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Rearing Milkfish (*Chanos chanos*) In Cadmium and Lead Contaminated Pond and its Effect on Alteration of Gill, Liver and Kidney

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ABSTRACT

This study was conducted to determine alteration of milkfish (*Chanos chanos*) organs (gills, liver and kidney) cultured in cadmium (Cd) and lead (Pb) contaminated ponds. The method used in this research was descriptive. Samples for research were obtained from Patuguran, Rejoso District, Pasuruan. The main parameters were the damage of the gills, liver and kidney of milkfish. Data were analyzed by analysis of variance (ANOVA), continued with least significant difference (LSD) and regression analysis. The result indicated that the heavy metals were present in water and sediment. Their concentrations were Pb 0.49 ppm and Cd 0.50 ppm, for water and sediment, respectively. The observation on organ histopathology revealed that gills experienced fusion, hyperplasia and necrosis. The liver showed cells degeneration, congestion and necrosis. Kidney experienced atrophy, cloudy swelling and glomerular hyalinization. The degree of organ damage was affected by length of rearing time. This research indicated that cadmium and lead polluted the fishpond in Rejoso District, Pasuruan and affected the organs of milkfish.

INTRODUCTION

Pasuruan regency, Indonesia, is located in the east of Surabaya, the capital city of East Java, next to Sidoarjo regency. This is an industrial area. Geographically, Pasuruan regency lies between longitudes 112° 33’ 55" E -113° 30’ 37" E and latitudes 7°32’34" S-8°30’20" S; the area is 147,401.50 ha. The detailed location is shown in Fig. 1.

Several industries have developed in the north part of Pasuruan regency. Hence, in the north part of Pasuruan, the waters are mostly polluted with heavy metals. Traditional fishponds are constructed near the industrial areas. Fishponds are mainly located near the Rejoso river estuary. This river is used as freshwater sources for traditional fishponds even though Rejoso, a sub-district, has been polluted with heavy metals such as cadmium (Cd) and lead (Pb) from several industries (unpublished work). The heavy metals in the water affects not only the biodiversity, but also the ecosystem. The accumulation of heavy metals in the fish increases the health risk of fish consumer, especially humans (Irwin et al. 1998).

Chanos chanos, a benthopelagic fish which usually eats at the bottom of water niche, is a perfect sample to analyze the effect of heavy metals on water organisms (Biuki et al. 2010). Milkfish is a famous commodity in Indonesia. It is mainly because of its straightforward rearing techniques. Furthermore, milkfish has relatively good immunity, resulted in the easiness to adapt with stressor such as harsh environment and pollution.

This research was aimed to investigate the alteration of the milkfish (*Chanos chanos*) organs due to the level of heavy metals, cadmium (Cd) and lead (Pb) in fishpond rearing water and sediments.

MATERIALS AND METHODS

Sample collection: Water and sediment samples were collected from three traditional fishponds. Each sample was stored in the sterilized bottles. The bottles were then stored in a cool box and transferred to the laboratory. Milkfish samples were collected from fishponds. The samples were collected after the fish was reared for 1, 2 and 3 months.

Water quality analysis: The main water quality parameters were analyzed. They were, the level of heavy metals, Pb and Cd; and the supporting parameters like temperature, DO, pH, ammonia and salinity.

Histology analysis: Fish were dissected and the gill, liver and kidney of the fish were stored in bottles containing Davidson’s solution. The histology of sample organs (gills, liver and kidney) was investigated. All of the samples were treated based on Short et al. (2000) method. The sample fixation needs a 24 hours process using 10% formalin solution. The next steps were dehydrating, clearing, paraffin infiltration, blocking, cutting/sectioning, staining and mounting. Organs observation was conducted by using a light microscope.

Data analysis: The data showing the histopathology abnormality were quantified by using scoring. The range of the score was based on Pantung et al. (2008). The analysis of histopathology data was done by using ANOVA, LSD, and regression analysis.

RESULTS AND DISCUSSION

The level of heavy metals in water and sediment: The level of heavy metals lead (Pb) and cadmium (Cd) in fishponds' water and sediment is depicted in Table 1.

The highest lead concentration was found in the sediment. Sediment was the main location where the heavy metals are stored. Similar result was reported by Shanbehzadeh (2014). In comparison to the allowed standard level of heavy metals in Indonesia (KMNLH 2004), lead (Pb) and cadmium (Cd) concentrations in the fishponds are above the limit, especially in the sediments. The high level of heavy metals is due to the location of the water source. It is clear that the Patuguran fishponds' water has been polluted.

Milkfish (*Chanos chanos*) gills: Gills histopathology was analyzed to determine the effect of the contaminated fishpond on the fish. The gill showed hyperplasia, fusion, and necrosis (Fig. 2).

Table 1: The heavy metals level in fishpond water and sediment.

