CHAPTER 4

RESULTS

4.1 Ecological works

4.1.1 Physico-chemical parameters of streams

Physico-chemical parameters including stream velocity, temperature, conductivity, TDS and pH of water at 1050, 900 and 700 mAMSL along Mae Sa Noi and the control streams during April 1999 - February 2000 were monitored. Analyzed data using ANOVA and Mann Withney U test to compare means indicated that some of these parameters were different between seasons, elevations and streams but some were comparable. The details are as following and show in Table 4.1.

Stream velocities of each elevation at the same season and stream were not clearly different, except for dry season at 900 mAMSL which stream velocity was significantly higher than that other elevations. In rainy season the velocity at 900 and 700 mAMSL of Mae Sa Noi stream was not significantly different but not at 1050 mAMSL which was similar in the control stream where velocity at 900 mAMSL and was similar with 700 mAMSL. Mean stream velocities were not significantly different between seasons. Most of the stream velocities of the control stream were higher than those of Mae Sa Noi though not significantly different.

Mean temperatures were not significantly different between streams and elevations but significantly different between seasons.

Table 4.1 Physico-chemical parameters in Mae Sa Noi and control streams at 1050, 900 and 700 mAMSL in dry and rainy season in 1999 to cold season in 2000

Season	Elevation	Stream velocity (Mean±SD) (m/s)		Temperature (Mean±SD) (°C)		Conductivity (Mean±SD) (µs/cm)	
	(mAMSL)	Mae Sa Noi	Control	Mae Sa Noi	Control	Mae Sa Noi	Control
Dry	1050	0.25 ± 0.02^{abcd}	0.24 ± 0.02^{abcd}	19.9 ± 0.1^{cde}	19.3 ± 0.0^{bcde}	88.1 ± 0.8^{cd}	57.2 ± 2.3^{a}
	900	0.45 ± 0.02^{efg}	0.67 ± 0.13^{h}	20.5 ± 0.1^{cde}	20.1 ± 0.0^{cde}	100.1 ± 0.6^{de}	469.0 ± 9.6^{k}
	700	0.24 ± 0.01^{abc}	0.31 ± 0.10^{abcde}	20.8 ± 0.1^{de}	21.2 ± 0.0^{e}	203.0 ± 1.0^{hi}	405.0 ± 5.0^{j}
Rainy	1050	0.16 ± 0.05^{a}	$0.36\pm0.15b^{cdef}$	16.6 ± 2.7^{b}	16.8 ± 2.6^{b}	73.3 ± 4.1^{abc}	58.8 ± 2.0^{ab}
	900	0.42 ± 0.11^{def}	0.52 ± 0.21^{fgh}	16.6 ± 1.8^{b}	17.6 ± 3.2^{bc}	106.9 ± 3.2^{e}	112.5 ± 17.5^{f}
	700	0.40 ± 0.11^{cdef}	0.60 ± 0.21^{gh}	17.9 ± 2.2^{bcd}	18.1 ± 2.9^{bcd}	190.7 ± 28.0^{h}	139.7 ± 14.2^{f}
Cold	1050	0.28 ± 0.02^{abcde}	0.20 ± 0.02^{ab}	9.7 ± 0.5^{a}	10.1 ± 0.1^{a}	75.2 ± 10.8^{bc}	66.4 ± 2.1^{ab}
	900	0.32 ± 0.01^{abcde}	0.36 ± 0.06^{bcdef}	9.9 ± 0.2^{a}	11.0 ± 0.1^{a}	113.0 ± 0.4^{e}	159.3 ± 1.2^{g}
	700	0.31 ± 0.04^{abcde}	0.38 ± 0.06^{cdef}	11.4 ± 0.1^{a}	11.5 ± 0.1^{a}	214.0 ± 6.5^{i}	164.9 ± 0.7^{g}

