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Mentum Deformities of Chironomid larvae in Huai Kakang stream (Northeastern Thailand) and Lead Exposure Effect

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Abstract

Deformities in the mouthparts of Chironomidae larvae, particularly of the teeth on the mentum, have been proposed as a bioindicator of anthropogenic impacts on water quality. Therefore, this study was used the deformities of Chironomid larvae for assessing water quality of Huai Kakang stream (Maha Sarakham Province, Thailand). The effect of lead exposure in the mentum deformities of *Chironomus* spp. was also studied to confirm the relationship between the toxicant and the deformities. Water, sediment and chironomids larvae, were collected from Huai Kakang stream. Water and sediment were analyzed for nitrate (NO_3^-), total phosphate (TP), chemical oxygen demand (COD) and heavy metals (i.e. Cd, Cu, Ni, Pb and Zn). Chironomids larvae were examined for the mentum deformity. For the bioassay test, the larvae were raised in 4 different concentrations of lead, including 0, 10, 20 and 30 mg_{Pb}/L. The results indicated that the lower stream site of Huai Kakang was polluted with the exceeding limits of Lead and cadmium. The 53.33% of chironomid larvae exhibited mentum abnormality. The bioassay test showed the high concentration of lead caused the larvae to reduce in number. The 50% lethal concentration (LC_{50}) was 34.74 mg_{Pb}/L. The experiment of 20 mg_{Pb}/L showed the highest percentage of deformity and bioaccumulation of chironomids.

Introduction

Most water quality monitoring tools employed in Thai legislation are mainly along Physicochemical parameters, with the exception of *Escherichia coli*. However, environmental protection agency and academic intuitions are working on using biological indicators in biomonitoring programs in the country. Most biological water quality monitoring tools employed in Thailand are based on community response to water quality impairments. However, community structure assessments are essentially measures of lethality and concentrations of contaminants must be high enough to result in the disappearance, or reduced abundance and diversity of sensitive taxa before the community response approach would detect water quality stressors (Odume et al., 2012). The morphological deformities in aquatic animals such as the gill abnormality of hydropsychids

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larvae (Vouri & Kokkonnen, 2002; Prommi & Thamsenanupap, 2013), or mouthpart deformity of chironomids larvae (Al-Shami et al., 2011; Odume et al., 2012; Thani & Prommi, 2017) were used and represent sub-lethal response to in-term pollutants and are considered early warning indicators of water quality deterioration.

The Chironomidae are considered to be the ideal bioassay organisms since they spend most of their life stage in water where they are exposed to toxicants in both water and sediments. When continuously exposed to the stress of pollution, late instar of chironomid larvae frequently develop deformities in their mouthparts, especially mentum (Al-Shami et al., 2011). Therefore, the application of chironomids' mentum deformity as bioindicators of pollution stress have been published and illustrated primarily for bioassessment purposes.

In Thailand, a few papers on mentum abnormality of chironomids were published, such as Thani & Prommi (2017) which studied the use of mentum deformity of Chironomidae to indicate environmental perturbation in freshwater habitats. No published paper was studied on the toxicity test. Therefore, this paper aims to 1) study the effect of anthropogenic impacts on the Huai Kakang stream based on the mentum deformity in Chironomidae and 2) conduct an experiment on lead exposure in *Chironomus* spp. and their effects on the mentum deformities. The experiment was conducted to clarify that the deformity caused by the pollutants was found in the site's survey.

Materials and methods

The research was conducted for 2 studies. The first study was conducted to survey the deformity of chironomids' mentum and together with water quality parameters to assess Huai Kakang stream quality. The second study was an experiment on lead exposure of *Chironomus* spp. The latter study was conducted in a purpose of explanation of mentum deformity and metals concentration relations. Details of methodology were as follows.

1. A survey on mentum deformities of *Chiromomus* spp. in Huai Kakang stream

1.1 Site Descriptions

Huai Kakang stream has an estimated 43 km length, and originates from Bann Kok Koh, Maha Sarakham Province and flows to Chi river at Roi-et Province, Thailand. This stream is the main water resource for local people for many activities, including agriculture, aquaculture, recreation and for receiving wastewater. In this study, the sampling sites were two sites at Haui Kakang stream, Maha Sarakham Province (Fig. 1). The first site represented the upper stream site, where was chosen for a reference site. This site is located at the coordination of 16°04'26.4"N 103°15'39.7"E, where is surrounded by agricultural area and small villages. The second site is the lower stream site, where is located at the coordination of 16°11'26.4"N 103°18'27.1"E. This site has directly received untreated community wastewater from Maha Sarakham municipality. Therefore, this site was chosen for an impacted site.

