CHAPTER 4
RESULTS AND DISCUSSION

Mollusk diversity and ecology were described in this chapter. Firstly, the species of mollusks and physico-chemical properties of the water samples, the heavy metals concentrations in mollusks and sediments were presented. Secondly, the results of the impact of heavy metals to mollusk kidney used by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) technique were discussed. Finally, the results of experiment of heavy metals accumulation in selected mollusk were presented.

4.1 Ecology and Diversity of Mollusk

The results of this study revealed 8 species of mollusks from five collecting stations (Table 1). They were *Clea Helena* (Hoy Jedee), *Filopaludina martensi* (Hoy Khom), *Lymnaea (Radix) auricularia rubiginosa* (Hoy Kun), *Pila polita* (Hoy Pung), *Pomacea canaliculata* (Hoy Cherry), *Corbicula* sp. (Hoy Lebma), *Pilsbryoconcha exilis* (Hoy Kab) and *Scabies crispata* (Hoy Sobnok) (Figure 7). The mollusks species found in every season were *Filopaludina martensi* (Hoy Khom), *Pomacea canaliculata* (Hoy Cherry), *Lymnaea auricularia* (Hoy Kun) and *Scabies crispata* (Hoy Sobnok). The sequential highest number of mollusks species were *Filopaludina martensi* (Hoy Khom) and *Pomacea canaliculata* (Hoy Cherry), respectively, thus, the most dominant species. These species showed to possess high tolerance to waste water. The affected area was 300 rais and there were plenty of water hyacinth. Sediments collected from the affected area were dark brown, viscous with the smell of H_2S. Diversity Index and Evenness of ecology in Bueng Jode wetland are shown in Table 2, with the following values from Station 1 to Station 5 as 1.343, 0.930, 1.112, 1.045, 0.171 and 0.749, 0.671, 0.691, 0.754, 0.446 respectively. The values of Diversity Index are closely related in every Station under studied. Evenness of ecology signified distribution of mollusks at various collecting Station.

Table 1. Species of mollusks from samples collected in three seasons

(+ : found, - : not found)

Mollusk species (Scientific Name)	Sample Collecting Station				
	1	2	3	4	5
Rainy season					
Gastropod					
Clea (Anentome) helena (Philippi, 1847)	-	-	-	-	-
Filopaludina martensi (Frauenfeld, 1865)	+	-	+	+	+
Lymnaea(Radix) auricularia rubiginosa (Michelin, 1831)	-	+	-	-	-
Pila polita (Deshayes, 1830)	-	-	-	-	-
Pomacea canaliculata (Lamarck, 1819)	+	+	+	+	+
Bivalve					
Corbicula sp. (Mühlfell, 1811)	-	-	-	-	-
Pilsbryoconcha exilis (Lea, 1839)	-	-	-	+	-
Scabies crispata (Gould, 1848)	+	-	-	-	-
Cold dry season					
Gastropod					
Clea (Anentome) helena (Philippi, 1847)	-	-	-	-	+
Filopaludina martensi (Frauenfeld, 1865)	+	+	+	+	+
Lymnaea(Radix) auricularia rubiginosa (Michelin, 1831)	-	-	+	-	+
Pila polita (Deshayes, 1830)	-	+	+	-	+
Pomacea canaliculata (Lamarck, 1819)	+	+	+	+	+
Bivalve					
Corbicula sp. (Mühlfell, 1811)	+	-	-	-	-
Pilsbryoconcha exilis (Lea, 1839)	-	-	-	-	-
Scabies crispata (Gould, 1848)	+	-	-	-	-
Summer season					
Gastropod					
Clea helena (Philippi, 1847)	-	-	-	-	-
Filopaludina martensi (Frauenfeld, 1865)	+	+	+	+	+
Lymnaea (Radix) auricularia rubiginosa (Michelin, 1831)	-	+	+	+	-
Pila polita (Deshayes, 1830)	-	-	-	-	-
Pomacea canaliculata (Lamarck, 1819)	+	+	-	-	+
Bivalve					
Corbicula sp. (Mühlfell, 1811)	+	-	-	-	-
Pilsbryoconcha exilis (Lea, 1839)	+	-	-	-	-
Scabies crispata (Gould, 1848)	+	-	-	-	-

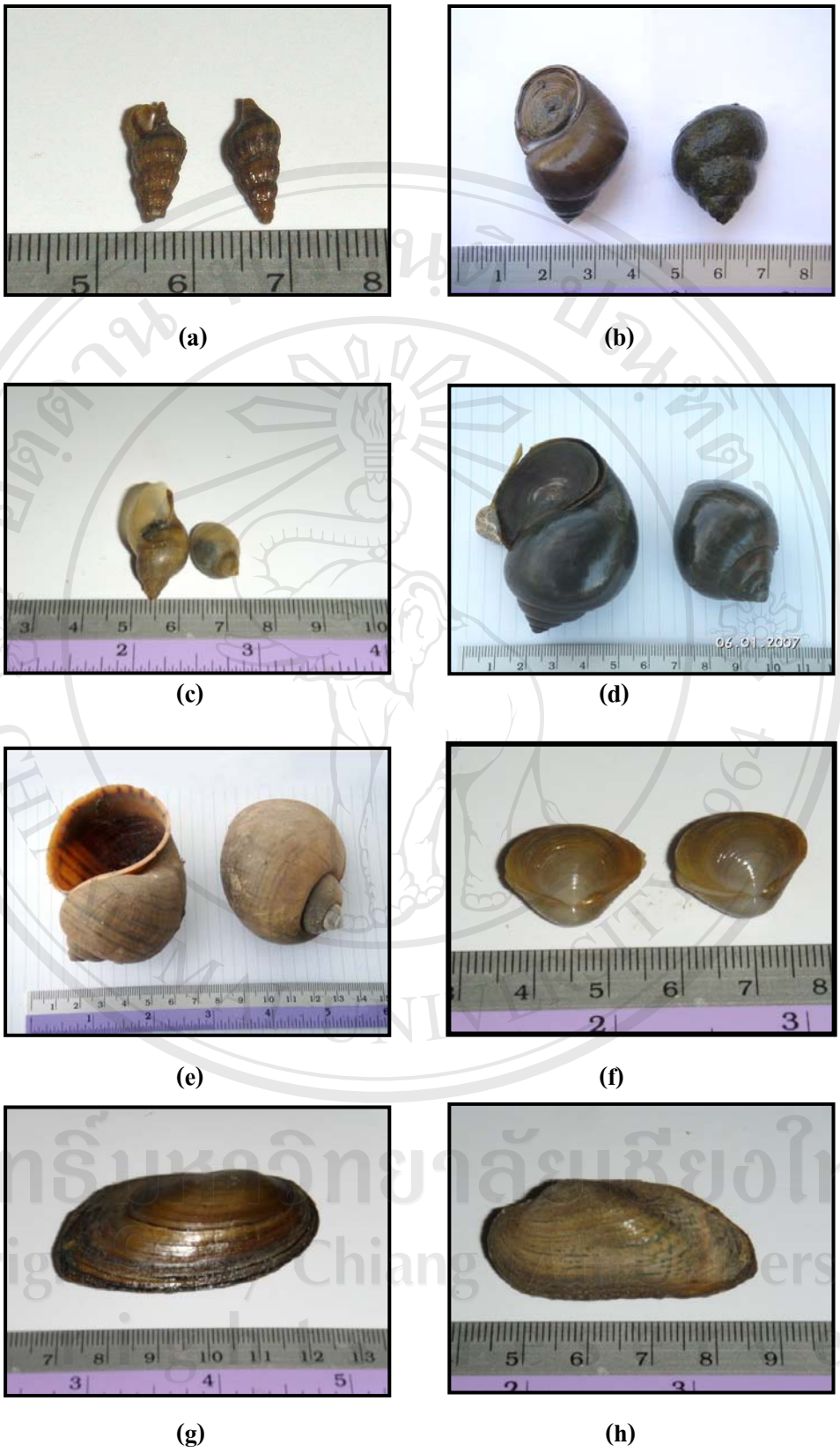


Figure 7. Species of mollusks in Bueng Jode wetland from five collecting stations: (a) *Clea helena*, (b) *Filopaludina martensi*, (c) *Lymnaea (Radix) auricularia rubiginosa*, (d) *Pila polita*, (e) *Pomacea canaliculata*, (f) *Corbicula* sp., (g) *Pilsbryoconcha exilis* and (h) *Scabies crispata*

Phylum Mollusca
Class Gastropoda
Subclass Prosobranchia
Order Neogastropoda
Superfamily Buccinacea
Family Buccinidae
Genus *Clea*
Species *helena*

Clea helena (Hoy Jedee)

Distribution: This species is widely distributed in Southeast Asia, Malaysia and Indonesia. In Thailand, the species is found in almost every province. This is the only Thai species which is not restricted to running water as it is also found in lakes and ponds (Brandt, 1974).

Phylum Mollusca
Class Gastropoda
Subclass Prosobranchia
Order Mesogastropoda
Superfamily Vivaparacea
Family Viviparidae
Genus *Fiopaludina*
Species *martensi*

Filopaludina martensi (Hoy Khom)

Distribution: This race of *Filopaludina martensi* is ubiquitous in Thailand. In Chiang Mai, it was replaced by *Filopaludina maekoki* and in the eastern and southeast provinces by other races. *Filopaludina martensi* are found in all freshwater habitats throughout the country (Brandt, 1974).

Phylum Mollusca
Class Gastropoda
Subclass Pulmonata
Order Basommatophora
Superfamily Lymnaeacea
Family Lymnaeidae
Genus *Lymnaea*

Lymnaea (Radix) auricularia rubiginosa (Hoy Kun) Species *rubiginosa*

Distribution: In Thailand, this species can be found everywhere except for the northernmost provinces. This species is found in all kinds of bodies of water, lakes, ponds, brooks, canals, ditches, rivers and even mountain creeks, but it seems to prefer still water (Brandt, 1974).

Phylum Mollusca
Class Gastropoda
Subclass Prosobranchia
Order Mesograstropoda
Superfamily Cyclophoracea
Family Cyclophoridae
Genus *Pila*

Pila polita (Hoy Pung) Species *polita*

Distribution: Common in the central, northeastern and northern provinces. Specimens have not been found in Prachuap-Khiri-khan or in the south. The central provinces from which *Pila polita* has been most collected are Bangkok, Prachin Buri, Chon Buri, Rayong, Phetcha Buri, Sing Buri, Lop Buri and Nakhon-sawan. The northeastern provinces are Loei, Udonthani, Sakonnakhon, Kkon Kaen, Kalasin, Chaiyaphum, Buri-ram, Surin, Si-sa-ket, Nakhon Ratchasima and Ubon Ratchathani. The northern provinces are Chiang-rai, Phayao, Lampang and Tak. *Pila polita* prefers standing water bodies to streams or irrigation canals. Its habitats are pools, ponds,

ditches and water reservoirs which are partly covered by aquatic plants (Keawjam, 1986).

Phylum Mollusca
Class Gastropoda
Subclass Prosobranchia
Order Mesograstropoda
Superfamily Cyclophoracea
Family Cyclophoridae
Genus *Pomacea*
Species *canaliculata*

Pomacea canaliculata (Hoy Cherry)

Distribution: In Thailand, *Pomacea canaliculata* is widely distributed everywhere throughout the country (Keawjam, 1986).

Phylum Mollusca
Class Bivavia
Subclass Heterodonta
Order Verneroida
Superfamily Corbiculoidea
Family Corbiculidae
Genus Corbicula

Corbicula sp. (Hoy Lebma)

Distribution: Species of *Corbicula* are a common inhabitat of the streams, rivers and lakes. The species has become an important component of the benthic communities in freshwater both lentic and lotic environments. In some environments its success has been extraordinary and attention has been paid to its detrimental impact on native freshwater bivalve species (Britton and Morton, 1982).

