CHAPTER 4

RESULTS

4.1 Ecological studies

4.1.1 Abundance and diversity

A total of 3073 ephemeropteran nymphs were found in 3 different rivers in Ulu Gombak, Selangor from August 2006 to July 2007. The relative abundance range from 443 to 1601 number of individuals with an estimated standard deviations ranging from 72.5 to 334.2. The mean abundance of ephemeropteran nymphs found in the rivers of Ulu Gombak Forest Reserve was between 73.8 and 266.8. Six genera of ephemeropteran nymphs found were *Baetis* sp. (232) (Baetidae), *Thalerosphyrus* sp. (925) (Heptageniidae), *Campsoneuria* sp. (1432) (Heptageniidae), *Neurocaenis* sp. (471) (Tricorythidae), *Platybaetis* sp. (12) (Baetidae) and *Prosopistoma* sp. (1) (Prosopistomatidae) (Figure 4.1 – Figure 4.6) (Table 4.1).

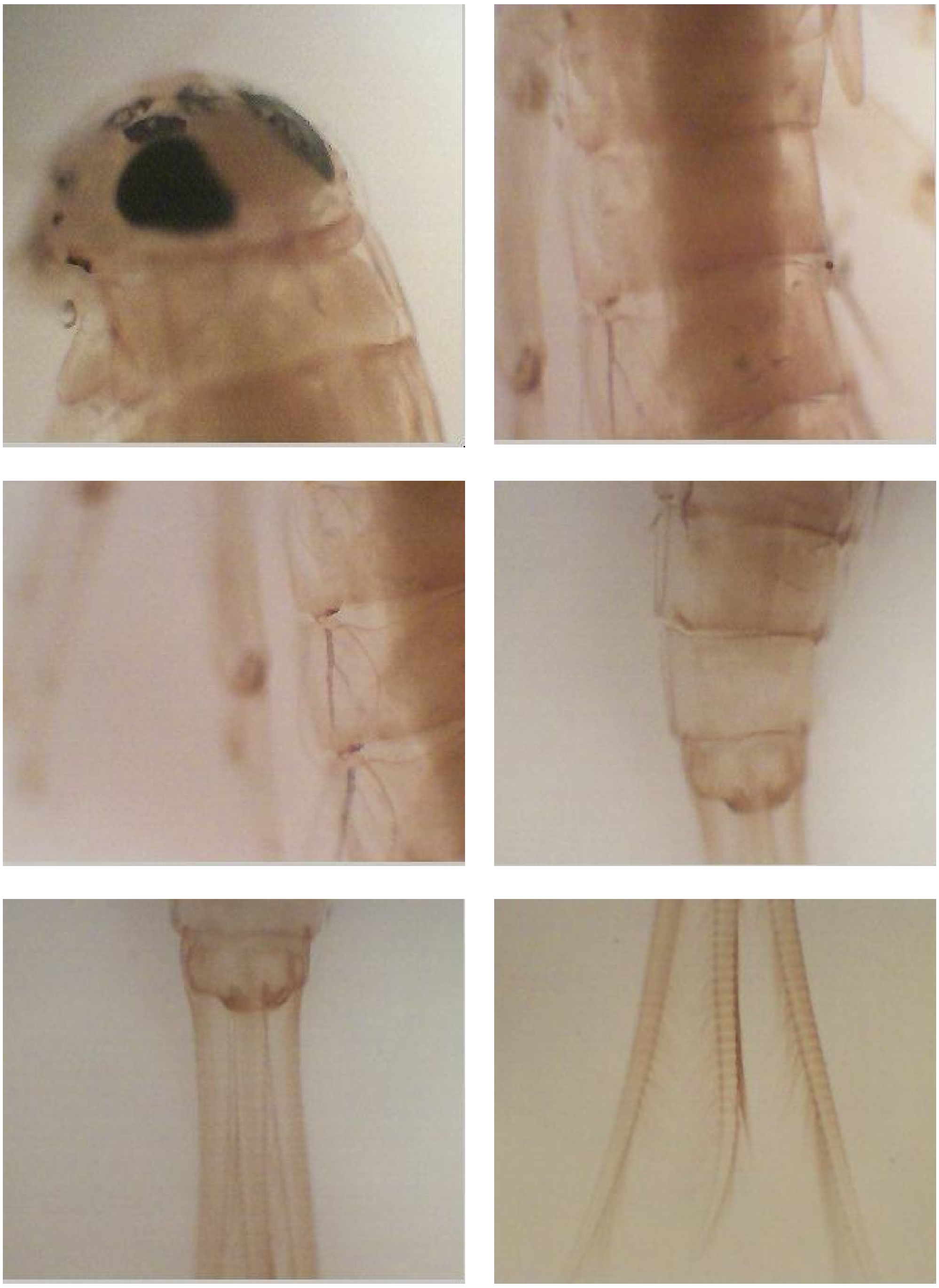

Figure 4.1. *Baetis* sp. (Baetidae)

Head hypognathous; slender, cylindrical body and small gills on its abdomen; the small gills indicate that it lives in moving water, and the shape of its body makes it well-suited for swimming against the current; body streamlined with mottled colouring; abdomen without posterolateral spines; middle tail shorter than outer ones; inner margins of caudal filaments fringed with setae (Khoo, 2004; Edmunds, 1982).

Figure 4.2. *Platybaetis* sp. (Baetidae)

Body dorso-ventrally flattened; head prognathous; gills on segments 2-7; gills lamellate or plate-like (Khoo, 2004; Edmunds, 1982).

Figure 4.3. *Thalerosphyrus* sp. (Heptageniidae)

Flat plate-like head, with dorsally situated eyes, concealing the mouthparts when viewed from above; body dorsoventrally compressed; spiniform lateral process on segments II-VIII well develop (Khoo, 2004; Edmunds, 1982).

Figure 4.4. *Campsoneuria* sp. (Heptageniidae)

Flat plate-like head, with dorsally situated eyes, concealing the mouthparts when viewed from above; body dorsoventrally compressed; fore femora with patches of stout setae on dorsal surface; gill VII with tracheal tufts (Khoo, 2004; Edmunds, 1982).

Figure 4.5. *Neurocaenis* sp. (Tricorythidae)

The gill plates on second abdominal segments have become enlarged to form a pair of opercula that protect the other gills from damage in silty environments (Khoo, 2004; Edmunds, 1982).

