

APPENDICES

Appendix 3.1. Data of protein standard curve based on Lowry *et al.* (1951)

BSA concentration ($\mu\text{g/mL}$)	Optical density (660 nm) Average \pm S.E
0	0.000
5	0.1283 \pm 0.0010
10	0.1972 \pm 0.0042
15	0.2864 \pm 0.0029
20	0.3703 \pm 0.0038
25	0.4303 \pm 0.0039
30	0.5121 \pm 0.0054
35	0.5683 \pm 0.0048
40	0.6527 \pm 0.0041
45	0.7086 \pm 0.0090
50	0.7718 \pm 0.0076
60	0.8578 \pm 0.0143
70	0.9625 \pm 0.0065
80	1.0691 \pm 0.0073
90	1.1421 \pm 0.0059
100	1.2597 \pm 0.0201

Appendix 3.2. Data of alpha naphthol standard curve

Average optical density (590nm)	Alpha naphthol concentration ($\mu\text{M/mL}$)
0.069 \pm 0.002	1.563
0.140 \pm 0.000	3.125
0.248 \pm 0.006	6.250
0.513 \pm 0.011	12.000
1.033 \pm 0.029	25.000
1.883 \pm 0.076	50.000

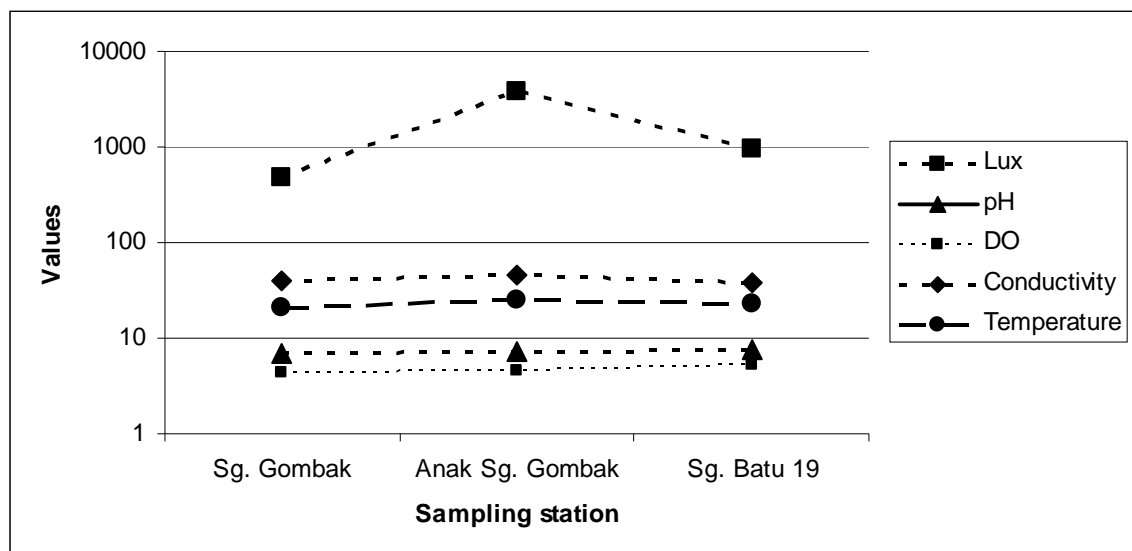
Appendix 4.1. Cumulative abundance of ephemeropteran nymphs in Ulu Gombak Forest Reserve

R= Rare (1 or 2 collected)

C= Common (3 to 9 collected)

A= Abundant (10 or more collected)

Sampling station	Taxon	Abundance
Sg. Gombak	<i>Baetis</i> sp.	A
	<i>Platybaetis</i> sp.	C
	<i>Thalerophyrus</i> sp.	A
	<i>Camponeuria</i> sp.	A
	<i>Neurocaenis</i> sp.	A
Anak Sg. Gombak	<i>Baetis</i> sp.	A
	<i>Platybaetis</i> sp.	C
	<i>Thalerophyrus</i> sp.	A
	<i>Camponeuria</i> sp.	A
	<i>Neurocaenis</i> sp.	A
Sg. Batu 19	<i>Baetis</i> sp.	A
	<i>Thalerophyrus</i> sp.	A
	<i>Camponeuria</i> sp.	A
	<i>Neurocaenis</i> sp.	A
	<i>Prosopistoma</i> sp.	R
	<i>Neurocaenis</i> sp.	A



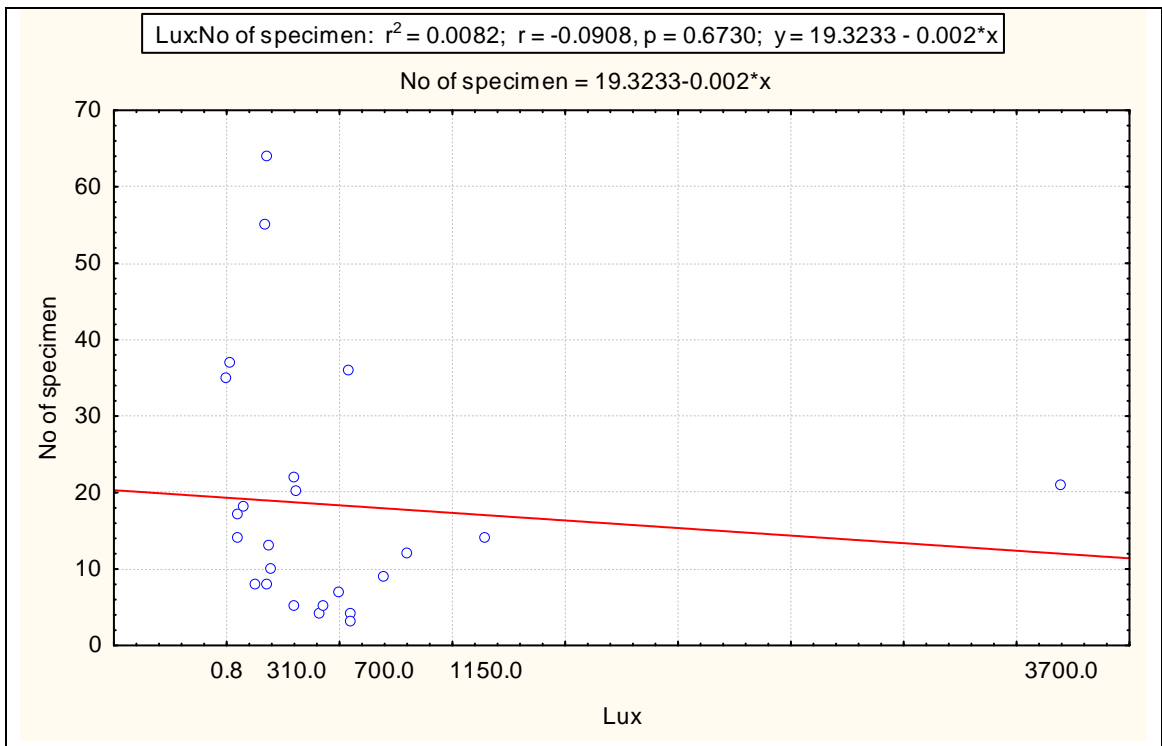
Appendix 4.2. Fluctuations of physical-chemical parameter along the sampling stations

Appendix 4.3. Significant comparison of ephemeropteran nymph abundance between Sg. Gombak, Anak Sg. Gombak and Sg. Batu 19 using student t-test at $p \leq 0.05$

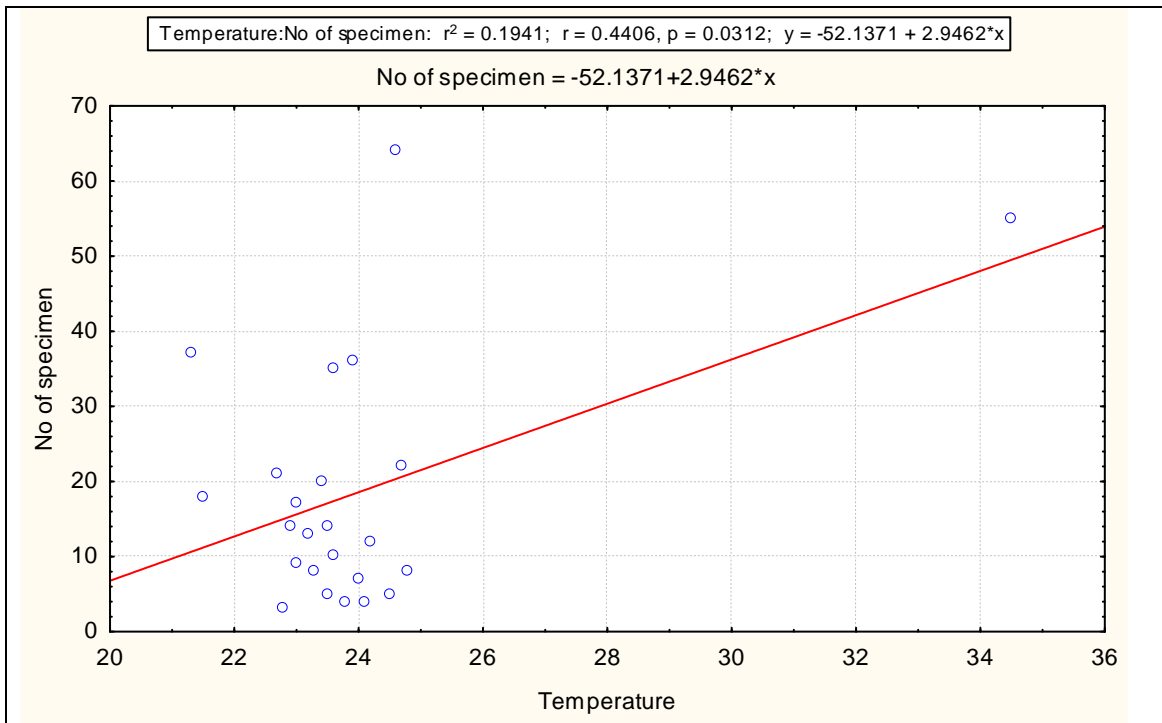
Taxon	t value	df	p value
<i>Baetis</i> sp.	3.21	2	0.09
<i>Platybaetis</i> sp.	1.92	2	0.20
<i>Thalerosphyrus</i> sp.	3.49	2	0.07
<i>Camponeuria</i> sp.	2.35	2	0.14
<i>Neurocaenis</i> sp.	4.52	2	0.05
<i>Prosopistoma</i> sp.	1.0	2	0.42

Appendix 4.4. Significant comparison of diversity indices between Sg. Gombak (downstream) and Sg. Batu 19 (upstream) using student t-test at $p \leq 0.05$

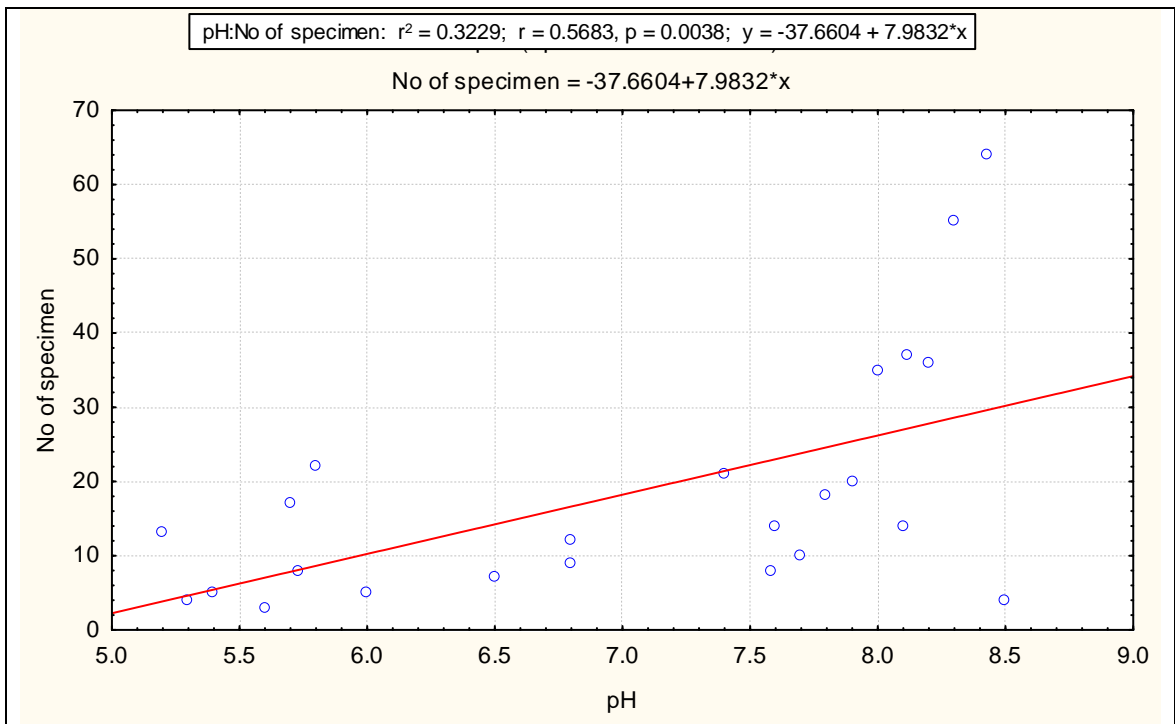
Diversity indices	t value	df	p value
Shannon's Diversity Index, H'	13.44	1	0.05
Shannon's Equitability, E_H	13.73	1	0.05
Simpson's Index of Dominance, D	8.50	1	0.07
Simpson's Index of Diversity, $1-D$	16.50	1	0.04
Simpson's Reciprocal Index, $1/D$	8.03	1	0.08
Simpson's Index of Evenness, E_s	7.38	1	0.09
Margalef's Richness Index, d	10.00	1	0.06



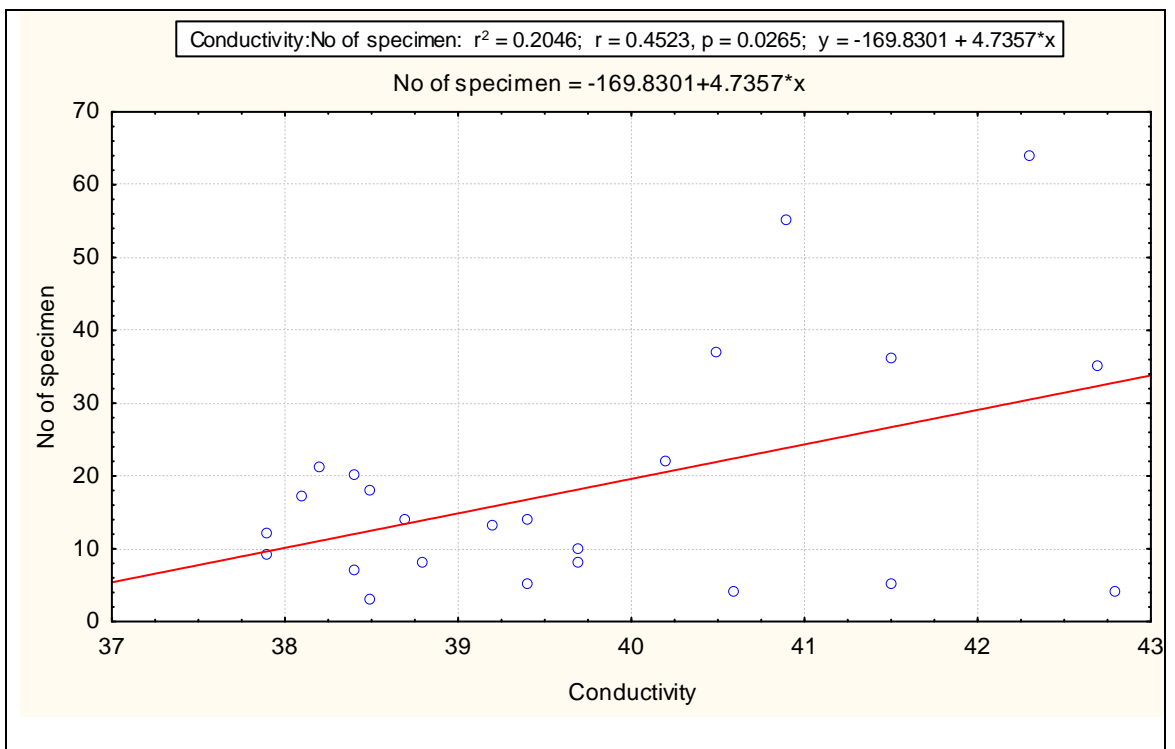
Appendix 4.5. Scatterplot of Ephemeropteran nymphs vs illuminance (Lux) at Sg. Gombak



