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Toxicity of cadmium and lead on tropical midge larvae, *Chironomus kiiensis* Tokunaga and *Chironomus javanus* Kieffer (Diptera: Chironomidae)

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ABSTRACT

Objective: To investigate the acute toxicity of cadmium and lead on larvae of two tropical Chironomid species, *Chironomus kiiensis (C. kiiensis)* Tokunaga and *Chironomus javanus (C. javanus)* Kieffer. **Methods:** Different larval instars (first-fourth) were exposed using a static non-replacement testing procedures to various concentrations of cadmium and lead. **Results:** In general, younger larvae (first and second instars) of both species were more sensitive to both metals than older larvae (third and forth instars). The toxic effects of the metals on *C. kiiensis* and *C. javanus* were influenced by the age of the larvae (first to fourth instars), types of metals (cadmium or lead) and duration of larval exposure (24, 48, 72 and 96 h) to the metals. **Conclusions:** Cadmium was more toxic to the chironomids than lead and *C. javanus* was significantly more sensitive to both metals than *C. kiiensis* ($P<0.05$).

1. Introduction

Good selection of test organisms in laboratory bioassays is an important criterion for a reliable assessment of the anthropogenic activities. Invertebrates which are either environmentally or economically important such as daphnids, amphipods, mayflies, stoneflies and chironomids are frequently used in aquatic assessments[1]. Among the invertebrates, Chironomidae (Insecta: Diptera) is a key family in most freshwater ecosystem. It accounted for 80% of total insect secondary production in northern Indiana stream[2] and a healthy population may reach a density of up to 70 000 larvae in a square meter[3]. In Malaysia, *Chironomus kiiensis (C. kiiensis)* is commonly found in paddy fields[4,5] and Chironomidae is the dominant aquatic insect family in river ecosystem[6-9].

Larvae of non-biting midges (Chironomidae: Diptera) are widely distributed, sensitive to many pollutants, relatively easy to culture and have a short life cycle.

These criteria make them suitable organisms for ecotoxicological monitoring[4,5]. Several species of *Chironomus* such as *Chironomus tentans (C. tentans)*, *Chironomus riparius (C. riparius)* and *Chironomus plumosus (C. plumosus)* are widely used for toxicity assessment in temperate countries and their culturing techniques have been well established[1,10]. However, they are not readily found in tropical areas as their occurrences are restricted to cooler areas[10]. *C. kiiensis* and *Chironomus javanus (C. javanus)*, however, are the dominant species of the genus *Chironomus* in South East Asian countries including Malaysia, inhabiting rivers, wetlands and rice agroecosystems[5,7] This two species had been previously shown to be suitable organism for bioassesment and environmental monitoring[4,7].

In the present study, culturing technique and the effects of cadmium and lead on larvae of two common Malaysian *Chironomus* species; *C. kiiensis* Tokunaga and *C. javanus* Kieffer were investigated. Their sensitivity to these metals would justify them for use as test organisms in future toxicity testing in tropical areas especially in Malaysia.

2. Materials and methods

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2.1. Laboratory culture

Stock cultures of *C. kiiensis* and *C. javanus* were initiated from egg masses and adults collected from Minden, Penang, Malaysia in 2007 following the described method of Ali *et al* [11]. They were maintained in aquariums (121 cm× 55 cm×41 cm) in the aquatic laboratory of Universiti Sains Malaysia in Penang. A pure culture of *C. kiiensis* and *C. javanus* were obtained by separating egg masses of these midges and placing them into two different culture.

The aquaria were half-filled with de-chlorinated water and covered with a nylon screen. Continuous gentle aeration was provided using an air pump to maintain optimum oxygen level. To maintain a good water quality, 30% of the water volume was replaced weekly or when the water was cloudy.

The breeding chambers were completely drained after three months, cleaned and refilled to remove detrimental materials accumulated in the chambers and to ensure optimal productivity[12]. The culture was kept in 12D : 12L at room temperature (30 to 34 ℃).

Shredded tissue papers were soaked overnight in acetone to eliminate impurities and rinsed with culture water to remove the acetone. These shredded papers were provided at the bottom of the aquarium for the *Chironomus* larvae to construct cases. The larvae were provided with fish food flakes (TetraMin, Tetra-Werke, Germany). The concentrations of the TetraMin fish food flakes were maintained at 0.04 mg dry solids/mL of water[10].

2.2. Bioassay design

A test chamber was made of an 800 mL styrene plastic container, 80 mm high, 120 mm diameter at the top and 90 mm at the bottom. Twenty one test chambers were prepared for testing six concentrations and a control of each of the metal with three replicates of each concentration tested. The concentrations of lead used were 0, 6.3, 15.8, 39.8, 63.1, 100.0 and 158.5 mg/L, while for cadmium, the concentrations were 0, 2.5, 6.3, 15.8, 25.1, 39.8 and 50.1 mg/L.

The static test was initiated with placing the organisms in the test chamber within 30 min after the test materials were added to the seasonal water that made up the final volume of 300 mL. Ten larvae (instars I to IV) were randomly selected and immediately placed into chambers.