Figure 4.6. *Prosopistoma* sp. (Prosopistomatidae)

Body smooth and hemispherical (beetle-like); body form extremely modified, oval in dorsal view, dorsal surface strongly convex, ventral surface flat; eyes depressed; all gills and much of the abdomen covered by a thoracic shield; legs not well developed with tarsi reduced and delicate; caudal filaments short, subequal (Khoo, 2004; Edmunds, 1982).

Table 4.1. Ephemeropteran nymphs in rivers at Ulu Gombak, Selangor, August 2006 to July 2007.

Family	**Taxon**	**Sg. Gombak**	**Anak Sg. Gombak**	**Sg. Batu 19**	**Total**
Baetidae	*Baetis* sp.	32	86	114	232
	Platybaetis sp.	5	7	0	12
Heptageniidae	*Thalerophyrus* sp.	153	313	459	925
	Campsoneuria sp.	162	414	856	1432
Tricorythidae	*Neurocaenis* sp.	91	209	171	471
Prosopistomatidae	*Prosopistoma* sp.	0	0	1	1
Total number of individuals:		443	1029	1601	3073
Mean:		73.8	171.5	266.8	
Standard deviation:		72.5	16.7	334.2	

The ascending patterns of ephemeropteran nymphs abundance along the sampling stations (Sg. Gombak to Sg. Batu 19) were shown in Figure 4.7. It distinguishes the segments of the forests stream into upstream (lotic environment), meandering intermittent pool (mix lotic-lentic environment) and downstream (lentic environment). Sg. Gombak represented the upstream environment while the intermittent pool was demarcated by Anak Sg. Gombak. Sg. Batu 19 represented the downstream environment. The abundance of ephemeropteran nymphs increased gradually from Sg. Gombak to Sg. Batu 19. Sg. Batu 19 shows the highest abundance while Sg. Gombak has the least abundance in Ulu Gombak Forest Reserve.

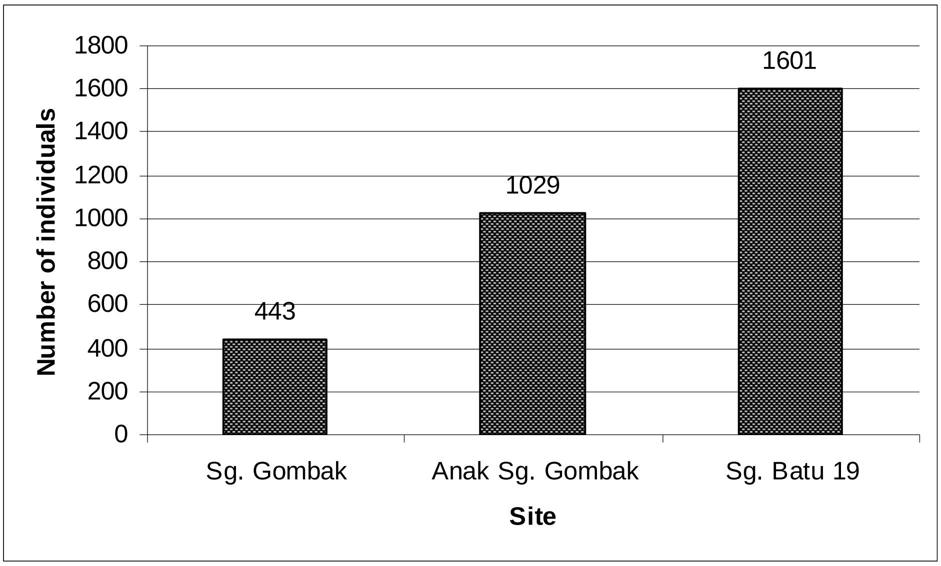

Figure 4.7. Relative abundance of ephemeropteran nymphs along Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19 from August 2006 to July 2007

Campsoneuria sp. has the highest abundance with a total of 1432 individuals. *Platybaetis* sp. was present only in Sg. Gombak and Anak Sg. Gombak while *Prosopistoma* spp. was found only in Sg. Batu 19. *Prosopistoma* sp. was also the least ephemeropteran nymphs to be found throughout the sample collection with only 1 individual. The most dominant family of ephemeropteran nymphs, as shown in Table 4.1 is Heptageniidae. *Neurocaenis* sp. (Tricorythidae) were the only nymphs that showed significant difference in abundance between all three streams ($t=4.52$, $p=0.05$) using student t-test ($p\leq0.05$).

4.1.2 Diversity Indices

Comparison of diversity indices, evenness index and richness indices between Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19 were presented in Table 4.2. All three streams were represented by the same number of species richness, with each stream consisting of five different species of ephemeropteran nymphs. The calculated values for Shannon's Diversity Index (H') ranged from 1.12 to 1.30 indicating low diversity. Shannon's Equitability (E_H)

components ranged from 0.70 to 0.81, indicating the nymphs were more or less evenly distributed. The diversity and evenness of the ephemeropteran nymphs were the highest in both Sungai Gombak and Anak Sg. Gombak. In Sg. Batu 19 over 54% of the nymphs belonged to one species, *Campsoneuria* sp. which was also the most abundant species in both Anak Sg. Gombak and Sg. Gombak, making up about 40% and 37% respectively. Therefore, the nymphs in Sg. Batu 19 were found to be the least diverse and equitably distributed in Ulu Gombak Forest Reserve.

The calculated values for Margalef's Richness Index ranged from 0.54 to 0.66. It is shown from the values obtained that Sg. Gombak has the highest richness of ephemeropteran nymphs (0.66). This coincides with the result obtained from the Shannon's Diversity Index as well as Simpson's Diversity Index. The difference between these indices can be attributed to the higher sensitivity of the Margalef's Richness Index to species in the sample represented by few or rare individuals.

It is clearly shown that the Shannon's Diversity Index (t=13.44, p=0.05), Shannon's Equitability (t=13.73, p=0.05) and Simpson's Index of Diversity (t=16.50, p=0.04) are significantly higher in Sg. Gombak (downstream) than in Sg. Batu 19 (upstream) using student t-test ($p \leq 0.05$).