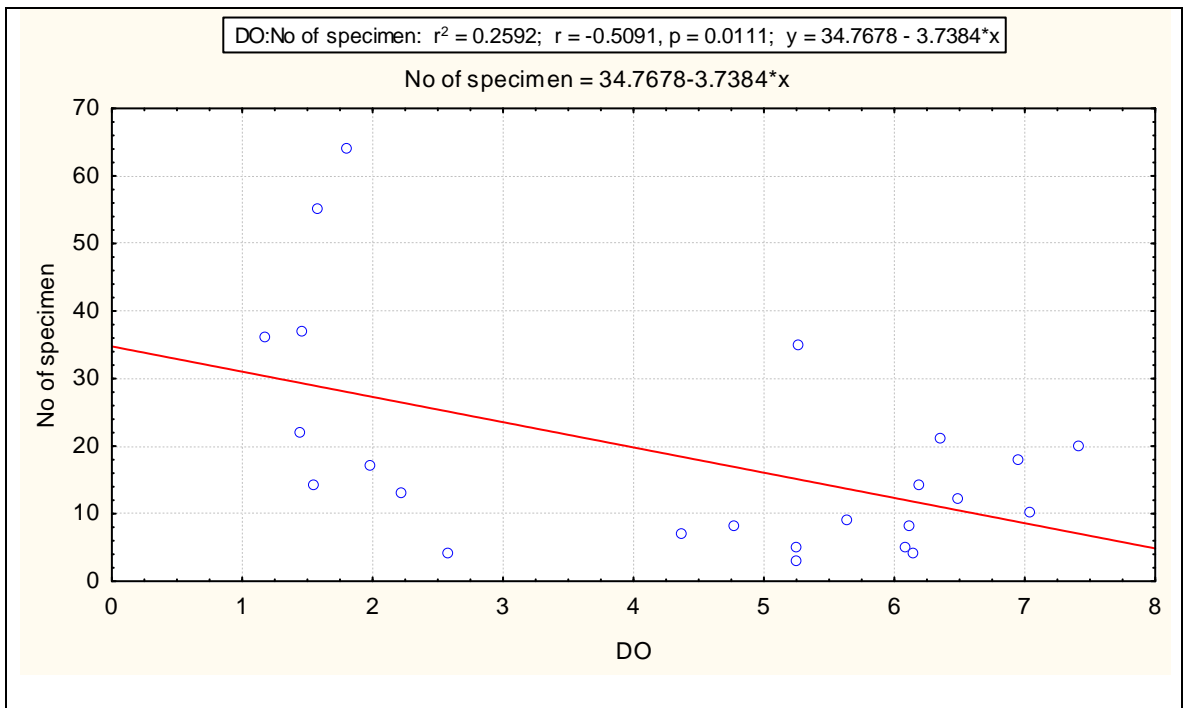
Appendix 4.6. Scatterplot of Ephemeropteran nymphs vs temperature at Sg. Gombak



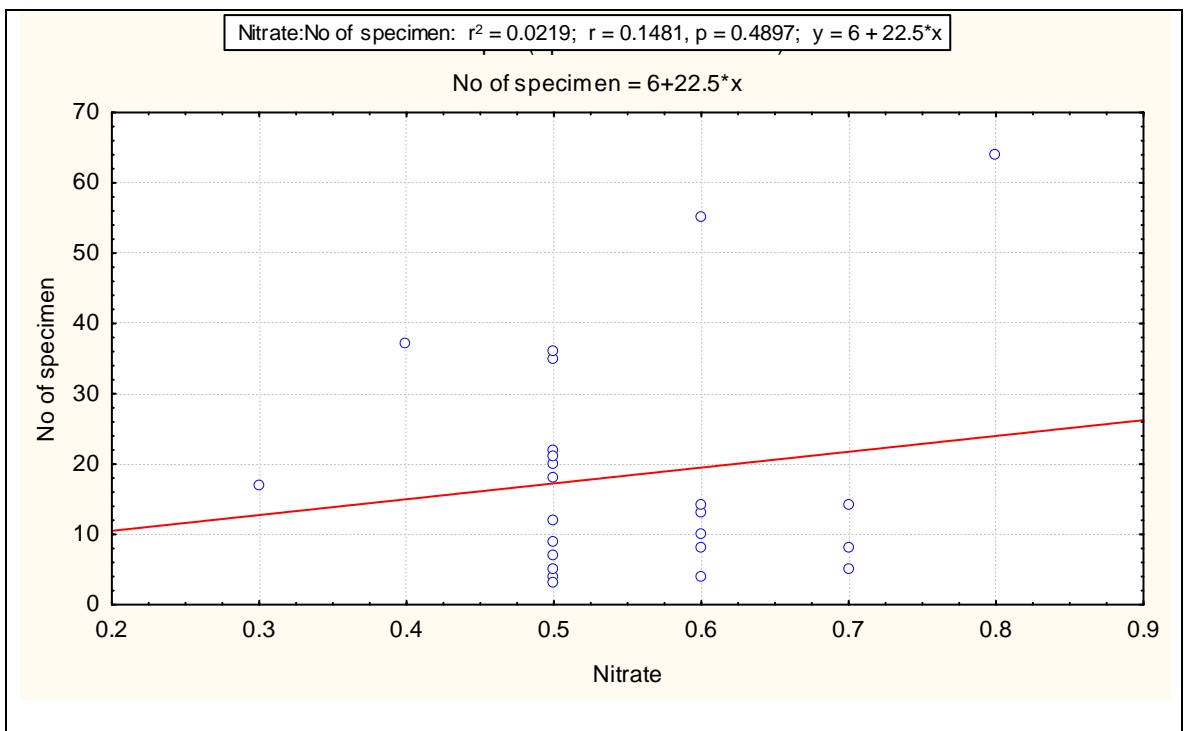
Appendix 4.7. Scatterplot of Ephemeropteran nymphs vs pH at Sg. Gombak



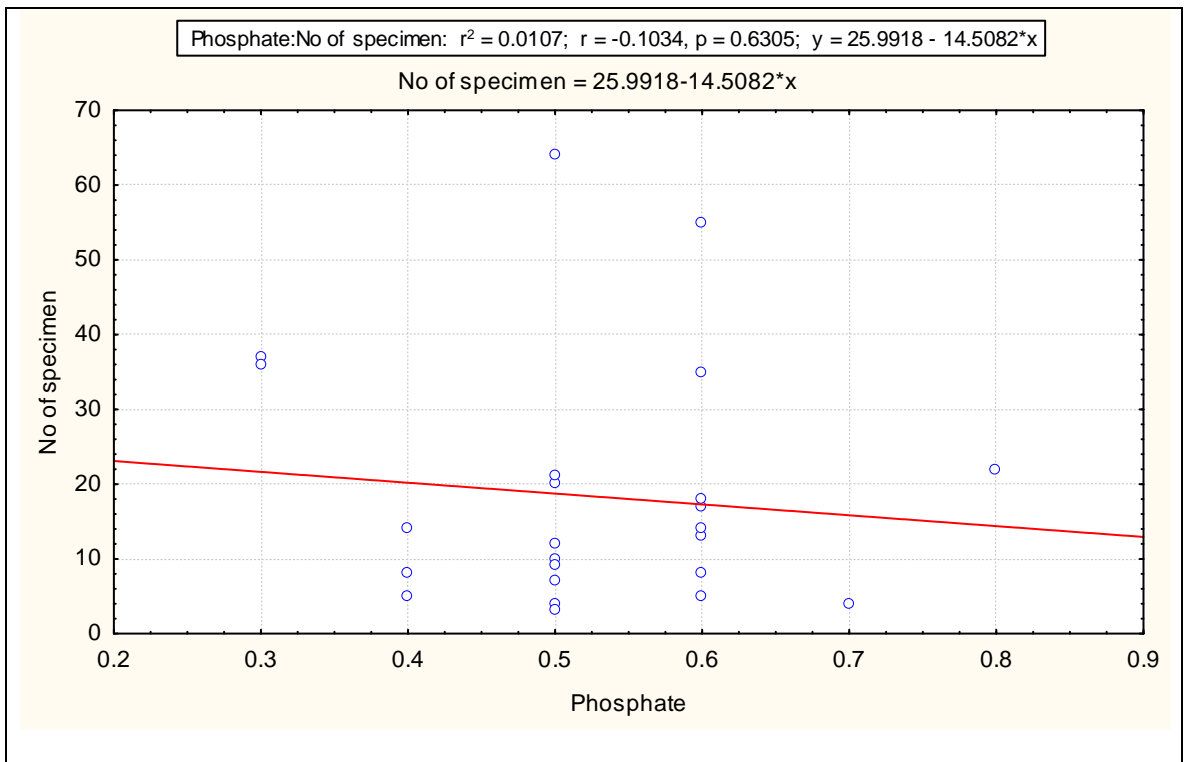
Appendix 4.8. Scatterplot of Ephemeropteran nymphs vs conductivity at Sg. Gombak



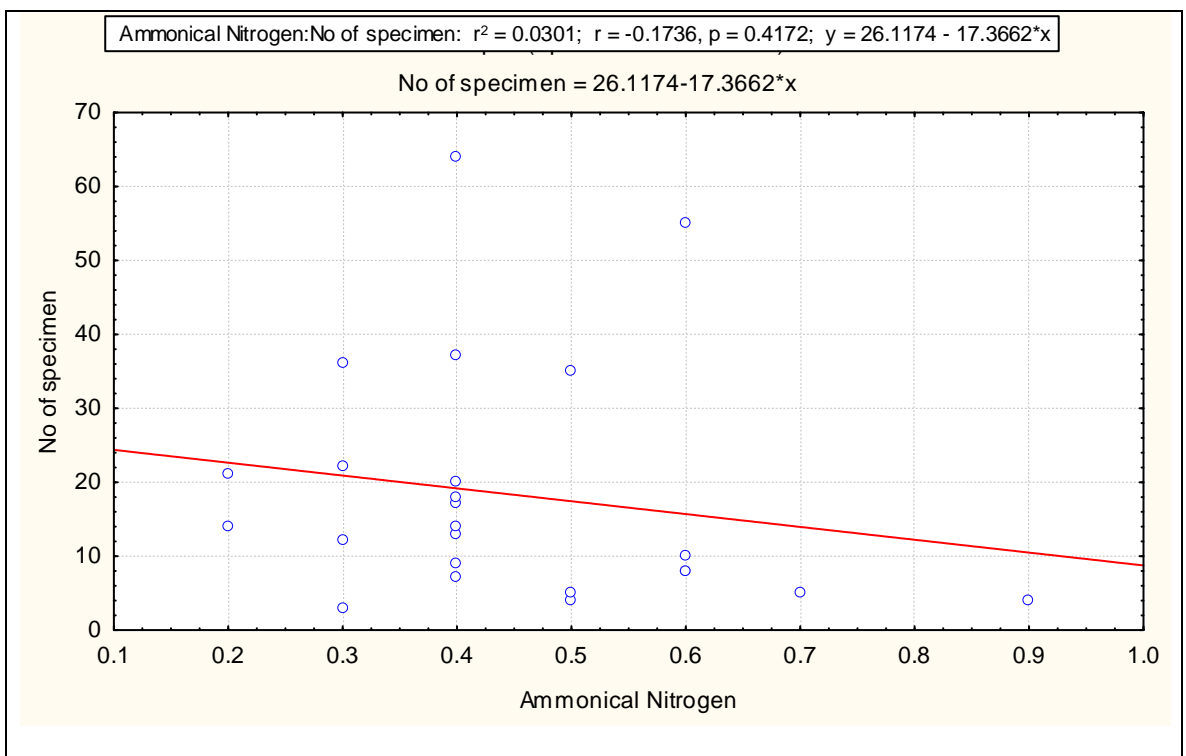
Appendix 4.9. Scatterplot of Ephemeropteran nymphs vs dissolved oxygen at Sg. Gombak



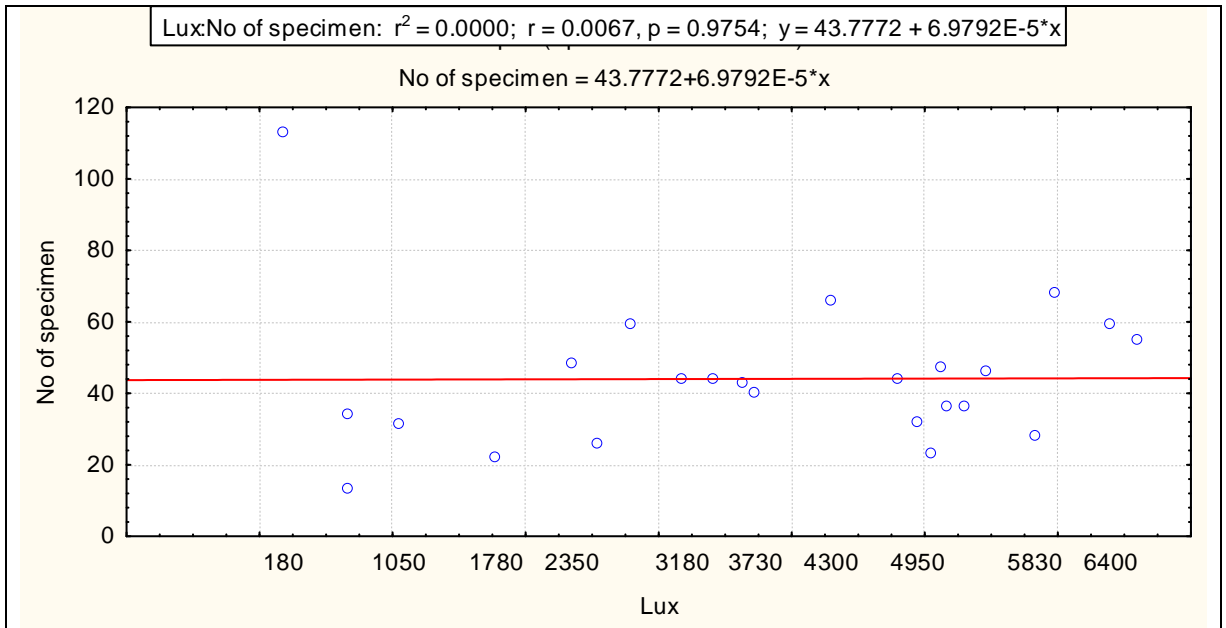
Appendix 4.10. Scatterplot of Ephemeropteran nymphs vs nitrate at Sg. Gombak



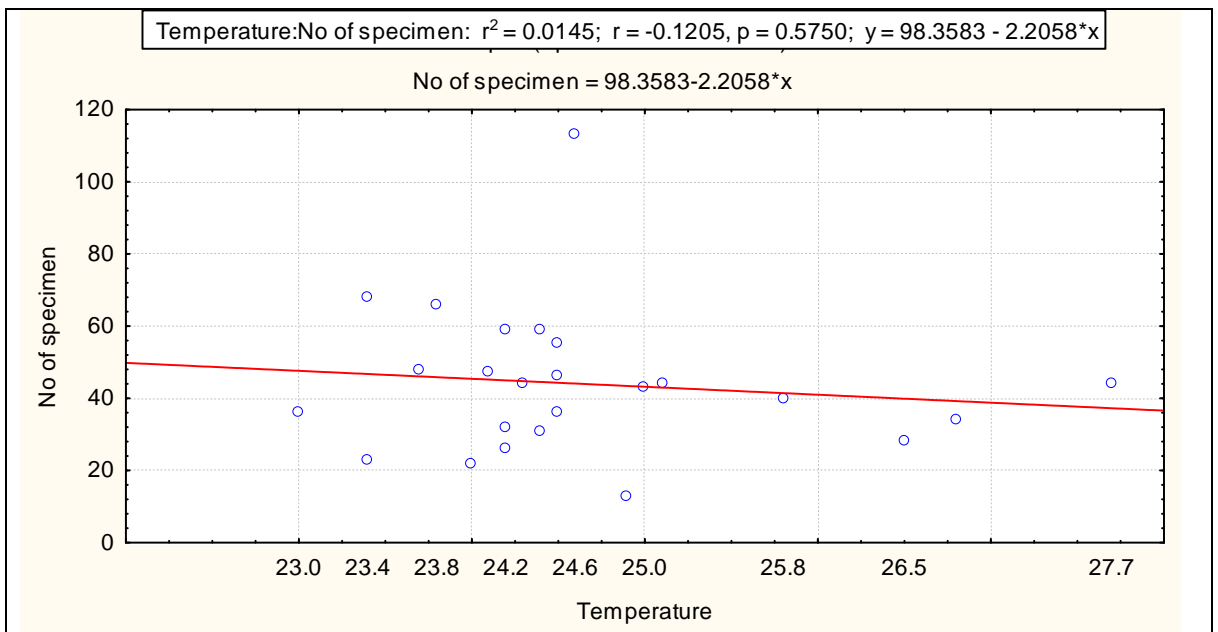
Appendix 4.11. Scatterplot of Ephemeropteran nymphs vs phosphate at Sg. Gombak