Pilsbryoconcha exilis (Hoy Kab)

Phylum Mollusca
Class Bivavia
Subclass Palaeoheterodonta
Order Unionoida
Superfamily Unionacea
Family Amblemidea
Subfamily Pseudontinae
Genus *Pilsbryoconcha*
Species *exilis*

Distribution: *Pilsbryoconcha exilis* has been found throughout South East Asean countries such as Thailand, Laos, Cambodia, South Vietnam, Malaysia, Sumatra, Java, Borneo. In Thailand, this species has been found in the plains of almost all provinces (Brandt, 1974).

Scabies crispata (Hoy Sobnok)

Phylum Mollusca
Class Bivavia
Subclass Palaeoheterodonta
Order Unionoida
Superfamily Unionacea
Family Amblemidea
Subfamily Parreysiinae
Genus *Scabies*
Species *crispata*

Distribution: *Scabies crispata* survives well in the drainage systems of all Thai rivers southwards to Pattalung River (Klong San). They are also found in the Mekong and its tributaries in Thailand, Burma, Laos and Cambodia, also in Vietnam and probably also in China (Brandt, 1974).

Table 2. Diversity Index and Evenness of ecology in Bueng Jode wetland

Sample	Shannon Index	Evenness	Number of species
Site 1	1.343	0.749	6
Site 2	0.930	0.671	4
Site 3	1.112	0.691	5
Site 4	1.045	0.754	4
Site 5	0.717	0.446	5

Remark: Calculation using MVSP program (Kovach, 1999)

4.2 Physicochemical characteristics of the water samples

Physical and chemical characteristics of the water samples were presented in Table 3. Temperatures of water were higher as compared to the surrounding air during rainy and cold dry season. In contrast, the temperatures in the air were higher than water during summer season (Figures 8 - 9). As can be seen that the water temperatures for all three seasons did not over-shoot the standard values of 40 ^{O}C from the Industrial Factories and Industrial Estate (Appendix C).

Table 3. Physical and chemical parameters of water from samples collected in three seasons

	Air t. (^{0}C)	Water t. (^{0}C)	pH	Conductivity (µS/cm)	DO (mg/L)	BOD (mg/L)	NH_3 (mg/L)	NO^-_3-N (mg/L)	PO^{3-}_4-P (mg/L)
Rainy season									
Station 1	29	30	7.56	356.00	5.85	4.60	ND	3.10	0.89
Station 2	29	30	7.48	550.00	4.60	3.45	ND	2.70	0.30
Station 3	31	30	7.48	432.00	5.35	4.25	ND	1.90	0.68
Station 3A	31	30	7.26	348.00	4.80	3.05	ND	2.30	0.62
Station 4	30	28	7.57	235.00	5.15	4.15	ND	1.70	0.20
Station 5	28	31	7.61	136.00	4.75	3.85	ND	1.60	0.05
Average	**30**	**30**	**7.49**	**342.83**	**5.08**	**3.89**	**ND**	**2.22**	**0.46**
Cold dry season									
Station 1	22	24	7.53	365.00	1.90	1.40	ND	0.40	0.15
Station 2	21	23	7.38	256.00	1.65	1.05	ND	0.40	0.01
Station 3	23	24	7.36	348.00	1.60	0.55	ND	0.20	0.10
Station 3A	23	24	7.95	350.00	2.00	0.65	ND	0.03	0.03
Station 4	25	24	8.08	324.00	2.10	0.55	ND	0.20	0.01
Station 5	24	26	7.97	238.00	2.05	0.45	ND	0.10	0.04
Average	**23**	**24**	**7.71**	**313.50**	**1.88**	**0.78**	**ND**	**0.22**	**0.06**
Summer season									
Station 1	33	29.00	7.43	167.00	6.10	5.55	ND	0.40	0.39
Station 2	31	30	7.88	174.00	6.70	5.75	ND	0.30	0.01
Station 3	31	31	7.95	805.00	6.60	5.15	ND	0.30	0.10
Station 3A	31	31	7.66	174.00	6.75	4.95	ND	0.30	0.14
Station 4	32	29	7.65	160.00	6.50	4.70	ND	0.20	0.13
Station 5	32	29	8.13	669.00	5.50	3.95	ND	0	0.04
Average	**32**	**30**	**7.78**	**358.17**	**6.36**	**5.01**	**ND**	**0.25**	**0.14**

Remark: *At Station 3A water level depth at 20 meters, t: temperature*

ND: Not detected; as measurement are below Limit of Detection (LOD) 0.02 mg/L

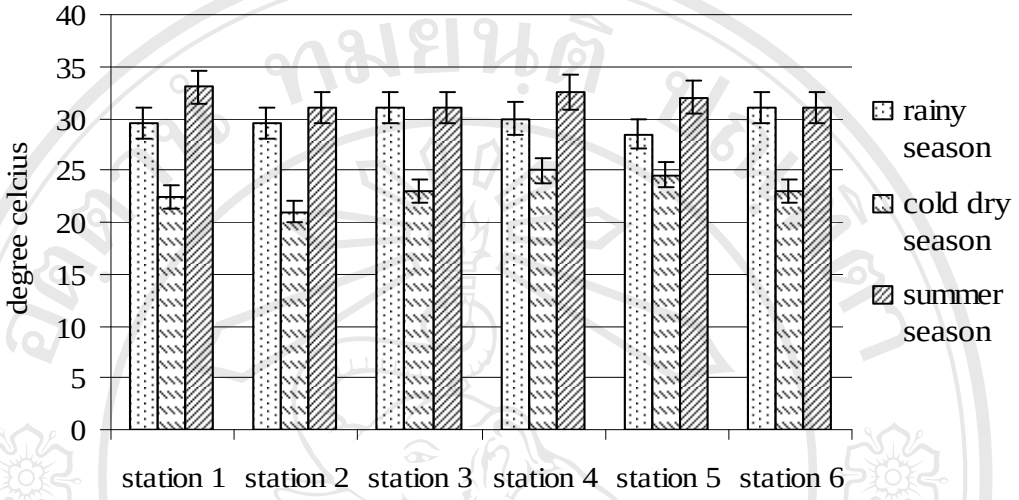


Figure 8. Air temperature of all collecting sites in three seasons

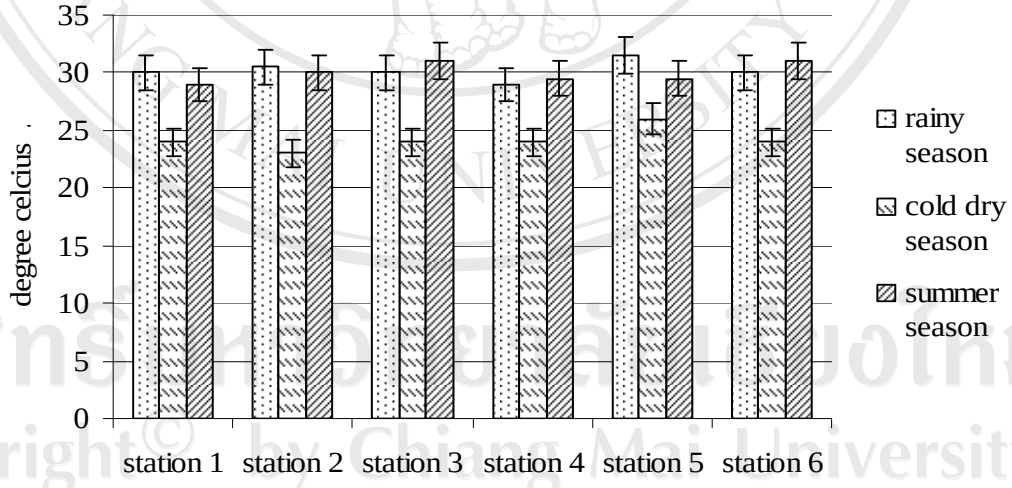


Figure 9. Water temperature of all collecting sites in three seasons

The pH of the water was neutral and was not greater than the standard with average values between 5.5 - 9 (Appendix C and Figure 10).

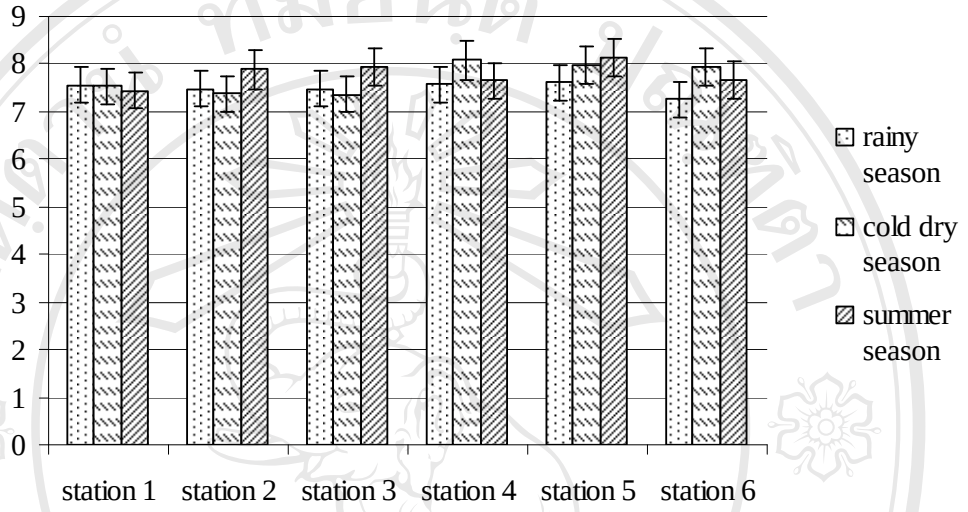


Figure 10. pH of all collecting sites in three seasons

Water conductivities showed possible salt contaminated in this water source, however the values were lower than that reported in earlier studies of water quality at Bueng-Jode water-log area by the group of Study for Integrated Management of the Pong River, Faculty of Engineering, Khon Kaen University, showing high water conductivities of 2,046µS/cm (Faculty of Engineering, 2003). This study showed that the water conductivity values during rainy season were 342.83 µS/cm, 313.50µS/cm in cold dry season and 358.17µS/cm in summer season (Figure 11). Mean dissolved oxygen was low concentration in cold dry season (1.88 mg/L) but showed normal levels in the rainy and summer season (5.08 mg/L and 6.36 mg/L, respectively) (Figure 12). The reasons for having low DO values during cold dry season could be due to infestation of water hyacinth on the water surface preventing oxygen to penetrate into the water body.