Table 4.1 continue

Season	Elevation	TDS (Mean±SD) (mg/l)		pH (Mean±SD)	
	(mAMSL)	Mae Sa Noi	Control	Mae Sa Noi	Control
Dry	1050	44.0 ± 0.6^{bc}	29.2 ± 2.1^{a}	4.94 ± 0.02^{a}	7.05 ± 0.09^{efg}
	900	50.5 ± 0.1^{cd}	243.0 ± 7.8^{j}	4.84 ± 0.02^{af}	7.67 ± 0.02^{h}
	700	101.3 ± 0.6^{gh}	209.3 ± 6.7^{i}	5.39 ± 0.04^{b}	7.35 ± 0.02^{fgh}
Rainy	1050	36.8 ± 2.1^{ab}	29.6 ± 0.7^{a}	6.48 ± 0.48^{c}	6.41 ± 0.28^{c}
	900	52.8 ± 2.3^{cd}	55.0 ± 10.0^{d}	6.70 ± 0.37^{cde}	6.74 ± 0.27^{cdf}
	700	94.8 ± 14.1^{g}	69.9 ± 7.0^{e}	7.40 ± 0.26^{gh}	7.00 ± 0.23^{def}
Cold	1050	37.8 ± 5.7^{ab}	33.3 ± 0.9^{a}	6.60 ± 0.20^{cd}	6.50 ± 0.47^{c}
	900	56.5 ± 0.2^{d}	79.8 ± 0.4^{f}	6.80 ± 0.26^{cde}	6.81 ± 0.35^{cde}
	700	110.0 ± 6.5^{h}	82.6 ± 0.4^{f}	7.30 ± 0.16^{fgh}	6.99 ± 0.39^{e}

Of both streams at each season conductivities and TDS changed significantly along the elevations, i.e., conductivities in Mae Sa Noi stream in rainy season were 73.3±4.1, 106.9±3.2 and 190.7±28.0 at 700, 900 and 1050 mAMSL reapectively, except for control stream at 900 and 700 mAMSL in dry season. In Mea Sa Noi stream were not significantly difference of conductivities and TDS at the same elavation but in control stream in dry season at 900 and 700 mAMSL had highest

values of them. Conductivities and TDSs of Mae Sa Noi stream were significantly higher than the control except at 900 mAMSL in all seasons and 700 mAMSL of dry season.

pH had tendency to increase following the reduction of elevations, except in dry season of both streams, this increasing was not significantly changed between elevation but the most significantly different were between 1050 and 700 mAMSL. In all seasons, pH of all elevations and streams were around 7 except in dry season of Mae Sa Noi stream, it was only 5. In rainy and cold seasons pH at Mae Sa Noi and control streams were not significantly different except at 700 while in dry season the control had significantly higher pH than Mae Sa Noi stream.

Mean physico-chemical parameters of the two streams were compared between elevations, seasons and streams using Mann-Whitney U test (Table 4.2). Most physico-chemical parameters of the study and control streams were similar except for the stream velocity and pH. The velocity and pH of the two streams were significantly different, i.e., p=0.0091 and 0.0026, respectively but comparable between elevations and seasons. Temperature was comparable between streams and elevations but significantly different between seasons (p=0.0000). Conductivity and TDS were comparable between streams but significantly different between elevations and seasons.

Table 4.2 Comparison of mean physico-chemical parameters between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams)

	Elevation at mAMSL			Season		
	1050	900	700	Dry	Rainy	Cold
Stream velocity (Mean ± SD) (m/s)	0.28±0.10^{a}	0.48±0.17^{b}	0.38±0.19^{ab}	0.36±0.17^{ab}	0.46±0.22^{a}	0.31±0.07^{b}
	p=0.0017			p=0.0208		
Temperature (Mean ± SD) (°C)	16.2±4.6^{a}	16.7±4.6^{a}	17.6±4.5^{a}	20.3±0.6^{a}	19.6±1.1^{b}	10.6±0.8^{c}
	p=0.6441			p=0.0000		
Conductivity (Mean ± SD) (µs/cm)	69.1±11.6^{a}	173.7±137.6^{b}	219.4±91.7^{c}	220.4±165.3^{a}	109.7±50.6^{b}	132.1±54.0^{b}
	p=0.0001			p=0.0061		
TDS (Mean ± SD) (mg/l)	40.0±8.8^{a}	87.6±75.0^{b}	98.2±54.9^{c}	112.9±86.2^{a}	51.5±18.4^{b}	61.5±26.3^{b}
	p=0.0048			p=0.0022		
pH (Mean ± SD)	6.36±0.72^{a}	6.63±0.91^{ab}	6.95±0.76^{b}	6.21±1.21^{a}	6.90±0.40^{a}	6.84±0.86^{a}
	p=0.0982			p=0.0166		