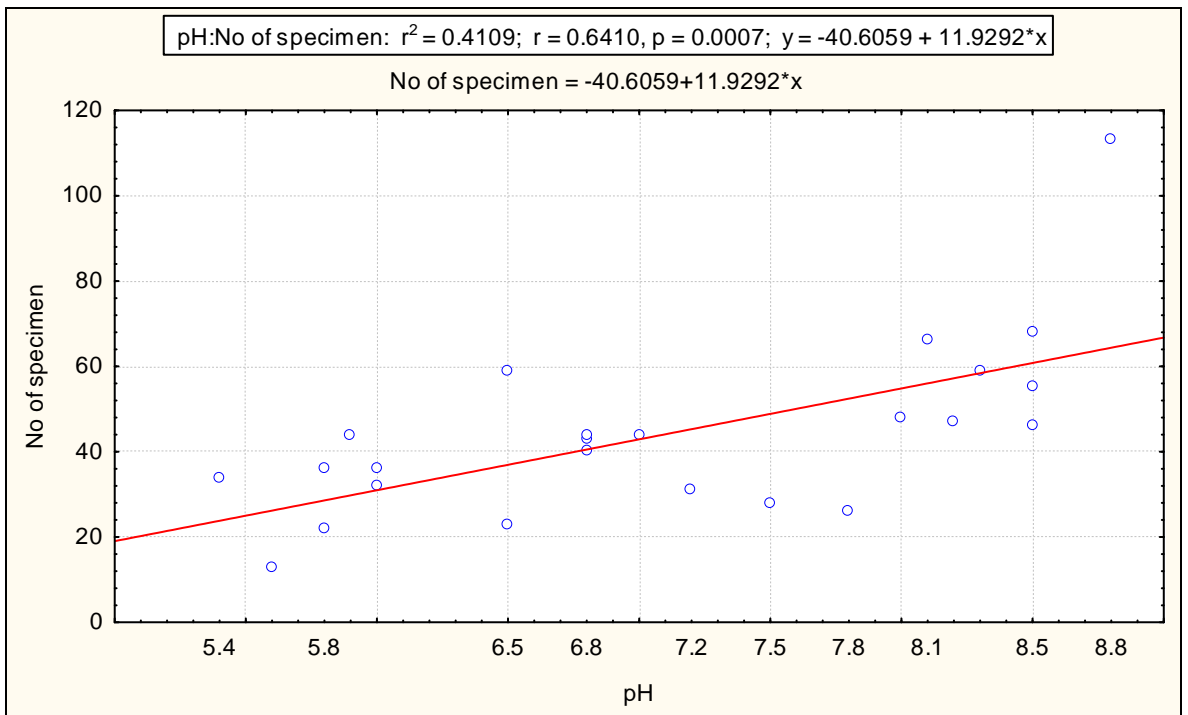
Appendix 4.12. Scatterplot of Ephemeropteran nymphs vs ammonical nitrogen at Sg. Gombak



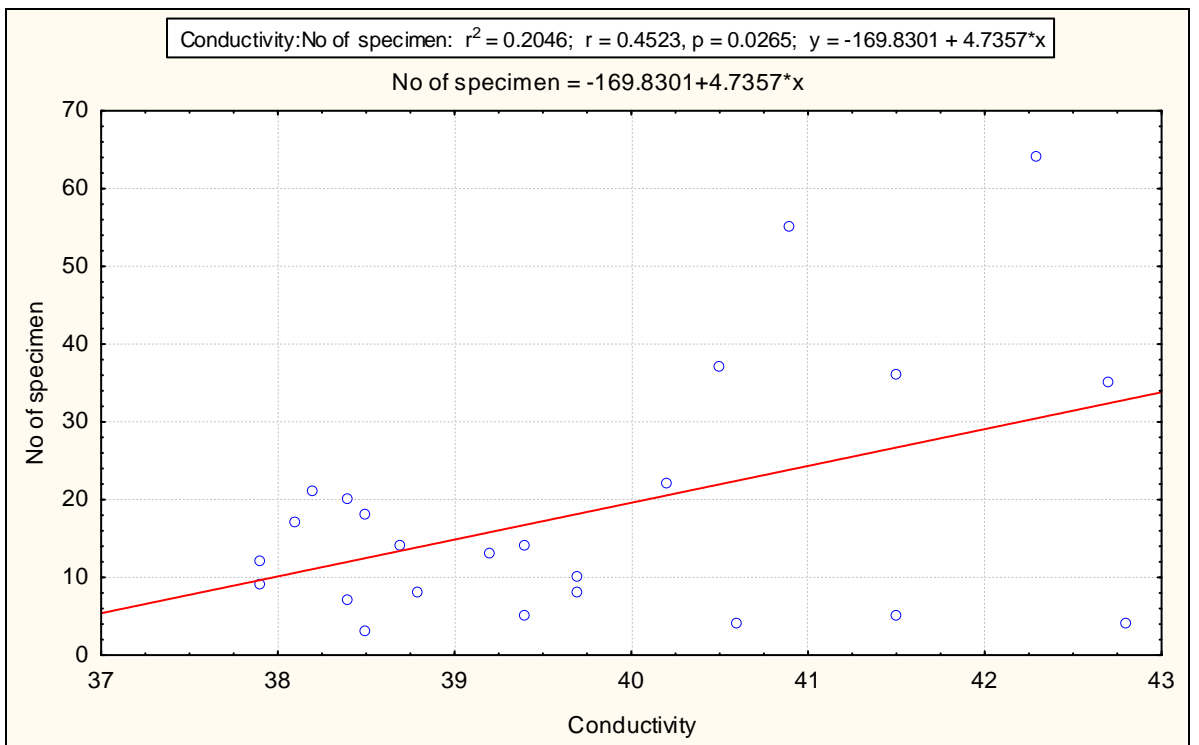
Appendix 4.13. Scatterplot of Ephemeropteran nymphs vs illuminance at Anak Sg. Gombak



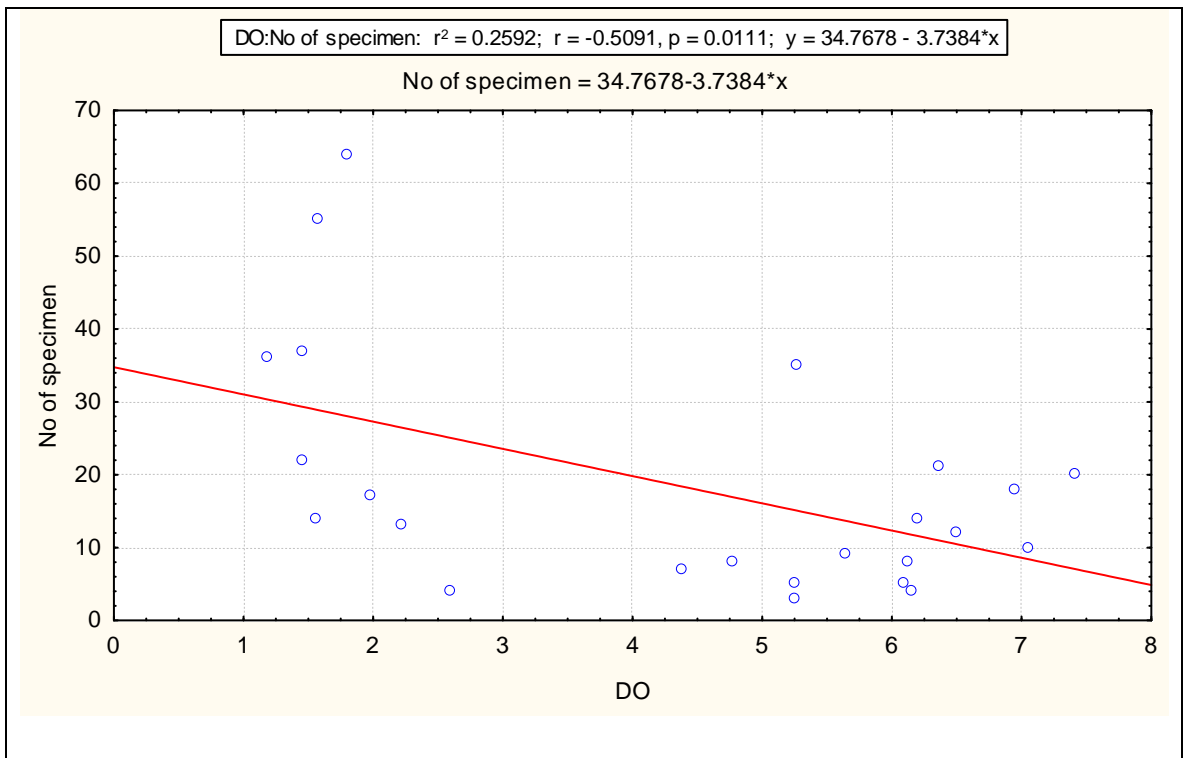
Appendix 4.14. Scatterplot of Ephemeropteran nymphs vs temperature at Anak Sg. Gombak



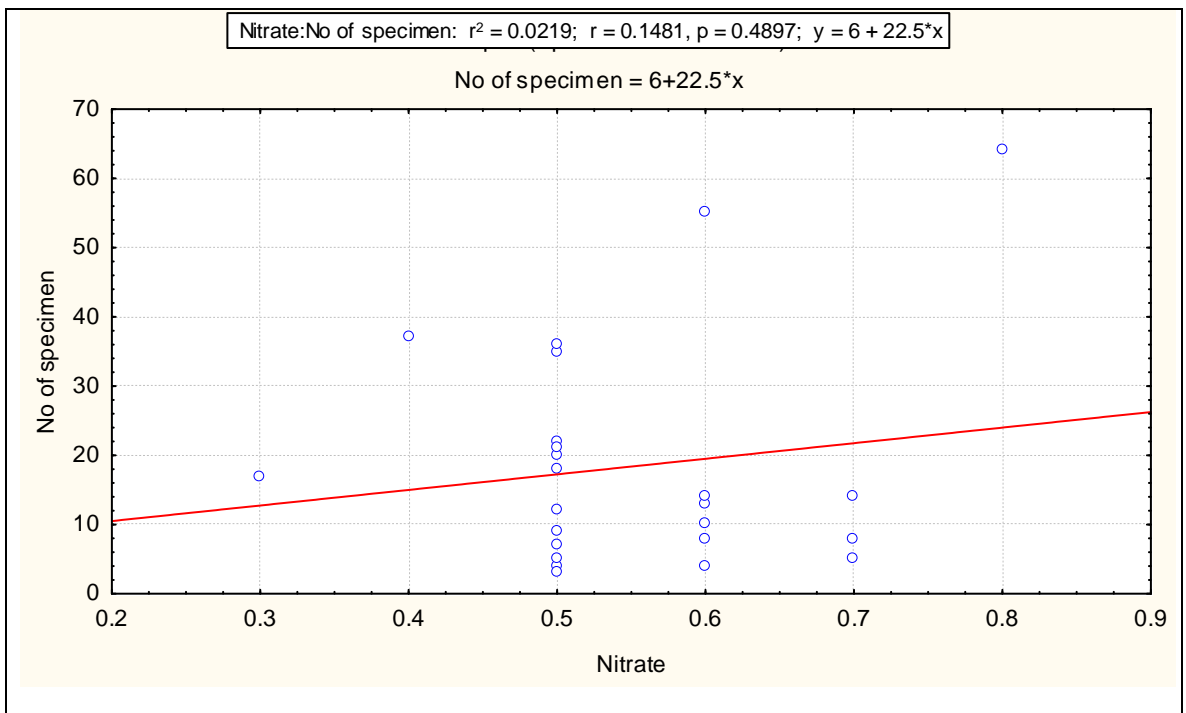
Appendix 4.15. Scatterplot of Ephemeropteran nymphs vs pH at Anak Sg. Gombak



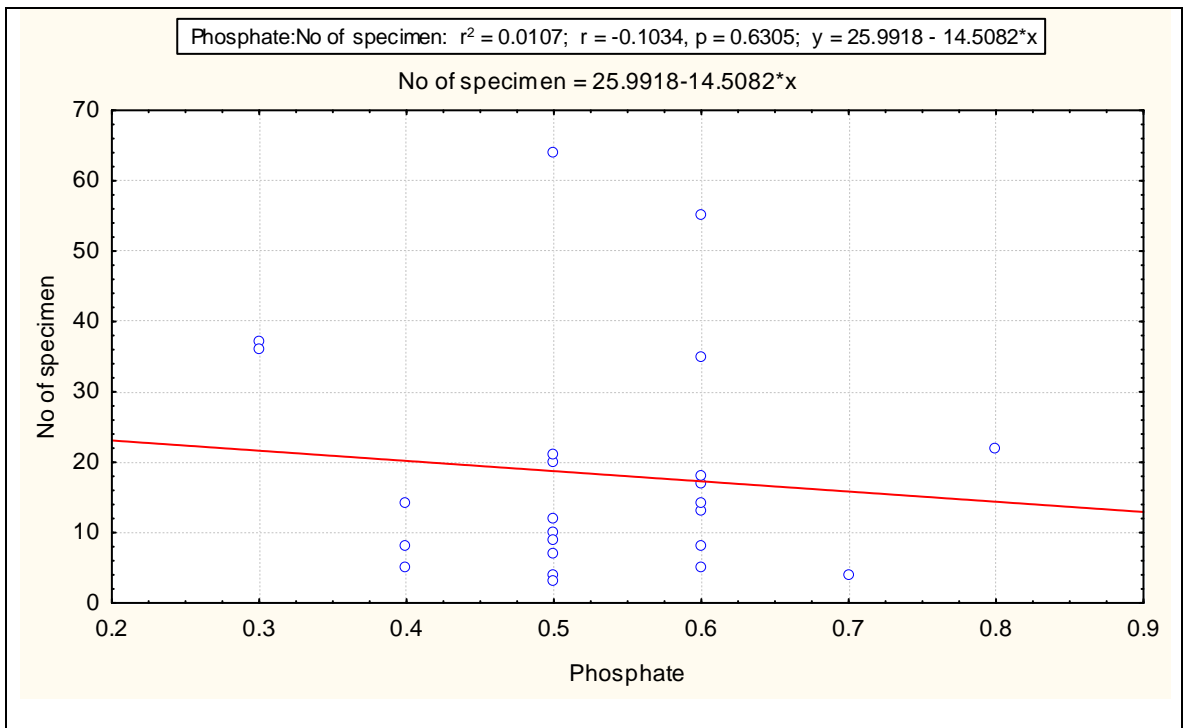
Appendix 4.16. Scatterplot of Ephemeropteran nymphs vs conductivity at Anak Sg. Gombak



