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Histological Effects of Contaminated Sediments in Mae Klong River Tributaries, Thailand, on Nile tilapia, *Oreochromis niloticus*

Piyanut Peebua[a], Maleeya Kruatrachue[a,b*], Prayad Pokethitiyook[a] and Pahol Kosiyachinda[a]

[a] Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand.
[b] Mahidol University International College, Mahidol University, Nakhon Pathom, 73170, Thailand.

* Corresponding author, E-mail: scmkt@mahidol.ac.th

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ABSTRACT: Histopathological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution. Nile tilapia, *Oreochromis niloticus* exposed for one month to sediments from the Mae Klong River, Samutsongkram province, South West Thailand, which contained elevated levels of heavy metals (lead and chromium), developed abnormalities of the gills, liver and kidney. In the gill filaments, cell proliferation, lamellar cell hyperplasia, and lamellar fusion were observed. In the liver, there was vacuolation of hepatocytes and nuclear pyknosis. Kidney lesions consisted of dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cell. No recognizable changes were observed in muscle tissue. Despite these histopathological changes, no firm correlation between levels of heavy metals in sediments and those in fish tissues could be established.

KEYWORDS: heavy metal, sediment, *Oreochromis niloticus*, histological effects.

INTRODUCTION

The Mae Klong River, one of the largest rivers in Thailand, is 140 km long, situated on the southwest side of Bangkok, in Samutsongkram province. It is a system of tributaries and the river itself flows into the Gulf of Thailand. The Mae Klong River supplies water for irrigation and supports aquaculture industries such as fish ponds and shrimp farms. The Mae Klong tributaries are occupied by agriculture fields such as rice fields, vegetable farms, fruit orchards and several industries such as chemical industries, paper factories, and storage battery factories. Agricultural runoff, aquacultural effluents, and domestic effluents directly enter into the river through drains and tributaries. In addition, the industries located in the vicinity of tributaries discharge effluents into the river. Pesticides such as endosulfan, beta-hexachlorocyclohexane (BHC), endrin, malathion, parathion, monocrotophos, carbofuran, and carbaryl are used for pest control programs in the agricultural lands adjacent to the Mae Klong River. Hence, the Mae Klong River and the tributaries are likely to be contaminated by pesticides and heavy metals[1,2,3].

Disturbance of living processes at the molecular and subcellular levels of biological organization by xenobiotics can lead to cell injury, resulting in degenerative and neoplastic diseases in target organs[4]. Therefore histopathological biomarkers have been proven to be useful indicators of toxicity in fish organs[5-6]. There are several reports on the impact of environmental toxicants on fish revealed by histopathological studies of vital organs such as gills, liver and kidney[7-13]. Several studies have also reported on the response of fish to sediments contaminated by pesticides, heavy metals, and persistent organic pollutants[14-20]. However, studies on ecotoxicological assessment of contaminants in sediments and their toxicities to fish is still lacking in Thailand. Therefore, one of the objectives of the present study was to quantify the heavy metals in sediment samples collected from the Mae Klong River tributaries[21]. The other objective was to investigate contaminant-related histopathology and metal accumulation in a commonly cultured fish, Nile tilapia or *Oreochromis niloticus*, exposed experimentally to these sediments.

MATERIALS AND METHODS

Sampling Stations

Five stations on tributaries of the Mae Klong River, namely Klong Khud Gum Non (KKGN), Klong Sapan Hun (KSH), Klong Phee Lork (KPL), Klong Sum Nga (KSN), and Klong Rang Tub Taeb (KRTT) were chosen for sediment sample collection (Fig. 1). At least 5 kg of sediment samples were collected from each sampling

site using a grab sampler. The samples were placed in high-density polyethylene bags, which were then tightly closed. They were kept in an ice-box prior to analysis in the laboratory. Sediments were analyzed for texture using the hydrometer method[22] at the laboratory of the Department of Soil Science, Ministry of Agriculture and Cooperatives, Bangkok, Thailand.