Table 4.2 continued

	Stream	
	Mae Sa Noi	Control
Stream velocity (Mean ± SD) (m/s)	0.30±0.08^{b}	0.45±0.21^{a}
	p=0.0091	
Temperature (Mean ± SD) (°C)	16.6±4.6^{a}	17.0±4.5^{a}
	p =0.7198	
Conductivity (Mean ± SD) (µs/cm)	129.9±57.8^{a}	178.1±147.1^{a}
	p=0.1192	
TDS (Mean ± SD) (mg/l)	69.3±22.9^{a}	81.3±80.2^{a}
	p=0.4592	
pH (Mean ± SD)	6.32±0.98^{b}	6.97±0.43^{a}
	p=0.0026	

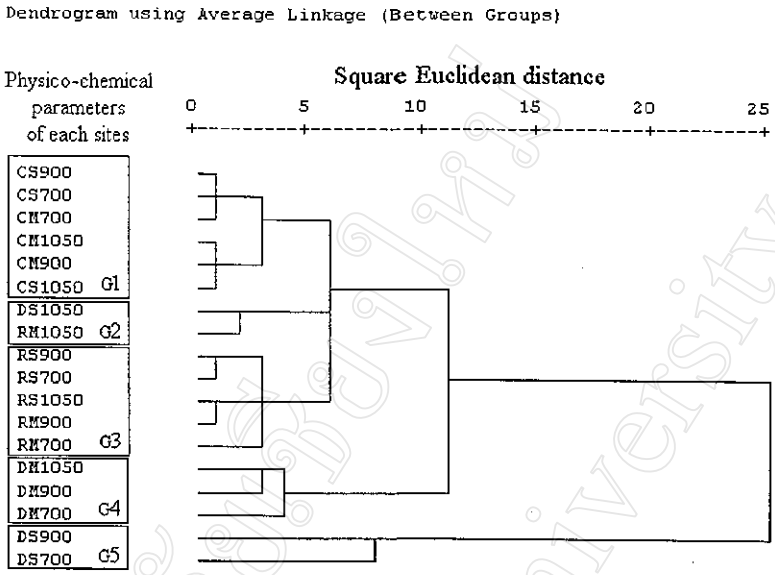

Fig 4.1 Dendrogram show the grouping of physico-chemical parameters of each site; G = group

4.1.2 Cluster analysis of physico-chemical parameters

Figure 4.1 showed 5 groups of physico-chemical parameters of study sites. There were trend of grouping by seasons, i.e.; groups 1, 3, 4 and 5. All members belonging to the same groups had closely values of physico-chemical parameters (velocity, temperature, conductivity, TDS and pH). The similar of physico-chemical parameters of the two streams were clearly occurred, e.g. CM1050, CM900 and CS1050 mAMSL were grouped at Square Euclidean Distance (SED) approximately 3 similar to the grouping of CS700, CS900 and CM700 mAMSL.

4.1.3 Biological components

Macroinvertebrate were seasonal randomly sampled at 1050, 900 and 700 mAMSL from Mae Sa Noi and the control stream using surber sampler for 10 samples per site. They were identified using McCafferty, (1989) and quantified in

laboratory, the communities characteristics were estimated, i.e., mean total population density, chironomid population density, richness index, diversity index and evenness. Cluster analysis (SPSS) was applied to find out the similarity of macroinvertebrate communities.