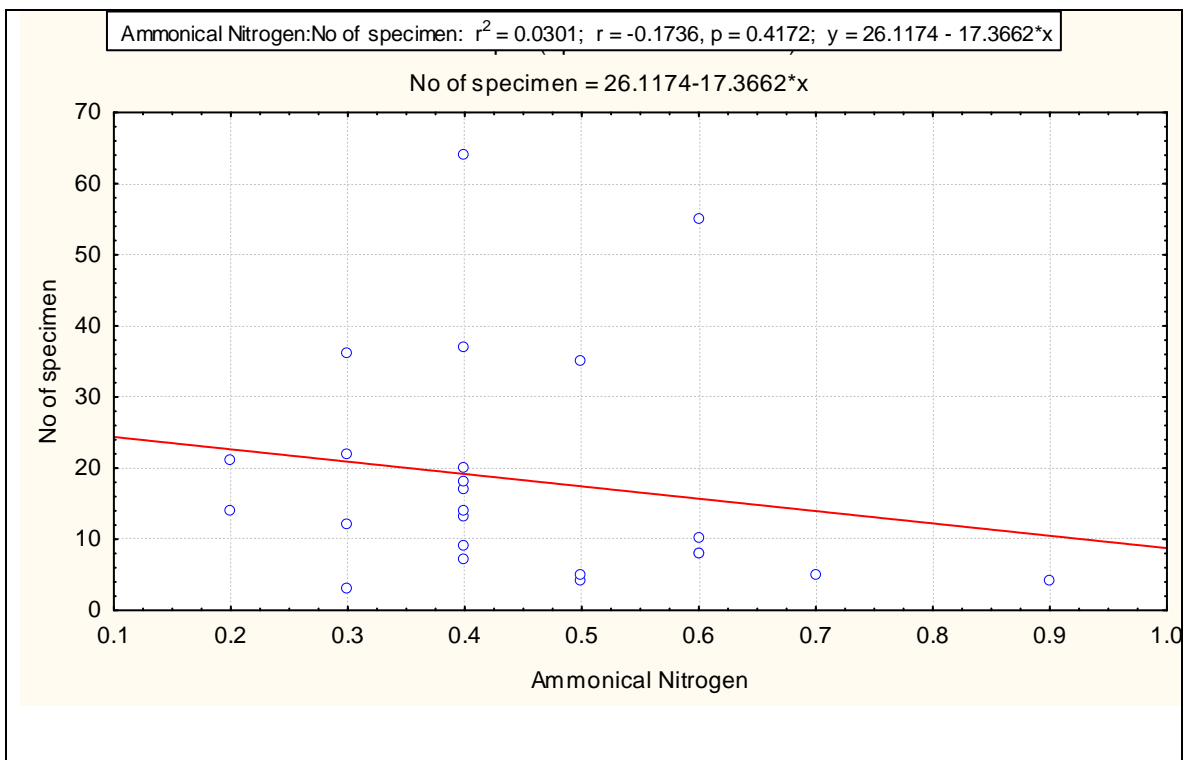
Appendix 4.17. Scatterplot of Ephemeropteran nymphs vs dissolved oxygen at Anak Sg. Gombak



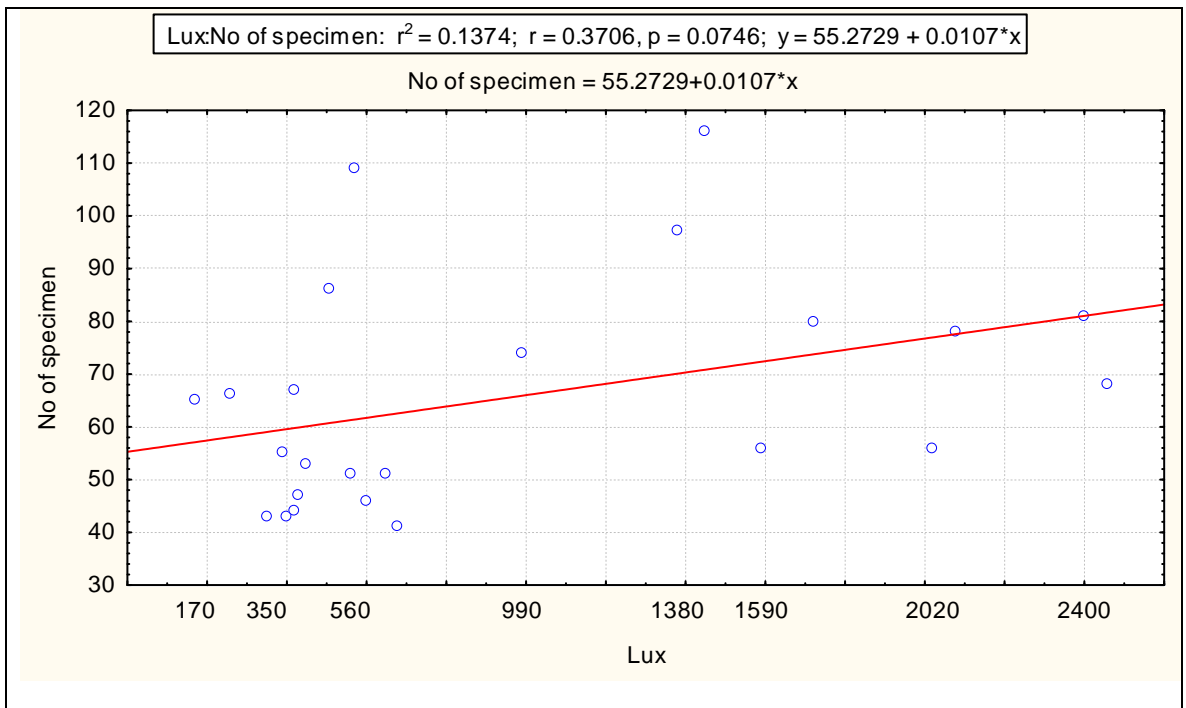
Appendix 4.18. Scatterplot of Ephemeropteran nymphs vs nitrate at Anak Sg. Gombak



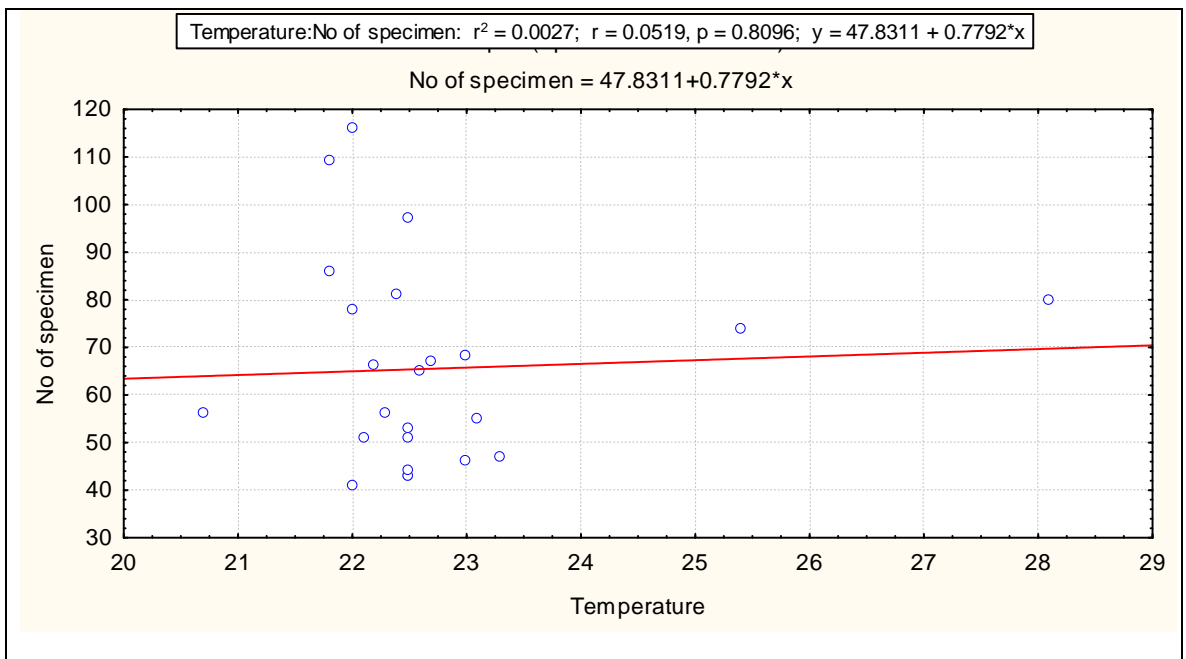
Appendix 4.19. Scatterplot of Ephemeropteran nymphs vs phosphate at Anak Sg. Gombak



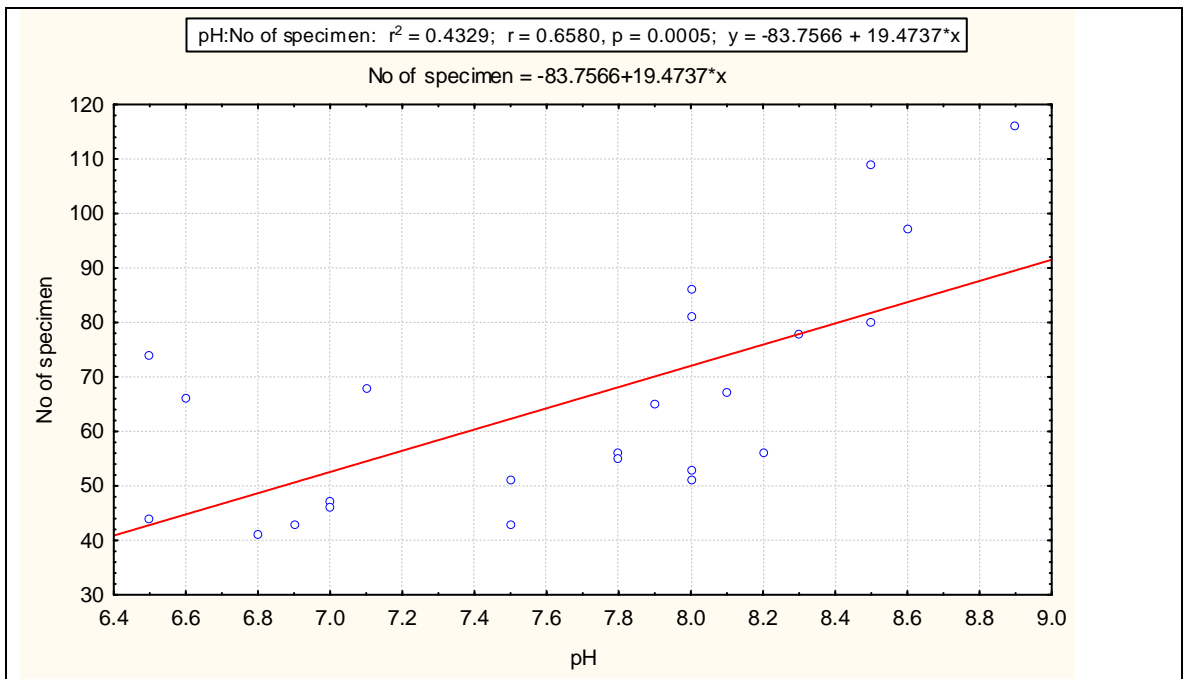
Appendix 4.20. Scatterplot of Ephemeropteran nymphs vs ammonical oxygen at Anak Sg. Gombak



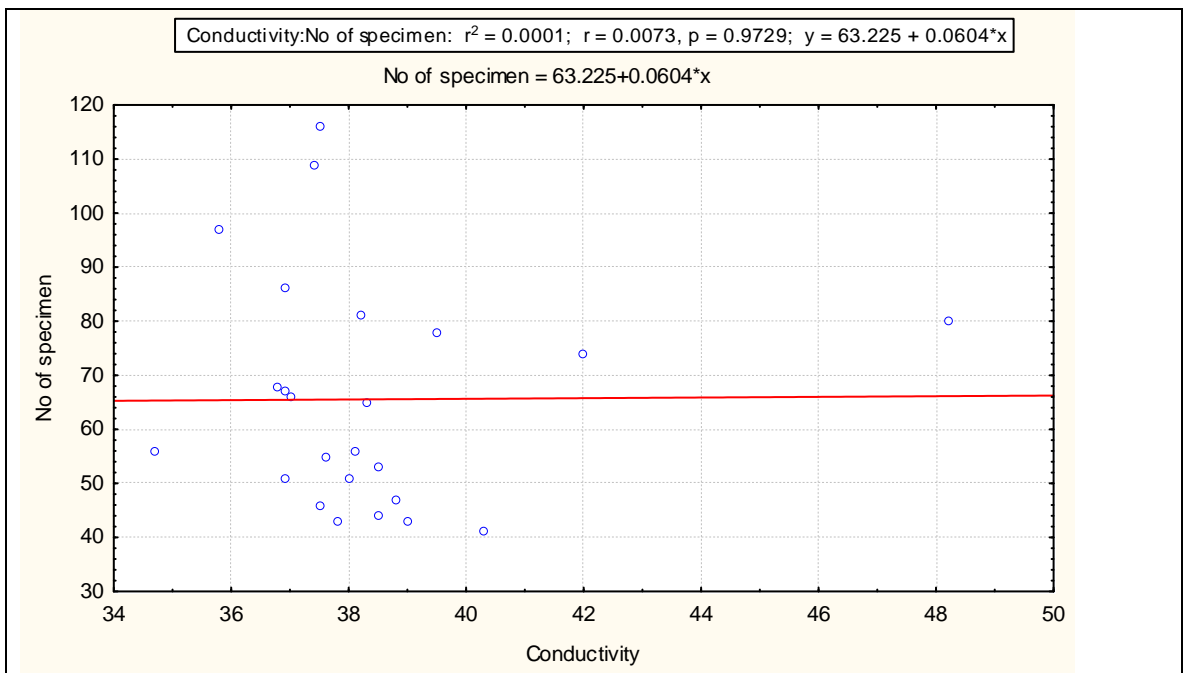
Appendix 4.21. Scatterplot of Ephemeropteran nymphs vs illuminance at Sg. Batu 19



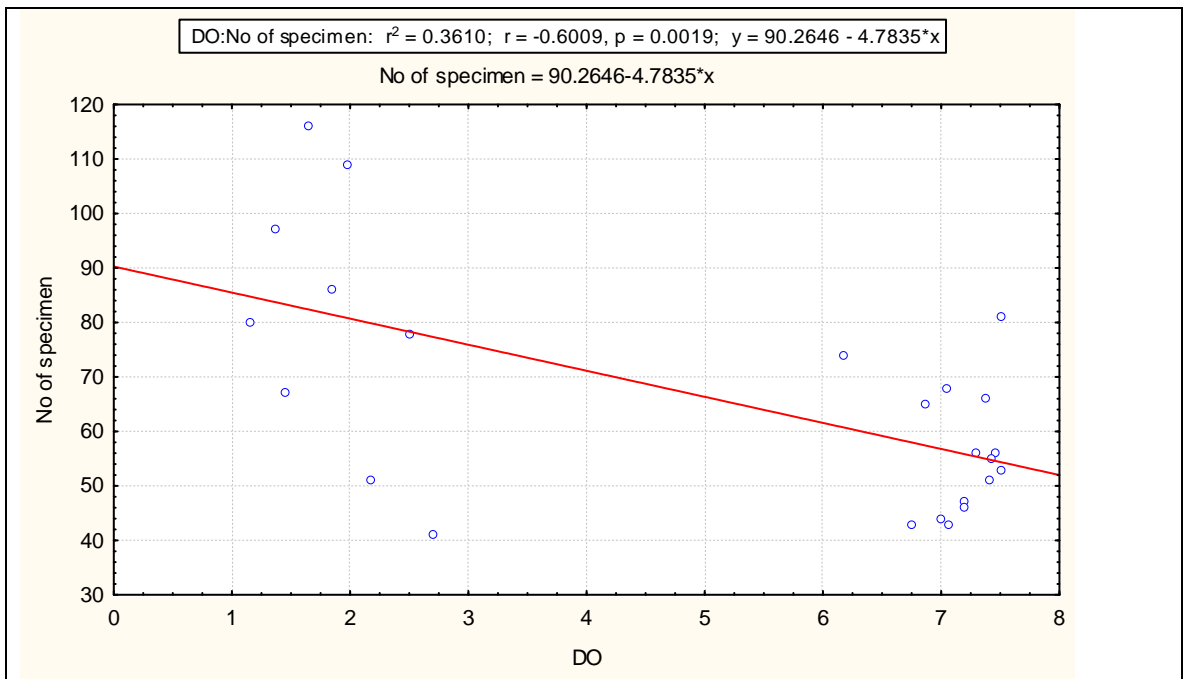
Appendix 4.22. Scatterplot of Ephemeropteran nymphs vs temperature at Sg. Batu 19