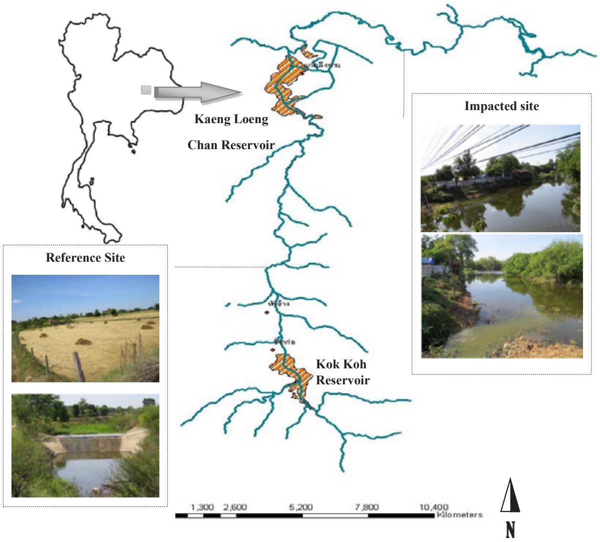

Fig. 1 Map of sampling site located at Huai Kakang stream in Maha Sarakham Province, Thailand.

1.2 Field Sampling and investigate

At each site, water was sampled using water sampler and placed in polyethylene bottle and refrigerated at 4°C. Physicochemical parameters were analyzed in laboratory at Faculty of Environment and Resource Studies, Mahasarakham University, comprised with nitrate (NO_3^-), total phosphate (TP), chemical oxygen demand (COD), and heavy metals (i.e. Cd, Cu, Ni, Pb and Zn) (APHA, 1992).

Sediment sample was randomly collected for 1 kg. at each site. The samples were air dried, ground and sifted using 0.2 mm. sieve in the laboratory and prepared for analyzing heavy metals (i.e. Cd, Cu, Ni, Pb and Zn) using Flame Atomic Absorption Spectrophotometer (AAS).

After finished water and sediment sampling, the chironomid larvae were also randomly sampled by hand picking technique in distance of 10-20 meters'of the stream's reach. A D-frame aquatic dip net was used for collecting the larval samples in a predominant find sand and silt habitats. The sampling was conducted to find as much as targeted chironomids in at least 30 minutes of investigate. The chironomid larvae were kept in 70% EtOH in glass vessel.

Each chironomids was transferred to a standard glass microscope slide. A head capsule was cut under the microscope and keeping the head capsule wet at all times by adding a drop or two drop of ethanol. The head capsule was transferred to 10%(w/v) NaOH in a beaker and soaked for 10-30 mins depending on size or recognized clear sample. The sample was move to slide with a drop of mounting media (i.e. resin) and then covered by a slip. Chironomid specimens were identified according to the number of ventral and/or anal tublues, setae, parapodia, paraligula, ligula and metum follow Simwiset et al. (2015). All menta were examined and evaluated for the presence of deformities and labeled. The type of deformity established according to the classification by Lenet (1993) and Reynolds & Ferrington (2001). Water, sediment and chironomid larvae were sampled 2 times during January and February 2016.

The incident of physicochemical parameters and mouthpart deformity of Chironomids was expected. The confirmation of this relation, laboratory bioassays was needed. Lead was the highest amount toxicant found exceeding the standard limits. Consequently, the experiment was on the exposure of *Chironomus* spp. to lead.

2. Experiment on lead exposure of *Chironomus* spp. and induction of mentum deformities

Before starting the experiment, chironomid larvae were cultured in concrete tube with 30 cm diameter and 40 cm height. One hundred grams of catfish feed were poured into the concrete tube, followed by 10 liters of freshwater. After chironomid female laid eggs for 2 weeks, 200 second instar larvae could be harvest for the experiment in one batch. The tube continued to cultivate the larvae in order to continue the experiment until it reached 2,000 individuals for use in the 10 batches of experiment. The larvae were fed using catfish feed once a week.