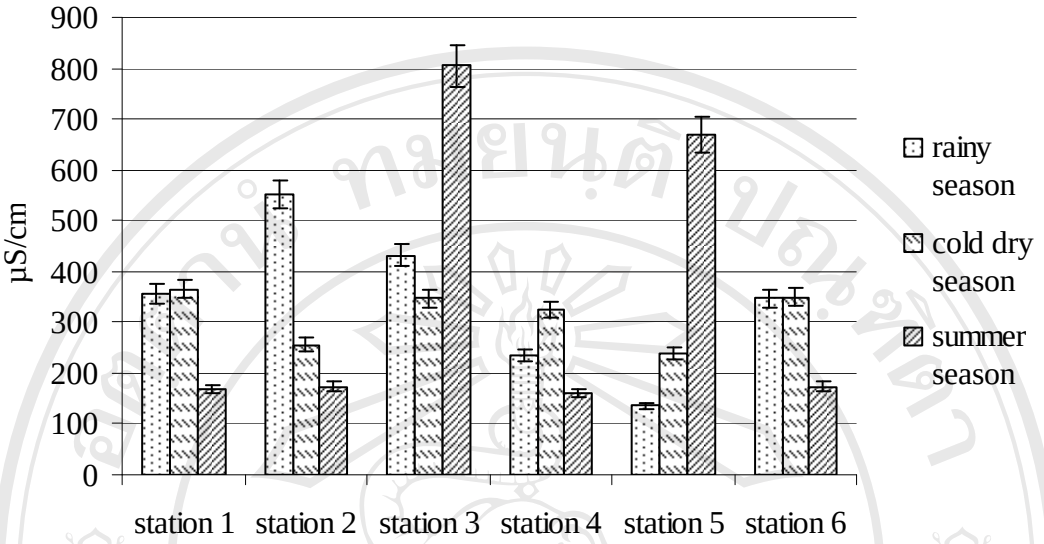


Figure 11. Conductivity of all collecting sites in three seasons

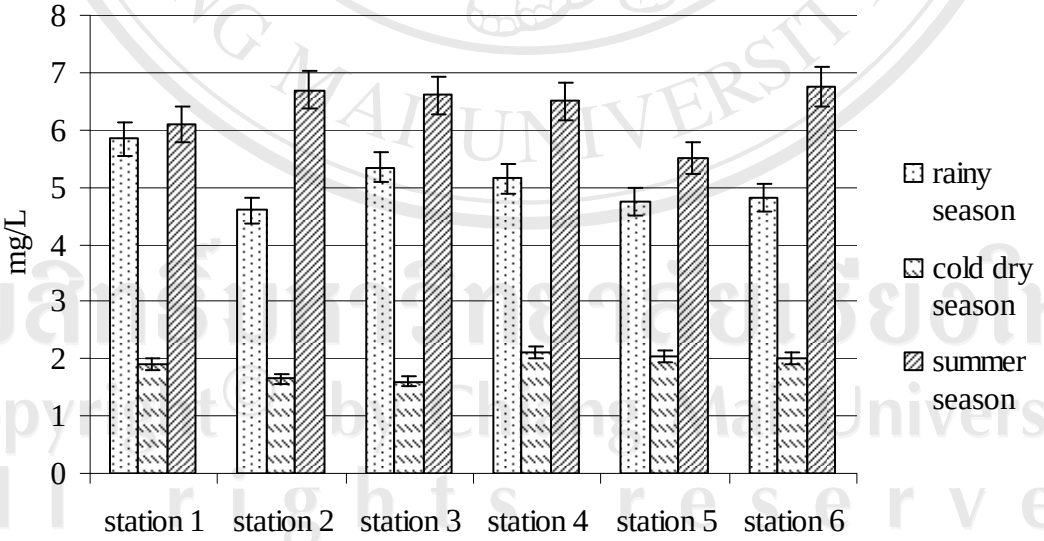


Figure 12. DO of all collecting sites in three seasons

It can be seen that dissolved oxygen value was low concentration during cold season, this must be due to lower dissolution of oxygen in cold water and also for utilization of oxygen in digesting organic matters in the form of BOD. The obtained biochemical oxygen demand (BOD) value was lower in cold dry season (0.78 mg/L) while in rainy and summer season were 3.89 and 5.01 mg/L, respectively (Figure 13). This study also showed mean BOD values of less than 20 mg/L for all three seasons which was below the stardardized values of wastewater stated by Pollution Control Department for Industrial Factories and Industrial Estate (Appendix C).

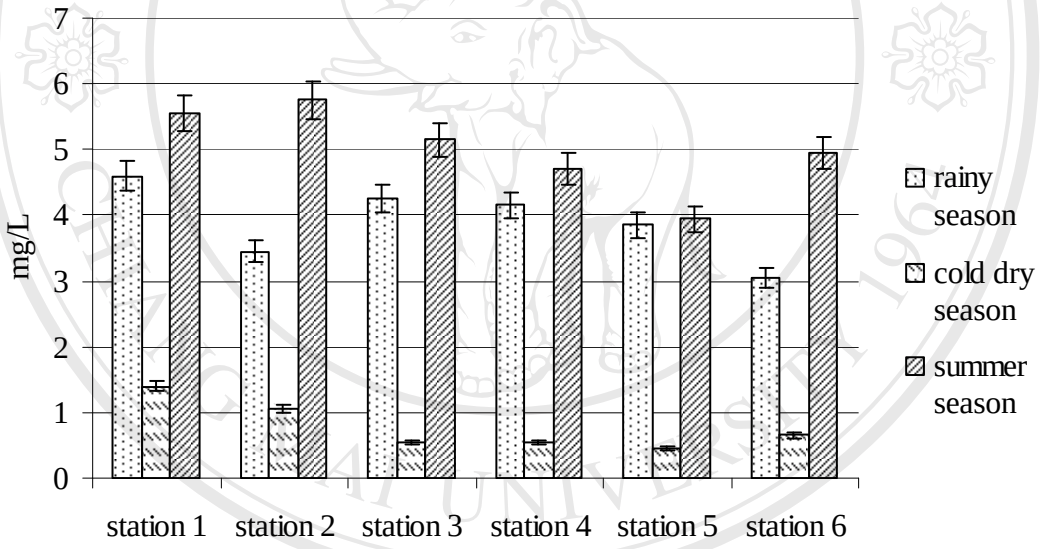


Figure 13. BOD of all collecting sites in three seasons

The average nitrate nitrogen was 2.22 mg/L (rainy season), 0.22 mg/L (cold dry season) and 0.25 mg/L (summer season) (Figure 14). The reason for not able to detect ammonia nitrogen might be due to the conversion of ammonia nitrogen into nitrate nitrogen, giving evidence of contamination of organic nitrogen in water source. This study also showed that there was high contamination of organic nitrogen during summer, as the water temperature was raised to about 30 ^{O}C facilitating ammonia nitrogen conversations to nitrate nitrogen. High values of nitrate nitrogen were probably due to waste discharge from local community.

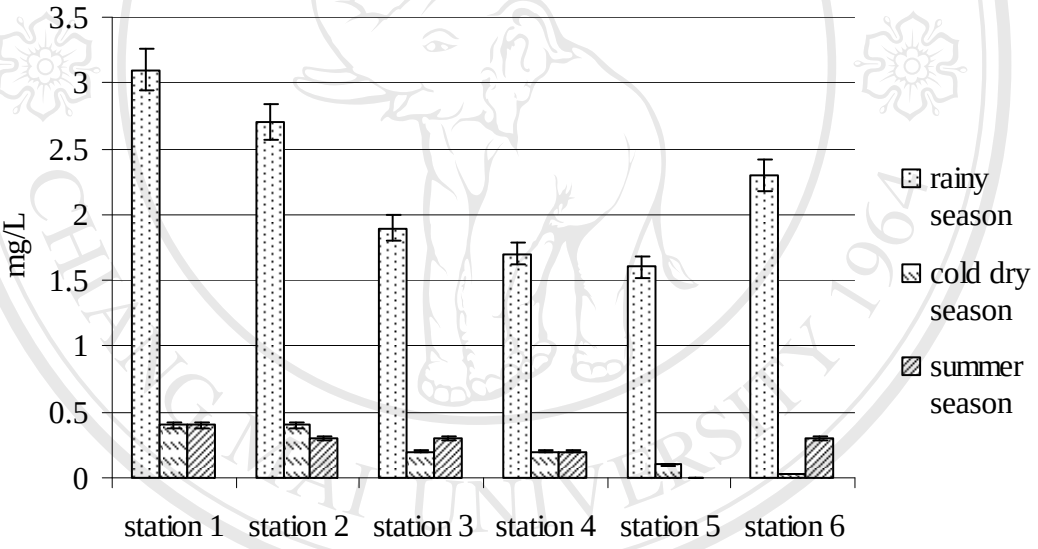


Figure 14. Nitrate nitrogen of all collecting sites in three seasons

Orthophosphates concentration was low in cold dry season (0.06 mg/L) while values were 0.46 and 0.14 mg/L in rainy and summer season, respectively (Figure 15). Finding of contaminated phosphates showed that the contamination of water-way was due to community activities surrounding Bueng Jode wetland. Phosphates contamination was higher value during rainy season due to washing of phosphates by rain water down into Bueng Jode resulting in higher value as stated previously. High values of phosphates were probably due to waste discharge from local community.

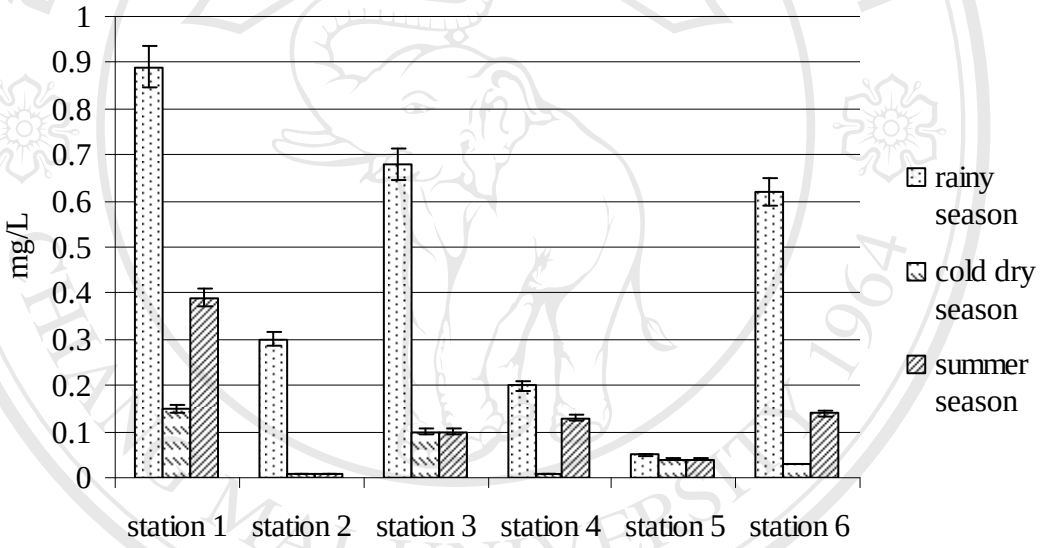


Figure 15. Orthophosphates concentration of all collecting sites in three seasons

4.3 Water Quality Assessment

Assessment of water quality was evaluated using the physico-chemical properties such as DO, BOD, conductivity, amonia nitrogen, nitrate nitrogen, orthophosphates as shown in Table 3, using AARL-PC Score (Peerapornpisal, 2006). The evaluation scores are shown in Table 4. Water quality in Bueng Jode wetland during the rainy season possesses moderate. In according to AARL-PC Score, water quality at Station 3 was poor. On the contrary, water quality at Station 1, 2, 3A and 4

possess moderate levels. As for the collecting Station 5, the water quality was with fairly clean. Water quality during the cold dry season showed fairly clean at every collecting Station, except at Station 1 was of moderate level. Water quality during the summer season showed fairly clean at every collecting Station. The data of this study illustrated unusually high values of waste water. This must be due to the over effluent discharge from industry, local community and agricultural land.

Table 4. Water quality assessment in Bueng Jode wetland

Study Site	Standard Score	Water Quality
Rainy season		
Station 1	3.5	moderate
Station 2	3.3	moderate
Station 3	5.4	poor
Station 3A	3.5	moderate
Station 4	3.6	moderate
Station 5	2.8	fairly clean
Average	**3.7**	**moderate**
Cold dry season		
Station 1	3.1	moderate
Station 2	2.8	fairly clean
Station 3	2.9	fairly clean
Station 3A	2.4	fairly clean
Station 4	2.6	fairly clean
Station 5	2.4	fairly clean
Average	**2.7**	**fairly clean**
Summer season		
Station 1	2.9	fairly clean
Station 2	2.4	fairly clean
Station 3	2.9	fairly clean
Station 3A	2.5	fairly clean
Station 4	2.4	fairly clean
Station 5	2.4	fairly clean
Average	**2.6**	**fairly clean**

Remark: *2.1-2.9 fairly clean, 3.0-3.8 moderate, 4.8-5.6 poor*

4.4 Concentrations of heavy metals in mollusks

Two mollusk species were collected from five collecting stations at Bueng Jode wetland. The two species were *Filopaludina martensi* (Hoy Khom) and *Pomacea canaliculata* (Hoy Cherry). Results from the analyses of metals in these species are given in Table 5.

