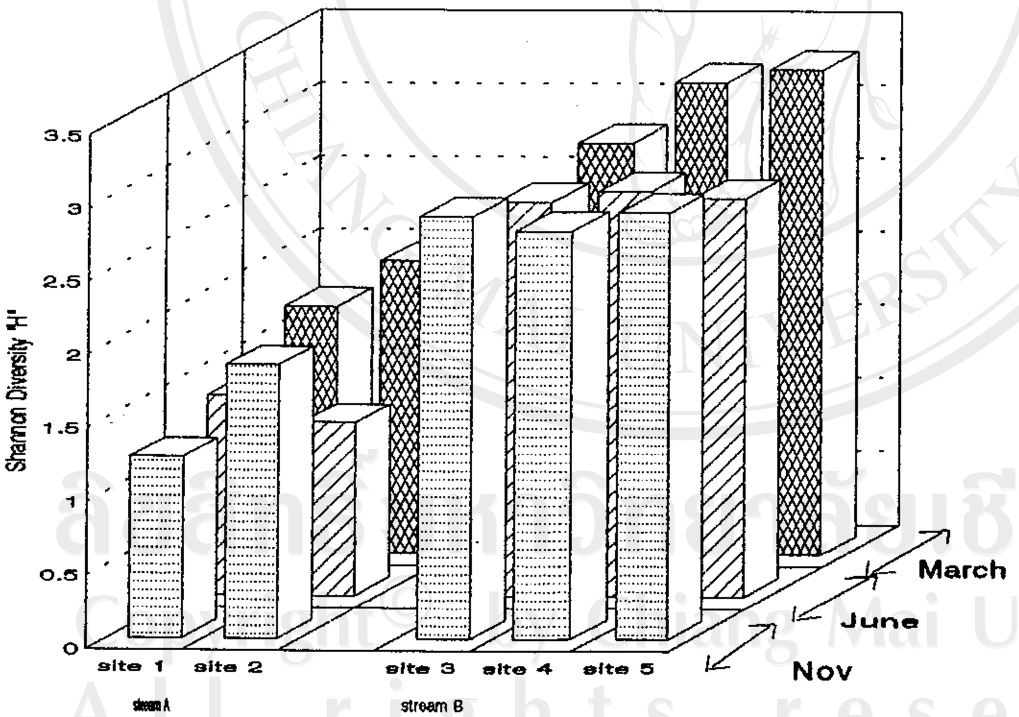


Fig.10 Graph showing the diversity of the families in each sites in different seasons. Site 1 and site 2 represent stream A and site 3, 4 and 5 represent stream B.

4.3. Community structure analysis

Community structure analysis using number of families recovered in each site of each stream showed that fewer families were present in stream A compared to the findings from stream B. The faunas found were relatively in small numbers in stream A during all the seasons.

Six major orders Plecoptera, Ephemeroptera, Coleoptera, Hemiptera, Diptera and Odonata were recovered in both streams. Insect orders Ephemeroptera, Diptera, Tricoptera, formed major part in the species composition in stream A followed by Coleoptera, Hemiptera and Plecoptera. Only one family Perlidae was recovered belonging to order Plecoptera in stream A. Higher number of each of the orders Ephemeroptera, Tricoptera, Diptera, Coleotera, Hemiptera, and Plecoptera were recovered in stream B . Order Diptera formed comparatively dominant group in stream A with lower number of the other families recovered where as the overall distribution pattern of faunas belonging to different orders gave more even distribution pattern in stream B. No prominent dominance is observed for any particular group of family at any season and site in stream B. The general community structure of the faunas in each of the stream is shown in the **Fig 11.** This community structure was produced by plotting the cumulative number of total families discovered in each stream at different sites over the sampling period of 9 months from March 1994 to November 1994.