Table 4.2. Calculated values of Diversity Indices for the three forest streams in Ulu Gombak Forest Reserve

Sampling station	Shannon's Diversity Index, H'	Shannon's Equitability, E_H	Simpson's Index of Dominance, D	Simpson's Index of Diversity, 1-D	Simpson's Reciprocal Index, 1/D	Simpson's Index of Evenness, E_s	Margalef's Richness Index, d
Sg. Gombak	1.30	0.81	0.30	0.70	3.34	0.67	0.66
Anak Sg. Gombak	1.29	0.80	0.30	0.70	3.31	0.69	0.58
Sg. Batu 19	1.12	0.70	0.38	0.62	2.60	0.51	0.54

4.2 Physical –Chemical Parameters and Water Quality Bioassessment

Table 4.3 tabulates the physico-chemical parameters at each sampling station in Ulu Gombak Forest Reserve while Figures 4.8 to 4.15 shows the fluctuations for the physical-chemical parameters along the sampling stations throughout the study period.

4.2.1 Illuminance

The mean illuminance reading for all sampling stations using a digital Lux meter ranged between 478.8±151.2 to 3789.6±394.3. Anak Sg. Gombak was found to have sunniest spots among all sampling stations. Illuminance measurements showed drastic fluctuations in all sampling stations throughout the study period (0.8 to 6600) (Figure 4.8).

4.2.2 pH

The mean value of pH ranged from 7.02±0.24 (Sg. Gombak) to 7.67±0.22 (Anak Sg. Gombak). The lowest measurement was 5.2 while the highest was 8.9 (Figure 4.9).

4.2.3 Temperature

For all sampling stations, the mean water temperature ranged from 22.7±0.3°C to 24.6±0.5°C. Sg. Batu 19 recorded the lowest measurement (22.7°C) due to its higher elevation among all sampling stations. Anak Sg. Gombak recorded the highest at 24.6°C. The range of water temperature for all sampling sites produced slight fluctuations throughout the study period (Figure 4.10).

4.2.4 Dissolved oxygen (DO)

The mean DO for all sampling stations ranged from 4.39±0.45 mg/L (Sg. Gombak) to 5.17±0.54 mg/L (Sg. Batu 19), indicating high level of DO throughout the year. DO values obtained from all sampling stations showed drastic fluctuations throughout the study period (1.09 mg/L to 7.5 mg/L) (Figure 4.11).

4.2.5 Conductivity

The mean conductivity values for all sampling stations ranged from 38.34±0.52 µS to 45.32± 0.48 µS. The highest and lowest measurement was recorded at Anak Sg. Gombak and Sg. Batu 19, respectively. Conductivity measurements from Anak Sg. Gombak showed drastic fluctuations throughout the study period (42.3 µS to 1087.8 µS) (Figure 4.12).

4.2.6 Nitrate

Both Sg. Gombak and Anak Sg. Gombak were recorded producing the same mean value of nitrate, 0.5±0.02 mg/L and 0.5±0.05 mg/L respectively while Sg. Batu 19 has the lowest mean value at 0.3±0.03 mg/L. The highest concentration of nitrate among all sampling stations was 1.3 mg/L at Anak Sg. Gombak and the lowest was at Sg. Batu 19 with 0.1 mg/L (Figure 4.13).

4.2.7 Phosphate

The highest mean value of phosphate was recorded at Sg. Gombak (0.5±0.02 mg/L) while the lowest was at Sg. Batu 19 (0.2±0.02 mg/L). The value of phosphate concentration in all sampling stations range from 0.1 mg/L to 1.0 mg/L (Figure 4.14).

4.2.8 Ammonical nitrogen

The mean concentration of ammonical nitrogen from all sampling stations ranged from 0.2±0.01 mg/L (Sg. Batu 19) to 0.5±0.07 mg/L (Anak Sg. Gombak). The highest and lowest value for ammonical nitrogen present in all sampling stations is 1.5 mg/L and 0.1 mg/L respectively (Figure 4.15).

Table 4.3. Variation of physical-chemical parameters in different locations in Ulu Gombak Forest Reserve.

Parameter	**Sampling Station**								
	Sg. Gombak			**Anak Sg. Gombak**			**Sg. Batu 19**		
	Min	**Max**	**Mean±S.E**	**Min**	**Max**	**Mean±S.E**	**Min**	**Max**	**Mean±S.E**
Illuminance (Lux)	0.8	3700	478.8±151.2	180	6600	3789.6±394.3	170	2460	956.3±148.4
pH	5.2	8.5	7.0±0.2	5.4	8.8	7.1±0.2	6.5	8.9	7.7±0.2
Water Temperature (^{0}C)	21.3	34.5	23.9±0.2	23.0	27.7	24.6±0.5	20.7	28.1	22.7±0.3
Dissolved Oxygen (mg/L O_2)	1.2	7.4	4.4±0.5	1.09	7.4	4.6±0.5	1.2	7.5	5.2±0.5
Conductivity (µS)	37.9	42.8	39.7±0.3	42.3	1087.7	45.3±0.5	34.7	48.2	38.3±0.5
Nitrate (mg/L)	0.3	0.8	0.5±0.02	0.3	1.3	0.5±0.05	0.1	0.6	0.3±0.03
Phosphate (mg/L)	0.3	0.8	0.5±0.02	0.1	1.0	0.4±0.04	0.1	0.4	0.2±0.02
Ammonical nitrogen (mg/L)	0.2	0.9	0.4±0.03	0.1	1.5	0.5±0.07	0.1	0.3	0.2±0.01

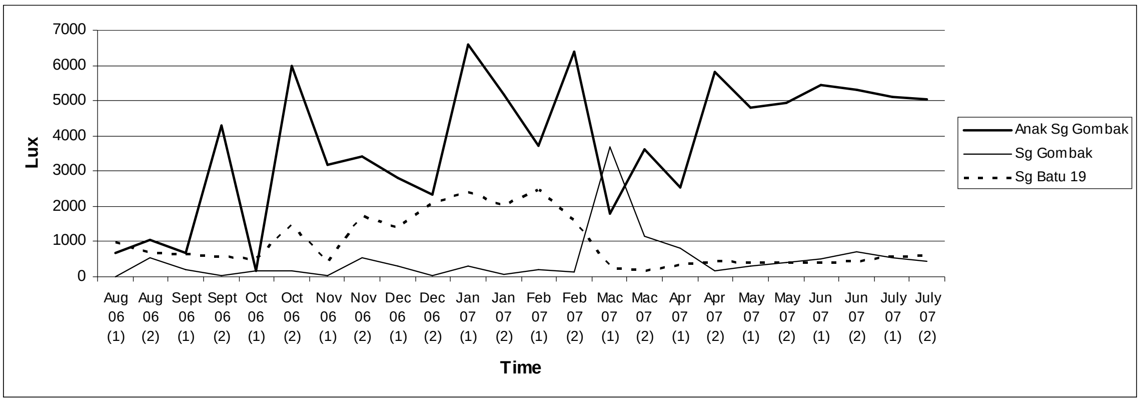

Figure 4.8. Illuminance measurements from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