Table 5. Concentrations of heavy metals in mollusks in three seasons
(% by dry weight)

Parameter	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Zn (mg/kg)
Rainy season					
Station1 (Hoy Cherry)	0.24	22.04	1.04	Not detected	125.85
Station 2 (Hoy Khom)	0.14	87.37	2.81	0.31	650.46
Station 3 (Hoy Cherry)	0.20	13.00	0.78	0.37	167.42
Station 4 (Hoy Cherry)	0.24	18.31	0.48	Not detected	157.69
Station 5 (Hoy Khom)	0.22	40.75	2.88	0.29	633.67
Cold dry season					
Station 1 (Hoy Cherry)	Not detected	2.18	0.20	Not detected	16.97
Station 2 (Hoy Khom)	Not detected	17.38	0.85	Not detected	108.29
Station3 (Hoy Cherry)	Not detected	1.46	0.22	Not detected	15.41
Station 4 (Hoy Cherry)	Not detected	0.46	0.30	Not detected	14.02
Station 5	-	-	-	-	-
Summer season					
Station 1 (Hoy Khom)	Not detected	12.81	0.26	Not detected	98.10
Station 2	-	-	-	-	-
Station 3	-	-	-	-	-
Station 4 (Hoy Cherry)	Not detected	3.24	0.40	Not detected	12.46
Station 5 (Hoy Khom)	Not detected	6.88	0.65	Not detected	110.11

Remark: *- : no mollusks were found in these stations.*

Not detected; as measurement are below Limit of Detection (LOD) 0.075 mg/kg for cadmium and 0.175 mg/kg for mercury

Concentrations of zinc in mollusks were high in all seasons. In addition, the concentration of zinc in mollusks in rainy season was much higher than that found in summer and cold dry seasons. Concentrations of zinc found in mollusks in rainy season at Station 1 to Station 5 were 125.85, 650.46, 167.42, 157.69 and 633.67

mg/kg, respectively. Concentrations of zinc in mollusks in cold dry season at Station 1 to Station 4 were 16.97, 108.29, 15.41 and 14.02 mg/kg, respectively. As for Station 5 no value of zinc was reported as there was no mollusk to be found. Concentrations of zinc in mollusks in summer season at Station 1, 4 and 5 were 98.10, 12.46 and 110.11 mg/kg, respectively. As for Station 2 and 3 no value of zinc was reported as there were no mollusks to be found. Concentrations of mercury and cadmium were similar values in rainy season while in cold dry and summer seasons could not be detected. It was found that concentration of zinc was higher than copper and lead in mollusks in three seasons respectively (Figure 16). For comparisons of data between two species of mollusks, the result found that concentrations of zinc in *Filopaludina martensi* (Hoy Khom) were higher than that of *Pomacea canaliculata* (Hoy Cherry) (Figure 17). This is due to the fact that *Filopaludina martensi* live in water longer than *Pomacea canaliculata*. These data showed that mollusk species of *Filopaludina martensi* could accumulate heavy metals in higher concentrations as compare to other species. Additionally, no differences were found on other heavy metals in these two species. Standard Values of cadmium and zinc in mollusks are 0.2-1.4 and 1,430 mg/kg, respectively (Merian *et al*, 2004). This study showed that the values of heavy metals from mollusks did not exceed standard values.

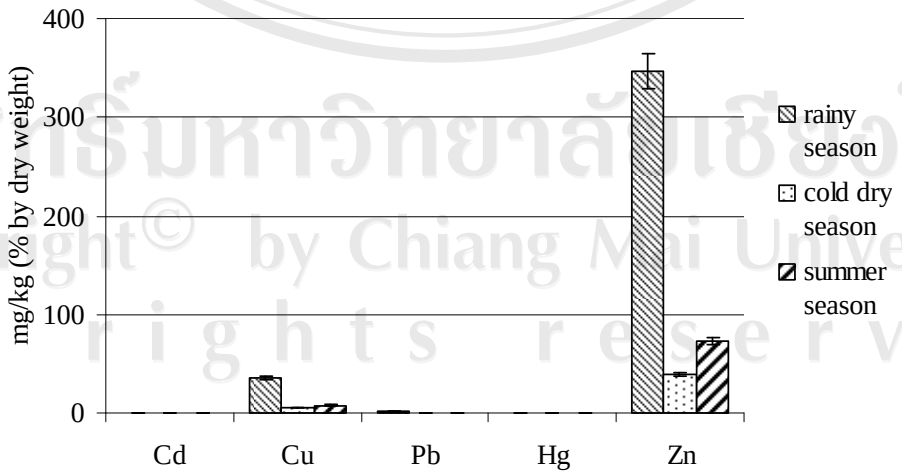


Figure 16. Concentrations of heavy metals in mollusks in three seasons

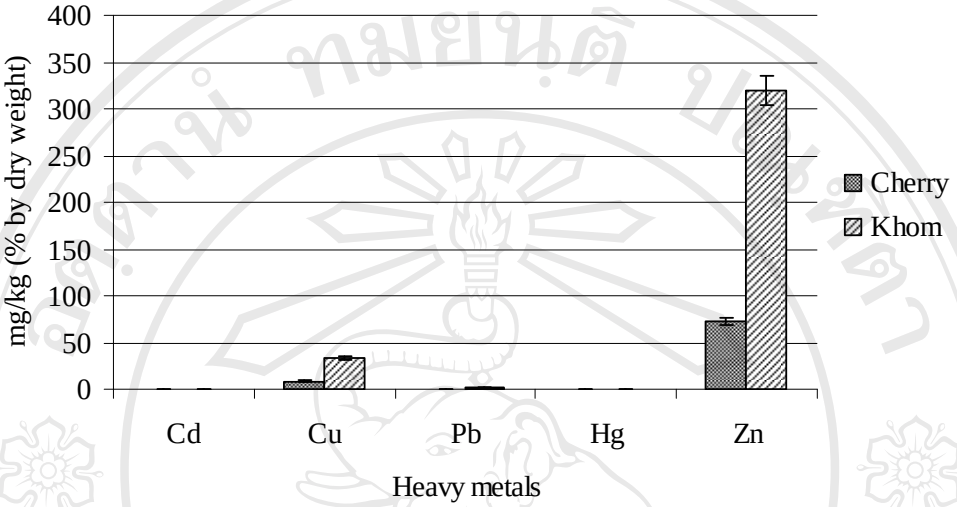


Figure 17. Concentrations of heavy metals in two mollusk species

4.5 Concentrations of heavy metals in sediments

Concentrations of heavy metals in sediments from five collecting stations within the affected area are illustrated in Table 6. The mean of zinc concentration was higher in three seasons compared with other remaining metals. In addition, the highest concentrations of zinc were detected in cold dry season, followed by summer and rainy season (34.58, 27.78 and 25.68 mg/kg, respectively). The concentrations of mercury could not be detected in three seasons (Limit of Detection = 0.175 mg/kg). The highest mean concentration of lead was detected in summer season followed by cold dry and rainy seasons (16.22, 12.43 and 9.91 mg/kg, respectively). The concentrations of copper were higher on rainy season than in summer and cold dry seasons while the concentrations of cadmium were detected with an average of 0.12 mg/kg in rainy season but could not be detected (less than 0.175 mg/kg) in cold dry and summer seasons. The mean concentrations of various heavy metals were compared in sequential order as zinc being higher than copper, lead, cadmium and

mercury respectively in sediments during rainy season, while in the case of remaining heavy metals were zinc higher than lead and copper respectively in sediments in cold dry and summer seasons (Figure 18). Standard Values of Soil Quality declared by the National Environmental Committee, Volume 25 (BC. 2547) stated that the value of cadmium should not exceed 37 mg/kg of soil, lead should exceed 400 mg/kg and mercury should not exceed 23 mg/kg. This study showed that the values of heavy metals from soil sediments were not exceed standard values for all three seasons.

Table 6. Concentrations of heavy metals in sediments in three seasons (% by dry weight)

Parameter	Cd (mg/kg)	Cu (mg/kg)	Pb (mg/kg)	Hg (mg/kg)	Zn (mg/kg)
Rainy season					
Station1	0.17	8.89	8.09	Not detected	28.31
Station 2	0.10	23.60	16.91	Not detected	38.00
Station 3	0.09	25.88	4.44	Not detected	21.91
Station 4	Not detected	18.53	14.15	Not detected	29.13
Station 5	Not detected	3.54	5.95	Not detected	11.03
Average	**0.12**	**16.09**	**9.91**	Not detected	**25.68**
Cold dry season					
Station 1	Not detected	4.75	6.87	Not detected	24.04
Station 2	Not detected	12.46	11.39	Not detected	41.85
Station 3	Not detected	7.01	10.92	Not detected	11.19
Station 4	Not detected	12.46	12.93	Not detected	24.43
Station 5	Not detected	3.13	20.04	Not detected	71.40
Average	Not detected	**7.96**	**12.43**	Not detected	**34.58**
Summer season					
Station 1	Not detected	7.67	10.82	Not detected	33.00
Station 2	Not detected	12.91	29.27	Not detected	27.63
Station 3	Not detected	14.28	17.18	Not detected	33.19
Station 4	Not detected	12.13	15.63	Not detected	26.85
Station 5	Not detected	4.77	8.19	Not detected	18.25
Average	Not detected	**10.35**	**16.22**	Not detected	**27.78**

Remark: *Limit of Detection (LOD) for Cd = 0.075 mg/kg, Hg = 0.175 mg/kg*

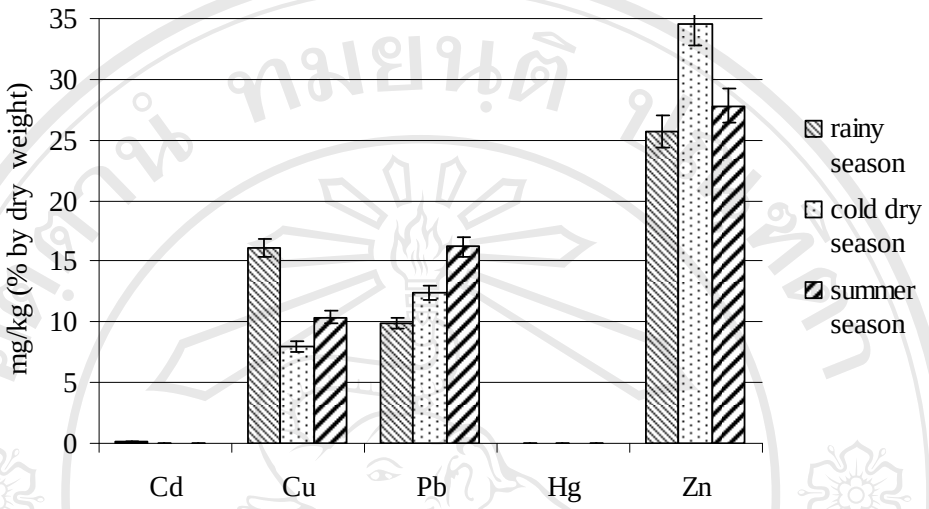


Figure18. Concentrations of heavy metals in sediment in three seasons

4.6 Statistical analysis (one-way ANOVA) in sediment and mollusk samples

ANOVA *p* values and significant level for analysis of metals concentrations in sediment and mollusk showed in Table 7. The concentrations of metals were slightly higher in mollusk than detected in sediment. There were significant differences in cadmium, lead and zinc concentrations (Cd, p=0.011; Pb, p=0.000; Zn, p=0.046) between mollusk and sediment samples all year. However, there was no significant differences in copper and zinc concentrations in each season (rainy, cold dry and summer season, respectively), except for lead (p<0.05).