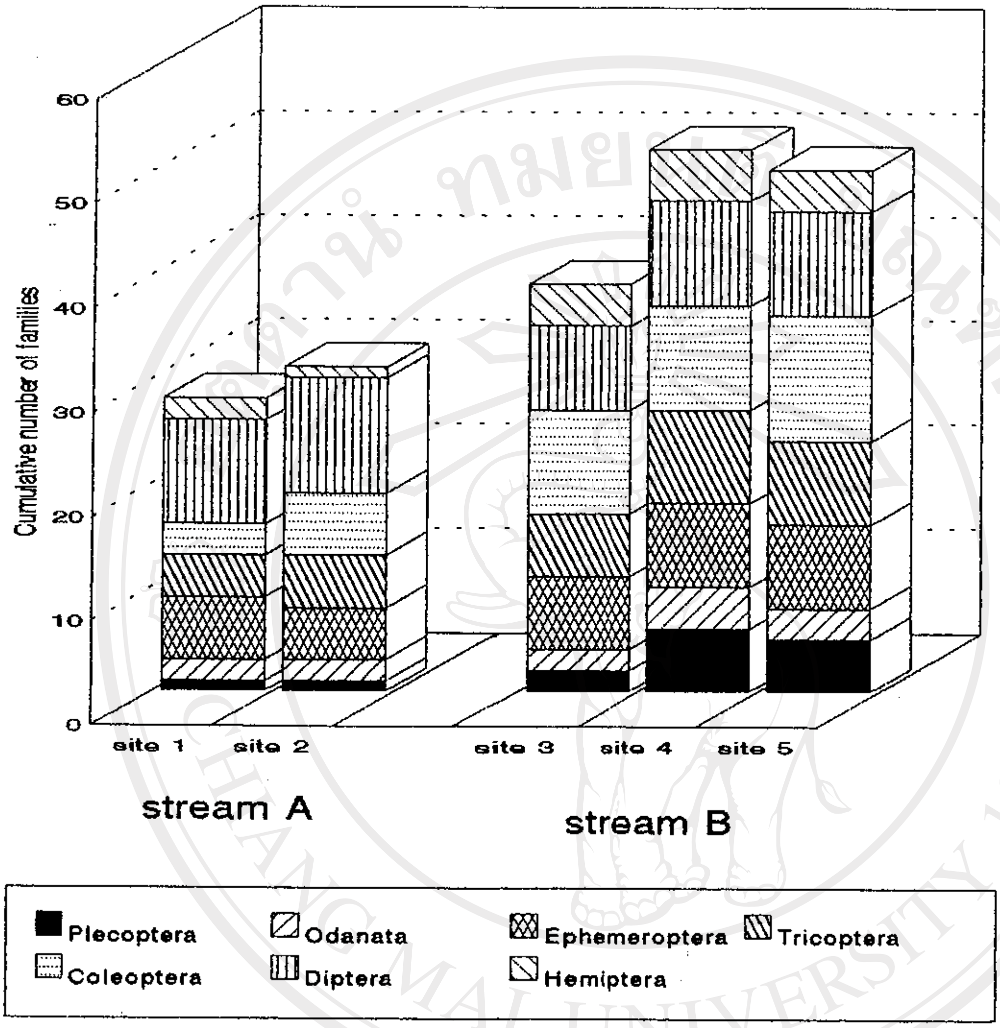


Fig. 11 : Graph showing the community structure of the benthic families in each site. Y axis represents cumulative number of families recorded over the total sampling period. Stream 1 and 2 representing stream A shows the community comprising fewer number of families compared to sites 3, 4 and 5 representing stream B.

4.4.4. Lognormal distribution of the families

The lognormal distribution of the families showed that the total number of families recovered in stream A were low in number compared to stream B. Only 21 families in stream A belonged to class I (0-10 families) compared with 37 families belonging to the class I in stream B. The distribution of other families in stream A showed as discontinuous and mainly dominated by three families Chironomidae, Hydropsychidae and Simuliidae. The overall look of the curve give shallow look for stream A compared to that of stream B with less steep slope representing lower number of rare faunas. Similar plot for the stream B gave different impression. The steeper slope formed signified the presence of higher number of rare families in stream B. (**Fig 12a, 12b**).

4.4.5 Ordination of families

The results from the varimax rotated factor using all the benthic community data extracted 12 factors with eigenevalues higher than 1. Factor 1 and 2 explained 34.8 % of the total variation of the families in the two stream under study (**Appendix - 9**). Since the extracted factors are considered to be independent and factor 1 to be explaining highest variance followed by factor 2 explaining next higher variance as explained in the methodology, the factor loadings from factor 1 and 2 were plotted against each other to find significant sub groupings which can represent the most of the commonality representing the streams.