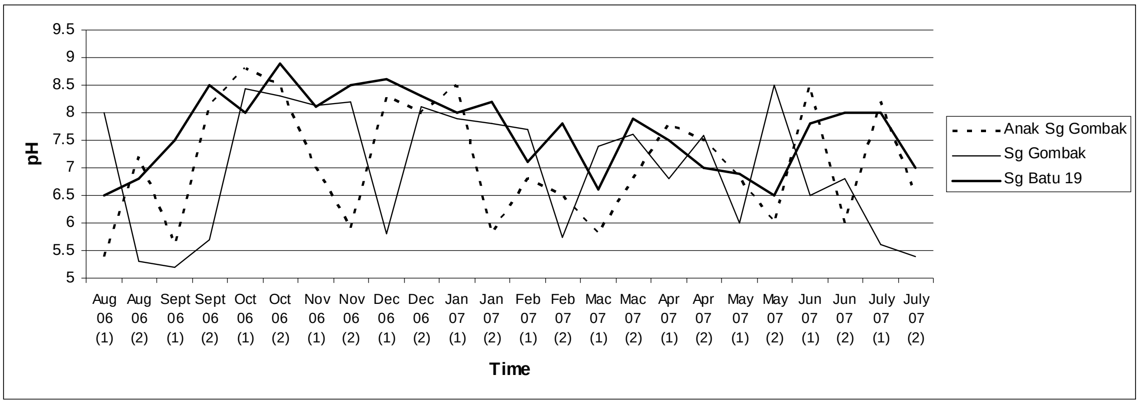

Figure 4.9. pH from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

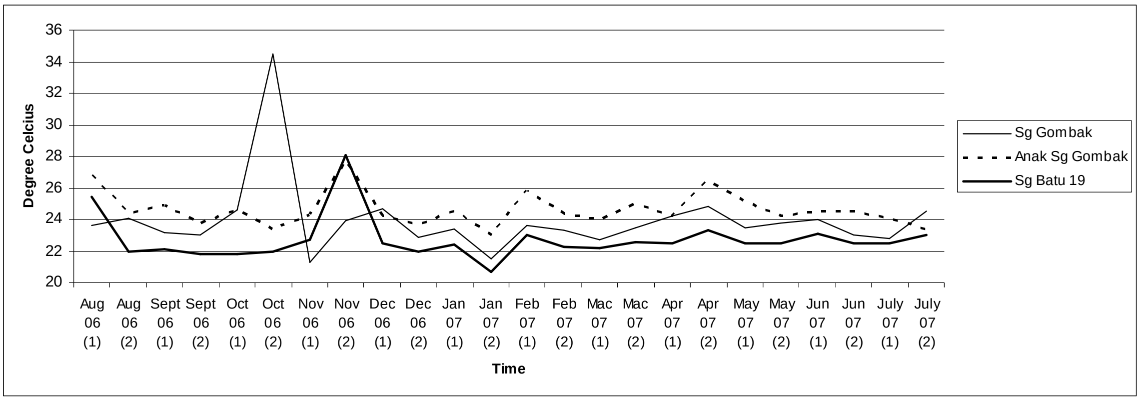

Figure 4.10. Water temperature from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

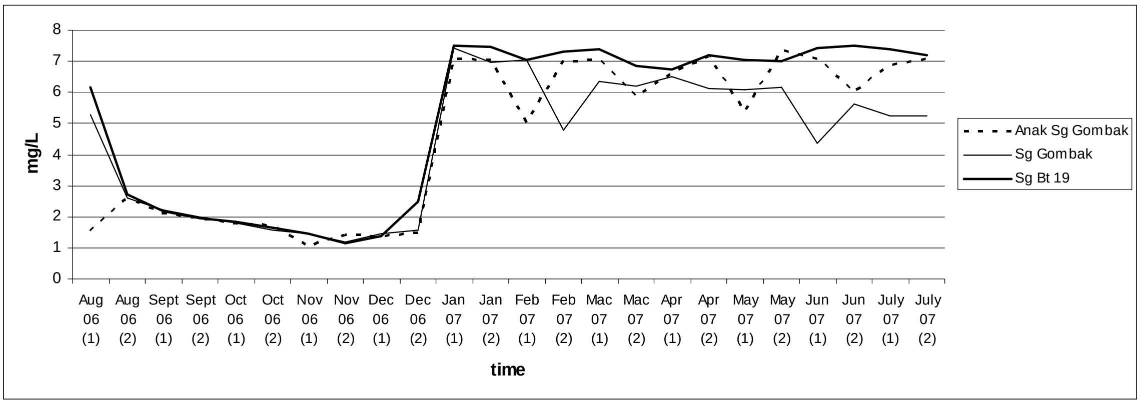

Figure 4.11. Dissolved oxygen from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

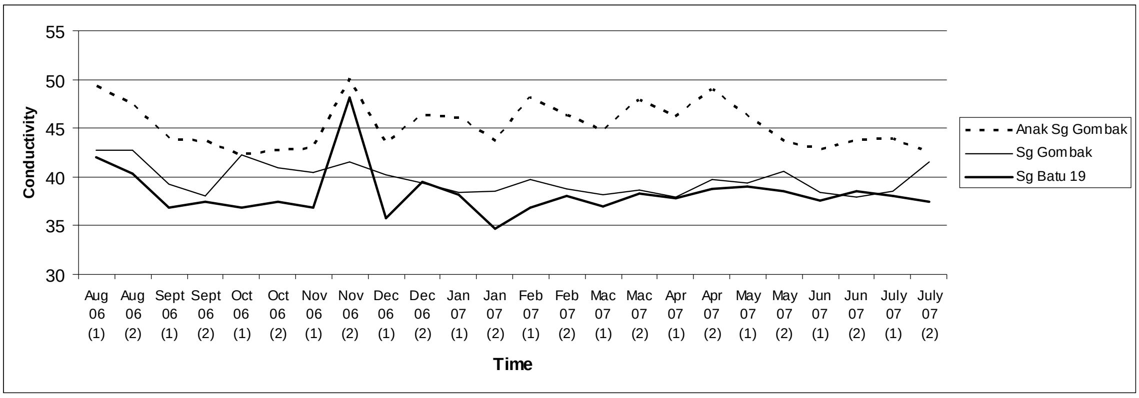

Figure 4.12. Conductivity from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