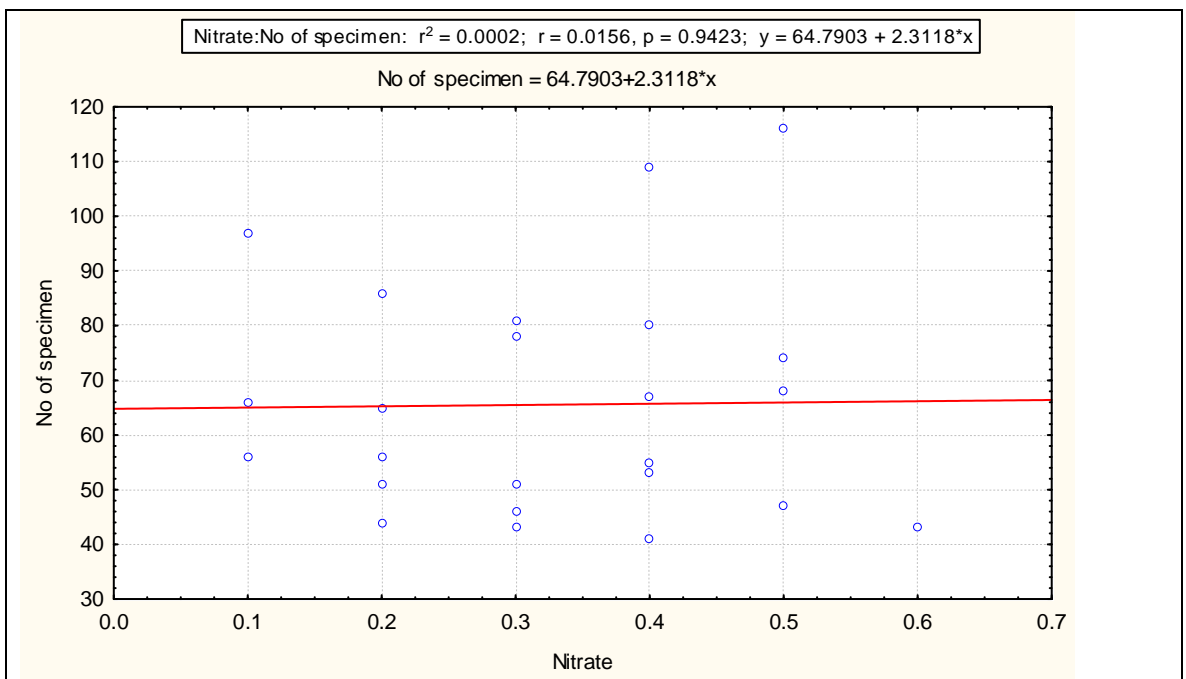
Appendix 4.23. Scatterplot of Ephemeropteran nymphs vs pH at Sg. Batu 19



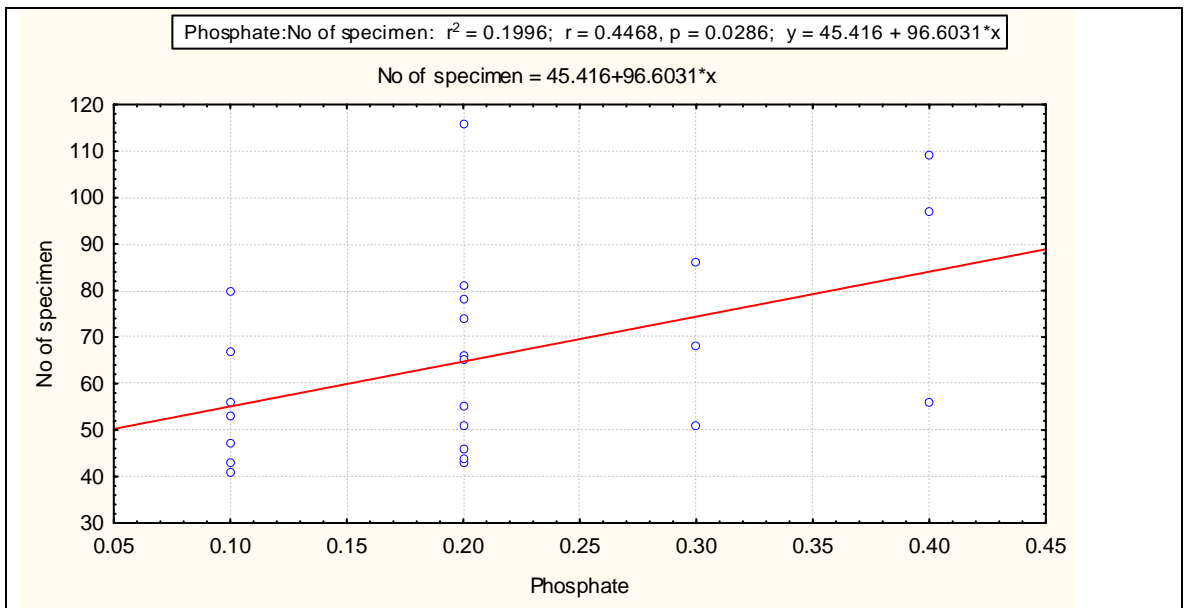
Appendix 4.24. Scatterplot of Ephemeropteran nymphs vs conductivity at Sg. Batu 19



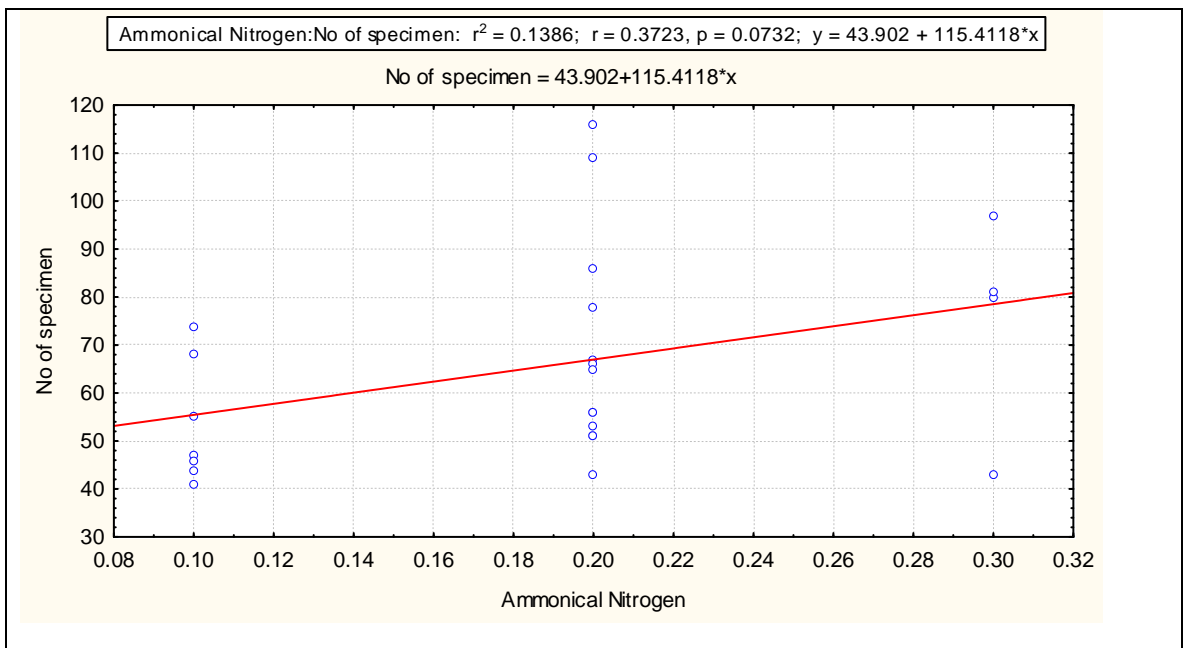
Appendix 4.25. Scatterplot of Ephemeropteran nymphs vs dissolved oxygen at Sg. Batu 19



Appendix 4.26. Scatterplot of Ephemeropteran nymphs vs nitrate at Sg. Batu 19



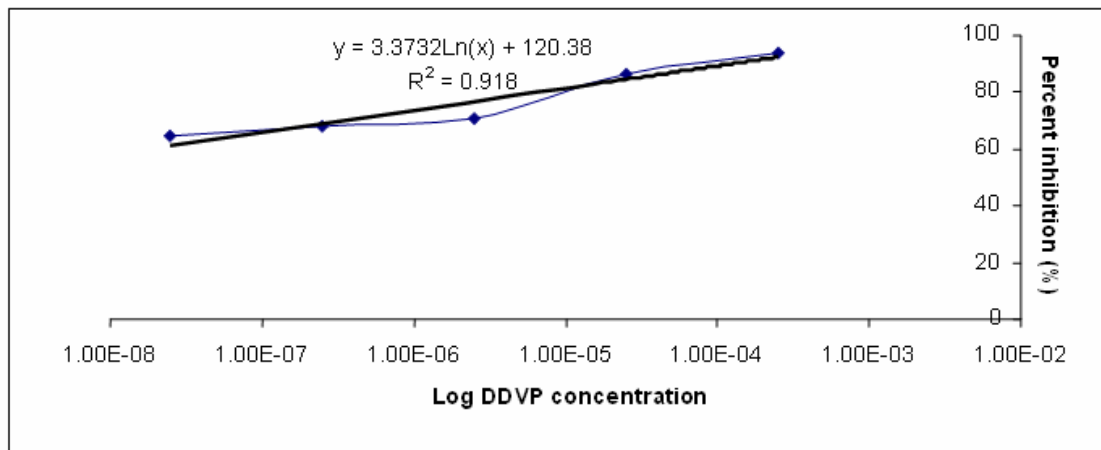
Appendix 4.27. Scatterplot of Ephemeropteran nymphs vs phosphate at Sg. Batu 19



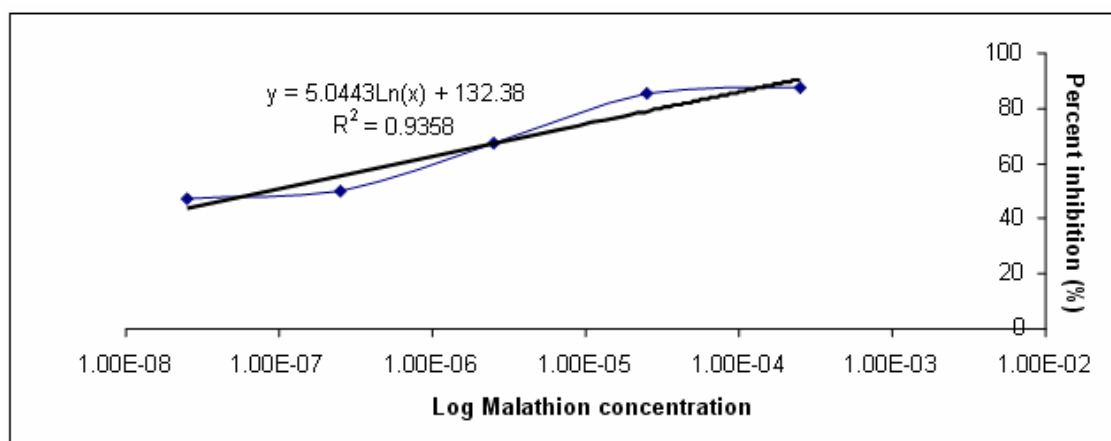
Appendix 4.28. Scatterplot of Ephemeropteran nymphs vs ammonical nitrogen at Sg. Batu 19

Appendix 4.29. Significant comparison of protein content and specific activity of non-specific esterase, acetylcholinesterase and glutathione-s-transferases from control strains of *Baetis* sp. and *Camponeuria* sp. using student t-test at $p \leq 0.05$

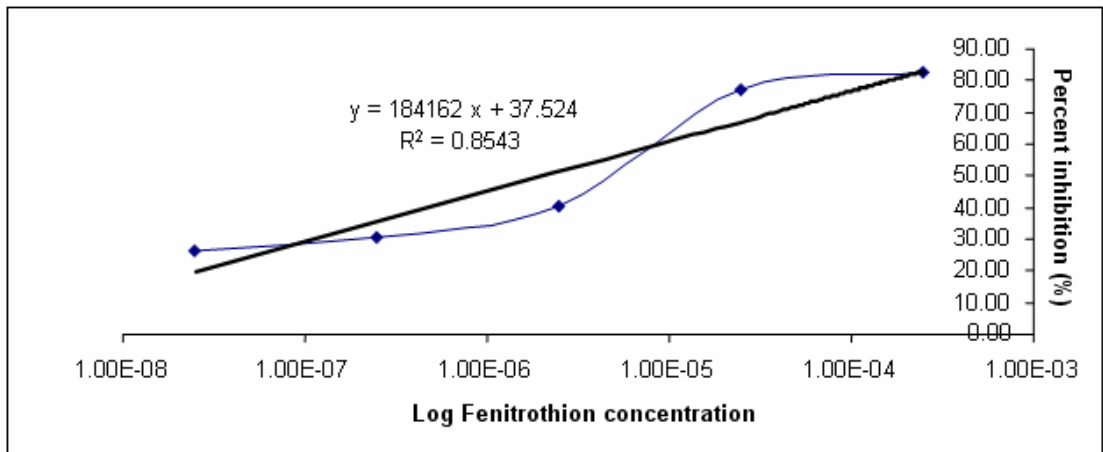
Specific activity	t value	df	p value
Non-specific esterase	2.13	1	0.28
Acetylcholinesterase	1.94	1	0.30
Glutathione- s – transferases	1.99	1	0.30
Protein content	2.07	1	0.29



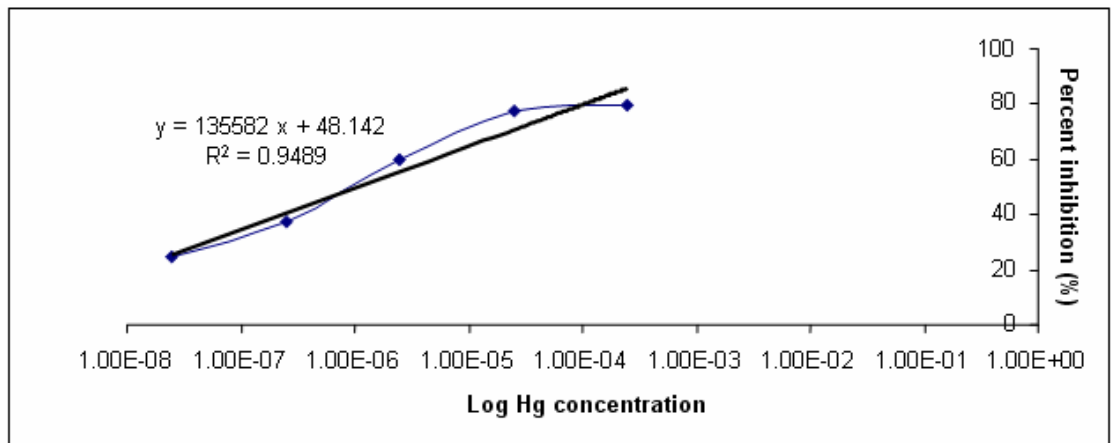
Appendix 4.30. The inhibitory effect of Dichlorvos (DDVP) on non-specific esterase of *Baetis* sp.