Mean total population density of macroinvertebrates was significantly highest at 900 mAMSL in all seasons of both streams, except for rainy season of Mae Sa Noi stream whereas population density at 1050 mAMSL was not significantly different with 700 mAMSL. Population densities at the same elevation and streams in 3 seasons were not significantly different although at 900 and 700 mAMSL of the control stream had significant difference in season. Almost population density at the same elevation and season of Mae Sa Noi stream were significantly lower than control stream. There was only 1050 mAMSL in rainy season of both streams that were significantly similar (Table 4.3).

Table 4.3 Mean biological components except for indices in Mae Sa Noi and control streams at 1050, 900 and 700 mAMSL in dry and rainy seasons in 1999 to cold season in 2000

Season	Elevation	Mean total population density (individual/m^2)		Mean chironomid density (individual/m^2)	
	(mAMSL)	Mae Sa Noi	Control	Mae Sa Noi	Control
Dry	1050	288 ± 223^{abcd}	163 ± 103^{ab}	0.47 ± 0.50^{a}	0.32 ± 0.21^{a}
	900	752 ± 533^{efg}	1028 ± 683^{gh}	0.22 ± 0.11^{a}	3.39 ± 2.60^{b}
	700	30 ± 42^{a}	531 ± 278^{cdef}	0.03 ± 0.05^{a}	0.54 ± 0.44^{a}
Rainy	1050	468 ± 207^{bcdef}	116 ± 66^{a}	0.19 ± 0.15^{a}	0.10 ± 0.15^{a}
	900	553 ± 401^{def}	1160 ± 640^{h}	0.39 ± 0.28^{a}	2.66 ± 1.40^{b}
	700	224 ± 153^{abc}	315 ± 132^{abcd}	0.19 ± 0.17^{a}	0.26 ± 0.32^{a}
Cold	1050	324 ± 278^{abcd}	123 ± 104^{a}	0.13 ± 0.11^{a}	0.06 ± 0.06^{a}
	900	728 ± 290^{efg}	1579 ± 862^{i}	0.34 ± 0.22^{a}	3.10 ± 2.27^{b}
	700	179 ± 32^{ab}	646 ± 142^{ef}	0.23 ± 0.08^{a}	0.33 ± 0.23^{a}

Table 4.3 continued

Season	Elevation (mAMSL)	Richness index (Margalef)		Diversity index (Shannon)		Evenness	
		Mae Sa Noi	Control	Mae Sa Noi	Control	Mae Sa Noi	Control
Dry	1050	4.02	3.68	2.41	2.17	0.78	0.75
	900	2.60	2.01	1.21	1.42	0.43	0.54
	700	2.04	1.38	1.73	1.45	0.89	0.66
Rainy	1050	3.34	2.56	1.86	1.98	0.62	0.79
	900	2.56	1.06	1.86	1.13	0.67	0.54
	700	2.63	1.70	2.00	1.54	0.76	0.67
Cold	1050	2.82	2.07	1.98	1.65	0.71	0.72
	900	2.29	1.02	1.60	1.23	0.59	0.59
	700	3.39	1.83	2.05	1.34	0.72	0.58

Chironomid population density of all elevations at the same season and stream were not significantly different except for 900 mAMSL of the control stream in all season where had highest density of chironomids. At the same elevation and stream, chironomid population density in each season were not significantly different. Chironomid population density in Mae Sa Noi stream were significantly lower than the control only at 900 mAMSL in all season (Table 4.3).

Total and chironomid population densities of the control stream at each elevation in the same season was significantly higher than that of Mae Sa Noi stream except at 1050 mAMSL in dry and rainy seasons for total population density. In both streams, total population density and chironomid population density were highest at 900 mAMSL in all season. Total population densities were clearly significantly different according to elevation except in rainy and cold seasons at Mae Sa Noi stream and in cold season at the control stream. In contrary to chironomid population density, there were similar between elevation at Mae Sa Noi but not clearly different at the control stream.