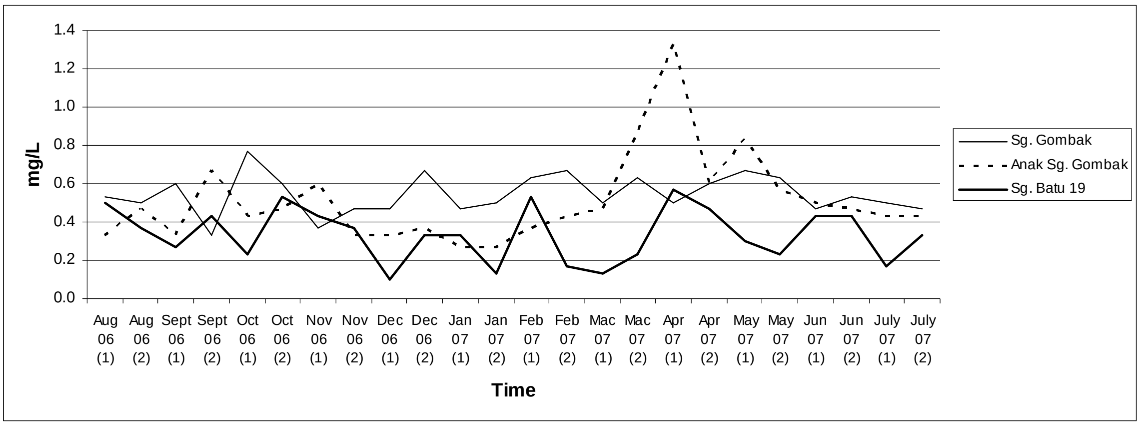

Figure 4.13. Concentration of nitrate from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

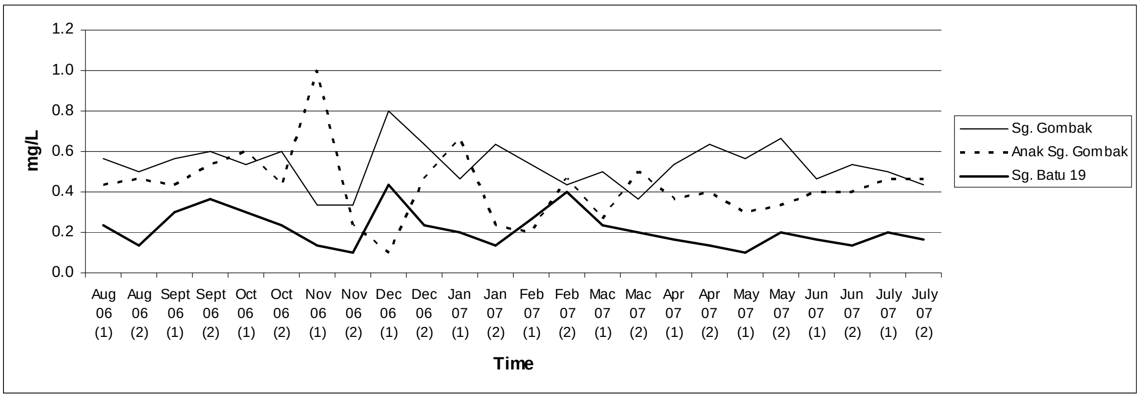

Figure 4.14. Concentration of phosphate from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

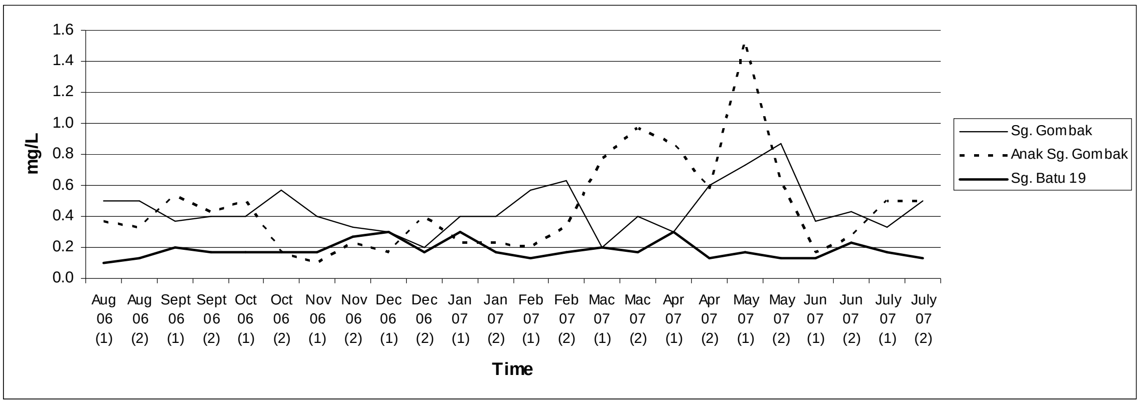

Figure 4.15. Concentration of ammonical nitrogen from selected sampling spots at Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19

4.3 Abundance of Nymphs In Comparison With Environmental Parameters

This part of the study includes results that indicated interactions between the abundance of mayfly nymphs and environmental parameters (Table 4.4). Scatterplots of Pearson's correlation coefficient for all three streams are shown in Appendix 4.5 to Appendix 4.28.

4.3.1 Sungai Gombak

An analysis using Pearson's correlation coefficient indicates a statistically significant weak positive linear association between three physical parameter and number of nymphs in Sungai Gombak: Temperature ($r=0.44$, $p\leq0.05$), pH ($r=0.57$, $p\leq0.05$) and conductivity ($r=0.45$, $p\leq0.05$). A significant weak negative correlation was concluded between dissolved oxygen and number of nymphs ($r=-0.51$, $p\leq0.05$). The mean for number of nymphs in Sungai Gombak was 73.8.