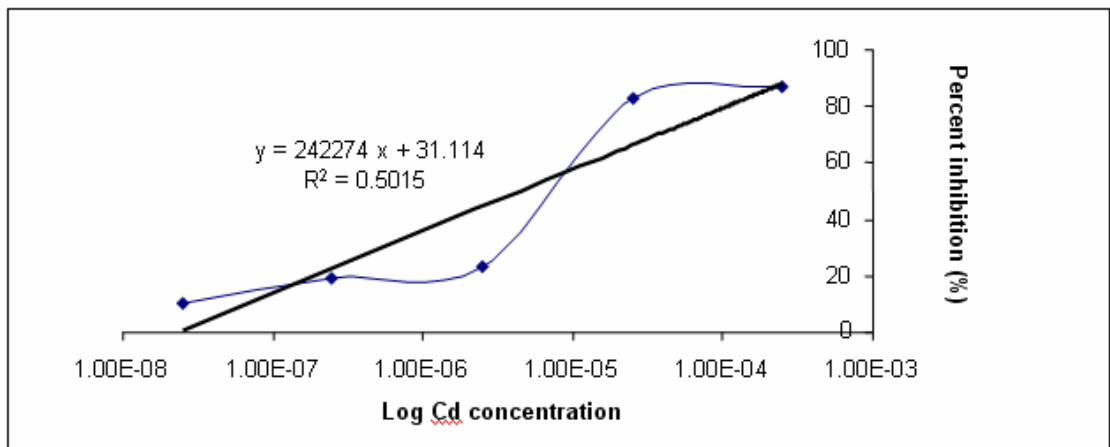
Appendix 4.31. The inhibitory effect of Malathion on non-specific esterase of *Baetis* sp.



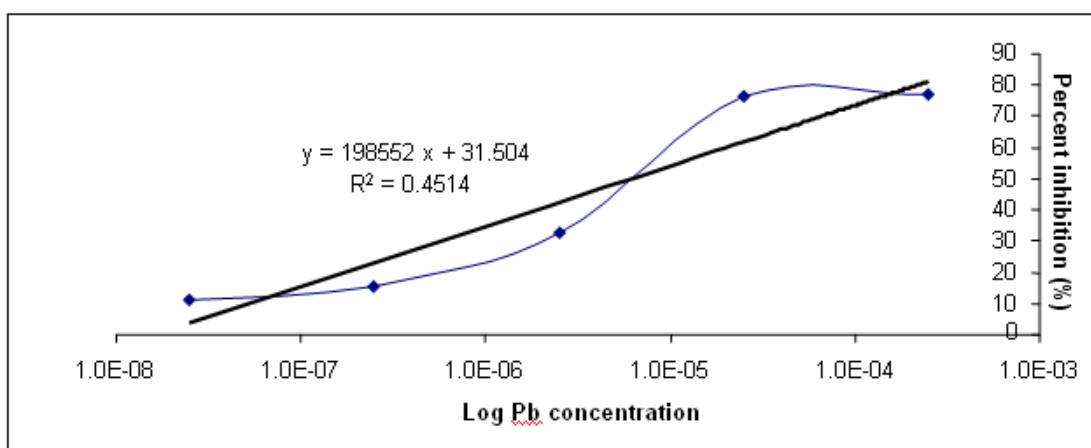
Appendix 4.32. The inhibitory effect of Fenitrothion on non-specific esterase of *Baetis* sp.



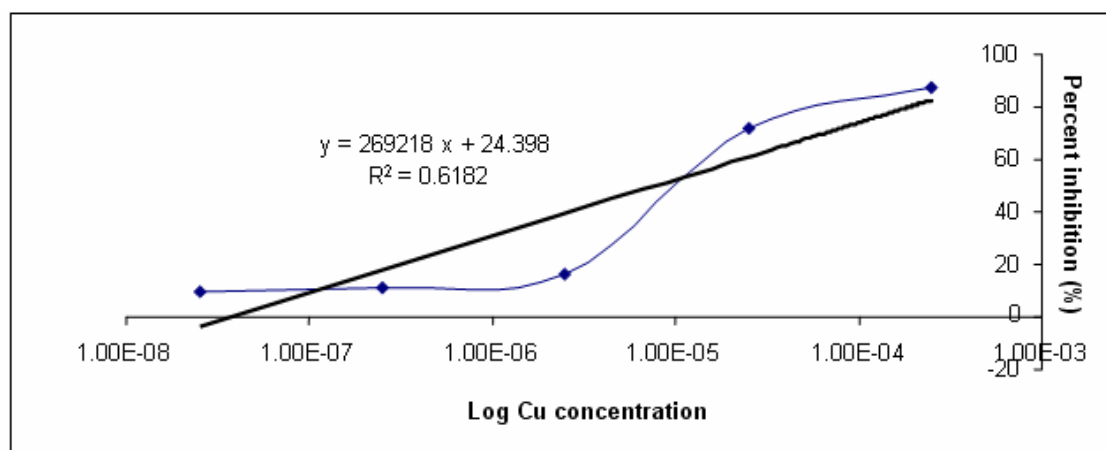
Appendix 4.33. The inhibitory effect of mercury (Hg) on non-specific esterase of *Baetis* sp.



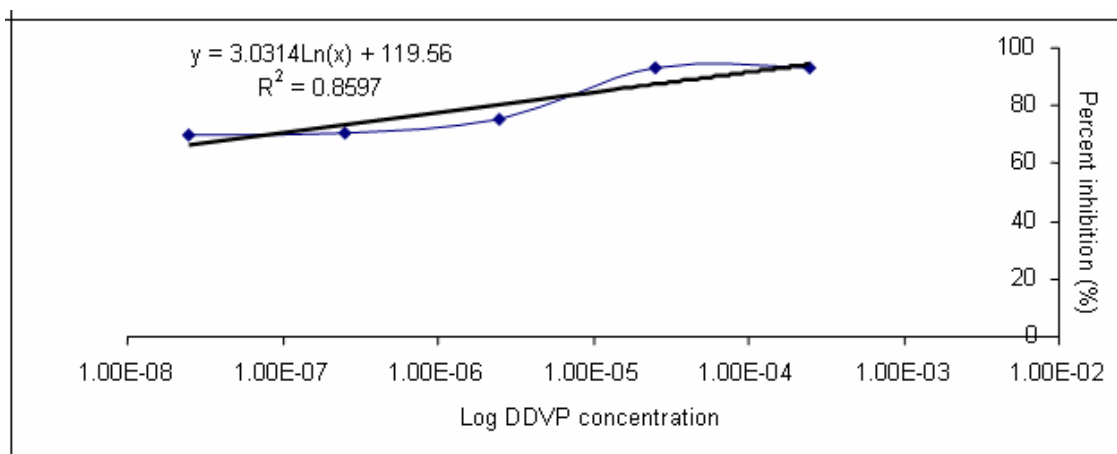
Appendix 4.34. The inhibitory effect of cadmium (Cd) on non-specific esterase of *Baetis* sp.



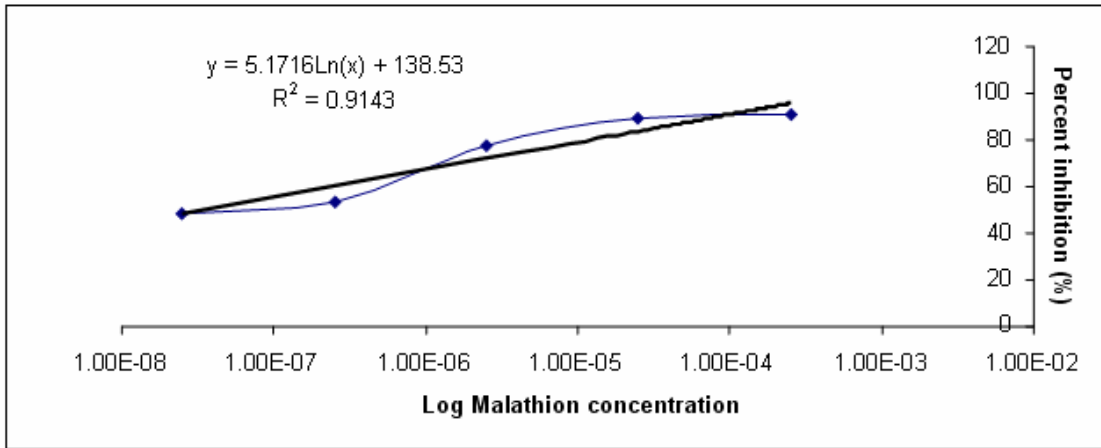
Appendix 4.35. The inhibitory effect of lead (Pb) on non-specific esterase of *Baetis* sp.



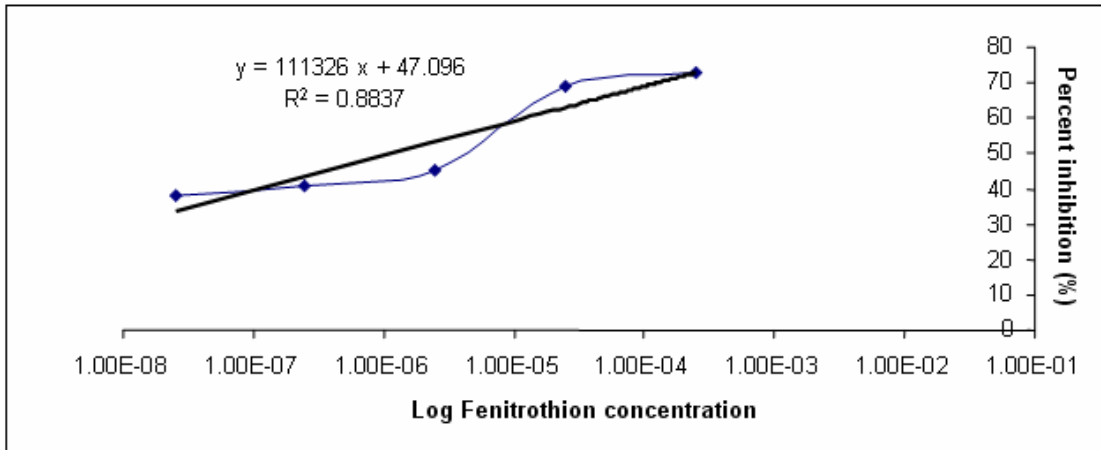
Appendix 4.36. The inhibitory effect of copper (Cu) on non-specific esterase of *Baetis* sp.



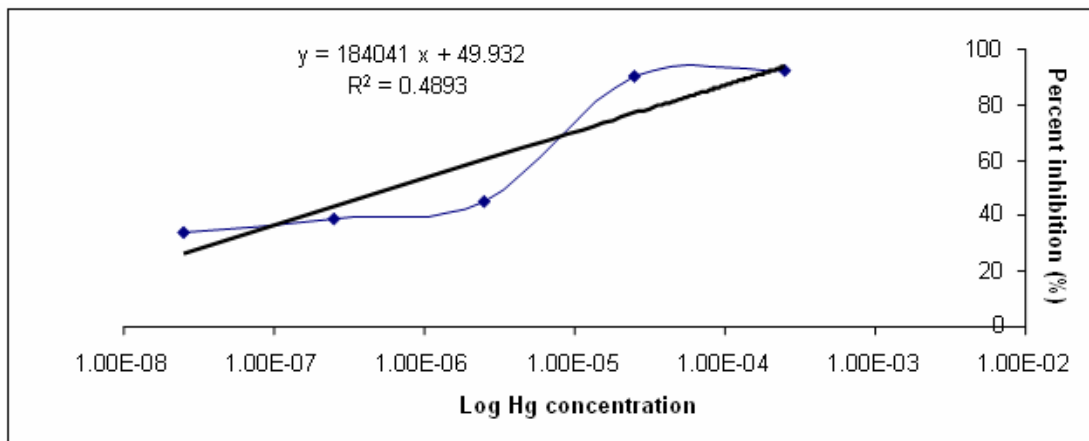
Appendix 4.37. The inhibitory effect of Dichlorvos (DDVP) on non-specific esterase of *Campsoneuria* sp.



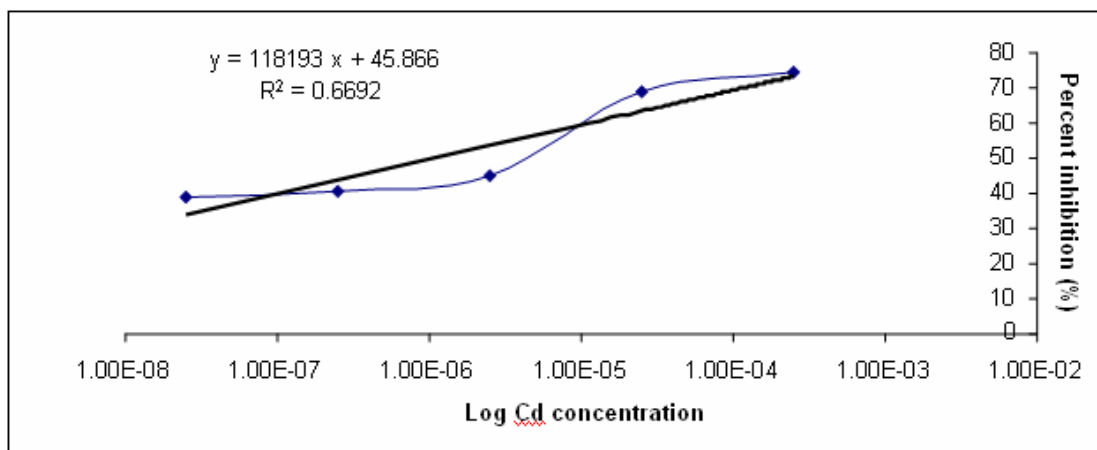
Appendix 4.38. The inhibitory effect of Malathion on non-specific esterase of *Campsoneuria* sp.



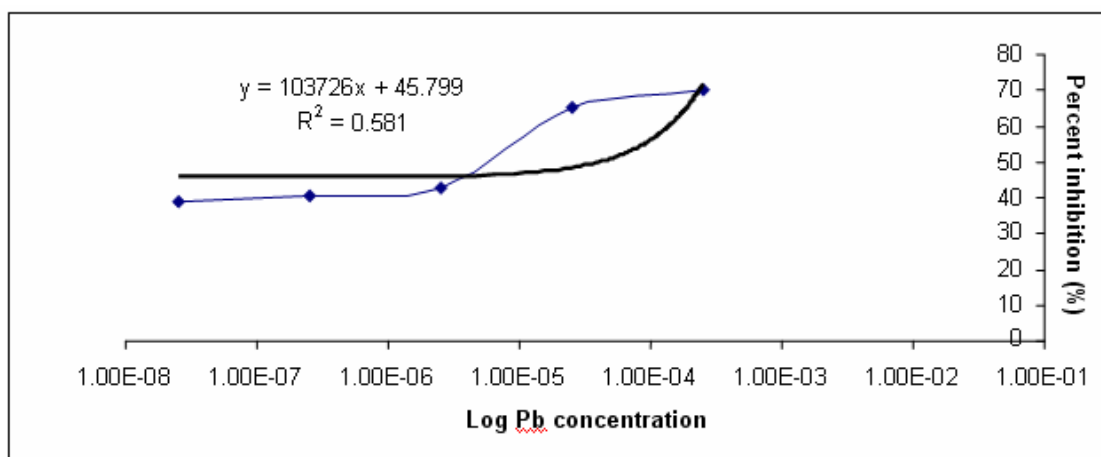
Appendix 4.39. The inhibitory effect of Fenitrothion on non-specific esterase of *Campsoneuria* sp.



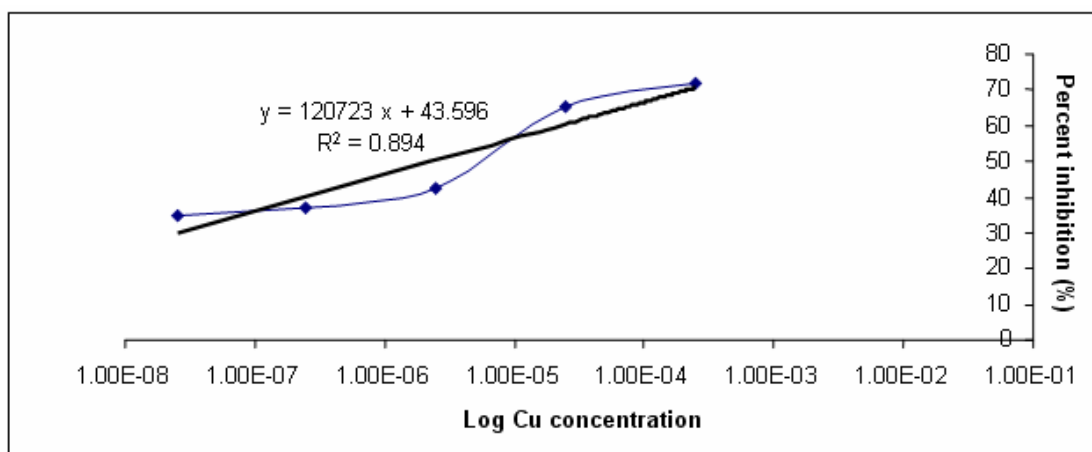
Appendix 4.40. The inhibitory effect of mercury (Hg) on non-specific esterase of *Campsoneuria* sp.