The ordination plotting factor I and factor II formed 3 sub groupings as shown in the **Fig 13.** The group I and II were formed at high correlation coefficient

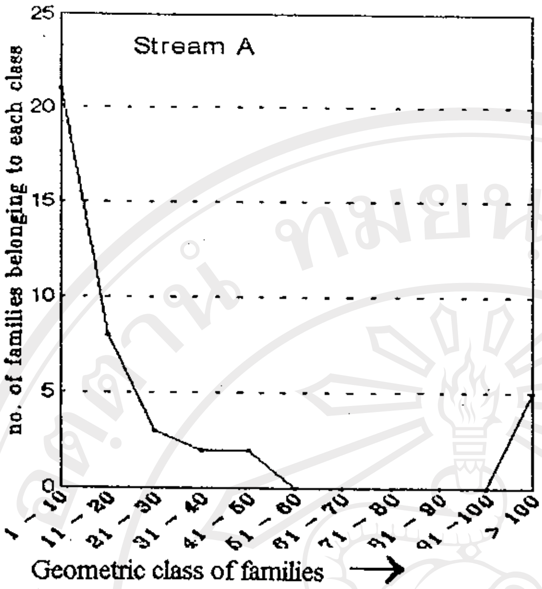


Fig 12a Graph showing the lognormal distribution pattern of families in stream A

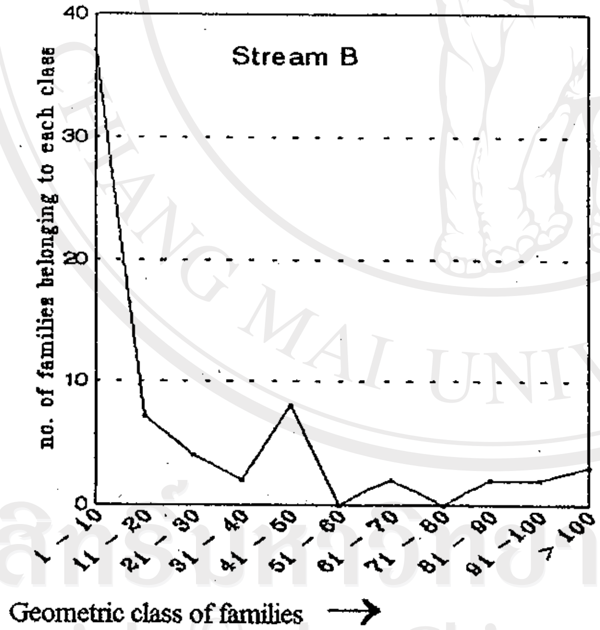


Fig 12b Graph showing the lognormal distribution pattern of families in stream B

Fig 12 Graphs showing the lognormal distribution of the families in each stream. The steep slope indicate higher number of rare families present in that ecosystem type. The distribution pattern of the families can be recognized as uneven in cae of stream A and comparitively even in case of stream B

level (>.85). But group III were formed at low correlation coefficient level ($\leq$ .2). Therefore, group III were not considered significant groupings and were not considered for further discussions. Group 1 consisted of the families belonging to stream A and B. This significant grouping represented only 18.4 % (**Appendix-9**)of the total variation of the benthic community in the two streams. Group II formed at high correlation coefficient level (> .85) represent the faunas absolutely from stream B. The faunas comprising the significant groupings of the families from factor 1 and factor II, altogether were taken for explaining the commonality between the two streams A and B. These groupings formed from the ordination are also supported from the results of cluster analysis.

Factor 1 Grouping

1. Philopotamidae
2. Hydrophilidae
3. Empididae
4. Glossomatidae
5. Psephenidae
6. Perlidae
7. Helodidae
8. Siphlonuridae
9. Leptophlebiidae
10. Ephemeridae
11. Pleidae
12. Lepidostomatidae

Factor 2 Grouping

1. Palingeniidae
2. Chloroperlidae
3. Ptilodactylidae
4. Notonectidae
5. Hydraenidae
6. Taeniopterygidae
7. Ametropodidae
8. Pteronarcydae
9. Anthericidae

The result from the factorial analysis was similar to that of cluster analysis. Similar groupings were formed in both the cases.