HeavyMetal	Water (ppm)	Sediment (ppm)	Standard Limit*)
Lead (Pb)	<0.0024	0.49	0.001
Cadmium (Cd)	<0.0044	0.50	0.008

*Standard limit for heavy metal concentration based on (KMNLH 2004).

Milkfish gill was close and without any space. It is an indication of mild hyperplasia, enlargement of gill tissue caused by an increase in the reproduction rate of its cells. In severe hyperplasia, the space between secondary lamella would be filled closely with new cells (Lakani et al. 2013). If exposed to heavy metals, fish gill will get cellular hypertrophy or hyperplasia in the epithelial layer of primary filaments (Hadi et al. 2012). Hyperplasia damage is because the gills are very dependent on the physical change. Chemical change might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance (Cengiz 2006). This abnormality is an indication of heavy metal pollution. Similar cases in freshwater fish were also reported due to the exposure to lead (Martinez 2004, Palaniappan 2008).

The gill also showed lamellar fusion (Fig. 2B). It is indicated by the wound which is covered by many interlamella cells after necrosis in epithelial cells. Lamellar fusion also identified by severe hyperplasia in secondary lamellae (Camargo et al. 2007, Oguz 2015). Lamellar fusion decrease

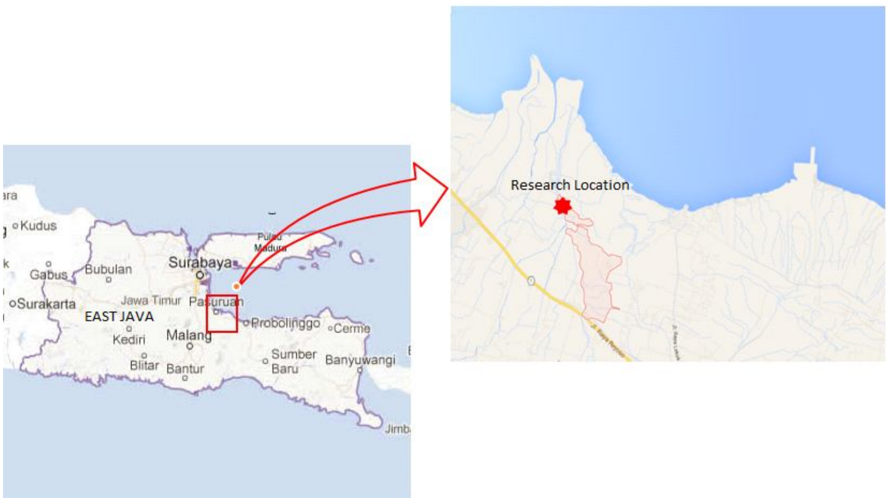

Fig. 1: Research location.

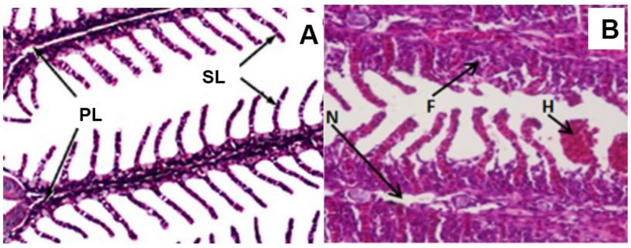

Fig. 2: Photomicrographs of the gill structure of Milkfish: [A] Normal aspect of the gill. (PL) Primary Lamellae. (SL) Secondary Lamellae. [B] Histopathology of Fish Gill. Hyperplasia (H). Fusion (F). Necrosis (N). The pictures were taken by using Olympus CX22 Binocular Microscope, 200X.

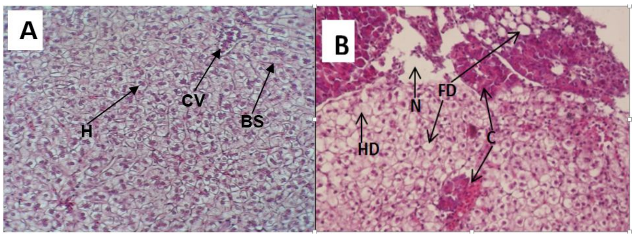

Fig. 3: Photomicrographs of the liver structure of Milkfish: [A] Normal hepatic liver tissue, showing hepatocytes (H), Blood Sinusoid (BS), and Central Vein (CV). [B] Histopathology of Fish liver. Hydropic Degeneration (HD). Fatty Degeneration (FD). Congestion (C). Necrosis (N). H-E staining, observed by using Olympus CX22 Binocular Microscope, 400X.

the space between lamellae and increase the thickness of water-blood barrier which reduces oxygen intake capacity leading to haemorrhage. Other cases, probably induced by the incidence of severe edema (Pane 2004, Schwaiger 2004). Lamellar fusion was a mechanism of defense of the fish that was exposed by several pollutants in the external environment. It is a barrier to the entrance of pollutants, such as a heavy metal (Poleksic et al. 1994, Fernandes et al. 2003).