Table 7. Comparison concentration of heavy metal in sediment and mollusk samples

Season	Cd	Cu	Pb	Zn
All Seasons	0.011***	0.331	0.000***	0.046***
Rainy Season	ND	0.192	0.024***	0.056***
Cold dry Season	ND	0.553	0.002***	0.867***
Summer Season	ND	0.420	0.017***	0.274***

Remark: *ND= no data for analysis, *** $p < 0.05$*

4.7 Toxicity of heavy metals to kidney cell of mollusks

4.7.1 Concentrations of heavy metal in *Filopaludina martensi*

Filopaludina martensi species were collected (Figure 19) from five collecting stations at Bueng Jode reservoir. Results of analyses for heavy metals in this species were given in Figure 20. Mean concentrations of zinc in *Filopaludina martensi* were high in all seasons. In addition, the concentration of zinc in rainy season was much higher than that found in cold and summer seasons (642.07, 108.29 and 104.11 mg/kg, respectively). Mean concentrations of mercury and cadmium were of similar values (0.30, 0.18 mg/kg, respectively) in rainy season while they could not be detected in cold and summer seasons. It was found that concentration of zinc was higher than copper and lead in mollusks in all three seasons.

Figure 19. *Filopaludina martensi* in Bueng Jode wetland

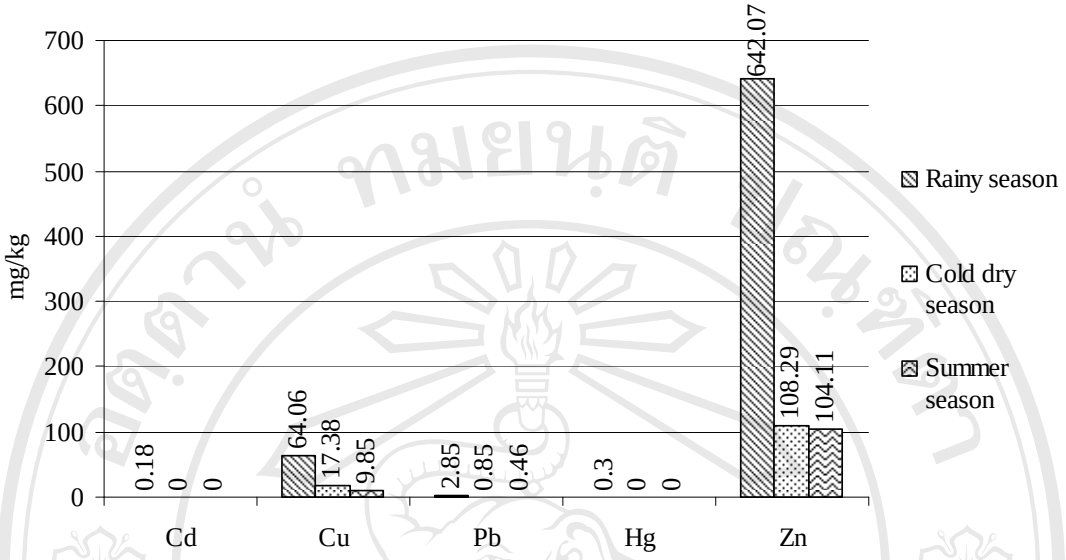
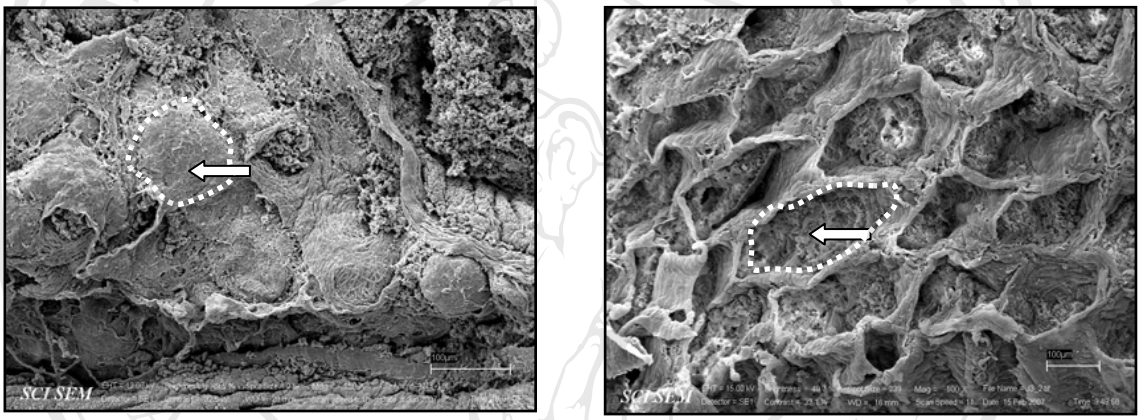


Figure 20. Concentrations of heavy metals in *Filopaludina martensi* on three seasons

4.7.2 Microscopical examination

The micrographs of kidney surface of *Filopaludina martensi* were shown in Figure 21. Kidney surface from natural pond appeared to be normal meanwhile kidney surface from polluted pond was damaged as clearly seen in indicated areas marked by the arrows. The kidney cells (Figure 22) were long, narrow columnar epithelial cells approximately 35 um in length. The nucleus was located basally and the basal membrane was finely infolded. The bases of the cells adjoin a blood sinus, in which amoebocytic blood cell. The apical membrane has brush border with microvilli. The outer surface of kidney cells has well-developed microvilli (Figure 22a). The most characteristic feature of a nephrocyte is the presence of a large apical vacuole. Nucleus of kidney of mollusk from natural pond revealed normal characteristic and it is close to mitochondria and they were not enlarged (Figure 22b-c). Once heavy metals accumulated in kidney cells, the result showed decline in the number of microvilli and nephrocyte. There was an increase in number of lysosomes, however the number of vacuole were decreased (Figure 22d), the morphology of nucleus kidney from polluted pond was disfigured (Figure 22e).

These results showed that cells must be death and microvilli were dissolved. Accumulation of heavy metals in kidney cells obviously revealed disfigurement of mitochondria. When the kidney cells accumulated heavy metals, the results showed an enlargement of mitochondria and nephrocyte was destroyed (Figure 22f). As previously stated by the work of Chelomin *et al.* (1995), where they showed that accumulated heavy metals in kidney had correlation with accumulation in other tissues. Therefore, it can be concluded that other tissues must be affected by heavy metals as well.



(a) Natural Pond (b) Polluted Pond

Figure 21. Scanning electron micrographs of outer kidney of *Filopaludina martensi* from natural and polluted pond. Arrows indicate surface of microvilli in *Filopaludina martensi* kidney with and without explosion to pollution

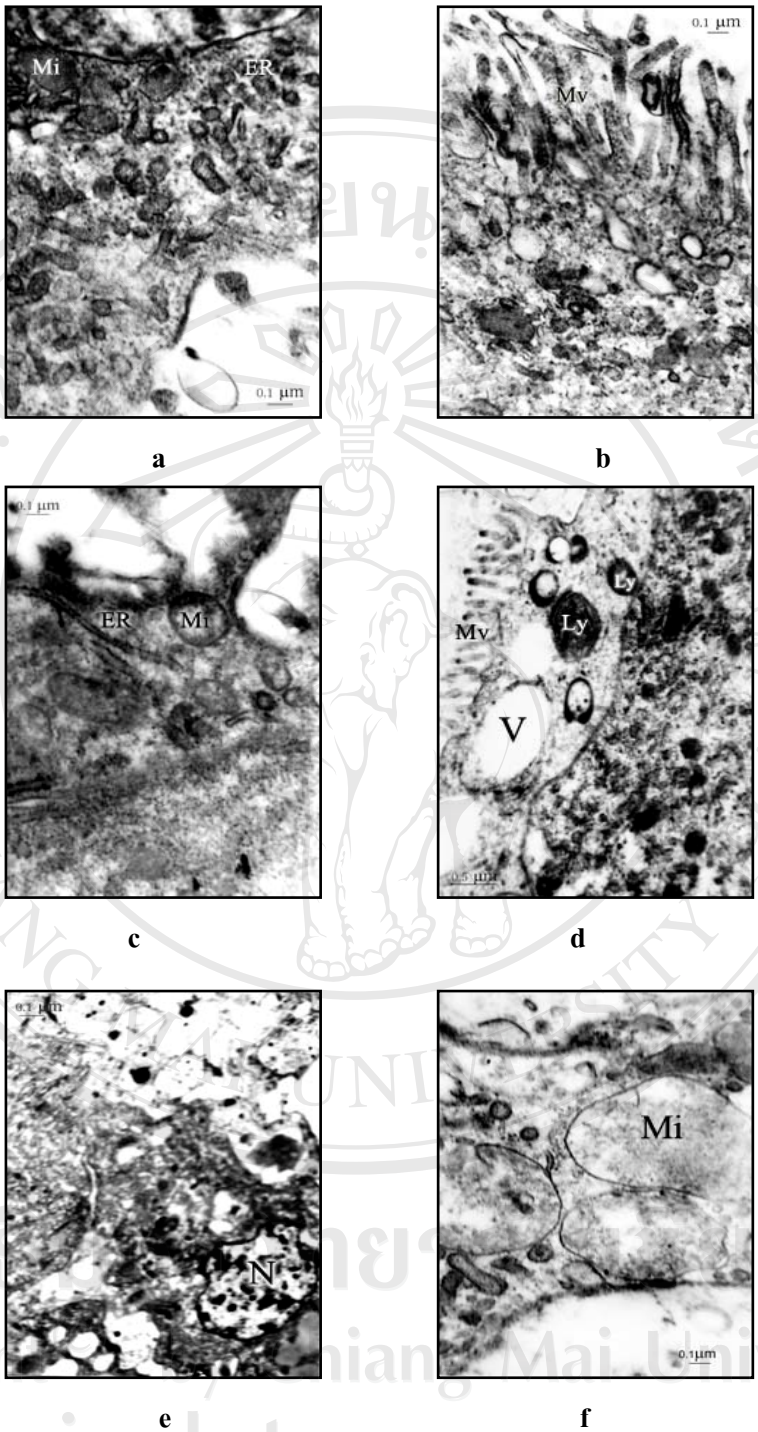


Figure 22. Transmission electron micrographs of nephrocyte in *Filopaludina martensi* (a), (b), (c) showing nephrocyte from natural pond and (d), (e), (f) showing nephrocyte from polluted pond
(ER= Endoplasmic Reticulum, Ly= Lysosomes, Mv= Microvilli, Mi= Mitochondria, N= Nucleus, V= Vacuole**)**

4.8 Heavy metal accumulation experiment

Heavy metals concentration data under this study showed to have higher values of zinc and lead, hence the two heavy metals were selected for laboratory investigation. The results are described below.

4.8.1 Lead and zinc concentrations in *Filopaludina martensi*

Filopaludina martensi species were collected from unpolluted area and were treated with lead and zinc in laboratory. The concentrations of lead were 0.5, 1.0 and 1.5 mg/L and zinc were 2.5, 5.0 and 10.0 mg/L. Analyses of heavy metals were taken at day 10, 20 and 30. In addition, control group were analyzed at day 0, 3 and 15**.** Results from the analyses of metals in these species were given in Table 8. Mortality rate of mollusks control was 6 % and mortality rate of mollusks which were treated with lead and zinc were 18 % and 16 % respectively.

As shown in Table 8, accumulation of lead in control group of mollusks at day 0, 3 and 15 were detected at 0.32, 0.37 and 0.39 mg/kg respectively. For the level of treated lead concentration at 0.5 mg/L at day 10, 20 and 30, the concentration of accumulated lead in mollusks were found at 0.36, 0.42 and 0.45 mg/kg respectively. Whereas at the level of treated lead concentration 1.0 and 1.5 mg/L at day 10, 20 and 30, the concentrations of accumulated lead in mollusks were found at 0.40, 0.52, 0.64 and 0.42, 0.54, 0.67 mg/kg respectively. At all values of lead treated concentration experiments at day 10 to day 30, the results showed that average accumulated lead in all mollusks under study were higher than that of control group, with the exception of lead treated concentration at the level of 0.5 mg/L where accumulated lead concentration in mollusks under study was equal to that found in control group at 0.32, 0.37 and 0.39 mg/kg. Otherwise the accumulated lead concentrations had the tendency of sequential increment during the time course (Figure 23).