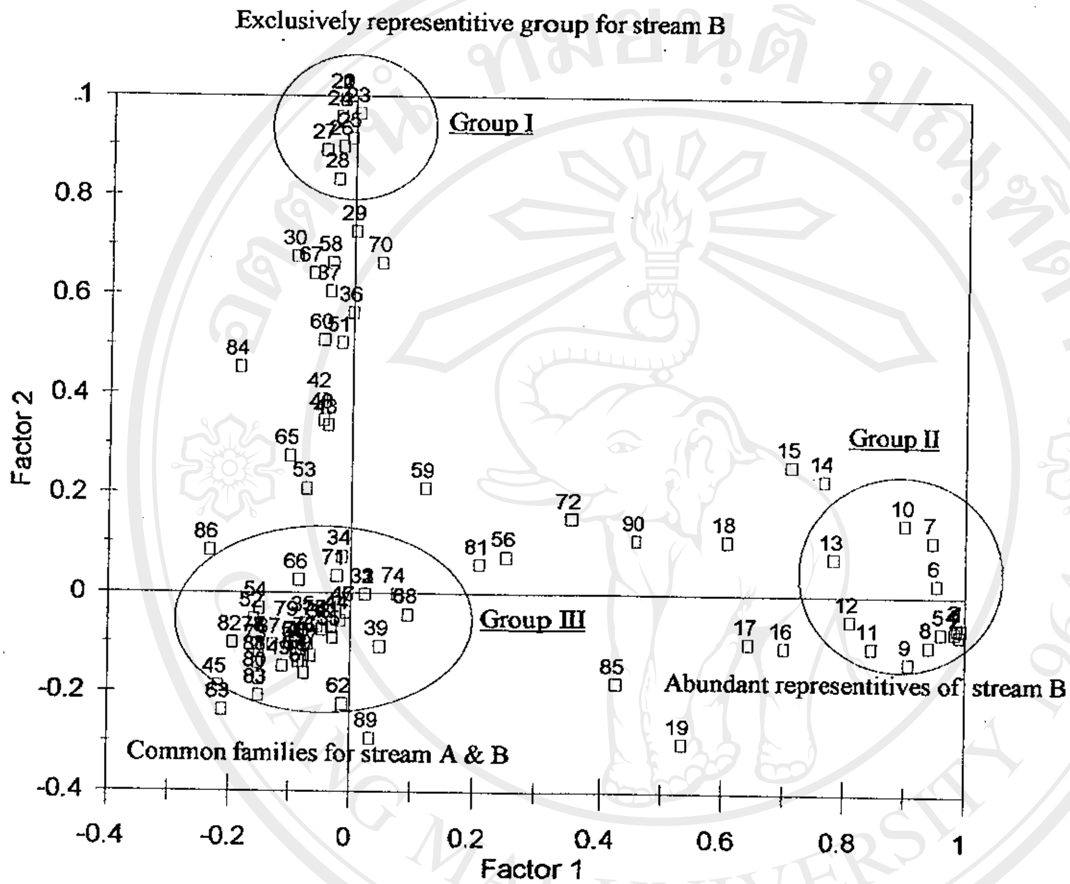


Fig. 13 Ordination of the benthic families using rotated varimax factor 1 and 2

4.5 Results from MANOVA

The results from multivariate analysis of variance (MANOVA) showed that there is no statistical difference between the two streams in terms of water quality. parameters.

No difference was found for the parameters alkalinity, ammonia, nitrate, percent saturation of oxygen, pH, phosphorous and velocity. The two stream differed significantly in terms of diversity, evenness and richness. Significant difference was also found for the substrate composition in each of the stream bed. The two stream differed significantly at composition of sand, stone (rocky substrate) and gravel. This can be supported from the findings of substrate composition (refer text. 4.2).

The parameters showing significant difference in the seasonal variation between the two streams were mainly ammonia, percent saturation of oxygen, pH and velocity. Seasonal variation was noticed for family richness in each of the sites in each stream. The variation of the families were insignificant within the sites of each stream A and B but the same data gave significant difference for family richness when compared to the two streams in each season (**Appendix. 10**).