Richness and diversity indices showed similar pattern (Table 4.3). These indices had maximum value at 1050 mAMSL, which were 2.07 to 4.02 and 1.65 to 2.41 for richness and diversity indices respectively. There was not significantly different of both indices seasonally. Both index values in Mae Sa Noi were higher than that of the control stream at the same elevation and season.

Evenness of community was around 0.6 to 0.9 (Table 4.3). The minimum of it was found at 900 mAMSL in all seasons and streams except in rainy season in Mae Sa Noi stream. Evenness was not different between seasons and streams.

Mean total population density and mean chironomid population density had similar pattern (Table 4.4). The population dendity at 900 mAMSL was significantly highest and at 1050 and 700 mAMSL were similar. Their value in Mae Sa Noi stream was significantly lower than the control stream (p=0.0266 and 0.0002, respectively). The total population density and chironomid population density were not significantly different seasonally.

Table 4.4 Comparison of means of biological components between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams)

	Elevation at mAMSL			Season		
	1050	900	700	Dry	Rainy	Cold
Mean total population density (individual/m^2)	247±215[a]	966±667[b]	321±257[a]	465±507[a]	473±466[a]	596±624[a]
	P=0.0000			p=0.3255		
Mean chironomid population density (individual/m^2)	0.21±0.27[a]	1.68±2.02[b]	0.26±0.29[a]	0.83±1.57[a]	0.63±1.08[a]	0.70±1.41[a]
	p=0.0000			p=0.7246		

Table 4.4 continued

	Stream	
	Mae Sa Noi	Control
Mean total population density (individual/m^2)	394±359[b]	629±651[a]
	p=0.0266	
Mean chironomid population density (individual/m^2)	0.24±0.25[b]	1.20±1.80[a]
	p=0.0002	

4.1.4 Cluster analysis of biological components

The grouping of communities presented with dendrogram analyzed with cluster analysis (Fig 4.2). There were 4 groups that most members of each group were communities of the same stream. Groups No. 1 and 3 were group of communities of Mae Sa Noi stream, groups 2 and 4 were groups of communities mostly were from the control stream.

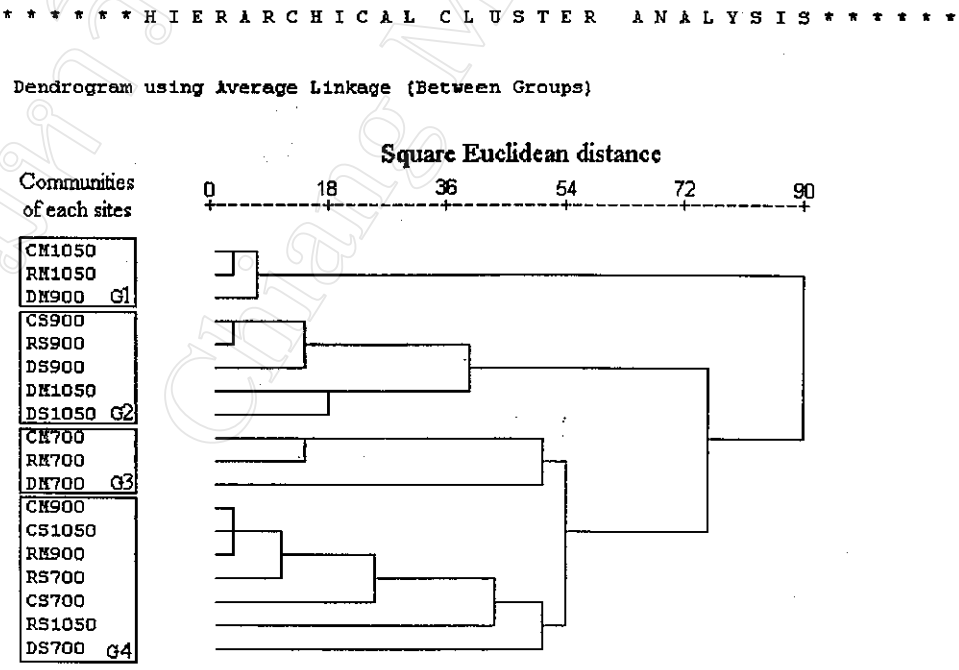

Fig 4.2 Dendrogram show the grouping of macroinvertebrate community of each site, G = group

4.1.5 Correlation between physico-chemical parameters and biological components

Both mean total population density and mean chironomid population density were significantly correlated with mean stream velocity (p=0.045 and 0.014 respectively), where as they were not significantly correlate with mean pH.