Animals

Nile tilapia (*O. niloticus*) weighing 10-12 g were reared in glass aquaria supplied with aerated water for at least 2 weeks. For the sediment exposure experiment, a static system was utilized. One kilogram of sediment collected from all five stations was placed in a 60 L tank and 30 L of water added. The tank was left for at least 24 h to allow the sediments to settle. Ten fish were then transferred to each tank filled with either dechlorinated tap water only (control) or water with sediments collected from the five stations (3 replicates for each station and one control; 18 tanks in total). Water quality parameters were monitored weekly: temperature (25 ± 2 °C), pH (6.8–7.3), dissolved oxygen (7.5-7.8 mg/L), ammonia (0.25–0.30 ppm), and alkalinity (65-70 mg/L). The fish were fed once daily with commercial fish-food. Fish were randomly sampled from all treatment groups at the end of the exposure period (day 30). They were anaesthetized in MS-222 (Finquel®) to mortality. Tissue samples from fish exposed to sediment in all treatment groups were analyzed for heavy metals and histological alterations.

Heavy Metal Analyses in Sediment and Fish Tissue

Gill, kidney, muscle and liver tissues (approximately 10-30 mg each) were removed, frozen in liquid nitrogen and stored at - 20 °C prior to chemical analysis. Heavy metal composition was analyzed according to US EPA RCRA SW-846 Method 6020 with some modifications[23]. A microwave acid digestion bomb (CEM model Mar5) was used for all microwave digestions. Approximately 0.3 g of sediment was weighed accurately into PTFE vessels. Five mL of concentrated nitric acid were added to each vessel. The sample vessels together with reagent blank vessels were sealed and digested using a microwave power progressively increasing up to 1200 W, 210 °C, over a 20 min duration. After cooling, all digestates were transferred into 50 mL calibrated flasks, and 1 mL of 1000 g/L in solution was added, and then diluted to 50 mL with deionized water. The sediment samples were analyzed by Inductively Coupled Plasma-Mass Spectrometry (Perkin Elmer SCIEX model ELAN 6000) using the isotopes ^{52}Cr, ^{114}Cd, ^{202}Hg and ^{208}Pb. Twenty grams of Rhodium (Rh) per liter were added as an internal standard. The concentrations of metals in fish tissues were determined with a graphite atomic

