Effects of cadmium on acetylcholinesterase activities and histopathology of African catfish (*Clarias gariepinus*) from contaminated fish farm in Mae Sot District, Tak Province, Thailand

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ABSTRACT: This study was aimed at evaluating the effects of cadmium (Cd) on bioaccumulation, acetylcholinesterase (AChE) activities and histopathological alteration in African catfish (*Clarias gariepinus*) from a contaminated fish farm in Mae Sot District, Tak Province, western Thailand. Along with water and sediment samplings, fish samples were collected from the contaminated fish farm after two, four and six months of cultivation. The Cd accumulation in liver and muscle of *C. gariepinus* were measured. Moreover, the effects of Cd on AChE activities in brain, kidney, liver, and muscle were studied. The results showed that Cd concentrations in water of Mae Tao Stream and fish pond ranged 0.001–0.038 mg/l, while in the sediments ranged 0.367–21.250 mg/kg. Cadmium concentration in the liver of *C. gariepinus* was higher than in the muscle, i.e., 0.190 mg/kg (range 0.150–0.270 mg/kg) and 0.030 mg/kg (range 0.020–0.040 mg/kg), respectively. AChE activities showed significant increase in the brain and the muscle and decrease in the kidney and the liver. In addition, after six months of fish cultivation, AChE activities were found significantly decreased in the liver. Furthermore, histopathological alterations were observed in the gills, the kidney and the liver after exposure, i.e., loss of all mucus membranes of the gills, distortion of capillaries of glomerulus in kidneys and large vacuoles in liver tissues. These results are useful for biomarker of Cd contamination in the aquatic environment.

KEYWORDS: cadmium, acetylcholinesterase, African catfish, histopathology, aquatic toxicology

INTRODUCTION

Cadmium (Cd) is a ubiquitous trace metal, biochemically classified as a nonessential element [1]. It occurs naturally in aquatic environments and is released as a result of anthropogenic activities such as mining [2]. Cd is a highly toxic metal to human and environment which can be contaminated in sediments and aquatic organisms [3]. The Cd contamination has been found in natural soils concomitantly with zinc (Zn) mining activities [4]. It can be exposed to human body through respiratory system and digestive tract by consumption of contaminated food and water [5], and eventually causing severe diseases (e.g. Itai-itai), renal destruction and death [6]. Moreover, Cd can be accumulated

in human body through the food chain, and inflows into aquatic ecosystems [4].

In Thailand, the highest Zn and Cd deposits were reported in Mae Sot District, Tak Province, a result of Zn mining activities that have been actively operating for more than 30 years [7]. Several researchers revealed that the concentrations of Cd in soil and sediment around Mae Tao Stream, Mae Sot District, Tak Province ranged from 0.84–7.86 and 3.40–284.00 mg/kg, respectively. The maximum Cd concentrations in those studies were about 100 times higher than the European Economic Community (EEC) Maximum Permissible (MP), and about 1800 times higher than the standard of Thailand [7, 8]. Moreover, Cd does not degrade easily into the environment, and can be bioaccumulated

in organism for several years after exposure to low levels [9].

Mae Tao Stream is the main water resource for agricultural crops of rice, soybean, corn, sugarcane and garlic cultivations in Mae Sot District, Tak Province, Thailand [10]. Moreover, the water resource is also used for domestic and aquaculture activities, especially fish farming. The heavy metals can be accumulated in fish tissues by the absorption along their gill surface and gastrointestinal tract wall, which can cause higher levels of toxic concentration in the body than their environment [11]. Cadmium usually accumulated less in gills since they are a temporary target organ of accumulation, unlike the digestive and reproductive organs [12]. Previous studies showed the alterations of AChE activities and histopathology in fish (e.g., Silver catfish [13] and White seabass [14]) depending on Cd concentration level. However, there are only few studies on the effects of Cd contamination in aquatic organism [7], especially, the effects of Cd on AChE activity and histopathological alteration in aquatic organisms due to Cd accumulation.