4.3.2 Anak Sungai Gombak

Pearson's correlation coefficient indicates a statistically significant weak positive linear relationship between pH and number of nymphs ($r=0.64$, $p\leq0.05$) at Anak Sungai Gombak. For these data, the mean for pH is 7.1 and number mean of nymph is 171.5.

4.3.3 Sungai Batu 19

There is a statistically significant weak positive correlation between two physico-chemical parameters and number of nymphs in Sungai Batu 19, pH ($r=0.64$, $p\leq0.05$) and phosphate ($r=0.42$, $p\leq0.05$). A significant weak negative association was concluded between dissolved oxygen and number of nymphs ($r=-0.65$, $p\leq0.05$). The mean for number of nymphs in Sungai Batu 19 is 266.8.

Table 4.4. Pearson's Correlation Coefficient and p value between parameter and number of nymphs in Ulu Gombak Forest Reserve.

Parameter	Sampling Station								
	Sg. Gombak			Anak Sg. Gombak			Sg. Batu 19		
	r	r^2	p	r	r^2	p	r	r^2	p
Illuminance	-0.0908	0.0082	0.6730	0.0067	0	0.9754	0.3593	0.1291	0.0847
pH	0.5683	0.3229	0.0038*	0.6410	0.4109	0.0007*	0.6367	0.4054	0.0008*
Water Temperature (^{0}C)	0.4406	0.1941	0.0312*	-0.1205	0.0145	0.5750	0.0341	0.0012	0.8744
Dissolved Oxygen (mg/L O_2)	-0.5091	0.2592	0.0111*	-0.3550	0.1260	0.0887	-0.6466	0.4181	0.0006*
Conductivity (µS)	0.4523	0.2046	0.0265*	-0.2545	0.0648	0.2301	0.0331	0.0011	0.8780
Nitrate (mg/L)	0.1481	0.0219	0.4897	-0.1364	0.0186	0.5252	0.0525	0.0028	0.8073
Phosphate (mg/L)	-0.1034	0.0107	0.6305	0.2039	0.0416	0.3394	0.4197	0.1762	0.0412*
Ammonical nitrogen (mg/L)	-0.1736	0.0301	0.4172	-0.2474	0.0612	0.2437	0.3688	0.1360	0.0761

*significant value at $p \leq 0.05$

4.4 Biochemical Analysis

4.4.1 Stress biomarker and protein content

Specific activity of the following stress biomarkers were measured spectrophotometrically for the samples of *Baetis* sp. and *Campsoneuria* sp. Enzyme bioassays conducted on these samples showed that esterases from *Campsoneuria* sp. showed higher affinity for alpha-NA than those of *Baetis* sp. The higher specific activity of *Campsoneuria* sp. esterases towards alpha-NA than *Baetis* sp. esterases is showed in Table 4.5. The difference in non-specific esterase specific activity values between both *Baetis* sp. and *Campsoneuria* sp. were found to be insignificant ($t=2.13$, $p=0.28$) using student t-test ($p>0.05$).

The specific activity of acetylcholinesterase for *Baetis* sp. and *Campsoneuria* sp. were $2.61\pm0.78\times10^{-7}$and $8.15\pm0.56\times10^{-7}$ ASChI µM/min/µg protein respectively. Acetylcholinesterase was the stress biomarker with the lowest specific activity among all other enzyme. Higher specific activity of *Campsoneuria* sp. acetylcholinesterase towards ASChI than that of *Baetis* sp. was observed, however, the difference were insignificant ($t=1.94$, $p=0.30$) using student t-test ($p>0.05$).

The specific activity of glutathione-s-transferases from *Campsoneuria* sp. strains were approximately 3-fold higher than that found in *Baetis* sp. samples (Table 4.5). However, statistical analysis showed that the difference in glutathione-s-transferases specific activity values between these two species were insignificant ($t=1.99$, $p=0.30$) using student t-test ($p>0.05$).

The levels of protein content for the tested ephemeropteran nymphs were 7.60 ± 0.68 µg/mL (*Baetis* sp.) and 2.65 ± 0.84µg/mL (*Campsoneuria* sp.), an average of 0.76 µg/mL and 0.27 µg/mL per nymph for *Baetis* sp. and *Campsoneuria* sp. respectively. The difference in protein content between both *Baetis* sp. and *Campsoneuria* sp. were found to be insignificant ($t=2.07$, $p=0.29$) using student t-test ($p>0.05$).

Table 4.5. Protein content and selected biomarker specific activity samples of *Baetis* sp. and *Campsoneuria* sp.

	Samples of ephemeropteran nymphs	
	***Baetis* sp.**	***Campsoneuria* sp.**
Protein[a]	7.60±0.68	2.65±0.84
Biomarker		
Non-specific esterase[b]	0.21±0.03	0.58±0.02
Acetylcholinesterase[c]	$2.61 \pm 0.78 \times 10^{-7}$	$8.15 \pm 0.56 \times 10^{-7}$
Glutathione-s-transferases[d]	$3.73 \pm 0.12 \times 10^{-4}$	$1.13 \pm 0.06 \times 10^{-3}$

[a] Values of protein content are mean±standard deviation µg/mL

[b] Values of non-specific esterase are mean±standard deviation alpha-naphthol µM/min/µg protein

[c] Values of acetylcholinesterase are mean±standard deviation ASChI µM/min/µg protein

[d] Values of glutathione-s-transferases are mean±standard deviation CDNA-ηmol/min/µg protein

4.4.2 Measurement of inhibitor potency

The sensitivity of non-specific esterase and acetylcholinesterase to the selected inhibitors (Dichlorvos, Malathion, Fenitrothion, mercury, cadmium, lead and copper) varied among the *Baetis* sp. and *Campsoneuria* sp. nymphs tested. The concentration of inhibitor that reduces enzyme activity by half is called the median inhibition concentration or I_{50}. I_{50} for each inhibitor was determined based on the percent inhibition of the respective biomarker versus log (inhibitor concentration) regression analysis (Appendix 4.30 to 4.57). The exposure concentrations of inhibitors required obtaining I_{50} values for *Baetis* sp. and *Campsoneuria* sp. are summarized in Table 4.6.