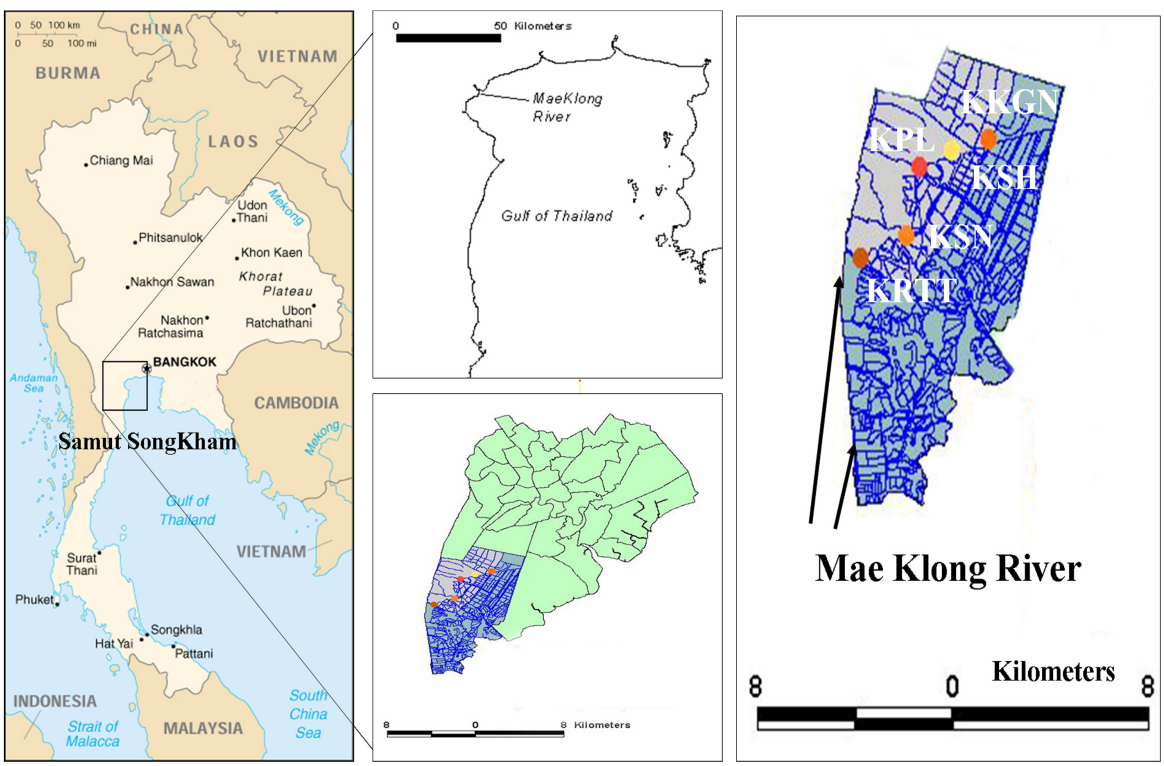

Fig 1. Map of Mae Klong River tributaries.

absorption spectrophotometer (Varian AAS model SpetrAA-55).

Histopathological Study

Samples of gill, liver, kidney and muscle were cut into approximately 0.5 cm^3 pieces and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C overnight. The tissues were then washed in 0.1 M sodium cacodylate buffer and postfixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer at 4 °C for one hour. Then, they were dehydrated in a graded series of ethyl alcohol, cleared in two changes of propylene oxide, infiltrated in a mixture of propylene oxide and Araldite 502 resin, and embedded in Araldite 502. Specimens were sectioned at 1-micron thickness on a Sorvall Porter-Blum MT-2 ultramicrotome, stained with 1 % methylene blue, and observed under an Olympus CH40 light microscope.

Semiquantitative Scoring

Six fish were randomly selected from each station (2 fish / replicate) and control. Approximately 30 serial sections were produced from each fish for analysis. Histopathology was determined based on severity of changes compared to control sections. Scores were based on the severity as well as the number of slides out of the total in which the histological changes were observed with: • = no histopathology in any field on the slides, + = mild histopathology present in <25% of the fields on the slides, ++ = moderate histopathology present in >75% of the fields on the slides; and + + + = all fields of the slides displayed severe histopathology.

Statistical Analysis

All data were presented as mean ± standard deviation. Variations in heavy metal concentrations in fish and sediment collected from different sites were tested by one-way analysis of variance (ANOVA) followed by Tukey Honest Significant Difference (HSD) as a comparison of means *post-hoc* Tukey-Kramer test. The level of significance was set at 0.05.

Results

Sediments taken from five sampling stations (KKGN, KSH, KPL, KSN, and KRTT) had different texture. Sediments from KKGN and KSH were silt, whereas sediments from KPL and KSN were silt-sand and that from KRTT was clay.

Heavy Metal Analyses of Sediment and Fish Tissue

Analysis of heavy metals was performed in sediments collected from five stations (Table 1). It was found that the sediment from KSH contained the highest level of Pb (12.45 µg/g dry wt) followed by KPL (10.56 µg/g), KRTT (7.22 µg/g), KSN (7.05 µg/g) and KKGN (6.25 µg/g). The highest levels of Cr were detected in KSH and KPL (26.22 and 25.61 µg/g, respectively) followed by KSN (18.09 µg/g), KKGN (13.87 µg/g) and KRTT (13.22 µg/g). Cd and Hg were not detected in the sediments at the minimum detectable limit (0.038 µg/g for Cd and 0.171 µg/g for Hg).

Table 1. Concentrations of heavy metals in sediments collected from the five stations on Mae Klong River tributaries.