African catfish (*Clarias gariepinus*) was chosen for this study because of its importance in the aquaculture industry of Thailand. In 2016, production of Thailand was around 122 418 mt which ranked second after Nigeria when the global production was 231 094 mt [15]. The fish was also reported to have the genetically distinct stocks that are useful for selective breeding program [16]. Since the local farmers use water from Mae Tao Stream to supply their fish ponds in dry season, therefore, the investigation of Cd concentration in water, sediment and tissue samples of African catfish *C. gariepinus*, as well as, their AChE activities and histopathological alteration, are highly required.

MATERIALS AND METHODS

Study sites

The two sampling sites on Mae Tao Streams (MT1 and MT2) and the selected fish farms were located in Phra That Padaeng Sub-district, Mae Sot District, Tak Province, Western Thailand (Fig. 1). Site MT1 is located at the upstream of Padaeng Zinc Mining (16°40′4.57″N, 98°42′4.89″E) and was defined as reference site. Site MT2 is located in the Mae Tao Stream (16°40′18″N, 98°37′10″E) which passes through Cd contaminated area from the Zn mining. The selected fish farm is operated near MT2 and uses water from the stream as its water resource.

Water, sediment and African catfish sampling

Two week old juvenile African catfish, with unknown whole body Cd concentration (detection was not performed), were released into the pond in June 2015. Three replicates of water and sediment samples were collected from the fish farm bimonthly between August and December 2015. Those water and sediment samples were preserved in polyethylene bottles at 4 °C until laboratory analysis of Cd concentration was performed [17]. Twenty fish samples were collected bimonthly between August and December 2015. Fish samples were kept in a cool box for determination of AChE activities and histopathological alteration in their tissues and compared with fish samples with no Cd contamination.

Analysis of cadmium in water and sediment samples

Water samples were digested in nitric acid and filtered. The filtrate was diluted to 100 ml with deionized water [18]. Sediment samples were dried at 140 °C in a hot air oven for four hours, and then 500 mg of the sample was digested in a 2:1 $HClO_4 : NHO_3$ using an open tube digestion method and block digester [19]. The digested samples were diluted to 25 ml with deionized water. Cd concentrations were determined by using an atomic absorption spectrophotometer at 228.8 nm (AAS with LOD 0.003 mg/kg) [17].

Analysis of cadmium in African catfish samples

Fish samples were dissected to separate liver and muscles. The liver and muscles were dried at 80 °C for two days to constant weight and then digested in concentrated nitric acid at 110 °C for three days. All digested samples were diluted to appropriate concentrations for analysis using inductively coupled plasma optical emission spectroscopy (ICP-OES) [20].