Table 4.5 Pearson correlation coefficient (r) between physico-chemical parameter; stream velocity and pH and mean total population density and mean chironomid population density

	Mean Stream velocity	Mean pH
Mean total population density (individual/m^2)	+0.4770 (p = 0.045)	+0.1596 (p = 0.527)
Chironomid population density (individual/m^2)	+0.5656 (p = 0.014)	+0.3002 (p = 0.226)

Note:

r = +1.0	Perfectly positive correlation
r = 0.0	No correlation
r = - 1.0	Perfectly negative correlation
p < 0.05	Statistically significant

4.2 Toxicological test

Chironomid sampled from study and control streams were measured for ChE activity using Ellman method with slight modification to study the impact of cholinesterase-inhibiting pesticides (i.e., OPs and CAs). Chironomid ChE activities from each site were measured and compared between streams. Inhibition test was further conducted by adding various concentrations of methyl-parathion; one type of repodtedly used organophosphate pesticides in Ban Mae Sa Mai, into chironomid supernatant to study the reduction of ChE activity.

4.2.1 ChE activity of chironomids

Mean ChE activities of chironomid were not significantly different between elevation in the same season and stream, except in rainy season at 1050 mAMSL which was significantly highest. In both rainy and cold seasons, they were not significantly different, except at 1050 mAMSL of both streams. Most chironomid ChE activities in Mae Sa Noi were lower than the control stream but not significantly, except at 1050 mAMSL in rainy season showed significantly lower of ChE activities in Mae Sa Noi.

At 1050 mAMSL, chironomid ChE activity was significantly higher than other elevations (Table 4.7). In rainy season, it was significantly higher than in the cold season ($p=0.0076$). Chironomid ChE activity in Mae Sa Noi stream was lower than the control but not significantly different ($p=0.0712$).

Table 4.6 Chironomid ChE activity in Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in rainy and cold seasons

Season	Elevation (mAMSL)	Chironomid ChE activity (Mean ± SD) (μM/min/mg protein)	
		Mae Sa Noi	Control
Rainy	1050	4.56±1.96^{b}	13.98±4.96^{a}
	900	2.39±1.24^{c}	2.53±1.02^{c}
	700	1.52±0.56^{c}	1.34±0.71^{c}
Cold	1050	1.87±0.24^{c}	2.94±0.47^{bc}
	900	1.39±0.31^{c}	1.60±0.30^{c}
	700	1.56±0.34^{c}	1.54±0.25^{c}

Table 4.7 Comparison of mean chironomid ChE activity between elevations (1050, 900 and 700 mAMSL), seasons (dry 1999, rainy 1999 and cold 2000) and streams (Mae Sa Noi and control streams)

	Elevation at mAMSL			Season		
	1050	900	700	Dry	Rainy	Cold
Mean chironomid ChE activity (μM/min/mg protein)	5.48±5.23^{a}	1.20±0.93^{b}	1.49±0.48^{b}	ND	4.11±4.67^{a}	1.82±0.61^{b}
	p=0.0000			p=0.0076		

Table 4.7 continued

	Stream	
	Mae Sa Noi	Control
Mean chironomid ChE activity (μM/min/mg protein)	2.22±1.45^{a}	3.70±4.65^{a}
	p=0.0712	

4.2.2 *In vitro* chironomid ChE activity inhibition test

Inhibition test, *in vitro,* was done because chironomid ChE activities sampled from Mae Sa Noi were not significantly lower than that from the control stream. Then methyl-parathion; one type of organophosphate pesticides often used in Ban Mae Sa Mai, was added at several of concentrations into chironomid samples for studying their inhibition.