Site	Sediment character	Cr	Cd	Hg	Pb
		(µg/g dry wt)			
KKGN	silt	13.87 ± 1.34[a]	N.D.	N.D.	6.25 ± 0.73[a]
KSH	silt	26.22 ± 2.48[b]	N.D.	N.D.	12.45 ± 1.15[b]
KPL	silt	25.61 ± 1.34[b]	N.D.	N.D.	10.56 ± 1.12[b]
KSN	sand	18.09 ± 0.98[c]	N.D.	N.D.	7.05 ± 0.09[a]
KRTT	clay	13.22 ± 0.79[a]	N.D.	N.D.	7.22 ± 1.60[a]

N.D. = less than detection limit.
The detection limits for Cd and Hg are 0.038 and 0.171 µg/g, respectively.
Data with the same superscript in the same column are not significantly different according to the results of one-way ANOVA followed by Tukey-HSD ($p<0.05$).

Table 2 shows heavy metal concentrations in Nile tilapia exposed to sediments from five stations. Pb concentrations were significantly different in the gills of fish exposed to sediments from all stations ($p<0.05$), while those in muscle were not significantly different ($p>0.05$). Cr concentrations in muscle were lower than 2.5 µg/g. Cr was not detected in the muscles of fish exposed to sediments from KSN, and KRTT, nor in the gills of fish exposed to sediment from all stations. There was no correlation between the levels of metals in sediments and those in fish tissues.

Histopathological Study

Similar histological alterations were observed in all fish exposed to sediments collected from all five stations. Table 3 shows semiquantitative scoring of gill, liver, and kidney histopathology. Most tissue samples showed

Table 2. Heavy metal concentrations in muscles and gills of Nile tilapia exposed experimentally to sediments collected from the five stations on Mae Klong River tributaries.

Site	Muscle		Gill	
	Cr(µg/g)	Pb(µg/g)	Cr(µg/g)	Pb(µg/g)
KKGN	0.89 ± 0.37[a]	4.16 ± 1.40[a]	N.D.	23.23 ± 0.29[a]
KSH	2.18 ± 2.79[a]	5.14 ± 1.05[a]	N.D.	16.26 ± 0.61[b]
KPL	0.93 ± 0.45[a]	4.86 ± 1.21[a]	N.D.	21.09 ± 0.60[c]
KSN	N.D.	8.41 ± 4.09[a]	N.D.	74.08 ± 1.51[d]
KRTT	N.D.	9.81 ± 4.68[a]	N.D.	28.18 ± 0.60[e]

N.D. = less than detection limit.
The detection limits for Cd and Hg are 0.038 and 0.171 µg/g, respectively.
Data with the same superscript in the same column are not significantly different according to the results of one-way ANOVA followed by Tukey-HSD ($p<0.05$).

Table 3. Semiquantitative scoring of gills, liver, and kidney in Nile tilapia, *O. niloticus*, exposed experimentally to sediments collected from the five stations on Mae Klong River tributaries.

	Lesion	Control	Site				
			KKGN	KSH	KPL	KSN	KRTT
Gill	Edema of epithelial cells	-	+ +	+	+ +	+ + +	+ + +
	Aneurism of secondary lamellae	-	+ +	+	+ +	+ + +	+ + +
	Breakdown of pillar cells	-	+ +	+	+ +	+ + +	+ + +
	Hypertrophy and hyperplasia of mucous and chloride cells	-	+ +	+	+ +	+ + +	+ + +
Liver	Blood congestion in sinusoid	-	+ +	+ + +	+	+ +	+ + +
	Hepatocytes:hydropic swelling lipid vacuoles necrosis	-	+ +	+ + +	+	+ +	+ + +
Kidney	Tubular cells:hydropic swelling hyaline droplets necrosis	-	+	+ +	+	+ +	+ + +
	Glomerular distortion	-	+	+ +	+	+ +	+ + +

N.D. (-) no histopathology; (+) mild histopathology (<25% of fields); (+ +) moderate histopathology (>75% of fields); (+ + +) severe histopathology (all fields).

lesions typical of chronic exposure. Severe lesions were observed in samples exposed to sediments from KSN and KRTT. These lesions correlated with higher Pb level detected in fish tissues (gills and muscle) as shown in Table 2.