As in the case of zinc concentration in control group of mollusks at day 0, 3 and 15 were detected at 80.61, 67.34 and 64.01 mg/kg respectively. For the level of treated zinc concentration at 2.5 mg/L at day 10, 20 and 30, the accumulated zinc concentration in mollusks were found at 70.70, 74.91 and 57.04 mg/kg respectively. Whereas at the level of treated zinc concentrations at 5.0 and 10.0 mg/L at day 10, 20 and 30, the concentrations of accumulated zinc in mollusks were found at 70.97, 74.33, 44.10 and 78.88, 80.15, 59.47 mg/kg respectively.

This is a well known fact that lead is a nonessential element, whereas zinc is an essential element. This study however showed that lead was being accumulated by mollusks during the time course. The results under this study showed that mollusks were unable to excrete lead out of their bodies and hence got accumulated (Figure 23). Unlike in the case of zinc, this element was accumulated at day 10 and day 20 and then at day 30 there was a decline in zinc concentration, this could be explained as zinc is an essential element as a co-enzyme of living organism hence mollusks probably utilized zinc for their living resulting in declining value of accumulated zinc at day 30 (Figure 24).

Table 8. Concentrations of heavy metals values during accumulation at days 10, 20 and 30 in *F. martensi* after treated by lead and zinc.

Treatment	Concentration of Pb			Concentration of Zn		
	0.5 mg/L	1 mg/L	1.5 mg/L	2.5 mg/L	5 mg/L	10 mg/L
10 days	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Replication 1	0.44	0.29	0.37	69.50	70.31	76.10
Replication 2	0.34	0.56	0.36	70.12	69.50	80.74
Replication 3	0.30	0.34	0.52	72.5	73.10	79.82
Average	0.36	0.40	0.42	70.70	70.97	78.88
20 days	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Replication 1	0.55	0.64	0.54	70.19	72.20	77.82
Replication 2	0.56	0.17	0.62	78.93	70.40	81.80
Replication 3	0.14	0.74	0.46	75.60	80.39	80.84
Average	0.42	0.52	0.54	74.91	74.33	80.15
30 days	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Replication 1	0.49	0.62	0.53	54.03	55.24	62.07
Replication 2	0.41	0.47	0.78	35.20	64.55	56.86
Replication 3	✶	0.82	0.71	43.06	51.34	✶
Average	0.45	0.64	0.67	44.10	57.04	59.47
Control		(mg/kg)			(mg/kg)	
0 day		0.32			80.61	
3 days		0.37			67.34	
15 days		0.39			64.01	

Remark: ✶ *: not sufficient number of mollusks remaining in the experiment*

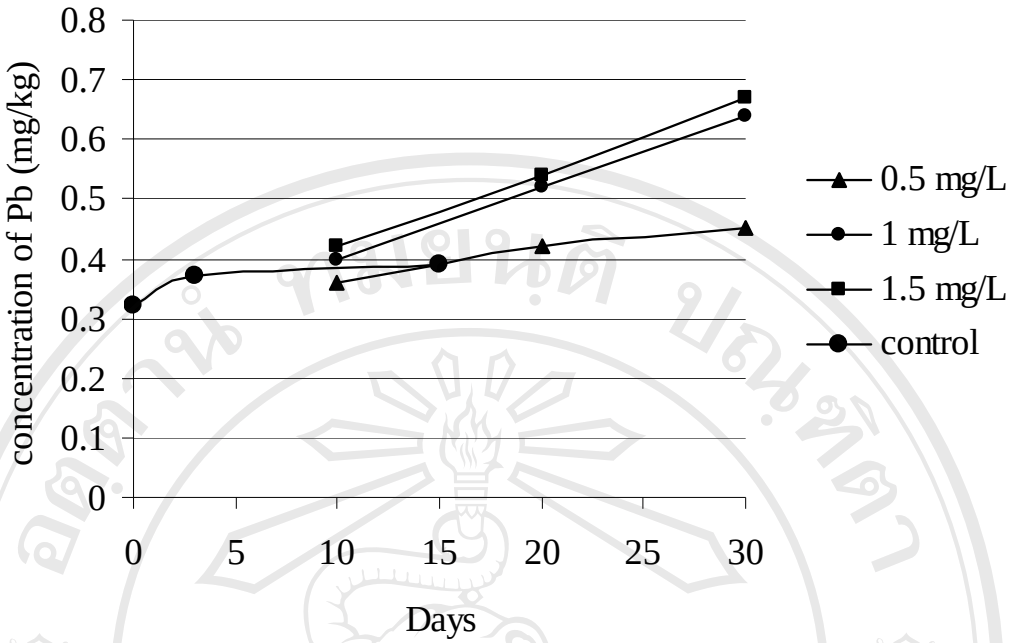


Figure 23. The concentration of accumulated lead within *F. martensi* at the given concentration of 0.5, 1 and 1.5 mg/L and that of lead concentration in control group

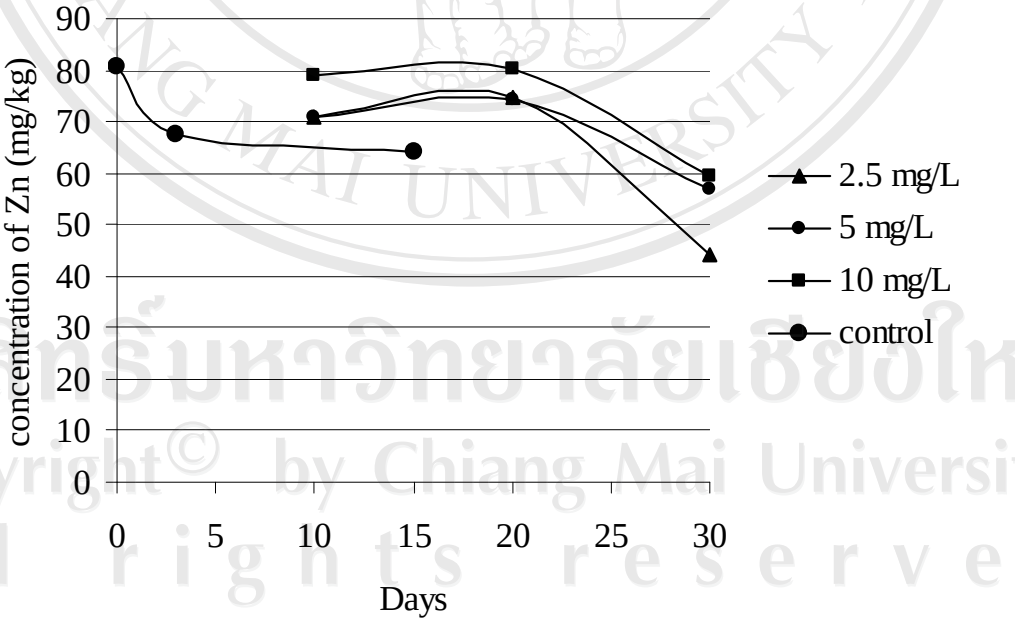


Figure 24. The concentration of accumulated zinc within *F. martensi* at the given concentration of 2.5, 5 and 10 mg/L and that of zinc concentration in control group

4.8.2 Effects of heavy metals towards esterases

Upon studies of the accumulation of lead and zinc in a group of mollusks in the laboratory revealed an increase in heavy metals accumulation with increase in time for treatments. Degree of accumulation depends on type of heavy metals which is essential for the survival of living organisms. In the case of zinc, the results showed increasing tendency of accumulation with increase in time, following a decrease level of accumulation as time progress. Such result was due to cellular utilization of zinc for their living. In case of lead, this heavy metal had been increasingly accumulated with time and cells were unable to expel the same out of the cells. Moreover, this study revealed that heavy metals had adverse effects on esterase activities which is essential for normal cellular activities in all living organisms including plants, animals and microorganisms. Esterases are widely distributed in animals, plants and microorganisms and could be distinguished from lipases in that the catalytic activities of the former are generally restricted to short-chain fatty acid esters (Krisch, 1971). With such rationality, this can be concluded that esterase is essential for normal growth of all living organisms, including group of mollusks under study. This study thus has an objective in studying the effects of heavy metals on esterase activity if the activity is on increment or lowering in mollusk *F. matensi*

By measuring the absorbance values of released p-nitrophenol from p-nitrophenyle acetate by esterase activity in mollusk. Results showed decrease in absorbance values following mollusk treatment with lead at differing levels of 0.5, 1.0 and 1.5 mg/L and with zinc at differing levels of 2.5, 5.0 and 10 mg/L after treating with for 10, 20 and 30 days. Prior to measuring esterase activity values, it is essential to analyze protein content in the cells of mollusk at day 10, 20 and 30 with the procedure used in the determination of esterase in tissue homogenates samples. The results were shown as absorbance values in Tables 9-11 and Tables 12-14.

Table 9. Protein concentrations as obtained from various samples at day 10

Tissue Homogenate Samples	Absorbance 595 nm			Average	Average standard-Average blank	Total protein (μg /ml)
	1	2	3			
Blank	0.718	0.666	0.627	0.670	0.000	
Control	1.255	1.334	1.280	1.290	0.620	1856.222
Control	1.312	1.294	1.349	1.318	0.648	1951.778
Pb 0.5 mg/L	1.291	1.272	1.286	1.283	0.613	1834.000
Pb 0.5 mg/L	1.259	1.282	1.281	1.274	0.604	1804.000
Pb 0.5 mg/L	1.241	1.208	1.243	1.231	0.561	1659.556
Pb 1.0 mg/L	1.258	1.240	1.287	1.262	0.592	1762.889
Pb 1.0 mg/L	1.250	1.210	1.274	1.245	0.575	1706.222
Pb1.0 mg/L	1.266	1.274	1.348	1.296	0.626	1877.333
Pb 1.5 mg/L	1.196	1.257	1.263	1.239	0.569	1686.222
Pb 1.5 mg/L	1.234	1.255	1.255	1.248	0.578	1717.333
Pb 1.5 mg/L	1.212	1.260	1.287	1.253	0.583	1734.000
Zn 2.5 mg/L	1.212	1.223	1.199	1.211	0.541	1595.111
Zn 2.5 mg/L	1.264	1.286	1.249	1.266	0.596	1778.444
Zn 2.5 mg/L	1.176	1.172	1.216	1.188	0.518	1517.333
Zn 5.0 mg/L	1.200	1.205	1.236	1.214	0.544	1602.889
Zn 5.0 mg/L	1.302	1.340	1.272	1.305	0.635	1906.222
Zn 5.0 mg/L	1.266	1.300	1.325	1.297	0.627	1880.667
Zn 10.0 mg/L	1.194	1.149	1.210	1.184	0.514	1505.111
Zn 10.0 mg/L	1.226	1.250	1.254	1.243	0.573	1701.778
Zn 10.0 mg/L	1.307	1.328	1.300	1.312	0.642	1929.556

Table 10. Protein concentrations as obtained from various samples at day 20

Tissue Homogenate Samples	Absorbance 595 nm			Average	Average standard-Average blank	Total protein (μg /ml)
	1	2	3			
blank	0.718	0.666	0.627	0.670	0.000	
control	1.255	1.334	1.28	1.290	0.620	1856.22
control	1.312	1.294	1.349	1.318	0.648	1951.78
Pb 0.5 mg/L	1.291	1.272	1.286	1.283	0.613	1834.00
Pb 0.5 mg/L	1.259	1.282	1.281	1.274	0.604	1804.00
Pb 0.5 mg/L	1.241	1.208	1.243	1.231	0.561	1659.56
Pb 1.0 mg/L	1.258	1.24	1.287	1.262	0.592	1762.89
Pb 1.0 mg/L	1.25	1.21	1.274	1.245	0.575	1706.22
Pb1.0 mg/L	1.266	1.274	1.348	1.296	0.626	1877.33
Pb 1.5 mg/L	1.196	1.257	1.263	1.239	0.569	1686.22
Pb 1.5 mg/L	1.234	1.255	1.255	1.248	0.578	1717.33
Pb 1.5 mg/L	1.212	1.26	1.287	1.253	0.583	1734.00
Zn 2.5 mg/L	1.212	1.223	1.199	1.211	0.541	1595.11
Zn 2.5 mg/L	1.264	1.286	1.249	1.266	0.596	1778.44
Zn 2.5 mg/L	1.176	1.172	1.216	1.188	0.518	1517.33
Zn 5.0 mg/L	1.2	1.205	1.236	1.214	0.544	1602.89
Zn 5.0 mg/L	1.302	1.34	1.272	1.305	0.635	1906.22
Zn 5.0 mg/L	1.266	1.3	1.325	1.297	0.627	1880.67
Zn 10.0 mg/L	1.194	1.149	1.21	1.184	0.514	1505.11
Zn 10.0 mg/L	1.226	1.25	1.254	1.243	0.573	1701.78
Zn 10.0 mg/L	1.307	1.328	1.3	1.312	0.642	1929.56