Analysis of AChE activities in African catfish samples

Acetylcholinesterase activity was measured following Ellman et al [21]. Individually, 20 mg of brain, kidney, liver and muscle tissues were weighed and placed in separate homogenizing tubes in ice during homogenization. The samples were homogenized with 0.1 M Phosphate buffer saline (PBS, pH 8.0) 1 ml and centrifuged at 3500 rpm for 10 min. Next, 20 µl of the supernatant was mixed with 1.3 ml of 0.2 mM 5,5′-Dithiobis (2-nitrobenzoic acid) and 63 µl of 0.75 mMATChI. The solution was removed
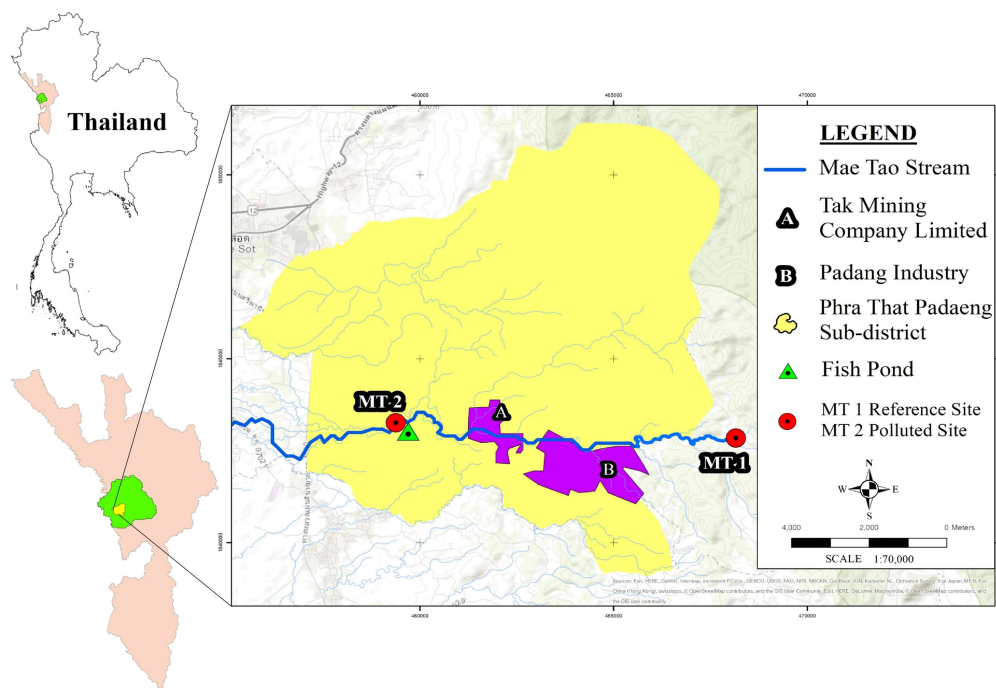
Fig. 1 Sampling sites on Mae Tao Stream and fish farm in Mae Sot District, Tak Province, Western Thailand.

to microplate 250 µl and analyzed at 405 nm every 2 min for 10 min using Elisa reader. All statistical analyses were accomplished by mean ± SD and one-way ANOVA using SPSS statistics version 17.0 (SPSS Inc., Chicago, IL, USA), with a significant threshold of $p < 0.05$.

Analysis of histopathological alteration in African catfish samples

Samples of gills, liver and kidney were dissected, separated and fixed in Bouin's fluid for 24 h. All tissues were washed several times in 70% ethanol, dehydrated in a graded series of ethanol, embedded in paraffin, and sectioned at 6–8 µm thickness. The sections were stained with hematoxylin and eosin staining, and then examined under light microscope [22].

RESULTS AND DISCUSSION

Cd concentrations in water, sediment and African catfish samples

Cd concentrations in water and sediment samples are shown in Table 1. The Cd concentrations in water ranged from 0.001 to 0.038 mg/l. The highest Cd concentration was found in fish farm. However, these concentrations did not exceed the values of the Surface Water Quality Standard of Thailand

(0.05 mg/l). The Cd concentration in sediments ranged from 0.367 to 21.250 mg/kg and the highest concentration was found in MT2 which was similar to other studies that Mae Tao Stream received contaminated sediment loading from Zn mining at the upstream into the downstream [7]. In addition, most of the sediments from Mae Tao Stream and fish farm were contaminated by Cd that clearly exceeded the European Maximum Permissible Levels (>3.0 mg/kg).

According to Cd concentrations in African catfish tissues, the Cd concentrations in muscle and liver of *C. gariepinus* were not exceeded the suggestion value (<0.5 mg/kg) from FAO [23]. However, these results indicated that Cd can be accumulated more in liver than muscle (Table 1). This agreed with several previous studies in *C. gariepinus*, *Rhamdia quelen*, and in *Tilapia nilotica* [1, 13, 24]. In this study, the highest Cd concentration was found in liver of *C. gariepinus* after rearing in the contaminated fish farm for two months and gradually decreased at four and six months of exposure. The increase of Cd level in liver was due to Cd detoxification by metallothioneins (MT) binding activity in the liver [1, 25]. Moreover, several studies have shown that the liver is the most important organ for Cd detoxification in fish [3, 12]. In the liver, Cd concentration increases rapidly until its capacity

Table 1 Cadmium concentration in water, sediment and fish organs collected from Mae Tao Stream and the fish farm.