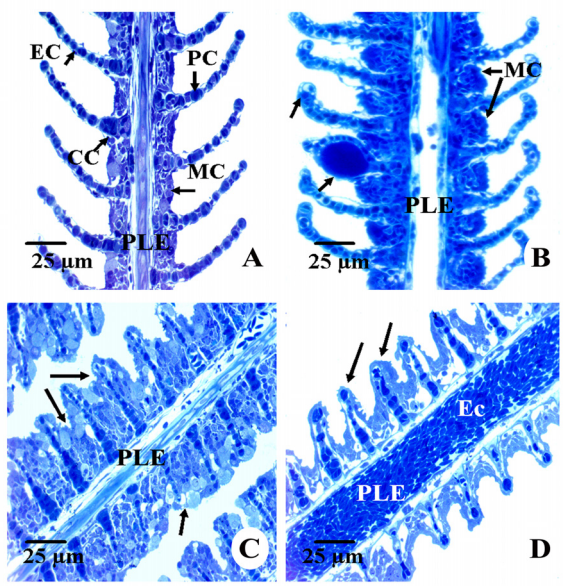

Fig 2. Light micrographs of a transverse section of Nile tilapia gill tissue. (A) Control group showing normal appearance of primary lamellar epithelium (PLE), chloride cell (CC), epithelial cell (EC), mucous cell (MC) and pillar cell (PC). (B) Treated group showing telangiectasia at the tips of secondary lamellae (arrows) and thickening of primary lamellar epithelium (PLE). MC, mucous cell. (C) Treated group showing thickening of primary lamellar epithelium (PLE) and fusion of secondary lamellae (arrows). (D) Treated group showing severe thickening of primary lamellar epithelium (PLE). Note the accumulation of erythrocytes (Ec) in primary lamella and the breakdown of pillar cell system (arrows).

Gills

Control group No recognizable changes were observed in the gills of control fish (Fig. 2(A)). There are four gill arches on each side of the buccal cavity. Each arch is composed of numerous gill filaments with two rows of secondary lamellae that ran perpendicular to each filament. Secondary gill lamellae were composed of a single layer of epithelial cells supported by pillar cells, which were contractile and separated the capillary channels. One to two erythrocytes were usually observed within each capillary lumen. Chloride cells were identified as large epithelial cells with light cytoplasm, usually present at the base of lamellae. Mucous cells were present in the epithelium of the filament at the base of lamellae, but they lacked the light cytoplasm and were smaller than chloride cells.

Treated groups After exposure to sediments, the fish gills showed extensive hypertrophy and hyperplasia of chloride cells and mucous cells at the base of the gill filament and secondary lamellae. Fusion of secondary lamellae was commonly observed (Fig. 2(B)). In more severe cases, the gill showed telangiectasis at the tip of the secondary lamellae (Fig. 2(B)). In addition, extensive edema of the epithelial cells, and blood congestion or aneurysm in many areas of secondary lamellae with the breakdown of the pillar cell system were observed (Figs. 2(C) and (D)). Semiquantitative scoring of gill lesion after exposure to sediment from five stations is shown in Table 3. The most severe lesions occurred in the fish exposed to sediments from KSN and KRTT.

Liver

Control group The livers of the control fish had a typical parenchymatous appearance (Fig. 3(A)). The parenchyma itself was primarily composed of polyhedral hepatocytes typically with central nuclei with densely stained chromatin. Venous blood entered the liver caudally from the intestine via the hepatic portal veins and branches into capillaries known as sinusoids. Sinusoids were lined with reticulo-endothelial

cells which were in turn surrounded by hepatocytes.