The order of fifty percent inhibitory concentrations for non-specific esterase of *Baetis* sp. was: Dichlorvos > Malathion > Mercury (Hg) > Fenitrothion > Cadmium (CD) > Lead

(Pb) > Copper (Cu), where the corresponding I_{50} values were 8.68×10^{-10} M, 8.08×10^{-8} M, 1.37×10^{-5} M, 6.77×10^{-5} M, 7.08×10^{-5} M, 9.3×10^{-5} M and 9.51×10^{-5} M, respectively. The order against *Campsoneuria* sp. was the same as *Baetis* sp., however with differing I_{50} values, 1.08×10^{-10} M, 3.68×10^{-8} M, 3.69×10^{-7} M, 2.61×10^{-5} M, 3.50×10^{-5} M, 4.05×10^{-5} M and 5.31×10^{-5} M, respectively. There were no significant difference found in the I_{50} values of the tested inhibitors between both ephemeropteran nymphs with Dichlorvos (t=1.28, p=0.42), Malathion (t=2.67, p=0.23), Mercury (Hg) (t=1.06, p=0.48), Fenitrothion (t=2.25, p=0.27), Cadmium (Cd) (t=2.63, p=0.23), Lead (Pb) (t=2.54, p=0.24) and Copper (Cu) (t=3.53, p=0.18) using student t-test (p>0.05) (Appendix 4.60).

Dichlorvos (DDVP) also exhibited high potency against *Baetis* spp. acetylcholinesterase followed by Malathion, mercury (Hg), Fenitrothion, cadmium (Cd),lead (Pb) and copper (Cu), where the corresponding I_{50} values were 8.32×10^{-9} M, 2.69×10^{-8} M, 2.09×10^{-7} M, 1.22×10^{-7} M, 1.87×10^{-5} M, 6.58×10^{-5} M and 1.21×10^{-4} M, respectively. The fifty percent inhibitory concentrations for acetylcholinesterase of *Campsoneuria* sp. was in the following order: Dichlorvos (DDVP) > Malathion > Fenitrothion > mercury (Hg) > cadmium (Cd) > lead (Pb) > copper (Cu), where the corresponding I_{50} values were 9.19×10^{-9} M, 4.44×10^{-8} M, 3.09×10^{-7} M, 3.23×10^{-7} M, 2.88×10^{-5} M and 8.10×10^{-5} M, respectively. Among all tested inhibitors, it appears that only I_{50} values of Dichlorvos (DDVP) between both ephemeropteran nymphs showed highly significant difference (t=20.04, p=0.03) (Appendix 4.61).

Overall, Dichlorvos (DDVP) was found to be the most potent inhibitor tested, for non-specific esterase and acetylcholinesterase from both species samples while copper (Cu) was the least toxic inhibitor for non-specific esterase and acetylcholinesterase from both species samples.

Table 4.6. Average comparative 50% inhibition of non-specific esterase and acetylcholinesterase from samples of *Baetis* sp. and *Campsoneuria* sp. by various pesticides and heavy metal in *in vitro* assays.

Inhibitors	I_{50} (M)			
	Baetis sp.		*Campsoneuria* sp.	
	EST	AChE	EST	AchE
Dichlorvos (DDVP)	8.68×10^{-10}	8.32×10^{-9}	1.08×10^{-10}	9.19×10^{-9}
Malathion	8.08×10^{-8}	2.69×10^{-8}	3.68×10^{-8}	4.44×10^{-8}
Fenitrothion	6.77×10^{-5}	2.09×10^{-7}	2.61×10^{-5}	3.09×10^{-7}
Mercury (Hg)	1.37×10^{-5}	1.22×10^{-7}	3.69×10^{-7}	3.23×10^{-7}
Cadmium (Cd)	7.08×10^{-5}	1.87×10^{-5}	3.50×10^{-5}	2.88×10^{-5}
Lead (Pb)	9.3×10^{-5}	6.58×10^{-5}	4.05×10^{-5}	8.10×10^{-5}
Copper (Cu)	9.51×10^{-5}	1.21×10^{-4}	5.31×10^{-5}	2.07×10^{-4}

4.4.3 Determination of acetylcholinesterase bimolecular inhibition rate constants

Dichlorvos (DDVP), Malathion, Fenitrothion, mercury (Hg), cadmium (Cd), lead (Pb) and copper (Cu) were all potential inhibitors of acetylcholinesterase for both *Baetis* sp. and *Campsoneuria* sp. Acetylcholinesterase bimolecular inhibition rate constants (K_i) were measured for each inhibitor compound with different concentrations. The concentration of inhibitors used for this procedure was as following: Dichlorvos (DDVP) (1×10^{-9} M), Malathion (1×10^{-8} M), Fenitrothion and mercury (1×10^{-7} M), cadmium and lead (1×10^{-5} M) and copper (1×10^{-4} M). Results show that the percentage of residual activity of AChE for all selected inhibitors gradually decreases with time as shown in Figure 4.16 and 4.17. The gradient obtained from the plot of the log of residual activity (AChE) against time and the values of K_i are shown in Table 4.7.

The order of sensitivity, based on K_i values of *Baetis* sp. to AChE inhibition, was Dichlorvos (DDVP) > Malathion > mercury > Fenitrothion > cadmium > copper > lead, while that for *Campsoneuria* sp. was Dichlorvos (DDVP) > Malathion > mercury > Fenitrothion > cadmium > lead > copper (Figure 4.13). The K_i value of Dichlorvos (DDVP) (t=21.44, p=0.03) for both *Baetis* sp. and *Campsoneuria* sp. were significantly higher ($p \leq 0.05$) than other tested inhibitors. The result showed the inhibiting power of acetylcholinesterase by Dichlorvos (DDVP) was the strongest among all other tested inhibitors for both, *Baetis* sp. and *Campsoneuria* sp. Similarly, there was also highly significant difference ($p \leq 0.05$) using student t-test in K_i values of Malathion (t=12.01, p=0.05) and cadmium (t=69.18, p=0.01) between both ephemeropteran nymphs.

