Research article

Development of the Indigenous Chironomid Species as Ecotoxicology Test: Tool for Water Quality Management in Thailand

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Abstract In Thailand, heavy metal contamination of the aquatic ecosystem is of concern because of the effect on water quality and toxicity to aquatic biota. The aim of this study was to develop ecotoxicology test protocols using a standard test organism the midge, *Chironomus striatipennis* Kieffer for measuring acute and chronic toxicology.. This species is an important indigenous freshwater invertebrate in Thailand. The acute effects of cadmium using cadmium chloride and lead as lead nitrate on midge larvae were investigated by static bioassay under laboratory conditions. The observed mortality data of the acute test for median lethal concentration 48 h LC_{50} of cadmium chloride on first, second, third and fourth instars of midge larvae were 25.1 (14.6-40.7), 201 (60.1-367), 579 (537-620) and 641 (564-717) mg/L, respectively. The 48 h LC_{50} of lead on first, second, third and fourth instars of midge larvae were 99.9 (14.7-209), 55.5 (266-897), 1,540 (1,200-1,870) and 3790 (2,890-4,410) mg/L, respectively. The results showed that cadmium chloride and lead nitrate were highly toxic to the first instar larvae followed by second, third, and fourth instar larvae, respectively. Sublethal studies of cadmium and lead on midge showed that both cadmium and lead had effects on the body length of larvae, the development time of larvae, dry weight and the number of female and male adults. The results from this study would be useful for developing a biomonitoring tool for heavy metal contamination assessment in freshwater ecosystem and water quality management in Thailand.

Keywords ecotoxicology test, heavy metal, freshwater management

INTRODUCTION

Aquatic ecosystem contamination with heavy metals has been an increasing concern in Thailand, due to their persistence and high toxicity to many living organisms when they exceed threshold concentrations (Chen et al., 2007; Be´chard et al., 2008). The impact of heavy metals on the ecosystemare significant as they cannot be completely removed from water by natural self purification in aquatic ecosystems and could accumulate in overlay water and in sediment and thus enter the food chain (Loska and Wiechuła, 2003) and subsequently cause ecotoxicological problems and then affect human health. Because of the great importance of this issue, there is an urgent need to have practical tools for water quality management and ecological risk assessment in Thailand. However, the study of ecotoxicology is quite new and has many limitations, especially the availability of ecotoxicological data on aquatic organisms in Thailand. As with many developing countries, we have relied upon overseas data (US.EPA, ATSM, OECD, etc.) for developing ecotoxicology test guidelines. However, these guidelines may not be suitable for Thailand. Because indigenous aquatic organisms in Thailand may be more or less sensitive to contaminants than surrogate species, the characteristic of sediment and water in Thai river systems may differ from other temperate locations.

Chironomid species have been adopted as test species for ecotoxicological assessments because they are an important component of freshwater ecosystems and represent one of most widely distributed group of benthic organisms. They are adapted to almost every type of aquatic ecosystem (Vos, 2000). These species are important as prey for juvenile and adult fish and aquatic birds and can be easily cultured under laboratory conditions with a relatively short life cycle (Taenzler, 2007). Two species of *Chironomus* are typically used in ecotoxicity testing: these are *C. tentans* and *C. riparius* (Watts and Pascoe, 2000). Unfortunately, these species are few in number in Thailand freshwater ecosystems. Therefore, the use of indigenous species to assess contaminated aquatic ecosystems should be investigated because the toxic response of such species represents more accurately the adverse effect of pollution on the tropical invertebrate species in Thailand. In addition, standardized protocols for acute and chronic aquatic toxicity testing should be developed using indigenous species.

The aim of this paper is to study the ecotoxicology of cadmium and lead on the indigenous freshwater invertebrate *Chironomus striatipennis*, a non-biting midge (Diptera; Chironomidae) found in Thailand. The result would be a useful component in developing a biomonitoring tool using an indigenous species test for evaluation heavy metal contamination in aquatic ecosystems and provide useful information for pollution control authorities involved in water quality management in Thailand.