The test organisms were fed daily with TetraMin fish food flakes, blended with culture water to form initial slurry. Every test chamber was provided with food concentration of 0.04 μg dry solids/ml of test water. Excess food was removed daily using a pipette. The tests were carried out under photoperiod of 12L : 12D.

Aeration was provided when the dissolved oxygen dropped to below 4.0 mg/L. Mortality data were recorded at 24, 48, 72 and 96 h after treatment with different metal concentrations and water quality parameters such as dissolved oxygen, temperature, pH and salinity were recorded daily. The organisms were considered dead when they did not respond to gentle prodding (gentle touching with a glass rod) and remained immobile.

2.3. Statistical analysis

Median lethal concentrations (LC_{50}) at different exposure periods (24 h, 48 h, 72 h and 96 h) were estimated using the Probit Analysis. The data obtained from the different experiments were analyzed for diference in mean using Analysis of Variance (ANOVA)[13].

3. Results

3.1. Water quality

Throughout the toxicity tests, pH and temperature were (7.3 ± 0.5) and (26.8 ± 0.5) ℃, respectively. The dissolved oxygen was maintained at (6 ± 1) mg/L.

3.2. Acute toxicity of lead

Survival in all controls exceeded 96% after the 96 h test period. In general, the larvae showed gradual tolerance to lead as they advanced in age (Appendix 1). The last (4th) instar larvae were the most tolerant stage for both species (Table 1). *C. kiiensis* was more tolerant than *C. javanus* as the LC_{50} values of *C. kiiensis* were generally much higher than that of *C. javanus*.

The LC_{50} values of the first instar decreased with time for both species from 137.29 mg/L (24 h) to 18.33 mg/L (96-h) and from 190.82 mg/L (24 h) to 17.30 mg/L (96 h) for *C. javanus* and *C. kiiensis*, respectively. Both species showed higher LC_{50} values for the second instars than the first instar but the difference was not statistically significant (F=0.305, P>0.05).

After 24 h exposure, the difference in LC_{50} values of the second instar of the two species was lower compared to the difference for the first instar (Table 1). For the third instar larvae, the difference was two-fold higher than the recorded value for the second instar.

This result revealed that from the third instar onward, the tolerance of these *Chironomus* to chemical exposures increased considerably. Comparing the response of all instars of both species at various exposure durations, *C. kiiensis* was more tolerant to lead than *C. javanus*. However, the second instar of *C. javanus* was more tolerant at 48 to 96 h exposures and its third instar was more tolerant than *C. kiiensis* at 96 h exposure. During the first 24 h of exposure, no mortality was recorded for *C. kiiensis* even at the highest lead concentration. As exposure time increased, the LC_{50} values for both species showed a gradual reduction, resulting in similar mortality at 96 h exposure.

These findings suggest that during prolonged exposure to lead, the second and third instars of *C. javanus* were more tolerant than *C. kiiensis*. The fourth instar of the later species was more tolerant to lead.

3.3. Acute toxicity of cadmium

The result of median lethal concentrations (24 h, 48 h, 72 h and 96 h LC_{50}'s) indicated vast differences between

Table 1
Toxicity of lead and cadmium to *C. kiiensis* and *C. javanus* larvae at different exposure periods (mg/L).

Exposure period (h)	Larval stage (instar)	Lead LC_{50} (95% CL)		Cadmium LC_{50} (95% CL)	
		C. kiiensis	*C. javanus*	*C. kiiensis*	*C. javanus*
24	1st	190.82 (122.25–424.92)	137.29 (98.73–233.99)	83.34 (58.58–146.99)	18.35 (15.41–21.90)
	2nd	214.23 (134.14–510.72)	206.33 (139.26–417.41)	243.23 (344.97–763.35)	161.78 (77.12–881.10)
	3rd	1 502.11 (538.54–1 526.70)	508.54 (294.32–711.32)	145.96 (85.50–1 569.71)	561.08 (109.65–2 568.00)
	4th	9 500.92 (*)	667.92 (347.68–790.81)	2 353.78 (*)	589.20 (196.66–1 062.26)
48	1st	66.21 (53.33–88.53)	50.27 (37.44–75.76)	44.95 (21.68–942.33)	6.96 (4.38–9.69)
	2nd	83.99 (66.98–115.59)	134.84 (79.58–415.53)	80.96 (58.54–135.45)	7.78 (3.86–12.11)
	3rd	594.81 (289.59–2 585.92)	463.97 (259.55–567.74)	116.81 (78.33–241.96)	20.20 (6.21–177.03)
	4th	6528.07 (1 062.37–6 574.01)	417.84 (240.99–549.33)	132.75 (83.35–603.21)	21.86 (18.21–26.62)
72	1st	35.51 (30.03–42.41)	26.61 (20.37–34.18)	17.97 (9.51–36.47)	4.65 (2.89–6.54)
	2nd	56.44 (45.69–74.13)	58.25 (41.47–99.27)	31.46 (22.69–49.85)	4.83 (2.01–7.82)
	3rd	223.61 (127.61–670.19)	118.48 (89.23–176.12)	80.03 (56.87–137.90)	8.69 (4.12–14.09)
	4th	289.04 (172.78–730.77)	190.87 (130.28–350.72)	221.87 (109.74–963.50)	10.98 (8.97–13.15)
96	1st	17.30 (10.26–24.61)	18.33 (14.41–22.37)	7.96 (2.41–14.72)	3.55 (2.13–4.93)
	2nd	27.65 (22.33–33.96)	28.37 (22.90–34.98)	13.70 (9.21–19.59)	4.02 (1.78–6.32)
	3rd	51.95 (37.79–74.64)	62.90 (51.30–79.12)	28.74 (23.79–35.93)	4.86 (2.64–7.18)
	4th	118.80 (87.93–182.48)	106.32 (78.95–161.29)	43.81 (32.99–65.61)	6.94 (3.59–10.54)