Treated groups The livers of fishes exposed to sediment showed several pathological changes. The general lesions were pyknotic nuclei and large lipid vacuoles in the cytoplasm of hepatocytes (Figs. 3(B) and (C)). Some liver areas showed focal necrosis and contained severe infiltration of leukocytes (Fig. 3(B)). In more severe cases, the liver showed a slight blood congestion in sinusoids and hydropic swelling of hepatocytes. Semiquantitative scoring of lesion of the liver after exposure to sediment from five stations is shown in Table 3. The most severe lesions occurred in the fish exposed to sediments from KSH and KRTT.

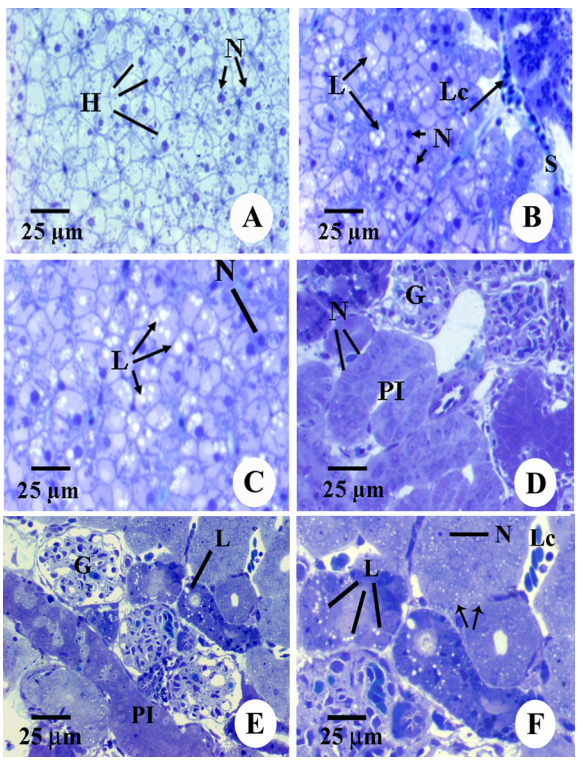

Fig 3. (A, B, C) Light micrographs of a transverse section of Nile tilapia liver tissues. (A) Control group showing hepatocytes (H) with central spherical nuclei (N). (B) Treated group showing moderate swelling of hepatocytes and presence of lipid vacuoles (L) and pyknotic nuclei (N), and severe infiltration of leukocytes (Lc) in sinusoid (S). (C) Treated group showing moderate swelling of hepatocytes and numerous lipid vacuoles (L) and pyknotic nuclei (N). (D, E, F) Light micrographs of a transverse section of Nile tilapia kidney tissue. (D) Control group showing normal appearance of glomeruli (G) and first proximal tubule (PI). N, nucleus. (E) Treated group showing mildly swollen PI epithelial cells, glomerular (G) distortion, and presence of large lipid vacuoles (L). (F) Higher magnification of treated group showing numerous lipid vacuoles (L), dilated nuclei (N), hyaline droplets (arrows) and infiltration of leukocytes (Lc).

Kidney

Control group No recognizable changes were observed in the kidneys of the control fish (Fig. 3(D)). The kidney was composed of numerous renal corpuscles with well developed glomeruli and a system of tubules. The proximal segment was covered by tall columnar epithelial cells with basal nuclei and brush border located along the cell apices. The distal segment was lined with large, relatively clear columnar epithelial cells with central nuclei and the brush border was reduced or absent. The collecting duct or glomerulus was larger in diameter than the distal segment, containing columnar epithelial cells with basal nuclei and no brush border.