MATERIALS AND METHODS

Test organism

Initially, a local chironomid species was found in Thailand, *Chironomus striatipennis* Kieffer, isolated from the field and cultured under control laboratory conditions at the Ecotoxicology and Environmental Sciences Laboratory, Faculty of Agriculture, Khon Kaen University, Thailand. *C. striatipennis* were cultured in 3 L glass tanks of 15 x 30 x 30 cm^3 in the aquarium and covered with netting to trap the emerging *C. striatipennis*. Overlying water was replaced every three days. Each egg mass was collected and placed in a 1 L beaker containing 500 mL of aerated water. When all eggs hatched, larvae were transferred in to a clean beaker and cultured until the larvae reached a suitable stage and were ready for use in experiments. The test species was given fish food at a rate of 0.5 g/L for food and conditions were maintained at 23±2 ^{o}C, on a 16:8 h light: dark regime with pH of 7-8 and a dissolved oxygen (DO) concentration of 5-7 mg/L.

Test chemicals

The test heavy metals were cadmium chloride ($CdCl_2$ 99.5%) and lead nitrate ($[Pb(NO_3)_2]$ 99%). Stock solutions were prepared by dissolving the heavy metal directly in distilled water and test solutions were prepared by appropriate dilution immediately before each experiment.

Acute toxicity test

Acute toxicity tests were conducted in order to calculate the Cd and Pb LC_{50} data of first, second, third and fourth instars of midge larvae. All experiments were performed according to the US.EPA (1991) method for determining 48 h LC_{50} values for *C. striatipennis*. Five dilutions were prepared for Cd: 200, 400, 600, 800 and 1,000 mg/L, and 6 for Pb: 100, 200, 300, 400 and 500 and 1,000 mg/L. Three replicates of 10 midge larvae per treatment plus a control were used. The larvae were exposed in 20 mL glass beakers containing 10 mL for each test concentration and control and were undertaken as static bioassay under laboratory conditions. Test organisms were not fed during the testing period. Observations were made after 24 and 48 h, and results recorded. For water quality purposes DO, pH, electrical conductivity (EC) and temperature were monitored at the beginning and end of the test. Mortality data were used to calculate 48 h LC_{50} values and 95% confidence intervals by Probit analysis with the Statistical Analysis System (SPSS) Version 12 statistical software.

Chronic toxicity test

Chronic toxicity testing for heavy metal effects on midge larvae followed the procedure recommended by USEPA document 6004-91/002 (1994). Based on the acute toxicity result, *C. striatipennis* were exposed to Cd concentrations of 0, 0.5, 2.5 and 5.0 mg/L. Lead concentrations of 0, 5, 25 and 50 mg/L were used.

The effects of Cd or Pb on the growth of midge larvae were evaluated by using 60 midge larvae and 300 eggs to study the effect on emergence of adult, length, the development time and dry weight of larvae. These were randomly sampled from each treatment and control and were exposed in 500 mL glass beakers for static bioassays containing 300 mL for each test concentration. Test organisms were fed with fish food 0.5 g/L. The water quality at the beginning and end of the test on control and treatments were measured. Data from chronic tests were analyzed using analysis of variance (ANOVA) to detect significant differences between treatment and control. Statistical analysis was performed using SPSS Version 12 statistical software.

RESULTS AND DISCUSSION

Acute toxicity

Table 1 shows the estimated 48 h LC_{50} for Cd and Pb that were calculated from standard toxicity tests on the first, second, third and fourth instars of *C. striatipennis*. The results of this investigation demonstrated that the 48 h LC_{50} values of first to fourth instars of *C. striatipennis* for Cd concentrations were between 25.1 - 641 mg/L and for Pb concentration between 99.9 - 3,720 mg/L, respectively. The 48 h LC_{50} value of Cd on second, third and fourth instars of *C. striatipennis* were 8, 23 and 25 greater than for first midge larvae, respectively. The 48 h LC_{50} value of Pb on second, third and fourth instars of C. striatipennis were 6, 15 and 31 fold greater than for first midge larvae, respectively.

On the basis of the experimental LC_{50} values from this study, *C. striatipennis* were more tolerant to the heavy metals Cd and Pb than some other Chironomid species (Bechard et al., 2008; CCME, 2006; Milani et al., 2003; USEPA, 1996; Watts and Pascoe, 2000). Comparison of the LC_{50} values showed that *C. striatipennis* was more sensitive to Cd and Pb than other aquatic insects such as stonefly and mayfly (William et al., 1985). Acute toxicity results from this study showed higher Cd toxicity on *C. striatipennis* than found for Pb and LC_{50}'s in similar reported findings (CCME, 2006; Suedel et al., 1997; US.EPA, 1996). The observed LC_{50} values of Cd and Pb showed high toxicity to the the first instar larvae followed by the second, third, and fourth instar larvae, respectively. The results were similar to other reported findings (Gills and Wood, 2007; Hooftman et al., 1989). This study showed that the second instars of midge larvae were a suitable stage organism to assess the ecotoxicology test because of its sensitivity as a test organism, suite size and being stronger than other instars of midge larvae. Williams et al. (1986) also reported that the second instar of midge was useful to assess the ecotoxicological test.