Sampling site	Duration time of exposure		
	Aug (2 months)	Oct (4 months)	Dec (6 months)
MT1			
water (mg/l)	0.001 ± 0.001	0.007 ± 0.003	0.004 ± 0.001
sediment (mg/kg)	1.117 ± 0.321	1.333 ± 0.161	0.367 ± 0.104
MT2			
water (mg/l)	0.008 ± 0.004	0.002 ± 0.001	0.006 ± 0.001
sediment (mg/kg)	18.900 ± 8.835	13.183 ± 1.249	21.250 ± 9.916
Fish farm			
water (mg/l)	0.038 ± 0.040	0.005 ± 0.004	0.004 ± 0.002
sediment (mg/kg)	6.833 ± 1.540	7.844 ± 0.920	3.611 ± 1.940
muscle (mg/kg)	0.037 ± 0.009	0.022 ± 0.002	0.028 ± 0.010
liver (mg/kg)	0.274 ± 0.150	0.146 ± 0.090	0.164 ± 0.080

Values are represented as mean ± standard deviation from three independent experiments.

limitation, then Cd could reach and accumulate in other organs such as muscle [3, 12, 26]. The high accumulation of Cd in liver was related to the fact that the liver plays a role in accumulation and detoxification [27]. However, after six months of exposure in contaminated fish farm, the Cd level in the liver of *C. gariepinus* slightly increased, and the reason is perhaps the ability of detoxification organs was decreased or became dysfunctional [28].

AChE activities in African catfish

Heavy metals cause different responses of AChE activities, such as inhibition [13] and increase [29]. In this study, AChE activities of African catfish after two months of exposure were significantly increased in brain and muscle, and significantly decreased in kidney and liver. As shown in Fig. 2a, AChE activities in brain and muscle were higher than kidney and liver, these results were similar to those suggested by Ventura et al [30]. AChE plays an important role in removing the neurotransmitter acetylcholine within the synapse through hydrolysis [31]. These results may affect fish behavior, i.e., motion balance, swimming or feeding, because it is related to AChE activity in brain and muscle [32, 33]. These results also showed biological responses toward Cd contaminant for survival in environmental stress.

AChE activities of African catfish after four months of exposure were not significantly decreased in any organs (Fig. 2b). The results were similar to a previous study in *Cyprinus carpio* [34]. However, AChE activities showed a decreasing tendency when compared with control group. The same results were reported in *Ramdia quelen* [13]. On the other hand, when fish are exposed to Cd, they synthesize the detoxifying protein such as metallothionein (MT), in kidney and liver [35]. Moreover, Dang and Wang [36] reported that Cd exposure to *Terapon jarbua* was correlated to the increase of MT concentration in its liver, suggesting the role of MT on metal detoxification. Thus, it could be expected that MT induction occurred and acted against the effect of Cd on AChE activity. Therefore, in four months, AChE activities were not significantly decreased in any organs.

AChE activities of African catfish after six months of exposure were not significantly decreased in kidney and muscle, whereas in the brain it was significantly increased and found to be significantly decreased in liver (Fig. 2c). Long term exposure to Cd resulted in neurotoxicity in brain and liver. AChE activities were interfered by high dose of Cd and time response, resulting in the accumulation of Cd in liver which caused liver dysfunction, and followed by Cd accumulation in other organs. Whereas AChE in brain was higher than the control, the same as a previous study [34]. The alteration of AChE activities may affect fish behavior such as swimming or feeding [13], and reduce survival leading to population-level effects as well [37].