Increasing concentration of methyl-parathion caused increasing of inhibition. In the case of %activity was minus it was mean 100% inhibition. There was not significantly different of methyl-parathion concentration, added into chironomid supernatant, in each elevation to cause inhibition for 25, 50 and 75% (Table 4.8). Chironomids from Mae Sa Noi stream required significantly lower concentration of methyl-parathion than the control stream at all elevations.

Inhibition detection in all elevations was not significantly different in methyl-parathion concentration added (Table 4.9). Chironomids from Mae Sa Noi stream used significantly lower concentration of methyl-parathion than that from the control stream at all elevations to cause the same %inhibition.

4.2.3 Cluster analysis of toxicological tests and some biological components

Cluster analysis (Fig 4.3) using ChE activity and inhibition test indicated the differences of the above mentioned toxicological tests of the two streams since those two toxicological tests of the same stream were grouped together. This agreed with the analysis using mean comparison (Tables 4.6-4.8).

Table 4.8 Concentration of methyl-parathion that caused inhibition of chironomid ChE activity at 25%, 50% and 75% in Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in cold season

% inhibition	Concentration of methyl-parathion (Mean ± SD) (μM)					
	At 1050 mAMSL		At 900 mAMSL		At 700 mAMSL	
	Mae Sa Noi	Control	Mae Sa Noi	Control	Mae Sa Noi	Control
25	55 ± 21^{ab}	115 ± 7^{c}	35 ± 21^{a}	108 ± 4^{c}	$80\pm10b^{c}$	103 ± 11^{c}
50	135 ± 21^{a}	218 ± 11^{b}	95 ± 21^{a}	195 ± 49^{b}	135 ± 7^{a}	195 ± 7^{b}
75	215 ± 21^{abc}	280 ± 28^{d}	168 ± 18^{a}	240 ± 42^{bcd}	175 ± 21^{ab}	253 ± 4^{cd}

Table 4.9 Comparison of methyl-parathion concentration (μM) that cause inhibition of chironomid ChE activity for 25%, 50% and 75% *in vitro* condition, chironomid sampling from Mae Sa Noi and the control stream at elevation 1050, 900 and 700 mAMSL in cold season

Inhibition	Methyl-parathion concentration (μM)				
	Elevation at mAMSL			Stream	
	1050	900	700	Mae Sa Noi	control
25%	85.0 ± 37.0^{a}	71.2 ± 43.7^{a}	91.2 ± 16.5^{a}	56.7 ± 25.0^{b}	108.3 ± 8.2^{a}
	p=0.7110			p=0.0036	
50%	176.2 ± 49.6^{a}	145.0 ± 65.6^{a}	165.0 ± 35.1^{a}	121.7 ± 24.8^{b}	202.5 ± 25.0^{a}
	p=0.6967			p=0.0039	
75%	257.5 ± 54.5^{a}	203.8 ± 49.6^{a}	213.8 ± 46.4^{a}	185.8 ± 27.6^{b}	264.2 ± 37.6^{a}
	p=0.3211			p=0.0065	