Table 11. Protein concentrations as obtained from various samples at day 30

Tissue Homogenate Samples	Absorbance 595 nm			Average	Average standard-Average blank	Total protein (μg /ml)
	1	2	3			
Blank	0.666	0.627	0.670	0.654	0.000	
Control	1.334	1.280	1.290	1.301	0.647	1948.07
Control	1.294	1.349	1.318	1.320	0.666	2012.15
Pb 0.5 mg/L	1.272	1.286	1.283	1.280	0.626	1878.44
Pb 0.5 mg/L	1.282	1.281	1.274	1.279	0.625	1874.00
Pb 0.5 mg/L	1.208	1.243	1.231	1.227	0.573	1701.41
Pb 1.0 mg/L	1.240	1.287	1.262	1.263	0.609	1820.30
Pb 1.0 mg/L	1.210	1.274	1.245	1.243	0.589	1753.63
Pb1.0 mg/L	1.274	1.348	1.296	1.306	0.652	1964.00
Pb 1.5 mg/L	1.257	1.263	1.239	1.253	0.599	1786.96
Pb 1.5 mg/L	1.255	1.255	1.248	1.253	0.599	1786.22
Pb 1.5 mg/L	1.260	1.287	1.253	1.267	0.613	1832.89
Zn 2.5 mg/L	1.223	1.199	1.211	1.211	0.557	1647.70
Zn 2.5 mg/L	1.286	1.249	1.266	1.267	0.613	1834.37
Zn 2.5 mg/L	1.172	1.216	1.188	1.192	0.538	1584.00
Zn 5.0 mg/L	1.205	1.236	1.214	1.218	0.564	1671.41
Zn 5.0 mg/L	1.340	1.272	1.305	1.306	0.652	1962.52
Zn 5.0 mg/L	1.300	1.325	1.297	1.307	0.653	1968.44
Zn 10.0 mg/L	1.149	1.210	1.184	1.181	0.527	1547.70
Zn 10.0 mg/L	1.250	1.254	1.243	1.249	0.595	1774.37
Zn 10.0 mg/L	1.328	1.300	1.312	1.313	0.659	1988.07

Table 12. Procedure used in determination of esterase in tissue homogenates samples at day 10

Samples	Volume of samples (µl)	Volume of Tris-EDTA (µl)	50 mM *p*-nitrophenyl acetate (µl)
control	10.8	979.2	10
control	10.2	979.8	10
Pb 0.5 mg/L	10.9	979.1	10
Pb 0.5 mg/L	11.1	978.9	10
Pb 0.5 mg/L	12.1	977.9	10
Pb 1.0 mg/L	11.3	978.7	10
Pb 1.0 mg/L	11.7	978.3	10
Pb1.0 mg/L	10.7	979.3	10
Pb 1.5 mg/L	11.9	978.1	10
Pb 1.5 mg/L	11.6	978.4	10
Pb 1.5 mg/L	11.5	978.5	10
Zn 2.5 mg/L	12.5	977.5	10
Zn 2.5 mg/L	11.2	978.8	10
Zn 2.5 mg/L	13.2	976.8	10
Zn 5.0 mg/L	12.5	977.5	10
Zn 5.0 mg/L	10.5	979.5	10
Zn 5.0 mg/L	10.6	979.4	10
Zn 10.0 mg/L	13.3	976.7	10
Zn 10.0 mg/L	11.8	978.2	10
Zn 10.0 mg/L	10.4	979.6	10

Table 13. Procedure used in determination of esterase in tissue homogenates samples at day 20

Samples	Volume of samples (µl)	Volume of Tris-EDTA (µl)	50 mM *p*-nitrophenyl acetate (µl)
control	12.9	977.1	12.9
control	11.4	978.6	11.4
Pb 0.5 mg/L	14.9	975.1	14.9
Pb 0.5 mg/L	13.9	976.1	13.9
Pb 0.5 mg/L	9.8	980.2	9.8
Pb 1.0 mg/L	12.0	978.0	12.0
Pb 1.0 mg/L	12.1	977.9	12.1
Pb1.0 mg/L	12.3	977.7	12.3
Pb 1.5 mg/L	12.3	977.7	12.3
Pb 1.5 mg/L	11.5	978.5	11.5
Pb 1.5 mg/L	11.8	978.2	11.8
Zn 2.5 mg/L	11.5	978.5	11.5
Zn 2.5 mg/L	10.5	979.5	10.5
Zn 2.5 mg/L	12.3	977.7	12.3
Zn 5.0 mg/L	14.9	975.1	14.9
Zn 5 .0mg/L	11.4	978.6	11.4
Zn 5.0 mg/L	16.7	973.3	16.7
Zn 10.0 mg/L	13.1	976.9	13.1
Zn 10.0 mg/L	14.6	975.4	14.6
Zn 10.0 mg/L	14.2	975.8	14.2

Table 14. Procedure used in determination of esterase in tissue homogenates samples at day 30

Samples	Volume of samples (µl)	Volume of Tris-EDTA (µl)	50 mM *p*-nitrophenyl acetate (µl)
control	10.3	979.7	10.3
control	9.9	980.1	9.9
Pb 0.5 mg/L	10.6	979.4	10.6
Pb 0.5 mg/L	10.7	979.3	10.7
Pb 0.5 mg/L	11.8	978.2	11.8
Pb 1.0 mg/L	11.0	979.0	11.0
Pb 1.0 mg/L	11.4	978.6	11.4
Pb1.0 mg/L	10.2	979.8	10.2
Pb 1.5 mg/L	11.2	978.8	11.2
Pb 1.5 mg/L	11.2	978.8	11.2
Pb 1.5 mg/L	10.9	979.1	10.9
Zn 2.5 mg/L	12.1	977.9	12.1
Zn 2.5 mg/L	10.9	979.1	10.9
Zn 2.5 mg/L	12.6	977.4	12.6
Zn 5.0 mg/L	12.0	978.0	12.0
Zn 5.0 mg/L	10.2	979.8	10.2
Zn 5.0 mg/L	10.2	979.8	10.2
Zn 10.0 mg/L	12.9	977.1	12.9
Zn 10.0 mg/L	11.3	978.7	11.3
Zn 10.0 mg/L	10.1	979.9	10.1

Table 15 shows absorbance values of end product of p-nitrophenyl acetate following the release action of esterase after being treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L and with zinc at concentration of 2.5, 5.0 and 10.0 mg/L at day 10. Mollusk in control group showed absorbance value at 0.514, while after treated with lead at concentrations of 0.5m 1.0 and 1.5 mg/L showed absorbance values at 0.470, 0.371 and 0.273, and after treated with zinc at concentration of 2.5, 5.0 and 10.0 mg/L showed absorbance values at 0.290, 0.306 and 0.262 respectively. It can be seen that absorbance values after being treated with lead and zinc showed lower value when compared with control values. This is an evidence showing the effects of heavy metals repressing the esterase activity in accordance with differing concentrations, the less concentration, more activity could be expressed, while at high concentration, less activity was expressed.

Statistical analysis at various concentrations of heavy metals versus esterase concentration at day 10 at differing lead concentrations at 1.0 and 1.5 mg/L showed definite significant adverse effects on esterase at 95% confidential limit. In the case of zinc concentrations affecting esterase lowered in concentration as compared to those in control groups which were not treated with heavy metals significantly at 95% confidential limit. Moreover, mollusks treated with differing concentrations of zinc significantly affected esterase concentration at 95% confidential limit. Comparing the affects of lead at 1.5 mg/L concentration and of zinc at 10.0 mg/L concentrations, both showed significant lowering of esterase concentration at 95% confidential limit (Appendix D).

Table 15. Absorbance values of p-nitrophenol released by esterase in mollusk samples after being treated with lead at the concentrations of 0.5, 1 and 1.5 mg/L and for zinc at the concentrations of 2.5, 5 and 10 mg/L at day 10

Sample	OD sample-OD blank			Mean	SD
control	0.431	0.425	0.445	0.514	0.090
	0.591	0.573	0.621		
Pb 0.5mg/L	0.501	0.503	0.538	0.470	0.042
	0.406	0.430	0.453		
	0.440	0.467	0.489		
Pb 1.0 mg/L	0.506	0.479	0.476	0.371	0.092
	0.340	0.337	0.346		
	0.266	0.282	0.303		
Pb 1.5 mg/L	0.247	0.269	0.279	0.273	0.032
	0.290	0.312	0.327		
	0.233	0.244	0.252		
Zn 2.5 mg/L	0.136	0.165	0.177	0.290	0.100
	0.365	0.368	0.381		
	0.331	0.324	0.366		
Zn 5.0 mg/L	0.304	0.303	0.327	0.306	0.013
	0.310	0.303	0.322		
	0.283	0.303	0.295		
Zn 10.0 mg/L	0.177	0.190	0.211	0.262	0.061
	0.248	0.261	0.284		
	0.344	0.312	0.334		

Remark: *OD =Absorbance*

Table 16 shows absorbance values of end product of p-nitrophenyl acetate following the release action of esterase after being treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L and with zinc at concentration of 2.5, 5.0 and 10.0 mg/L at day 20. Mollusks in control group showed absorbance value at 0.908, while after treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L showed absorbance values at 0.624, 0.999 and 0.558, and after treated with zinc at concentration of 2.5, 5.0 and 10.0 mg/L showed absorbance values at 0.619, 0.504 and 0.436, respectively. It can be seen that absorbance values after being treated with lead and zinc showed lower value when compared with control values, except for lead at the concentration of 1.0 mg/L showing absorbance value of 0.999 which was higher than the control group. This is an evidence showing the effects of heavy metals repressing the esterase activity in accordance with differing concentrations, the less concentration, and more activity could be expressed, while at high concentration, less activity was expressed.

Statistical analysis at various concentrations of heavy metals versus esterase concentration at day 20 at differing lead concentrations at 1.0 and 1.5 mg/L showed definite significant adverse effects on esterase at 95% confidential limit. In the case of zinc concentrations affecting esterase lowered in concentration as compared to those control groups which were not treated with heavy metals significantly at 95% confidential limit. Moreover, mollusks been treated with differing concentrations of zinc at concentrations of 2.5, 5.0 and 10.0 mg/L significantly affected esterase concentration at 95% confidential limit. Comparing the affects of lead at 1.5 mg/L concentration and of zinc at 10.0 mg/L concentrations, both showed significant lowering of esterase concentration at 95% confidential limit (Appendix D).