Histopathological alteration in African catfish

The histopathological alterations were observed in gills, kidney and liver of *C. gariepinus* from the contaminated fish farm when compared to the control from non-contaminated fish farm. The normal gill tissues included secondary lamellae lined up along both sides of the primary lamellae. The primary
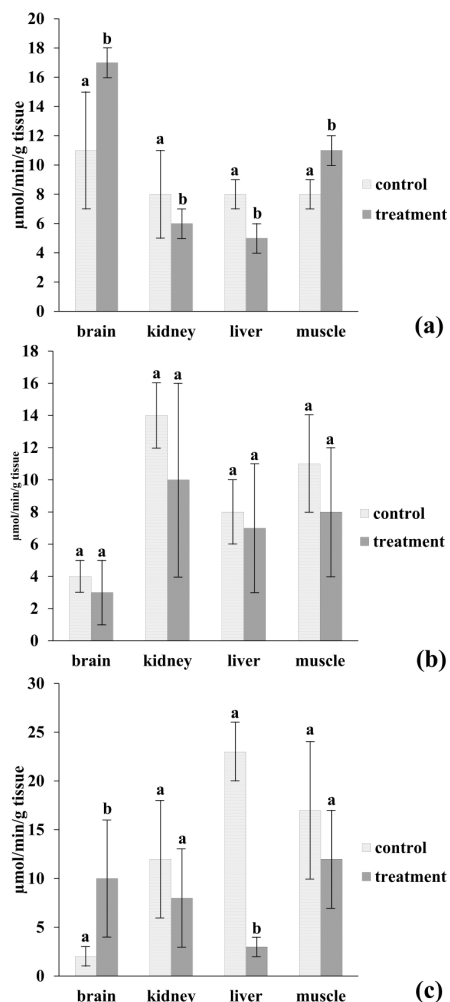
Fig. 2 Acetylcholinesterase activities in African catfish after (a) 2 months of exposure, (b) 4 months of exposure, and (c) 6 months of exposure in brain, kidney, liver and muscle.

Fig. 3 Transverse section of African catfish's primary lamellae gill: (a) control, (b) 2 months of exposure, (c) 4 months of exposure, and (d) 6 months of exposure. Cc, chondrocyte; PL, primary lamellae; SL, secondary lamellae; Mc, mucus cells; Ec, epithelial cells; yellow arrow, hyperplasia of epithelial cell; and red arrows, loss of epithelial cells.

lamellar were covered with epitheliual tissue and mucus membrane with epithelial cells inside and not separated from chondrocyte. Higher magnification (Fig. 3a) showed epithelial cells and large number of mucus cells. Whereas, gills of *C. gariepinus* exposed in contaminated fish farm for two and four months showed histopathological alterations i.e. losses of epithelial tissue in secondary lamellae and less mucus cells. Moreover, secondary lamellae showed hyperplasia of epithelial cell (yellow arrow). The secondary lamellae also showed destruction of either epithelial cells or a few lamellae were curled, leading to congestion and hemorrhage of gills (Fig. 3b). Whilst, the secondary lamellae
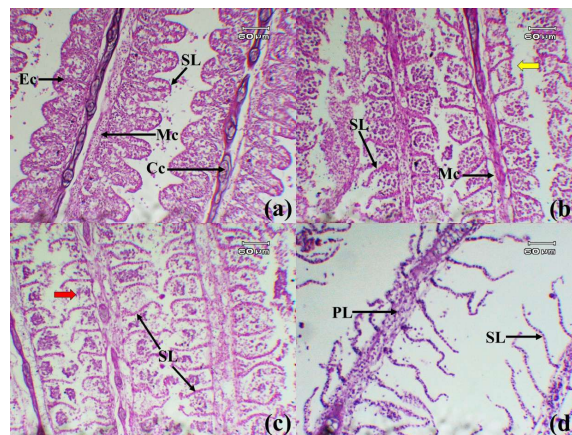
at four months of exposure (Fig. 3c) showed the curled and loss of epithelium cells (red arrow). These results agreed with previous studies that reporting that the gills of fish exposed to Cd showed thickening of the primary lamellar epithelium and clubbing of secondary lamellae [14, 22]. Moreover, serious symptoms were observed in gills after six months of exposure i.e. loss of all mucus membrane and epithelial cells (Fig. 3d). These alterations may negatively affect gill function causing decrease efficiency of osmotic regulation, ion regulation and respiration [14].