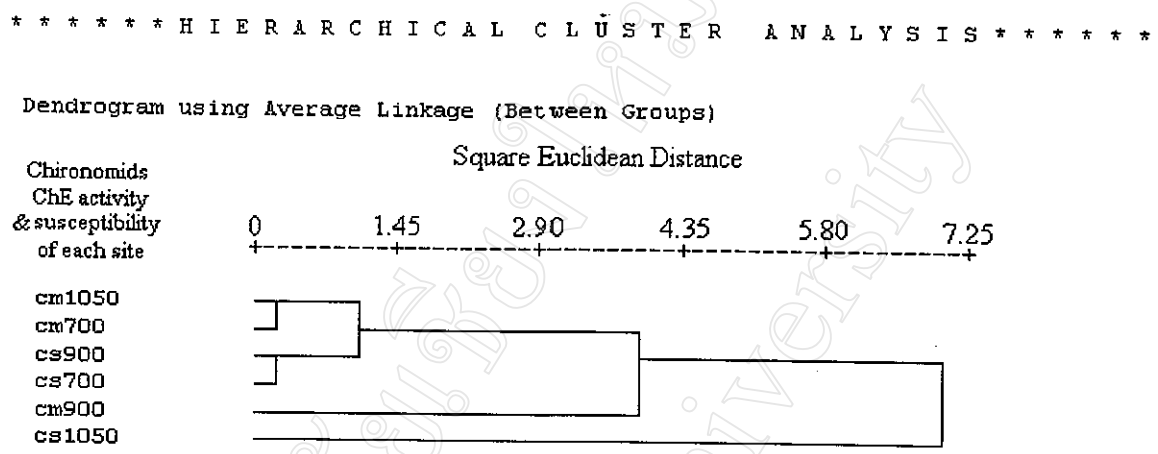

Fig 4.3 Dendrogram show the grouping of ChE activities and susceptibility of all sites in cold season

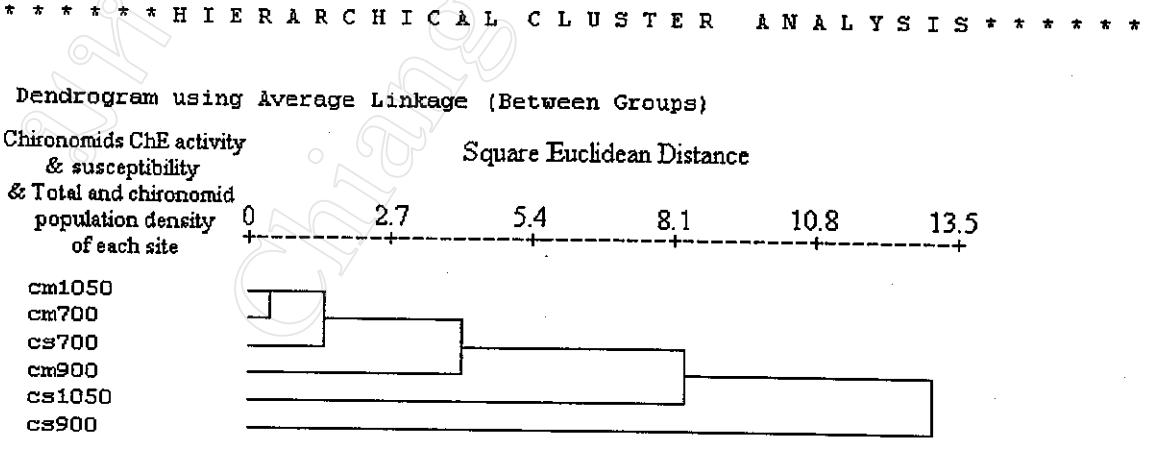

Fig 4.4 Dendrogram show the grouping of ChE activities, susceptibility, mean total population density and mean chironomid population density of all sites in cold season

Dendrogram using chironomid ChE activities and susceptibility, i.e., the concentration of methyl-parathion causing 50% inhibition of each site showed the grouping of the same stream, e.g., CM1050 with CM700 and CS900 with CS700 at the same level of SED, 0.25. CM900 and CS1050 joined at the high value of SED.

The cluster analysis showed in fig 4.4 was reproduced from the cluster in fig 4.3 by adding was total mean total and mean chironomid population densities in the evaluations. The cluster in fig 4.4 agreed with fig 4.3 cluster that showed the study sites by the grouping of study site of the same stream were grouped together, i.e., CM1050 and CM700 at approximately 0.8 of SED. It showed clearly that there were differences between streams.

By summarize, figure 4.3 indicated the similarity of ChE activities and susceptibility to methyl-parathion of chironomid from each study site of the same stream. There were significantly different between Mae Sa Noi and the control stream. When these parameters together with mean total and chironomid population densities were used in cluster analysis (Fig 4.4), those of Mae Sa Noi, i.e., CM1050 and CM700 were in the same group, indicated the similar values of these parameters of the sites along the same stream.