LC_{50} = 50% lethal concentration
CL= Confidence limits
* = The 95% confidence limits were not available
Note: mean results of 9 replicates from 3 different experiments with 10 larvae per treatment.

instars and species (Table 1). Significant differences were found between the LC_{50} values of cadmium for the two species at equivalent exposure periods, with *C. javanus* being the more sensitive species to cadmium (F=12.353, P<0.05). Different life stages of *C. javanus* and *C. kiiensis* showed different response to cadmium exposure.

The first instar of *C. javanus* was the most sensitive among all instars tested. All larval stages of *C. javanus* and *C. kiiensis* showed similar trends in their responses at 24 h, 48 h, 72 h and 96 h after treatment at different concentrations of cadmium. As expected, the 96 h LC_{50} values were generally lower than those obtained at 24 h. This was obvious for the last instar of both species (Table 1).

4. Discussion

Body size and life stage usually have a significant influence on toxic responses. Basiclly, later larval stage of larger body sizes showed lower sensitivity to toxicants. The 96 h LC_{50} values recorded in the current study were found to have similar pattern with previous studies which indicated that the values decrease over exposure period with the lowest values for the earlier life stages. Study of Khangarot and Ray[14] using *C. tentans* proved part of this statement as the LC_{50} values were found to decrease with increase in the exposure time. Information on lead toxicity to chironomids is scarce.

In this study, both *C. kiiensis* and *C. javanus* larvae were tolerant to lead which is in agreement with several previous studies that reported LC_{50} values of 50 to 350 mg/L[15,16]. The late instar larvae of both species were resistant to cadmium compared to the other earlier life stages. This finding is in agreement with previous toxicity studies using different age groups within the same species. Brix *et al*[17] reported that the fourth instar of *C. riparius* was approximately 952 times more resistant than the first instar according to the 24 h LC_{50} values. The LC_{50} value was recorded 2 000 mg/L for the fourth instar and 2.1 mg/L for the first instar. However, the difference in LC_{50} between larval stages of *C. kiiensis* and *C. javanus* in the present study was lower over the exposure period. For *C. kiiensis*, the fourth instar was approximately 28 times more resistant than the first instar and 32 times for *C. javanus* based on the 24 h LC_{50} values.

The resistance ratio of the fourth to first instar was reduced after 96 h of exposure as the fourth instar was approximately 5 times more tolerant than the first instar for *C. kiiensis* and about 2 times for *C. javanus*.

The result from the acute toxicity test demonstrated that the toxicity was dependant on the toxicant type and concentration. The larvae of *C. javanus* and *C. kiiensis* were found to be tolerant to lead compared to cadmium. Moreover, the fourth instar of both species was the most tolerant among larval stages tested. Previous studies have shown that smaller individuals have higher concentration of metals than larger individual because smaller organisms have larger surface area to volume ratio which can lead to faster chemical uptake per unit weight.

A similar trend was found in several annelids, such as polychaetes. Bryan and Hummerstone[18] found that smaller polychaetes such as *Heteromastus filiformis* tend to accumulate more metals per unit body weight than larger species, *Perinereis aibuhitensis*. In our study, *C. javanus* and *C. kiiensis* showed tolerant behaviour as they reached later larval stages and the younger instars were more vulnerable to lead and cadmium exposure.

The results of the comparative study using *C. javanus* and *C. kiiensis* indicated that both species can withstand the acute exposure of cadmium and lead. Even though the body size of *C. javanus* is bigger and longer than *C. kiiensis*, the latter species was found to be more tolerant to cadmium and lead exposure. This inverse relationship between the weight of animal soft tissue and metal has been found in some other species. Mouneyrac *et al*[19] analyzed cadmium, copper and zinc or metallothionein–like protein concentrations in the whole soft tissues of oysters, *Crassostrea gigas* and found a positive correlation with body size. Similarly, Martin[20] analyzed various elements in whole–body samples of the crab *Cancer irroratus* and found that only Mn exhibited a significant positive correlation with body size.

The pattern of response in *C. javanus* and *C. kiiensis* was similar although there were slight differences in the LC_{50} values. This does not hinder the application of either species in the toxicity tests because the determination of species sensitivity was the instar stages and toxicants rather than the species[21].

Conflict of interest statement

We declare that we have no conflict of interest.

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