Change in Two-Spot Catfish Histopathological Liver (Mystus nigriceps) Accumulated with Heavy Metal Cadmium (Cd) in Ketingan Estuary, Sidoarjo - East Java, Indonesia

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Abstract. Two-spot catfish (Mystus nigriceps) is freshwater aquatic organism that can survive against the change in a polluted aquatic environment. The aim of this study is to determine the content of heavy metal cadmium (Cd) in sediment, water, and organ of two-spot catfish (Mystus nigriceps) and to examine the histopathological changes in its liver of two-spot catfish living in the waters of Ketingan Estuary river, Sidoarjo, East Java. The results showed that there were damages in liver of 9 samples of two-spot catfish taken from 3 stations. Those damages were were hepatocyte congestion and swelling on the liver. The observations on heavy metal content i.e. cadmium (Cd) in the water, sediment and the two-spot catfish liver showed that they were above the specified threshold. The water quality, i.e. temperature, pH, DO, and brightness were well within the required range.

Keywords: Two-Spot Catfish, Heavy Metal Cadmium, Histopathology.

1. Introduction

Cadmium is soft, bluish-white metal, insoluble in base, easy to react, and can form Cadmium Oxide when heated. Cadmium (Cd) is generally found in combination with chlorine (Cd Chloride) or sulfur (Cd Sulfite). Cadmium forms an unstable Cd2+. Cd has an atomic number of 40, an atomic weight of 112.4, a melting point at 321°C, boiling point at 767°C and density (near r.t.) of 8.65 g/cm³ (Widowati et al., 2008). Two-spot catfish (Mystus nigriceps) is a typical fish found in a large population in Ketingan Estuary. It is freshwater organism that can survive against changes in polluted aquatic environment, including low oxygen level and high turbidity. Two-spot catfish is omnivorous and tends to be carnivorous. As one of the top predators in aquatic chain-food, two-spot catfish risk to take in heavy metals in their feed (Ulfah, 2014). According to the Ministry of Maritime Affairs and Fisheries (2011), Indonesia’s fisheries 2010 recorded a total of fish catches 1,121 tons with an average increase of 10.33% in 2009-2010 and the highest catch was in Java with 479 tons. The biggest fish catches in Java is East Java 339 tons, followed by Central Java 124 tons, West Java 13 tons, and DI Yogyakarta 3 tons. This study aims to determine the content of heavy metal cadmium (Cd) in the sediments, water, and organ of two-spot catfish (Mystus nigriceps) and histopathological changes in the liver in the waters around Ketingan Estuary Sidoarjo, East Java.

The case of cadmium pollution in Japan (Itai-itai Disease) was first found in a highly polluted area in the Jinzu river valley, Toyama Prefecture. The disease showed symptoms of nephropathy and
osteomalacia. Both of these symptoms arose due to cadmium in the body. The local health office or the Toyama Public Welfare Office identified that the area had been polluted with Cd since 1967. The office confirmed that 97% of the 132 deaths were victims of the disease (Kawano et al, 1984).

In 1967, the government identified the content of cadmium, zinc and copper in 34 irrigation areas that utilized Jinzu river as their irrigation system and other 16 irrigation areas used as irrigation systems. Jinzu River irrigation area was the one with the highest content of heavy metal. In 34 paddy fields around Jinzu river, there was 4.04 ppm of heavy metal in the water at the entry area, 2.42 ppm of heavy metal in the middle of the rice field area, and 2.24 ppm in the irrigation outlet area. The cadmium concentration was less than 1.0 ppm in all rice fields. It was hypothesized that cadmium found in the human body came from the rice produced in the region contaminated with cadmium. All rice studied had various Cd concentrations, i.e. ranging from 1.0 ppm to 6.88 ppm (Nogawa and Suwazono, 2011).

If we relate the cadmium case (Cd) of heavy metal pollution in Japan to Indonesian legislation, the case shall will fall under the category of heavy pollution. The content of heavy metal cadmium in rice found in the polluted areas ranged from 1.00 ppm to 6.88 ppm, and 4.04 ppm in Jinzu river. This is not in accordance with the LH Ministerial Regulation of 2010 in that the industrial standard wastewater quality standard is 0.1 mg/l.