In the experiment, the second instar larvae were raised in 4 different concentration of lead mixtures, including 0, 10, 20 and 30 mg/L for 10 batches. The solutions were prepared from $Pb(NO_3)_2$. At each batch, 200 second instar larvae were separated for 4 concentrations (i.e. 50 individuals per treatment) and placed into 1 Liter glass jar covered with lid. The larvae were feed 2 grams of catfish feed per week. The experiment was conducted until the larvae grew up to the fourth instar. Then, lead solution and chironomids larvae were harvested and examined. Lead concentration in the solution and the larvae were analyzed for the residue concentration and bioaccumulation using Flame Atomic Absorption Spectrophotometer. The larvae were kept in 70% EtOH and examined under microscope. The harvested larvae were examined and identified to species and use for bioassay experiment based on Simwiset et al. (2015). All menta were examined and evaluated for the presence of deformities and labeled.

Growth rate, 50% lethal concentration (LC_{50}) and bioaccumulation factor (BAF) were calculated. LC_{50} was calculated by regression equation. While bioaccumulation factor (BAF) calculation was followed Demina et al. (2009)- i.e. concentration of lead in organisms divided by concentration of lead in water.

Results

1. A survey on mentum deformities of *Chiromomus* spp. in Huai Kakang stream

1.1 Analysis of Physicochemical parameters

The main physical and chemical characteristics of the sampling station are shown in Table 1. According to the general water quality parameter, they were not in excess of the standard limits established by the National Standard Surface Water. However, the values were closed to the limits in both sites, especially COD and orthophosphorus, which indicated polluted water.

Upon analysis of the heavy metals in the water, all metals were detected, only Pb and Cd were detected at the sampling sites exceeding the limits specified as the maximum permissible established by the National Standard on Freshwater Sediment.

All the metals analyzed were detected in the sediments- i.e. Pb, Zn, Cd, Cu and Ni. The metals detected at both sites were not distinct different. Therefore, the water quality assessment indicated that Huai Kakang stream was polluted from the upper through the downstream. And the upper stream could not be called for the reference site.

Table 1 Physicochemical variables of water and sediment, average and standard deviation (SD), measured at the two sampling sites in the Huai Kakang stream

Parameters	Standard limits	Mean ± S.D.	
		Upper stream site	Lower stream site
pH in water	5.0-9.0	6.56 ± 0.09	7.54 ± 0.08
DO	Not lower than 6.0 mg/l	1.95 ± 1.70	3.53
Water temperature	-	31.05 ± 1.48	33.35 ± 0.3
COD (mg/L)	Not above 120 mg/l	111.80 ± 36.10	77.76 ± 5.40
Nitrate (mg/l) in water	Not above 0.5 mg/l	-	-
Orthophosphate (mg/L) in water	0.01-0.1 mg/l	0.51 ± 0.50	0.61 ± 0.50
Pb in water	Not above 0.05 mg/L	0.19 ± 0.01	0.19 ± 0.01
Pb in sediment	Not above 400 mg/Kg	16.8 ± 10.1	14.25 ± 5.5
Zn in water	Not above 1.00 mg/L	0.28 ± 0.03	0.25 ± 0.03
Zn in sediment	-	31.95 ± 24.40	31.50 ± 19.80
Cd in water	Not above 0.005 mg/L	0.07 ± 00	0.07 ± 00
Cd in sediment	Not above 37 mg/Kg	3.60 ± 00	3.60 ± 00
Cu in water	Not above 1.00 mg/L	0.13 ± 00	0.12 ± 00
Cu in sediment	-	12.50 ± 00	13.05 ± 7.00
Ni in water	Not above 0.1 mg/L	0.09 ± 00	0.09 ± 00
Ni in sediment	Not above 1,600 mg/Kg	7.70 ± 00	6.90 ± 2.80

1.2 *Chironomus* spp. throughout a polluted gradient

In this study the following 5 species were found in Huai kakang stream, including *Chironomus calligaster, C. circumdatus, C crassiforceps, C. javanus, C. kiiensis.* At the upper stream site, 3 species- i.e. *C. calligaster, C. crassiforceps, C. kiiensis* were found, while all 5 species were found at the lower stream sites.

Total 956 of *Chironomus* spp. larvae from Huai Kakang were counted and analyzed, and shown in table 2. *Chironomus* spp. were found at the upper stream site in higher number (i.e. 656 individuals) than the lower stream site (i.e. 300 individuals). As to mentum deformity in *Chironomu*s spp., individual from the upper stream site (n = 458) had no alteration in their mount structure, while 171 individuals exhibited metum deformity (26.07%). At the lower stream site, 300 individuals

Table 2 Summary of the number of larvae sampling and the number and percentage of mentum deformity registered at the Huai Kakang stream

	No. of *Chironomus* spp. in the upper stream site		No. *Chironomus* spp. in the lower stream site	
	Normal	Deformities	Normal	Deformities
1st sampling	190 (63.33%)	110 (36.67%)	194 (64.67%)	106 (53.33%)
2nd sampling	295 (79.67%)	61 (20.33%)	-	-
Total	485 (73.93%)	171 (26.07%)	194 (64.67%)	106 (53.33%)

collected, 106 individuals exhibited mentum abnormality (53.33%).