There were some parts of gill tissue, which did not absorb the stain. It is because some cells died from necrosis. Necrosis is caused by hyperplasia, severe secondary lamella fusion and unattached cells to its supporting cells. It is indicated by the gill that it was damaged by the pollution during the rearing period. Necrosis was the result of high concentration of lead. There was continuous absorption of lead ions inside the gill tissues causing damage to lamella. Pb causes necrosis in fish in a small amount (Tresnati & Djawad 2012).

Milkfish (*Chanos chanos*) liver: Alteration of liver histopathology of milkfish which was exposed to heavy metals Cd and Pb is showed in Fig. 3. Fish liver showed fatty degeneration, hydropic, congestion and necrosis.

There were changes in cell shape of milkfish liver due to the effect of heavy metals Pb and Cd pollution in water (Fig. 3B). Enlargement in cytoplasm cell which caused membrane cell damage is called fatty degeneration and hydropic. It is the secondary response from hypoxia, toxin, free radicals, virus and bacteria (Mc Gavin et al. 2007).

Fatty degeneration is the effect of hydropic degeneration, a condition when cells could not perform fatty metabolism, thus gets accumulated inside the cells. Fatty degeneration occurred as the result of the cells' inability to bind the energy which is caused by mitochondria damage. Continuous fatty degeneration would make cells die. According to Cheville (1999), cells enlargement or cells degeneration is reversible, if the toxic exposure does not happen in long period, the cells will back to normal. However, if toxic exposure happens continuously, the cells will not be able to tolerate the damage caused by toxic substances.

The liver also showed a liver congestion. It is a blockage of blood due to the disturbance of tissue circulation, which is lack of oxygen and nutrient; thus will narrow the sinusoid. It makes the liver looks bigger and cause impaired capillary circulation of blood vessels (Takashima & Hibiya 1995, Doaa et al. 2013).

Liver also showed necrosis. The cellular degeneration and necrosis may be due to the accumulative effect of metals in hepatic tissue. Contaminants induce biological disarrangement of the molecular organization of the cell membranes. Fish commonly accumulate greater concentrations of elements in liver than flesh and gills (Al-Yousuf et al. 2000). Sanad et al. (1997) reported that liver cell necrosis due to the inhibition of synthesis of DNA needed for the growth and maturation of the liver.

The most common histological changes observed in the liver of fish due to heavy metals are hepatocytes vacuolization, fatty degeneration, metabolic abnormality, changes in liver parenchyma and necrosis (Poleksic et al. 1995).

Milkfish (*Chanos chanos)* kidney: An alteration of kidney histopathology of milkfish which was exposed to heavy metals Cd and Pb is shown in Fig. 4. The pollutant caused atrophy degradation, cloudy swelling and hyaline.

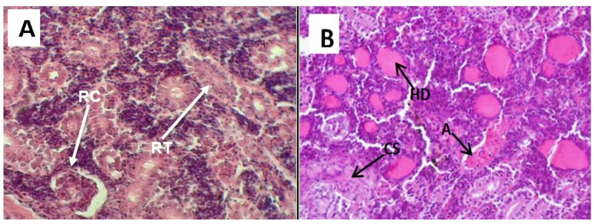

Fig. 4: Photomicrographs of kidney structure of Milkfish. [A] The Normal kidney. Normal renal corpuscle showing the glomerulus and the Bowman's space well defined (RC) Renal Tubules (RT). [B] Histopathology of Fish kidney. Atrophy (A), Cloudy Swelling (CS), Hyaline Degeneration of glomerulus (HD). HE Staining. The pictures were taken by using Olympus CX22 Binocular Microscope, 400X.