Chronic toxicity

Table 2 shows that the effect of sublethal Cd and Pb concentrations on emergence of the adult were reduced significantly ($P<0.05$) and that the number of females and males at 2.5 mg Cd/L and 50 and 5 mg Pb/L, respectively, were reduced. The LOEC of the number of emergent female and male was 2.5 mg Cd/L and NOEC was 0.5 mg Cd/L. The LOECs of Pb concentration for the number of emergent females and males were 50 and 25mg Pb⁄L; a NOEC of 25 mg Pb⁄L was obtained for the number of emergent females.

Table 1 Acute toxicity (Medium lethal concentration [LC_{50}]) of Cd and Pb on larvae of *C. striatipennis* at 48 h

Larvae instar	48h LC_{50} of Cd (95% C.I)	48h LC_{50} of Pb (95% C.I)
First	25.10 (14.61 - 40.67)	99.89 (14.68 - 209.13)
Second	201.30 (60.05 - 367.03)	555.49 (265.69 - 896.62)
Third	579.31 (536.74 - 620.07)	1,539 (1,201- 1,874)
Fourth	641.12 (564.30 -717.21)	3,720 (2,890 - 4,405)

Table 2 Effect sublethal Cd and Pb concentrations on the number of female and male *C. striatipennis* (n=300)

Chemical	Concentration	Number of emergence (number)		Emergence (%)	
	(mg/L)	Female	Male	Female	Male
Cd	Control	6.83 ± 0.75^{c}	7.17 ± 0.75^{b}	2.2	2.39
	0.5	6.50 ± 1.05^{c}	6.83 ± 1.17^{b}	2.17	2.28
	0.6	5.50 ± 0.55^{b}	5.33 ± 0.82^{a}	1.83	1.78
	5.0	4.50 ± 0.55^{a}	5.50 ± 0.84^{a}	1.50	1.83
Pb	Control	6.83 ± 0.75^{b}	7.17 ± 0.75^{bc}	2.28	2.39
	5	6.83 ± 1.47^{b}	7.50 ± 1.05^{c}	2.28	2.50
	25	6.67 ± 0.82^{b}	6.33 ± 0.82^{ab}	2.22	2.11
	50	4.83 ± 0.75^{a}	5.50 ± 0.55^{a}	1.61	1.83

Note: Values are mean ± standard deviation. Means with the same letter in the same column are not significantly different (P>0.05)

The effect of sublethal Cd and Pb concentrations on length, development time and dry weight of *C. striatipennis* are shown in Table 3. Length of midge larvae in the control and 3 additional concentrations of 0.5, 2.5 and 5 mg Cd/L and 5, 25 and 50 mg Pb/L showed no significant reduction (P>0.05) in length of the first to fourth instars of *C. striatipennis*.

The development time of the second, third and fourth instars of *C. striatipennis* was reduced significantly (P<0.05) at 0.5 mg Cd⁄L and higher concentrations. Lead concentrations at 0.5, 5 and 25 mg⁄L significantly reduced (P<0.05) the development time of the first, third instars, and second and fourth instars of *C. striatipennis*, respectively (Table 3). The development time is also an important endpoint for the chironomid as it reflects larval growth to development emergence data that were also similar to those found in other studies (Sikanchadra and Crane, 2000; Suedle et al., 1997). For the development time at each Cd concentration on the second, third and fourth instar of *C. striatipennis*, a lowest observed concentration (LOEC) of 0.05 mg⁄L was obtained and for Pb concentrations on the second and third instars, a LOEC of 5 mg and 50 mg⁄L was found while the LOEC of the third instar was 50 mg⁄L and no observed effects of concentration (NOEC) for Pb at 25 mg⁄L were obtained.

No difference in reduction of the dry weight (P>0.05) of *C. striatipennis* was observed between control and treatment with Cd concentration but in the fourth instars of *C. striatipennis* there was a significant reduction on dry weight (P<0.05) with Pb at 5 and 25 mg⁄L (Table 3). The LOEC of Pb concentration on fourth instar was 50 mg⁄L.