2. Experiment on lead exposure of *Chironomus* spp. and induction of mentum deformities

Two thousand larvae were used in the experiments. They were identified into 4 species, comprise with *C. circumdatus, C. kiiensis, C. crassiforceps* and *C. javanus. C. circumdatus* was the most abundant species, and also showed the highest percentage of mentum deformities (Table 4).

The chironomid mouthpart deformities were clearly induced after being exposed to lead solutions. All abnormal menta showed the same characters of deformity, such as distinct asymmetry, large gap or missing teeth. At treatment of 20 mg/L of lead concentration, the larvae had the highest percentage of deformity.

The growth of chironomids showed a decrease in population. Therefore, the death rate was calculated. The death rate of chironomids in concentration of 0, 10, 20 and 30 mg_{Pb}/L were -0.06617, -0.06911, -0.07962 and -0.11568 individual/day, respectively. It indicated that the high concentration of lead caused the larvae to reduce in number (Table 3). The 50% lethal concentration (LC_{50}) was 34.74 mg_{Pb}/l, which was calculated by regression equation shown in Fig 2. The LC_{50} value closed to the experiment of 30 mg_{Pb}/L.

Table 3 Number and percentage of Chironomids larvae exposed to the different lead concentration found dead, emerged, normal mentum and deformed mentum

Lead concentration (mg_{Pb}/L)	Dead		Emerged		Normal		Deformed		Total number (ind.)	Death rate (ind./day)
	no.	%	no.	%	no.	%	no.	%		
0	302	60.4	72	14.4	100	20.0	26	5.2	500	-0.06617
10	310	62.0	47	9.4	110	22.0	33	6.6	500	-0.06911
20	336	67.2	45	9.0	83	16.6	36	7.2	500	-0.07962
30	401	80.2	35	7.0	51	10.2	13	2.6	500	-0.11568

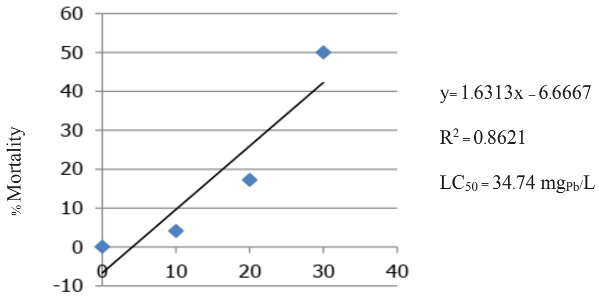

Fig. 2 Regression model on lead concentration (mg_{Pb}/L) against the percentage of mortality

The concentration of lead accumulated in the larvae was shown in table 5 and indicated that the deformed mentum larvae accumulated more high concentration than the normal larvae, especially the experiment of 20 and 30 mg_{Pb}/L. While the lower concentration (0 and 10 mg_{Pb}/L) showed less number of deformed menta larvae than the normal larvae.

The larvae in the experiment of 0 mg/L was also found the accumulated lead concentration. These may be caused by many issue such as feed and substrates used in the experiment (Mergalli et al., 2000).

Table 4 Incidences of mentum deformities among Genus Chironomus exposed to the different lead concentration

Lead concentration (mg_{Pb}/L)	Chironomid species	Normal		Deformities	
		No. of larva sampled	%	No. of larva sampled	%
0	*C.kiiensis*	14	73.68	5	26.32
	C.javanus	1	1.00	0	0
	C.cassiforceps	4	80.00	1	20.00
	C.circumdatus	81	80.20	20	19.80
	Total/average	**100**	**79.36**	**26**	**20.64**
10	*C.kiiensis*	9	81.82	2	18.18
	C.javanus	4	80.00	1	20.00
	C.cassiforceps	1	100.00	0	0
	C.circumdatus	96	76.19	30	23.81
	Total/average	**110**	**76.19**	**30**	**23.81**
20	C.kiiensis	6	66.66	3	33.34
	C.javanus	7	77.77	2	22.23
	C.cassiforceps	7	77.77	2	22.23
	C.circumdatus	63	68.48	29	31.52
	Total/average	83	69.75	36	30.25
30	C.kiiensis	4	80.00	1	20.00
	C.javanus	0	0	0	0
	C.cassiforceps	1	100.00	0	0
	C.circumdatus	46	79.31	12	20.69
	Total/average	**51**	**79.69**	**13**	**20.31**

Bioaccumulation of lead in *Chironomus* spp. larvae, which showed incidences of mentum deformities, were shown in Table 5. The experiment of 20 mg_{Pb}/L showed the highest lead concentration in the larvae of 24,863.64 mg_{Pb}/Kg wet weight/individual. The calculation result of bioaccumulation factor was 3,477.43.