Table 16. Absorbance values of p-nitrophenol released by esterase in mollusk samples after being treated with lead at the concentrations of 0.5, 1 and 1.5 mg/L and for zinc at the concentrations of 2.5, 5 and 10 mg/L at day 20

Sample	OD sample-OD blank			Mean	SD
control	0.941	0.887	0.734	0.908	0.092
	0.995	0.962	0.926		
Pb 0.5 mg/L	0.609	0.552	0.591	0.624	0.118
	0.477	0.538	0.543		
	0.715	0.779	0.815		
Pb 1.0 mg/L	1.384	1.391	1.420	0.999	0.301
	0.777	0.779	0.783		
	0.774	0.807	0.880		
Pb 1.5 mg/L	0.544	0.536	0.553	0.558	0.076
	0.461	0.481	0.499		
	0.609	0.658	0.679		
Zn 2.5 mg/L	0.680	0.688	0.701	0.619	0.062
	0.525	0.554	0.573		
	0.607	0.617	0.623		
Zn 5.0 mg/L	0.660	0.703	0.713	0.504	0.175
	0.502	0.535	0.551		
	0.281	0.289	0.306		
Zn 1.0 mg/L	0.371	0.382	0.402	0.436	0.040
	0.461	0.463	0.478		
	0.452	0.456	0.463		

Remark: *OD =Absorbance*

Table 17 shows absorbance values of end product of p-nitrophenyl acetate following the release action of esterase after being treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L and with Zn at concentration of 2.5, 5.0 and 10.0 mg/L at day 30. Mollusks in control group showed absorbance value at 0.379, while after treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L showed absorbance values at 0.408, 0.317 and 0.253, and after treated with Zn at concentration of 2.5, 5.0 and 10.0 mg/L showed absorbance values at 0.350, 0.217 and 0.279 respectively. It can be seen that absorbance values after being treated with lead and zinc showed lower value when compared with control values, except for lead at the concentration of 0.5 mg/L showing absorbance value of 0.408 which was higher than the control group. This is an evidence showing the effects of heavy metals repressing the esterase activity in accordance with differing concentrations, the less concentration, and more activity could be expressed, while at high concentration, less activity was expressed.

Statistical analysis at various concentrations of heavy metals versus esterase concentration at day 30 at differing lead concentrations at 1.0 and 1.5 mg/L showed definite significant adverse effects on esterase. In the case of zinc concentrations significantly affecting esterase lowered in concentration depending on zinc concentrations at 5.0 mg/L and 10.0 mg/L at 95% confidential limit. On comparing the affects of lead at 1.5 mg/L concentration and of zinc at 10.0 mg/L concentrations, both showed significant lowering of esterase concentration at 95% confidential limit (Appendix D).

Table 17. Absorbance values of p-nitrophenol released by esterase in mollusk samples after being treated with lead at the concentrations of 0.5, 1 and 1.5 mg/L and for zinc at the concentrations of 2.5, 5 and 10 mg/L at day 30

Sample	OD sample-OD blank			Mean	SD
control	0.299	0.292	0.292	0.379	0.094
	0.434	0.473	0.484		
Pb 0.5 mg/L	0.510	0.534	0.525	0.408	0.087
	0.341	0.336	0.341		
	0.349	0.367	0.373		
Pb 1.0 mg/L	0.312	0.316	0.319	0.317	0.011
	0.329	0.321	0.338		
	0.302	0.312	0.307		
Pb 1.5 mg/L	0.169	0.170	0.171	0.253	0.063
	0.305	0.307	0.312		
	0.277	0.276	0.286		
Zn 2.5 mg/L	0.355	0.370	0.381	0.350	0.022
	0.306	0.333	0.338		
	0.348	0.353	0.365		
Zn 5.0 mg/L	0.197	0.200	0.204	0.217	0.014
	0.224	0.226	0.236		
	0.213	0.224	0.227		
Zn 10.0 mg/L	0.249	0.279	0.262	0.279	0.019
	0.287	0.286	0.317		
	0.271	0.275	0.286		

Remark: *OD =Absorbance*

Table 18 shows comparison of absorbance values of end product of p-nitrophenyl acetate following the release action of esterase after being treated with lead at concentrations of 0.5, 1.0 and 1.5 mg/L and with zinc at concentration of 2.5, 5.0 and 10.0 mg/L at day 10, 20 and 30. Results showed the absorbance values of end product released by esterase of the control group being high values at day 20 and decreasing at day 30. Such observation could be explained that at day 10, the mollusk continued to express esterase activity. With time passing to day 30, there was a reduce concentration of esterase. This could be due to the environment of culturing mollusks in distilled water and feeding on feed pellets. This was due to the condition in laboratory differing to that in natural habitat. Comparison of absorbance values on end-product released by esterase after being treated with lead at concentrations of 0.5 1.0 and 1.5 mg/L at day 10, 20 and 30.

Table 18. Comparison of absorbance values of p-nitrophenol released by esterase in mollusk samples after being treated with lead at the concentrations of 0.5, 1 and 1.5 mg/L and for zinc at the concentrations of 2.5, 5 and 10 mg/L at day 10, 20 and 30

		Day 10	Day 20	Day 30
control	Mean	0.514	0.908	0.379
	Std. Deviation	0.090	0.092	0.094
Pb 0.5 mg/L	Mean	0.470	0.624	0.408
	Std. Deviation	0.042	0.118	0.087
Pb 1.0 mg/L	Mean	0.371	0.999	0.317
	Std. Deviation	0.092	0.301	0.011
Pb 1.5 mg/L	Mean	0.273	0.558	0.253
	Std. Deviation	0.032	0.076	0.063
Zn 2.5 mg/L	Mean	0.290	0.619	0.350
	Std. Deviation	0.100	0.062	0.022
Zn 5.0 mg/L	Mean	0.306	0.504	0.217
	Std. Deviation	0.013	0.175	0.014
Zn 10.0 mg/L	Mean	0.262	0.436	0.279
	Std. Deviation	0.061	0.040	0.019

Results showed to day 20 that there was continual increment of esterase activity and was reduced on day 30 (Figure 25). However, all absorbance values were lower than that of control group for all period of the experiment. The results were similar to that with lead (Figure 26). Comparison of absorbance values for end-product released by esterase in group of mollusks being treated with lead had higher values than a group of mollusks being treated with zinc (Figure 27). This must be because a group of mollusks being treated with lead did not got utilized esterase in their normal living condition, hence showing higher absorbance values than those mollusk being treated with zinc. Mollusks being treated with zinc showed lower absorbance values. This might be due to zinc being essential element catalyzing normal growth and was important in supporting esterase activity.

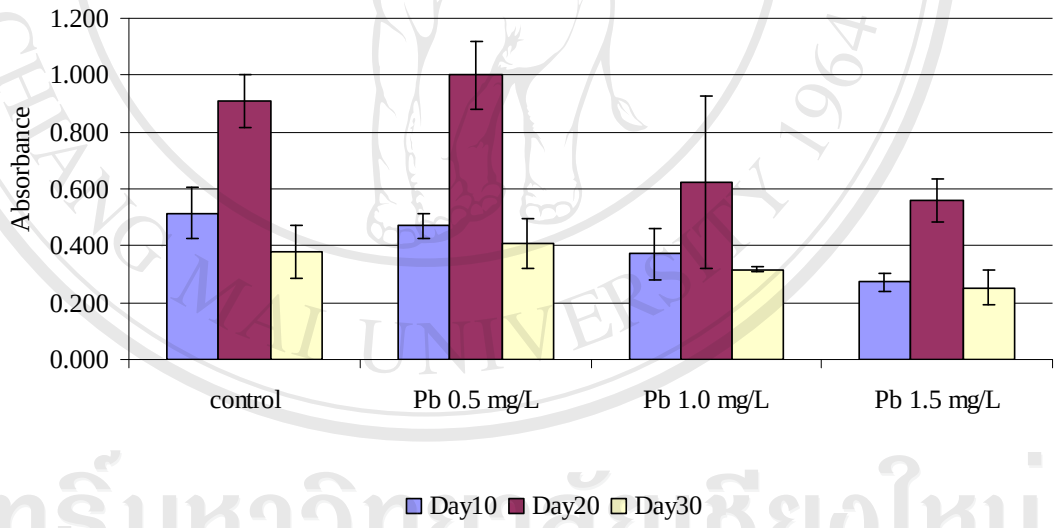


Figure 25. Absorbance values of end-product released by esterase after group of mollusk under study being treated with differing concentrations of lead at 0.5, 1.0 and 1.5 mg/L at day 10, 20 and 30

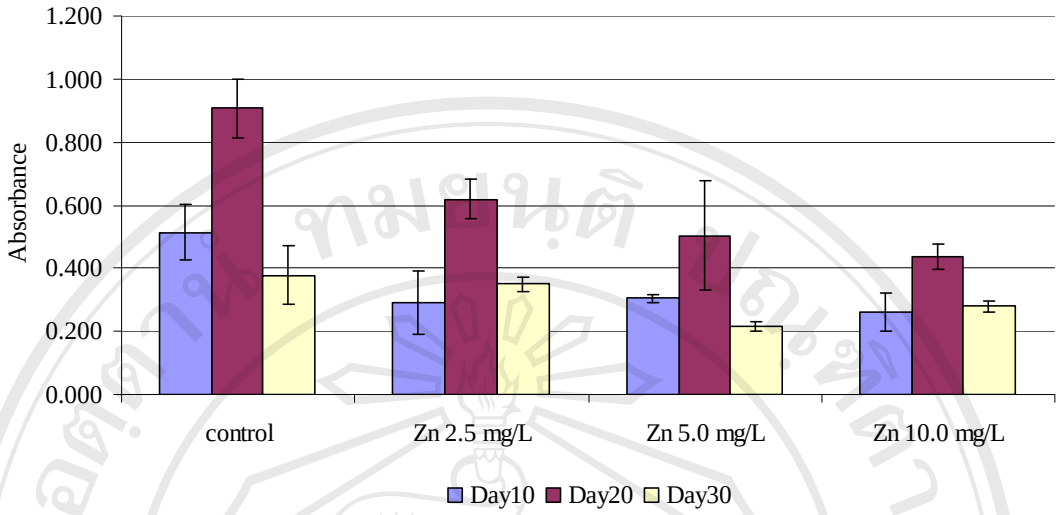


Figure 26. Absorbance values of end-product released by esterase after group of mollusk under study being treated with differing concentrations of zinc at 2.5, 5.0 and 10.0 mg/L at day 10, 20 and 30

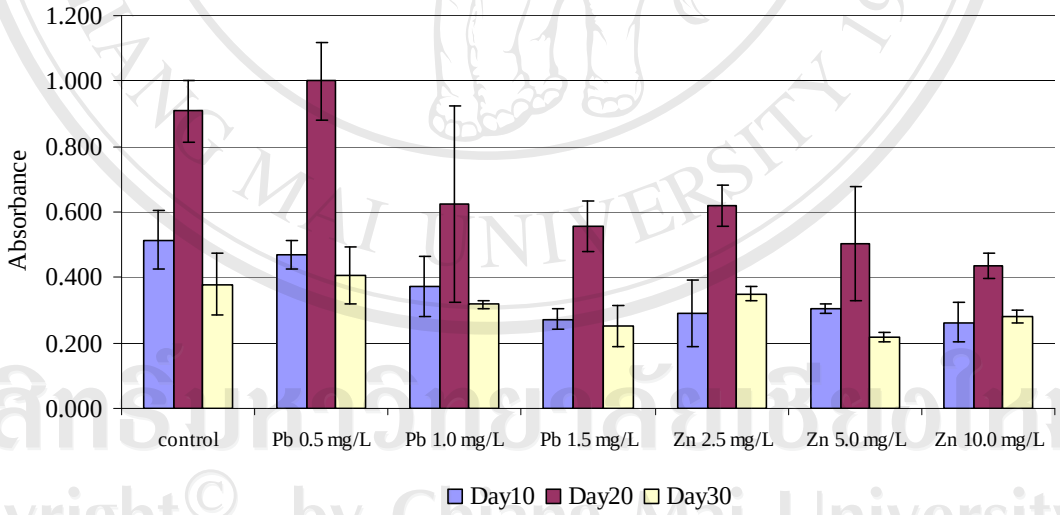


Figure 27. Absorbance values of end-product released by esterase after group of mollusk under study being treated with differing concentrations of lead at 0.5, 1.0 and 1.5 mg/L and zinc at 2.5, 5.0 and 10.0 mg/L at day 10, 20 and 30