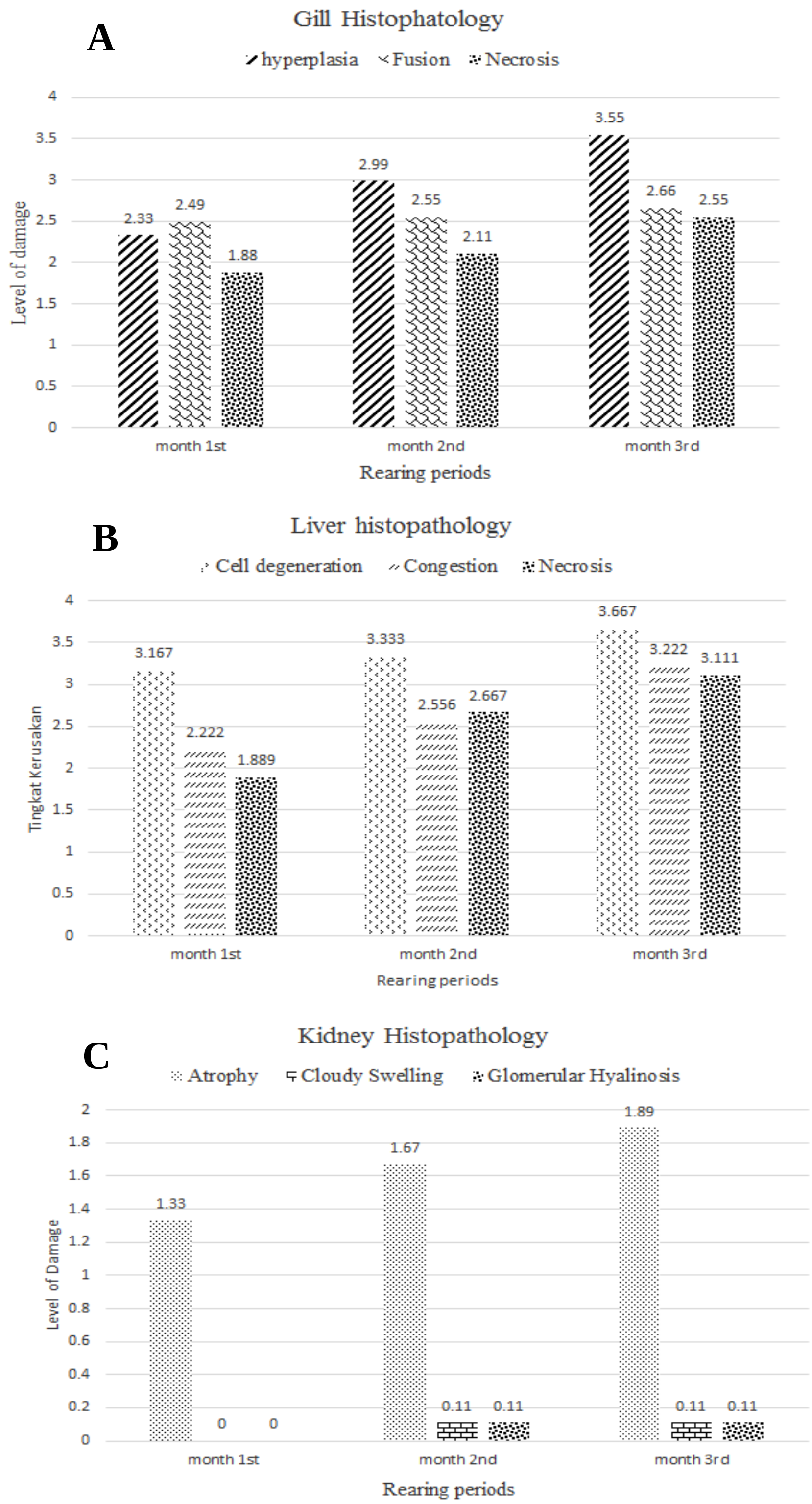

Fig. 5: Level damage scoring of organs histopathology. [A]. Gills, [B]. Liver, [C]. Kidney

Atrophy and cloudy swelling could be seen in Fig. 4B. Cells shrinkage occurred in the kidney tissue as a result of toxic absorption from polluted water. According to Takashima & Hibiya (1995), atrophy is a condition when the amount and the volume of cells are under the normal limit. Continuous atrophy was the main cause of death of cells. The glomerulus in normal kidney structure was neat and clear. However, it is clear that in polluted water, precipitation in the glomerolus surface was caused by polluting substances inside the cell. Interference in the glomerulus production process is called hyaline degeneration of glomerulus (Takashima & Hibiya 1995).

Effect of rearing periods: Milkfish rearing period in the polluted area of Pb and Cd affected the scoring of all alteration of organs. Scoring analysis revealed that the longer rearing period, the more severe the organ damage (Fig. 5).

All abnormalities in gills increased dependently with the rearing periods. In the gills, hyperplasia damage was significantly different compared for 1, 2 and 3 months cultivation ($p < 0.01$). The damage can be categorized based on the level of gill tissue's changing, starting from minor to major. Those are hypertrophy, proliferation, hyperplasia, rupture and necrosis (Oguz 2015). In this research, the damage level of tissue had reached major damage.

In the liver, similar phenomena was also found. Rearing period in Pb and Cd polluted water obviously affected the liver throughout the damage level of cells degeneration, congestion and necrosis. The damage of kidney was also appeared. Atrophy damage is the main alteration of fish kidney.

CONCLUSION

Concentration of the heavy metals (Cd and Pb) in the fishpond were above the permissible limits. The level of heavy metals in the water and sediments of fishponds gave a damage effect on the cultured fish. In this case, the organs gills, liver and kidney of the fish were altered from the normal form due to heavy metal contamination. This result suggested that the water quality of the fishpond was not appropriate for rearing milkfish (*Chanos chanos*).

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