Table 5 Bioaccumulation of lead in mentum deformity *Chironomus* spp. larvae and in water

Lead concentration (mg_{Pb}/L)	Bioaccumulation of Lead in Chironomids (mg_{Pb}/Kg wet weight/ individual)		Actual Lead Concentration in water (mg/L)		Bioaccumulation Factors of deformity larvae (BAF)
	Normal	Deformity	Before Experiment	After Experiment	
0	654.52	636.48	1.54	4.89	130.16
10	1420.95	657.18	8.29	5.89	117.77
20	7,964.14	24,863.64	15.75	7.15	3,477.43
30	2,435.78	4,114.13	18.08	8.67	280.94

Discussion

Many publications –e.g. Thani & Prommi (2017), Di Veroli et al. (2010), Odume et al. (2012)- indicated the relations of the presence of pollutants in freshwater habitats and mouthpart deformities. The result of this study showed the same trends of those publications in strongly supported that the deformity can be used in the bioassessment work. The screening *Chironomus* communities for the deformities in Huai Kakang stream indicated the habitat perturbation by community activities caused notable impact on water quality at all stream reach as seen by physicochemicals parameter and percentage of the deformities at the upper stream site. The percentage of deformities found in the upper stream and the lower stream sites, were 26.07% and 53.33%, respectively. This number were much higher than a deformity of Chironomids found in the freshwater ditches at Nakhon Pathom province (6-12%) (Thani & Prommi, 2017).

Many different pollutants suspected to be the majors concern of the deformities, i.e. orthophosphate (PO_4^{3-}), ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N), dissolved oxygen (DO), pH and water temperature (Thani & Prommi, 2017), heavy metals and pesticides (Di Veroli et al., 2010). In this study, metals-especially lead and cadmium, were found exceeding the limits specified as the maximum permissible and were suspected to be a major pollutant to cause the deformities in chironomids. Consequently, we conducted the experiment on lead exposure of *Chironomus* spp. and their mentum abnormality. On the experiment, *Chironomus circumdatus* was the common species in Maha Sarakham province, which showed the relationship with level of contaminant and the percentage of mentum deformity. We suggested using this species as a bioindicator for minoring Huai Kakhang stream water quality. Meregalli et al. (2000) said that assessing the presence of pollutants in the stream by monitoring mouthpart deformities is more suitable than using endpoints such as growth and survival. Our research showed the same results. The growth rate and LC_{50} studies indicated that the highest lead concentration caused the highest deformities of the mentum. While the percentage of deformities was found the highest percentage in the experiment of 20 mg_{Pb}/L, which the *Chironomus* spp. accumulated the highest bioconcentration of lead in the same experiment. The frequency of mentum deformity has been reported to reach 60% by Vermeulen et al. (1998), where as in the present in Huai Kakang stream was 53.33% in the impacted site, and in experiment the maximal number was 30.25% in the experiment of 20 mg_{Pb}/L. This suggested that pollution level at the Huai Kakang stream survey's sites were almost extreme. The bioaccumulation of lead concentration showed the same trend as the deformity. Again, this confirmed that biomonitoring using the mouthpart deformity of *Chironomus* spp. is more suitable. Surprisingly, Chironomids had the bioaccumulation factor (BAF) value of 3,477.43 it means a larva can accumulate lead into its body for about 3,400 times higher than water. That is why the scientist classified the Chironomidae as bioaccumulator. Another surprise was that high amount of lead accumulated in the larvae, which was much higher than the added amount. Still, we could not find a good explanation for this situation.

Conclusion

In conclusion, the stress of anthropogenic pollutions in Huai Kakang stream could be distinctly detected and may cause the problem for user in many activities, such as fishery, agriculture (normally use for rice and vegetable growing) and recreation. Therefore, the community wastewater management is urgently need for Huai Kakang stream.

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