The level of significance for each of the variables measured are shown in the **table 3.**

Table: 3 Table showing the statistical difference between the two streams in terms of physical and chemical parameters of water quality, community (families) and percent substrate composition in each stream A and B using multiple analysis of variance. Variables indicate the parameters accounted for each of the constants. Sig. F indicate the significant value at 0.05 confidence level. The values below 0.05 are considered to be significant.

constants	variables	Sig. F
Water Quality	Alkalinity	.246
	Ammonia	.985
	Nitrate	.608
	Percent Saturation	.777
	pH	.693
	Phosphate	.336
	Velocity	.692
Community (tax)	Diversity	.000
	Evenness	.004
	Richness	.001
Substrate	Sand	.002
	Stone	.000
	Gravel	.001
seasonal variation (between stream A and stream B)	Alkalinity	.52
	Ammonia	.000
	Nitrate	.073
	Percent Saturation	.003
	pH	.028
	Phosphate	.420
	velocity	.000

4.6 Correlation between physico-chemical, biological and percent substrate composition in each stream

The correlation coefficient matrix using physico-chemical, biological parameters like diversity, evenness, richness and percent substrate composition, for streams A and B is shown in the (**Appendix 11** and **Appendix 12**). The organochlorine pesticides detected were too low in concentration to cause any environmental damage to the benthic communities, therefore these variables were not considered for correlating with the biotic indices along with other environmental variables. This was taken only as possible indication of pesticide impact on the experimental stream A.

Since, diversity is the combination of richness and evenness, it was not taken for representing the biotic community. Richness, represented absolute number of species encountered in a sampling effort with consistency in sampling design, sampling effort and sampling intensity. Therefore, the correlation measure of this biotic indices with several abiotic factors was expected to give idea about the early warning for different kinds of stresses posed on that ecosystem and was taken for explaining the impact of other environmental variables.

In stream A, significant correlation was found for richness with depth, ammonia, velocity, conductivity and factor-1 (significance level ≤ 0.05). This extracted factor-1 was found significantly correlated with depth, velocity, conductivity, richness and negative significant relation with ammonia. This in turn was supported by the positive factor loading parameters from the rotated factor loading matrix that the positive factor loading parameters are also depth, conductivity, velocity. The result of the correlation matrix table for the stream B showed that the richness was significantly correlated with dissolved oxygen which can be reflected from the rich faunal groupings with high diversity, high abundance and higher sensitive groups. No other positive significant relation could be found for any other environmental variables with the richness. Significant negative correlation was found for richness with ammonia in both the stream ecosystem.

4.7 Regression analysis

The regression analysis between the environmental variables, percent substrate coverage and the biotic factors for two streams A and B gave different results. The most significant linear correlation in stream A was found for richness with factor 1 only (**Appendix- 13**). Thus extracted factor 1 for the physico- chemical parameters of stream A was linearly correlated with pH and conductivity (**Appendix 14**) .

The most significant correlated parameter for richness in stream B was found with dissolved oxygen with richness (**Appendix-15**) and, significant negative linear relationship was found with ammonia in both the streams. Considering the two streams to be separate ecosystems, the regression equation for family richness in each of the streams were drawn as follows:

Stream A Richness = 1. 76 Factor 1 + 19. 83 (r= 0.82)

where, Factor 1 = 0.16 conductivity (μs/ cm) - 1.06 pH + 2.64 (r= 0.97)

Stream B Richness = -5 dissolved oxygen (mg/l) + 77 (r = 0.81)

Since the two streams were considered to be similar in bio-physical and chemical parameters, the combined regression equation for the two streams should provide an idea about the factors determining the decling or inclining state of community structure for future monitoring programmes. Therefore combined regression equation were drawn combining the datas from the two stream together (**appendix 16**).

Family richness = .38 percent stone coverage - 11.5 Ammonia (mg/l) + 21.82

(r= .84)

The estimation of family richness drawn from this equation could be established for determining the state of community health at any period of time. The deteriorating or improving state of the streams could be evaluated using this equation. Monitoring purpose need further confirmations through further study.