Treated groups The kidneys of fishes exposed to sediments, showed hydropic swelling and hypertrophy of tubules with dilated nuclei (Figs. 3(E) and (F)). Glomerular alteration was also observed (Fig. 3(E)). In more severe cases, the tubular cells showed hyaline droplet accumulation (Fig. 3(F)). Some tubules were dilated and necrotic (Fig. 3(F)). Semiquantitative scoring of kidney lesion after exposure to sediment from five stations is shown in Table 3. The most severe lesions

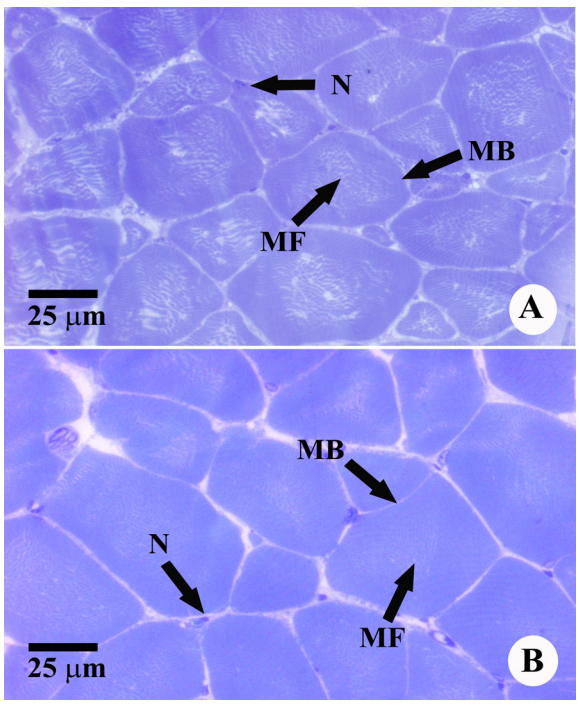

Fig 4. (A, B) Light micrographs of a transverse section of Nile tilapia muscle tissue. (A) Control group showing normal muscle bundle and muscle fibers with nuclei. (B) Treated groups showing muscle tissue without pathological alterations. MB, muscle bundle; MF, muscle fiber; N, nucleus.

occurred in the fish exposed to sediment from KRTT.

Muscle

Control group No recognizable changes were observed in the muscle of the control fish (Fig. 4(A)). The multiple nuclei lay at the periphery of the muscle fibers. Groups of the fibers were surrounded by a large pale area with loose connective tissues, the perimysium. The whole muscle or muscle bundle was surrounded by a denser connective tissue, the epimysium (Fig. 4(A)).

Treated groups No recognizable changes were observed in the muscle of exposed fish. The muscle bundles and muscle fibers had a normal appearance (Fig. 4(B)).

Discussion

Human activities generate contaminants that are incorporated into the Mae Klong River and tributaries. The main contaminants, coming from the fruit canning industries, are organic matter of vegetable origin and toxic organic substances (e.g. fungicides and pesticides). The remaining industries (e.g. chemical industries, paper factories and storage battery factories) are potential sources of inorganic (i.e. heavy metals) and organic (e.g. hydrocarbons and detergents) contaminants. Sediment analysis for heavy metals showed that the highest concentrations of heavy metals was found at KSH and KPL (Cr>25 µg/g; Pb>10 µg/g). Sediments collected from KSH were most likely to be contaminated with Cr and Pb because the open rubbish dump and battery factories are located along these tributaries. The dumping of used batteries in these areas explains the relatively high values for Cr and Pb. In general, Cr and Pb concentrations in water should not exceed 0.05 µg/L and 0.05 µg/mL, respectively[24]. The relatively high concentrations of Cr and Pb in the sediments from KSH and KPL indicated pollution by these metals in the Mae Klong River.

Omnivores such as tilapia are mostly bottom dwellers and have a high contact with the sediments, which act as a sink for heavy metals[25]. In the present study, we found high Pb concentration in fish gills (74.08 µg/g), whereas Cr accumulation in muscle was less than 2.5 µg/g and it was not detected in the gill. This high Pb concentration in fish muscle and gill exceeds the standard consumption level of 0.5 µg/g [26]. People who consume the fish are exposed to the residues of heavy metals such as Pb and Cr. These residues are bioconcentrated through the food chain. The high accumulation potential of heavy metals was thought to be due to their close association with the transformation pathway. Accordingly, heavy metals enter the blood circulation through gill epithelia and finally accumulate in the liver[27].