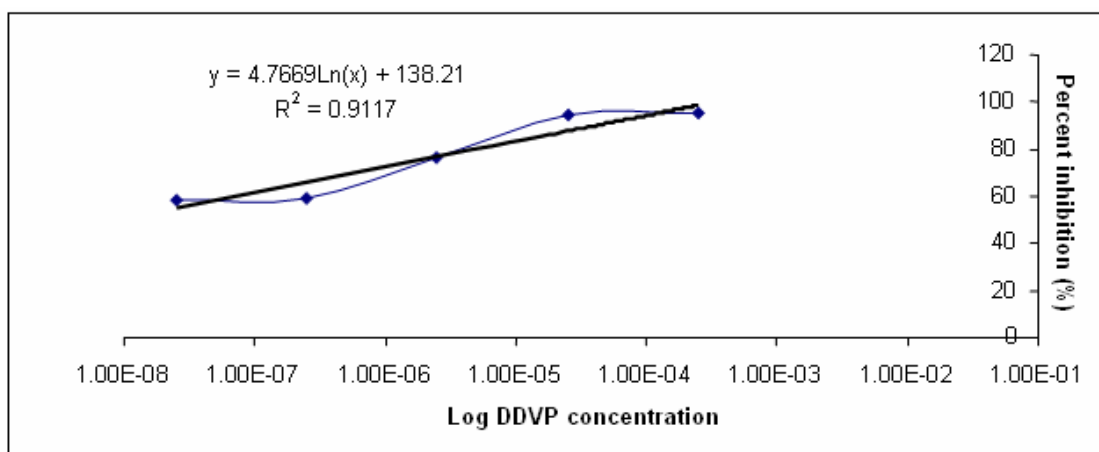
Appendix 4.41. The inhibitory effect of cadmium (Cd) on non-specific esterase of *Campsoneuria* sp.



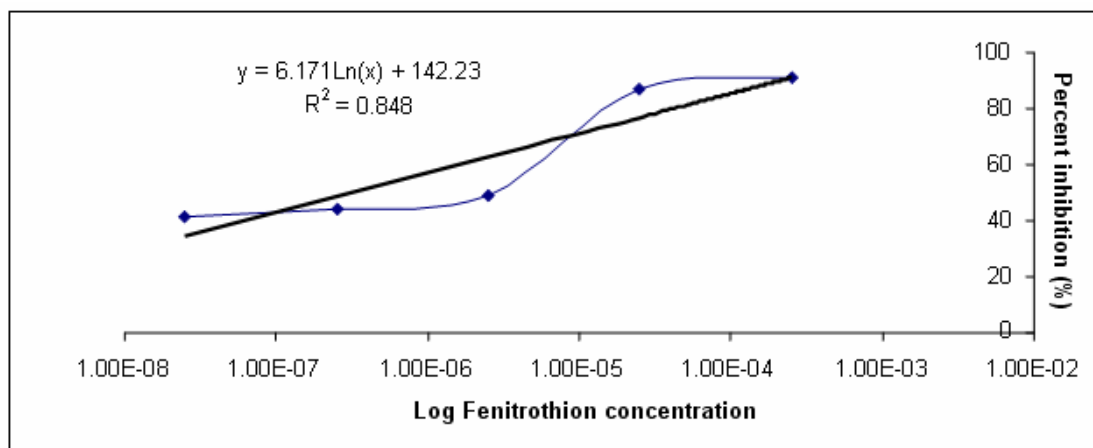
Appendix 4.42. The inhibitory effect of lead (Pb) on non-specific esterase of *Campsoneuria* sp.



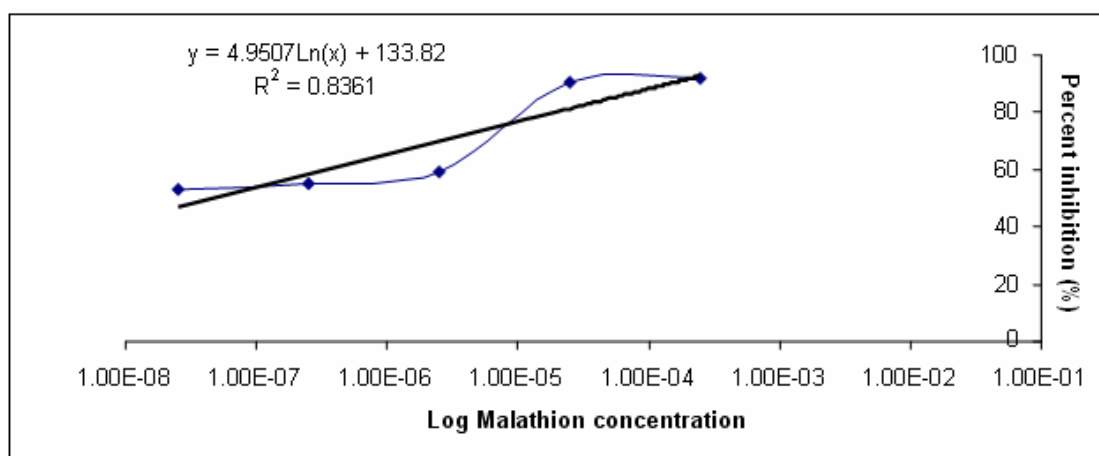
Appendix 4.43. The inhibitory effect of copper (Cu) on non-specific esterase of *Campsoneuria* sp.



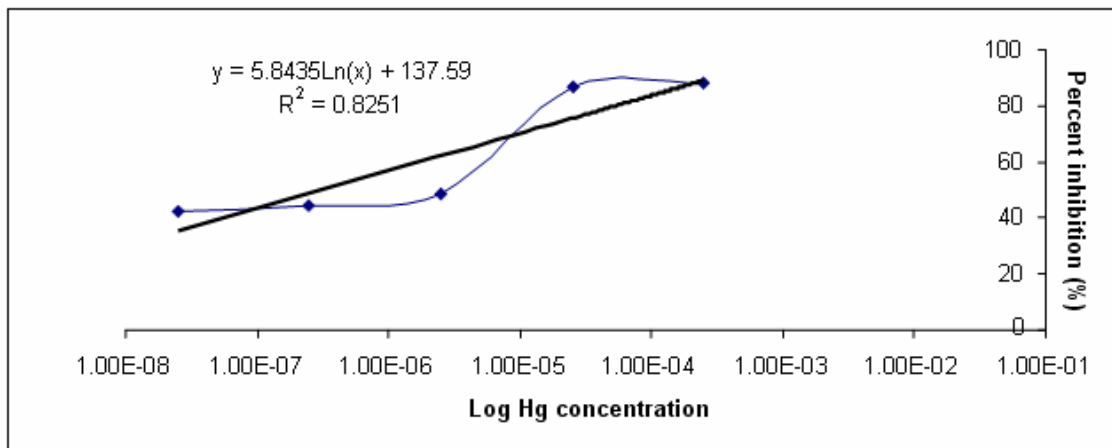
Appendix 4.44. The inhibitory effect of Dichlorvos (DDVP) on acetylcholinesterase of *Baetis* sp.



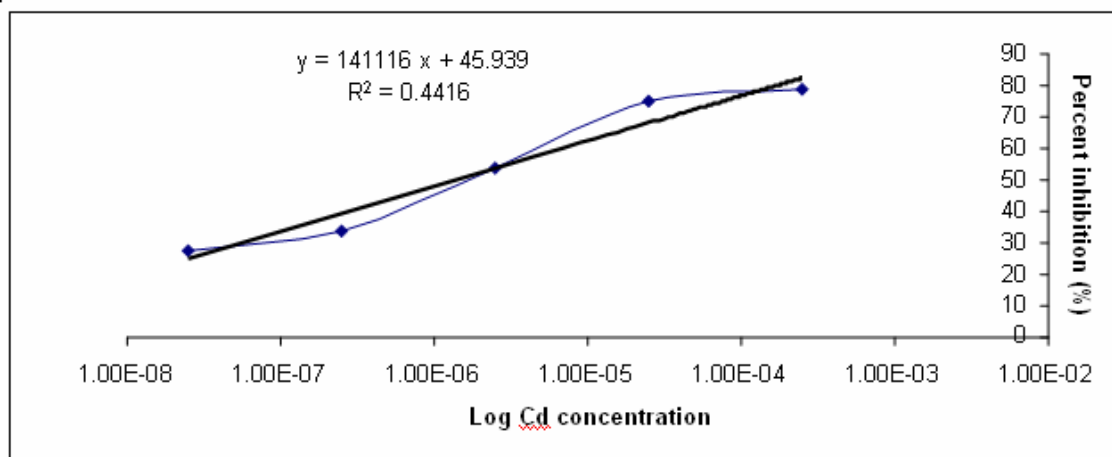
Appendix 4.45. The inhibitory effect of Fenitrothion on acetylcholinesterase of *Baetis* sp.



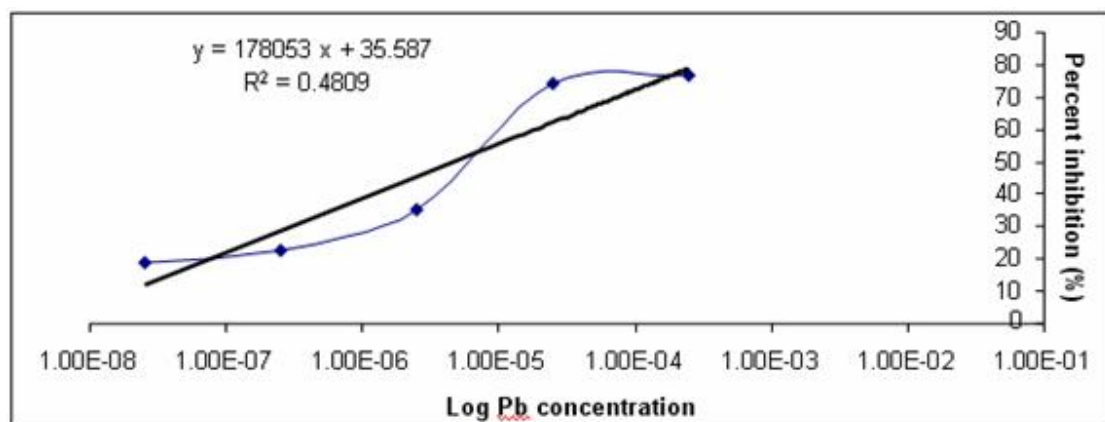
Appendix 4.46. The inhibitory effect of Malathion on acetylcholinesterase of *Baetis* sp.



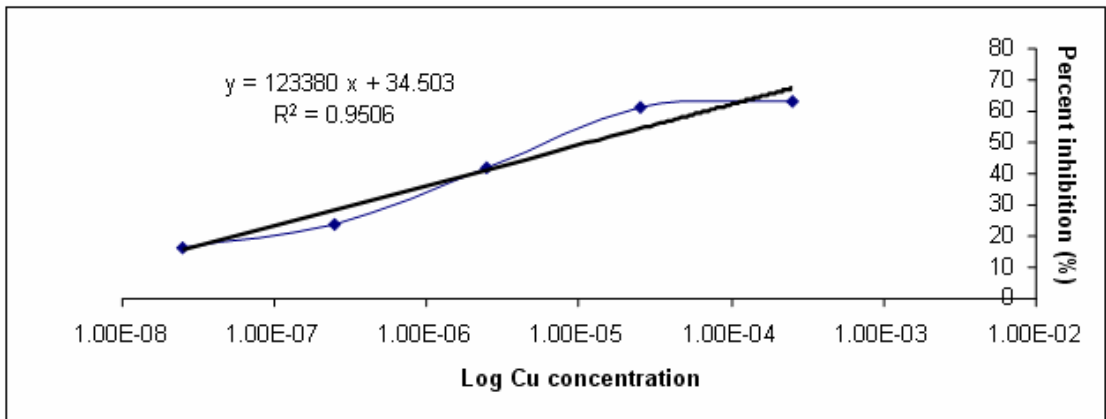
Appendix 4.47. The inhibitory effect of mercury (Hg) on acetylcholinesterase of *Baetis* sp.



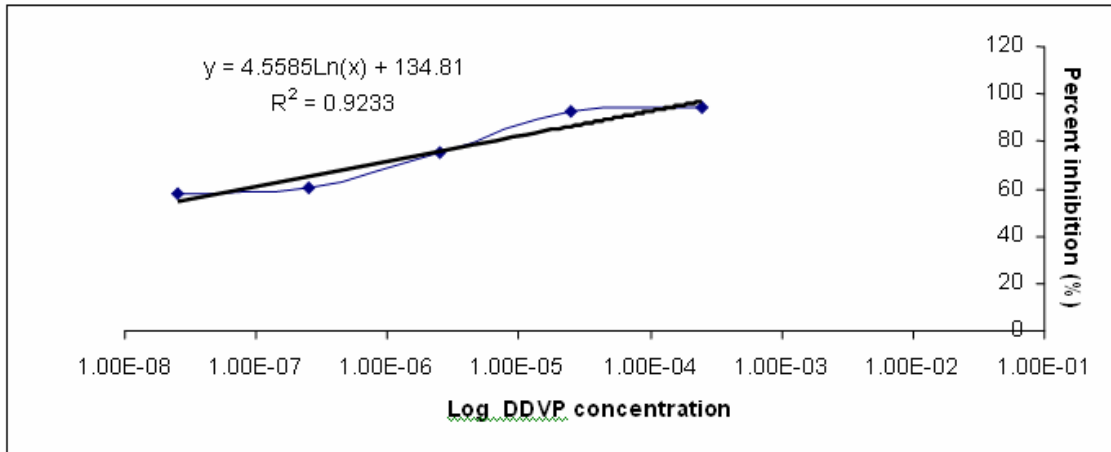
Appendix 4.48. The inhibitory effect of cadmium (Cd) on acetylcholinesterase of *Baetis* sp.



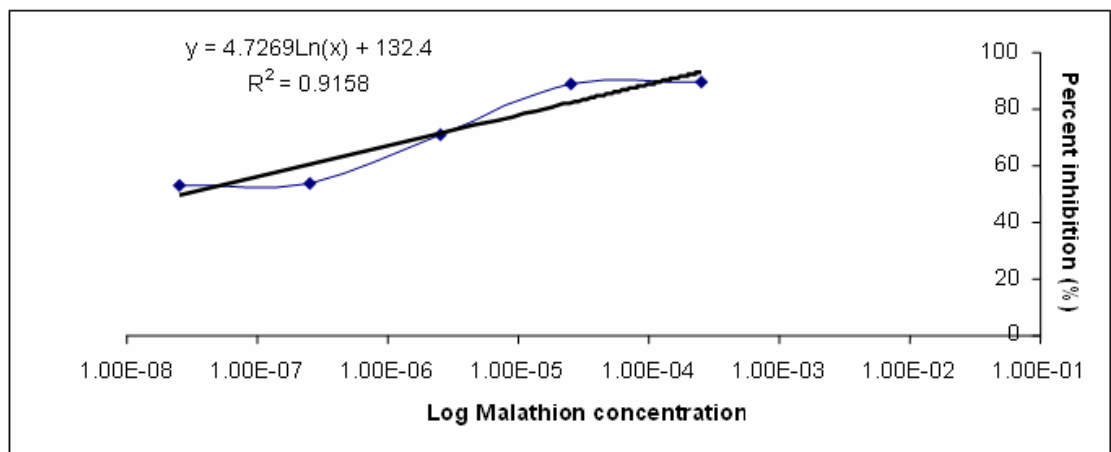
Appendix 4.49. The inhibitory effect of lead (Pb) on acetylcholinesterase of *Baetis* sp.