Table 3 shows that the effect of sub lethal Cd and Pb concentrations significantly reduced emergence of the adults (P<0.05) and that the number of females and males at 2.5 mg Cd⁄L and 50 and 5 mg Pb⁄L, respectively, were reduced. The LOEC of the number of emergent females and males was 2.5 mg Cd⁄L and NOEC was 0.5 mg Cd⁄L. The LOECs of Pb concentration for the number of emergent of female and male were 50 and 25mg Pb⁄L; a NOEC of 25 mg Pb⁄L was obtained for number of emergent females.

Table 3 Effect of sub lethal Cd and Pb concentrations (mg/L) on the body length (mm), the development time (days) and the dry weight (mm) of instar larvae of *C. striatipennis*

(Effect)	Concentration of Cd (mg/L)				Concentration of Pb (mg/L)			
Instar	Control	0.5	2.5	5	Control	5	25	50
(Length)								
1st	2.21±0.46	1.21±0.30	1.17±0.24	1.20±0.30	1.18±0.25	1.21±0.29	1.13±0.22	1.04±0.13
2nd	3.88±1.17	2.31±0.42	2.16±0.41	2.27±0.40	2.21±0.46	2.24±0.37	2.06±0.31	1.88±0.34
3rd	8.60±1.83	4.00±1.26	3.41±0.73	3.61±1.14	3.88±1.17	3.68±1.06	3.24±0.78	3.01±0.51
4th	6.65±0.47	8.72±1.80	7.91±2.03	8.17±2.08	8.60±1.83	8.22±1.95	7.61±2.16	7.59±2.09
(Development time)								
1st	6.65±0.47	6.63±0.66	6.62±0.51	6.62±0.42	7.00±0.47[d]	6.50±0.50[c]	6.00±0.23[b]	5.00±0.12[a]
2nd	3.17±0.38[a]	3.50±0.50[b]	3.50±0.50[b]	3.50±0.50[b]	3.17±0.38[b]	3.00±0.12[a]	3.00±0.00[a]	3.15±0.36[b]
3rd	4.33±0.48[d]	4.00±0.22[c]	3.83±0.13[b]	3.67±0.48[a]	4.33±0.48[b]	4.33±0.75[b]	4.17±0.91[b]	3.67±0.48[a]
4th	8.67±0.75[c]	8.17±0.38[b]	7.50±0.97[a]	7.33±1.11[a]	8.67±0.75[c]	7.17±0.38[b]	7.00±0.11[a]	7.18±0.39[b]
(Dry weight)								
1st	0.03±0.01	0.03±0.00	0.04±0.00	0.04±0.01	0.03±0.01	0.03±0.01	0.04±0.00	0.05±0.00
2nd	0.10±0.00	0.07±0.02	0.15±0.06	0.11±0.05	0.10±0.01	0.05±0.01	0.08±0.01	0.08±0.01
3rd	0.11±0.03	0.16±0.00	0.12±0.05	0.10±0.00	0.11±0.03	0.16±0.03	0.26±0.01	0.17±0.11
4th	0.30±0.01	0.26±0.02	0.24±0.02	0.21±0.10	0.30±0.01[a]	0.21±0.10[b]	0.31±0.00[a]	0.42±0.05[b]

Note: Values are mean ± standard deviation. Means with the same letter in the row are not significantly different to control ($P>0.05$)

CONCLUSION

The development of a method using an indigenous Chironomid species, *C. striatipennis*, as a bioindicator and aquatic organism for ecotoxicology tests is reported as a biomonitoring tool for evaluation of heavy metal contamination in the aquatic ecosystem of Thailand. Compared to lead, cadmium had higher acute toxicity to the first instar larvae followed by second, third, and fourth instar larvae of *C. striatipennis*, respectively. Sub lethal effects of Cd and Pb concentration significantly reduced the length of larvae, the development time for larvae, dry weight and the number of emergent females and males. These endpoints are also important for Chironomids because they reflect larval growth and the development of the emergent Chironomid species and its abundance in the aquatic ecosystem.

The results showed that *C. striatipennis* was sensitive to Cd and Pb concentrations. Thus this species is suitable as a bioindicator and as a test organism for aquatic ecosystem assessment. However, revision of water quality guidelines is required to provide more precise and conclusive toxicology data for heavy metals on other indigenous aquatic organisms.

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