In the present study, Nile tilapia exposed to contaminated sediment showed several histological alterations. The livers displayed the highest prevalence of histological changes, with necrosis representing the dominant structural alteration. Hepatic necrosis and inflammation, indicative of infection or toxic injury by contaminants, was prevalent in greenback flounders (*Rhombosolea tapirina*) exposed to contaminated marine sediments[28]. In addition, hepatocytes of Nile tilapia exposed to contaminated sediment showed hydropic swelling, and nuclear pyknosis. In more severe cases, there was a slight blood congestion in sinusoids. Similar alterations were observed in the hepatocytes of Nile tilapia exposed to glyphosate herbicide[29] and in white seabass (*Lates calcarifer*) exposed to Cd[30]. Other fish species such as estuarine fish (*Platichthys flesus, Pomatoschistus minutus, and Zoarces viviparous*) captured from contaminated sites also showed these alterations which are often associated with a degenerative-necrotic condition[4, 33].

The kidney of Nile tilapia exposed to contaminated sediment showed similar histopathology to that of liver. In the proximal tubular cells, hydropic swelling, hypertrophy, nuclear pyknosis, and accumulation of hyaline droplets occurred. These lesions were very similar to those observed in Nile tilapia exposed to glyphosate[29], and white seabass exposed to Cd[30]. The hyaline droplets likely represented protein reabsorbed from the glomerular filtrate[29]. In addition, there was an alteration of the glomerulus in the kidneys of fish in the present study. Other fish species exposed to PAH and natural petroleum also displayed dilation of blood capillaries in the glomeruli and granulomatous inflammation[4, 14].

The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion[31]. They are vulnerable to pollutants in water because of their large surface area and external location. For this reason, they are considered to be appropriate indicators of water pollution levels[32]. In the present study, Nile tilapia exposed to contaminated sediment for one month showed high concentrations of Pb in the gills. Several investigators have reported histopathological changes in the gills of different fish species exposed to pesticides, petroleum hydrocarbon, PCB, PAH, and heavy metals[4, 11, 13, 18, 20, 29-32]. These included several alterations similar to those of Nile tilapia exposed to contaminated sediment in the present study, such as hyperplasia and hypertrophy of chloride cells and mucous cells, edema of epithelial cells, clubbing of gill filament, and aneurysm. Lamellar fusion and clubbing of lamellae may have been protective in that they could diminish the vulnerable gill surface area[9]. These histopathological changes of the gills likely resulted in hypoxia, respiratory failure problems with

ionic and acid-base balance[32].

Histopathological alterations in various organs of Nile tilapia exposed experimentally to contaminated sediments from Mae Klong River tributaries implies exposure and uptake of at least one or more xenobiotic contaminants. The induction of tissue lesions indicates bioavailability of contaminants released from the sediments. The sub-lethal effects detected in the histological alteration of tissues are important in relation to the health of tilapia exposed to contaminated sediment. These responses indicate that biochemical alterations were severe enough to lead to structural changes at the tissue level. If exposure to contaminated sediment in the field extends to a longer term, physiological impairment of the individual would most likely increase with increasing prevalence and severity of pathologic changes. Consequently, exposure to contaminated sediments from Mae Klong River tributaries may present a significant risk to tilapia. This study provided some baseline data on the levels of heavy metals contaminated in sediments of Mae Klong River tributaries. It has demonstrated a combined approach of chemical and biological assessment of heavy metal contamination in field samples. The study was ecologically relevant, demonstrating the potential routes and toxicological impacts of environmental contaminants to fish. Our study highlighted that no single investigative approach is able to measure the adverse effects of environmental contaminants. Ecotoxicological studies using both chemical and biological methods are essential. Future investigations on the histopathological alterations and accumulation of heavy metals of wild fish collected from Thai rivers should be pursued in order to determine the contaminant level in the water.

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