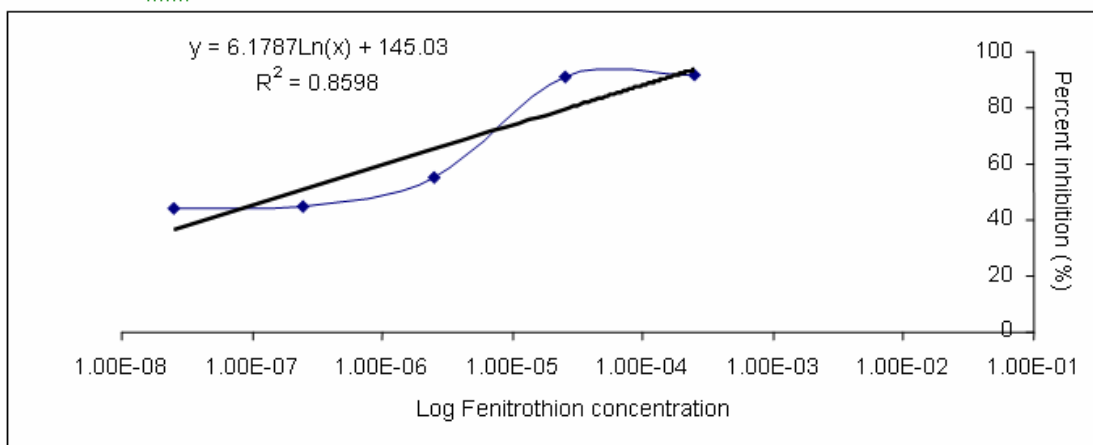
Appendix 4.50. The inhibitory effect of copper (Cu) on acetylcholinesterase of *Baetis* sp.



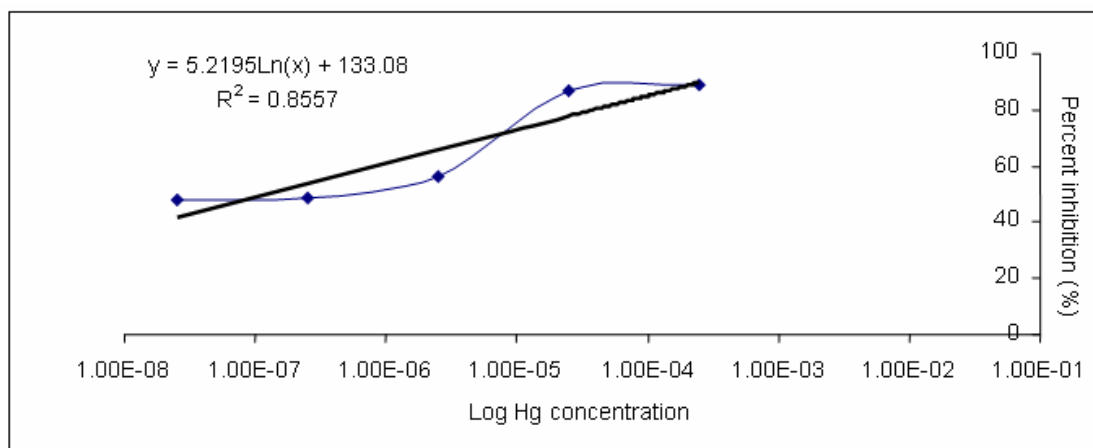
Appendix 4.51. The inhibitory effect of Dichlorvos (DDVP) on acetylcholinesterase of *Campsoneuria* sp.



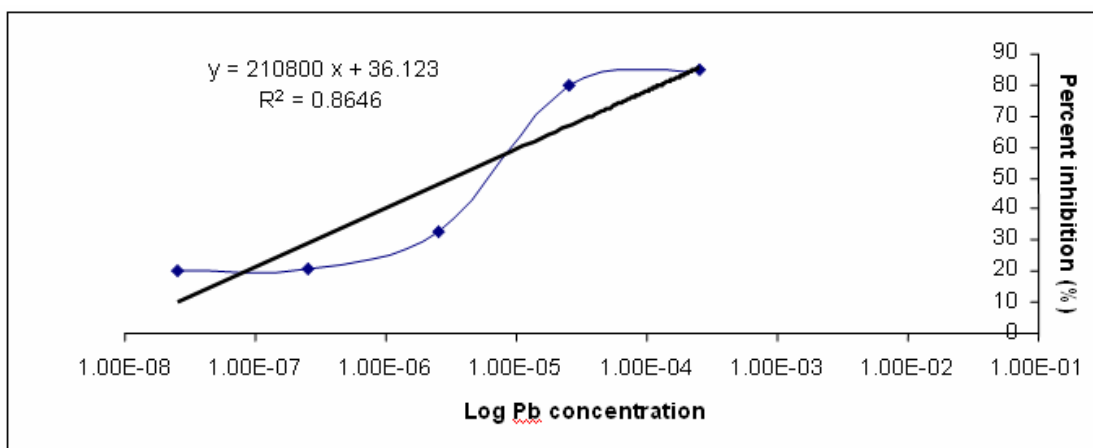
Appendix 4.52. The inhibitory effect of Malathion on acetylcholinesterase of *Campsoneuria* sp.



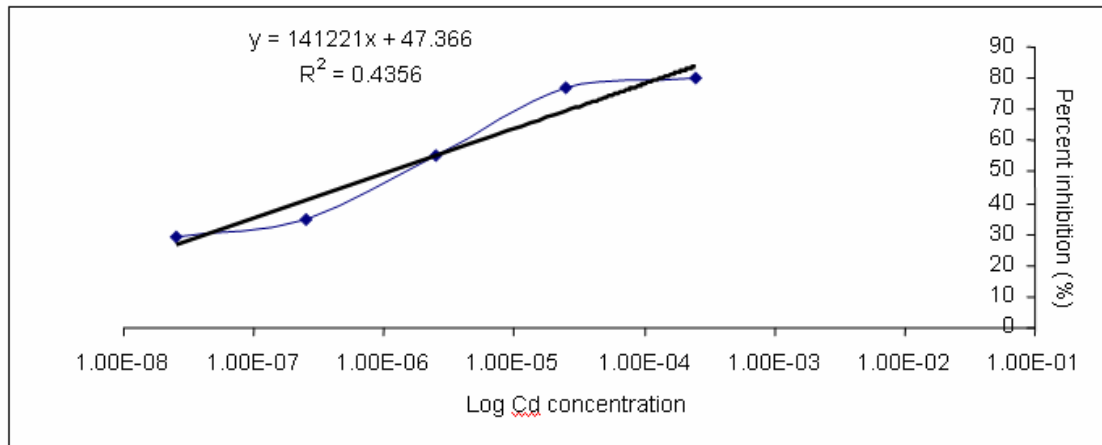
Appendix 4.53. The inhibitory effect of Fenitrothion on acetylcholinesterase of *Campsoneuria* sp.



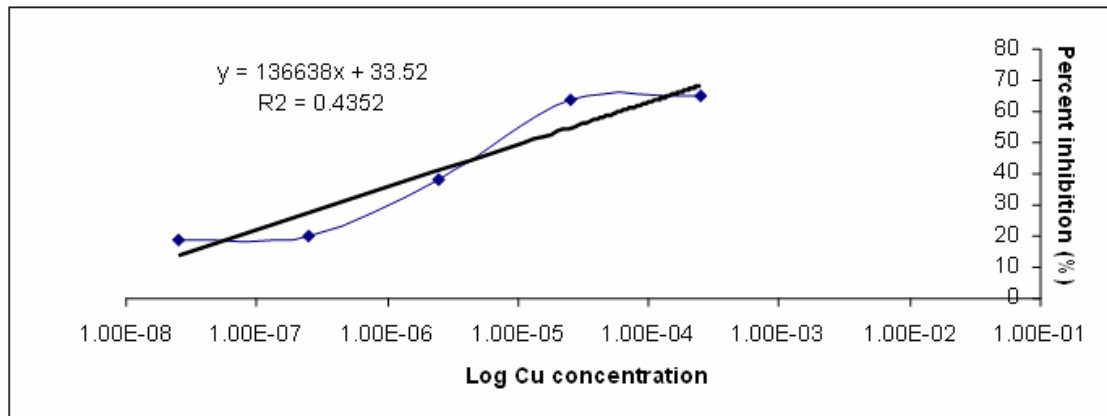
Appendix 4.54. The inhibitory effect of mercury (Hg) on acetylcholinesterase of *Campsoneuria* sp.



Appendix 4.55. The inhibitory effect of cadmium (Cd) on acetylcholinesterase of *Campsoneuria* sp.



Appendix 4.56. The inhibitory effect of lead (Pb) on acetylcholinesterase of *Campsoneuria* sp.



Appendix 4.57. The inhibitory effect of copper (Cu) on acetylcholinesterase of *Campsoneuria* sp.

Appendix 4.58. Data of inhibition percentage of selected inhibitors on non-specific esterase from strains of *Baetis* sp. and *Camponeuria* sp. by *in vitro* assays.

Inhibitor	Concentration (M)	Inhibition percentage (%I)	
		<i>Baetis</i> sp.	<i>Camponeuria</i> sp.
Dichlorvos (DDVP)	10 ⁻⁴	94.15	93.09
	10 ⁻⁵	86.61	93.43
	10 ⁻⁶	70.96	75.54
	10 ⁻⁷	68.00	70.63
	10 ⁻⁸	64.62	69.59
Malathion	10 ⁻⁴	87.30	90.47
	10 ⁻⁵	85.08	88.77
	10 ⁻⁶	65.57	77.89
	10 ⁻⁷	49.69	53.31
	10 ⁻⁸	46.92	48.66
Fenitrothion	10 ⁻⁴	82.37	73.04
	10 ⁻⁵	77.03	69.04
	10 ⁻⁶	40.21	45.21
	10 ⁻⁷	30.43	41.03
	10 ⁻⁸	26.15	38.09
Mercury	10 ⁻⁴	79.34	92.44
	10 ⁻⁵	77.47	90.21
	10 ⁻⁶	60.18	45.16
	10 ⁻⁷	36.99	38.82
	10 ⁻⁸	24.39	34.15
Cadmium	10 ⁻⁴	87.19	74.36
	10 ⁻⁵	83.08	68.87
	10 ⁻⁶	23.35	44.75
	10 ⁻⁷	19.10	40.61
	10 ⁻⁸	10.15	39.09

Lead	10^{-4}	77.17	70.08
	10^{-5}	76.35	65.25
	10^{-6}	32.61	42.81
	10^{-7}	15.71	40.57
	10^{-8}	10.84	39.09
Copper	10^{-4}	87.74	71.95
	10^{-5}	71.78	65.14
	10^{-6}	16.23	42.20
	10^{-7}	11.32	37.23
	10^{-8}	9.7	35.00

Appendix 4.59. Data of inhibition percentage of selected inhibitors on acetylcholinesterase from strains of *Baetis* sp. and *Campsoneuria* sp. by *in vitro* assays.

Inhibitor	Concentration (M)	Inhibition percentage (%I)	
		<i>Baetis</i> sp.	<i>Campsoneuria</i> sp.
Dichlorvos (DDVP)	10 ⁻⁴	95.04	94.00
	10 ⁻⁵	94.83	93.00
	10 ⁻⁶	76.51	75.01
	10 ⁻⁷	59.24	60.00
	10 ⁻⁸	58.00	58.02
Malathion	10 ⁻⁴	92.07	90.00
	10 ⁻⁵	90.76	89.00
	10 ⁻⁶	59.15	71.03
	10 ⁻⁷	54.78	54.06
	10 ⁻⁸	53.06	53.06
Fenitrothion	10 ⁻⁴	91.14	89.00
	10 ⁻⁵	87.17	87.00
	10 ⁻⁶	49.02	56.00
	10 ⁻⁷	44.32	49.00
	10 ⁻⁸	41.52	48.00
Mercury	10 ⁻⁴	88.51	92.00
	10 ⁻⁵	86.97	91.00
	10 ⁻⁶	48.71	55.00
	10 ⁻⁷	44.27	44.90
	10 ⁻⁸	42.59	43.90
Cadmium	10 ⁻⁴	78.60	80.00
	10 ⁻⁵	75.12	77.00
	10 ⁻⁶	53.71	55.01
	10 ⁻⁷	33.77	35.02
	10 ⁻⁸	27.71	29.02

Lead	10 ⁻⁴	76.71	85.01
	10 ⁻⁵	74.22	80.05
	10 ⁻⁶	35.07	33.03
	10 ⁻⁷	22.53	21.04
	10 ⁻⁸	18.87	20.04
Copper	10 ⁻⁴	62.92	65.00
	10 ⁻⁵	61.27	64.00
	10 ⁻⁶	42.00	38.00
	10 ⁻⁷	24.05	19.84
	10 ⁻⁸	16.54	18.90

Appendix 4.60. Significant comparison of I₅₀ of selected inhibitors on non-specific esterase from strains of *Baetis* sp. and *Camponeuria* sp. using student t-test at p≤0.05

Inhibitors	t value	df	p value
DDVP	1.28	1	0.42
Malathion	2.67	1	0.23
Hg	1.06	1	0.48
Fenitrothion	2.25	1	0.27
Cd	2.63	1	0.23
Pb	2.54	1	0.24
Cu	3.53	1	0.18

Appendix 4.61. Significant comparison of I₅₀ of selected inhibitors on acetylcholinesterase from strains of *Baetis* sp. and *Camponeuria* sp. using student t- test at p≤0.05

Inhibitors	t value	df	p value
DDVP	20.04	1	0.03
Malathion	4.08	1	0.15
Hg	2.30	1	0.26
Fenitrothion	4.67	1	0.13
Cd	4.71	1	0.13
Pb	9.69	1	0.07
Cu	3.82	1	0.16

Appendix 4.62. Percentage of residual activity of acetylcholinesterase for *Baetis* sp. used in determining the value of Ki

Time (min)	%							
	Control	DDVP	Malathion	Fenitrothion	Hg	Cd	Pb	Cu
0	100	100	100	100	100	100	100	100
1	100	82.4	85.6	89.9	88.4	91.5	93.4	95.4
2	100	79.6	84.5	88.8	87.2	90.8	92.5	94.8
3	100	75.5	81.9	86.4	85.3	89.2	91.6	92.9
4	100	74.2	78.6	84.1	82.8	86.5	90.2	90.4
5	100	70.9	77.1	83.1	80.6	84.3	89.4	88.5
6	100	68.6	74.1	78.2	76.5	80.9	87.5	86
7	100	64.2	72.3	76.5	75.2	78.4	85.8	86.8
8	100	61.4	68.4	74.8	72	77	83.5	84.9
9	100	57.9	64	70.9	67.9	71.5	82.4	81.5
10	100	54.8	61.8	67.2	65.9	70.4	81.5	83.4

Appendix 4.63. Percentage of residual activity of acetylcholinesterase for *Camponeuria* sp. used in determining the value of Ki

Time (min)	%							
	Control	DDVP	Malathion	Fenitrothion	Hg	Cd	Pb	Cu
0	100	100	100	100	100	100	100	100
1	100	80.2	85.9	87.4	82.5	90.8	93.5	96.4
2	100	78.4	83.4	85.1	80.2	88.5	90.3	92.8
3	100	77.9	80.5	82.9	74.3	85.4	89.2	90.7
4	100	76.3	75.8	79	72.8	83.9	88	87.3
5	100	75.9	70.5	75.8	70.9	82.1	87.4	85.2
6	100	70.6	68.2	73.9	67.8	81.8	86.5	82.7
7	100	63.2	65.2	68.4	64.5	75.5	84.1	81.6
8	100	55.4	62.9	63.4	60.2	73.9	78.3	80.1
9	100	54.2	59.6	62.1	57.9	70.4	75.8	79
10	100	52.3	58.4	60.5	55.6	68.4	70.4	78.4

Appendix 4.64. Significant comparison of AChE K_i of selected inhibitors on acetylcholinesterase from strains of *Baetis* sp. and *Camponeuria* sp. using student t-test at $p \leq 0.05$

Inhibitors	t value	df	p value
DDVP	21.44	1	0.03
Malathion	12.01	1	0.05
Hg	9.07	1	0.07
Fenitrothion	7.82	1	0.08
Cd	69.18	1	0.01
Pb	5.01	1	0.13
Cu	7.90	1	0.08