With regard to kidney and liver tissues, the histopathological alterations were observed after four months of exposure. The alteration of kidney tissues (Fig. 4c-d) was represented by wider lumen of proximal and distal tubules (PT and DT), distortion and broadening of Bowman's capsule (BC), distortion of glomerulus (G) capillaries. Whereas those alterations were not observed in both the control and the two months of exposure (Fig. 4a-b). Kidney tissues such as renal tubular epithelium is particularly sensitive to poisons. The highly metabolic proximal tubules are most seriously affected by toxin. In *Tilapia mossambica*, after it was exposed to Cd, the cell size of kidney was reduced, and the glomerular tissues remained more or less intact. Also damage of interstitial edema and renal tubes in several areas were observed. In addition, the hydrophobic degeneration of renal tubes in the
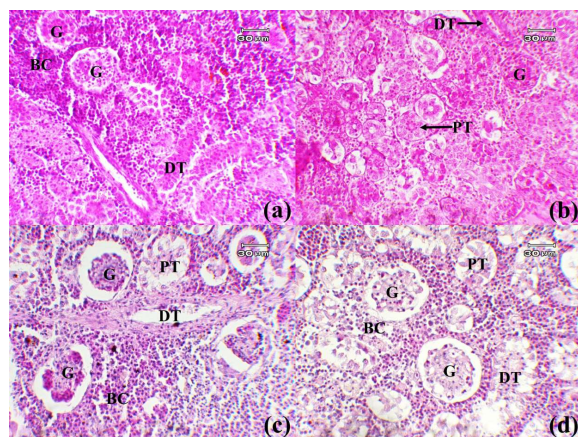
Fig. 4 Transverse sections of African catfish's kidney: (a) control, (b) 2 months of exposure, (c) 4 months of exposure, and (d) 6 months of exposure. G, glomerulus; PT, proximal tubules; DT, distal tubules; and BC, Bowman's capsule.
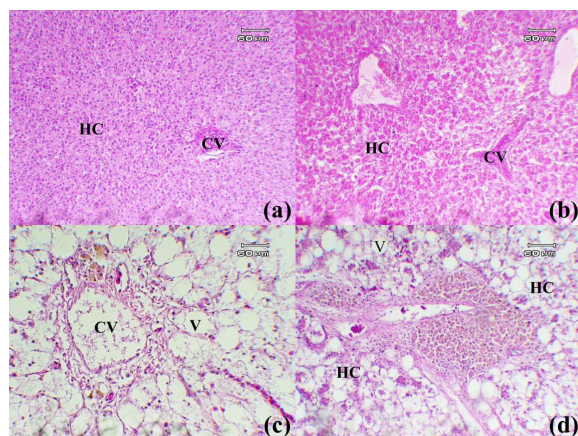

Fig. 5 Transverse sections of African catfish's liver: (a) control, showing normal exo-structure of hepatic cells (HC); (b) 2 months of exposure, showing hemorrhagic tissues and central vein (CV); (c) 4 months of exposure, showing sponge condition and large vacuole (V); and (d) 6 months of exposure, showing large number of vacuoles.

glomerular tissues has been observed [38].

Histopathological effects of Cd on liver tissue, such as hemorrhage, were observed in two months of exposure (Fig. 5b), and severe damages in four and six months of exposure (Fig. 5c-d), i.e., losing of hepatic cells (HC), tissue converted into sponge and large vacuoles (V), and central vein (CV) exhibiting wider, when compared to the control (Fig. 5a). These results were similar to those previously re-

ported in Mosquito fish (*Gambusia affinis*) that Cd caused liver tissue alterations [39]. These alterations cause serious modification of detoxification pathways in liver and kidney of fish [22]. All of the histopathological observations indicated that Cd contaminated farm can cause destructive effect in gill, kidney and liver tissues of *C. gariepinus*.

CONCLUSION

In this study, only Cd concentrations in sediment samples were higher than fish and water samples, which exceeded the standard of Thailand. In fish samples, Cd concentration in liver was higher than muscle. The inducing of AChE activities in brain and muscle of juvenile *C. gariepinus* could be used as biomarker for Cd pollution. Whereas, the decreasing of AChE activities in liver of *C. gariepinus* indicated long term exposure of Cd causing accumulation of Cd in the liver and organ's dysfunction. Moreover, histopathological alterations could be used as biomarkers of Cd contamination in the aquatic environment.

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