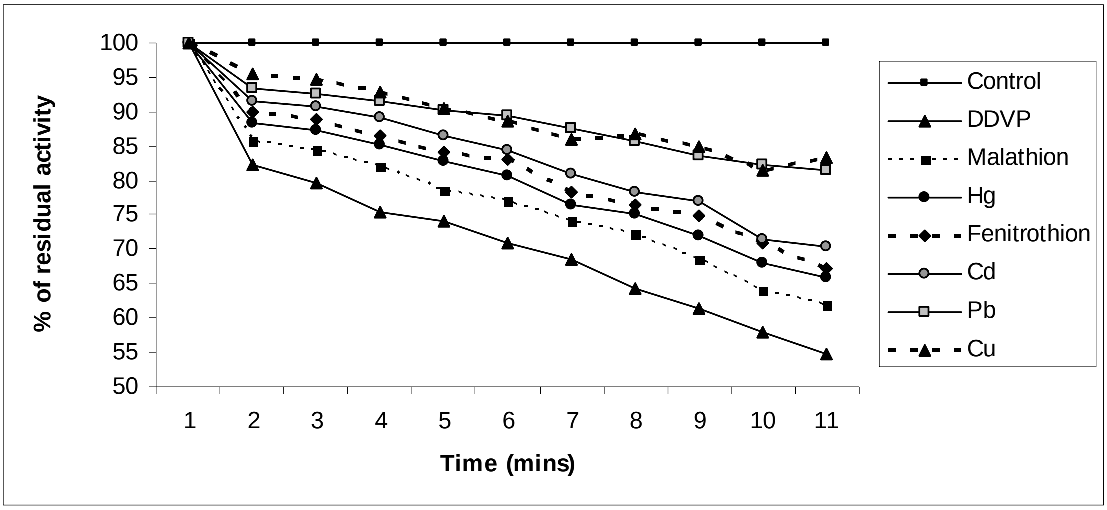

Figure 4.16. Percentage of residual activity of AChE versus time (minutes) for *Baetis* sp.

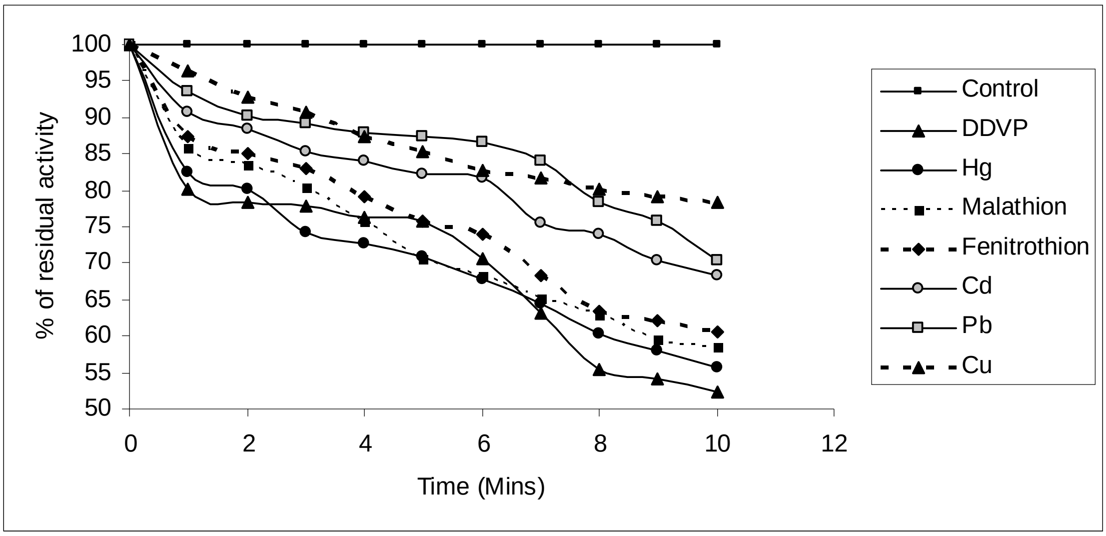

Figure 4.17. Percentage of residual activity of AChE versus time (minutes) for *Campsoneuria* sp.

Table 4.7. Calculated values of Ki of selected inhibitors on acetylcholinesterase from strains of *Baetis* sp. and *Campsoneuria* sp.

	***Baetis* sp.**		***Campsoneuria* sp.**	
Inhibitor	**Regression**	**Ki**	**Regression**	**Ki**
DDVP	y = -3.6982x + 93.962	8.52	y = -4.06x + 95.669	9.35
Malathion	y = -3.1764x + 96.176	7.32	y = -3.7536x + 96.195	8.64
Fenitrothion	y = -2.7973x + 98.593	6.44	y = -3.6173x + 97.931	8.33
Hg	y = -2.9509x + 97.869	6.80	y = -3.6818x + 93.609	8.48
Cd	y = -2.6964x + 99.86	6.21	y = -2.7755x + 98.535	6.39
Pb	y = -1.6164x + 98.589	3.72	y = -2.4227x + 100.31	5.58
Cu	y = -1.6809x + 99.595	3.87	y = -2.1682x + 99.755	4.99

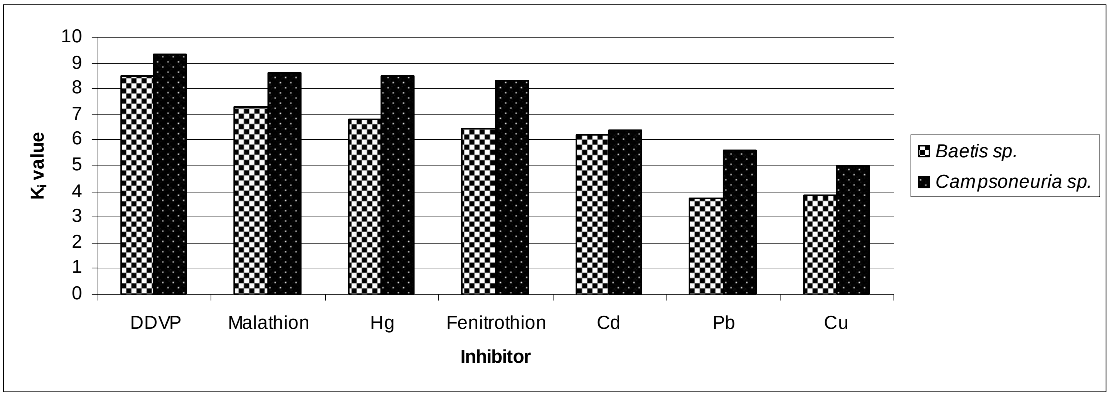

Figure 4.18. Bimolecular inhibition rate constant (K_i) value of selected inhibitors to acetylcholinesterase for *Baetis* sp. and *Campsoneuria* sp.