2. Research materials and method
2.1. Determination of research station
The research was conducted in May - July 2017. Sampling was carried out at Ketingan Estuary, Sidoarjo, East Java. We conducted survey on the geography condition and activities around the research environment to determine the water sampling stations, marine sediments and fish (Hadi, 2007). Based on the result of a the survey, we determined three stations as sampling stations. Sampling map/location is illustrated in Figure 1. Preparation and test samples was conducted at the Laboratory of the Faculty of Fisheries and Marine Airlangga University Surabaya, Surabaya Industrial Research and Consultation Hall, and Surabaya Fish Quarantine Center.

![Figure 1. Location Map](image)

Description: Station 1 = coastal waters that are located near residential areas.
(7°28’17.4"S - 112°48’26.6"E)
Station 2 = coastal waters that are located near the downstream of the river
(7°29’35"S - 112°49’18.2"E)
Station 3 = coastal waters region located near the boat port
(7°28’30.5"S - 112°48’34"E)

2.2. Sampling
Method fish sampling: the fish nets were spread to the waters for 40 minutes (Haerunnisa, 2014). The fish sampling was based from their size i.e. 15-20 cm -the adult size of two-spot catfish. Hee (2002)
stated that the average length of adult two-spot catfish is 16.3-20.7 cm. Sediments were taken using paralon pipes and sediments were taken at 10-15 cm from the surface of the sediment (Kitong et al. 2012). The amount of water sample for heavy metal testing was 250 ml. The water was then stored in the cool box for heavy metals analysis in the laboratory.

2.3. Chemical analysis using Atomic Absorption Spectrophotometry (SSA)
Chemical analysis was carried out to determine the concentration of cadmium (Cd) in the sediment, water, and fish liver. We analyze the cadmium (Cd) in fishery products using the AAS method (Atomic Absorption Spectrophotometry) based on ISO 2354.5: 2011, prepared wet samples of 5 grams or dry samples of 0.5 grams on a porcelain. The initial step was the evaporation on a hot plate with temperature of 100° C until it was dried. Next, we inserted the sample and the oven ashing into a closed section. The temperature of the oven was increased gradually to 100° C every 30 minutes until it reaches 450° C and it lasted for 18 hours, then we cooled it down. After it was cooled down, we added 1 ml of 65% HNO3, stirred it carefully so that all the ash dissolved in the acid. Next, the initial stage was repeated but the temperature was maintained for only 3 hours. After the white ash was completely formed, the sample was cooled at room temperature. Then we added 5 ml of 6 M HCl to each sample and stirred it carefully so that all the acid did not dissolve the ash. Next, we evaporated it on a hot plate at 100° C to dry and added 10 ml of 0.1 M HNO3, cooled it down until room temperature and left it for 1 hour. We transferred the solution into a 50 ml tube and added modifier solution polypropylene matrix, set it to mark the boundaries using 0.1 M HNO3; next, inside vortex: sequentially add 5 ml - 10 ml of 65% HNO3 and 2 ml H2O2; we sublimated it by setting the microwaves program (adjust the microwave used); transferring the dissolution of 50 ml ashes as an added matrix modifying solution, adjusting it to mark boundaries with deionization of water; preparing a standard Cd working solution of at least 5 concentration points; read standard work solutions, samples and spikes on furnace graphite atomic absorption spectrophotometers at 228.8 nm length.

3. Result and discussion
In this study there were three observation stations, namely stations near residential areas, stations near fishing boat ports, and downstream river stations.

| Code | Sediment | Water | Liver |
|------|----------|-------|-------|
| St. 1.1 | 7.91     | 2.42  | 0.13  |
| St. 1.2 | 7.08     | 1.98  | 0.11  |
| St. 1.3 | 8.15     | 3.02  | 0.12  |
| St. 2.1 | 6.88     | 2.03  | 0.14  |
| St. 2.2 | 5.90     | 1.74  | 0.16  |
| St. 2.3 | 5.18     | 2.90  | 0.15  |
| St. 3.1 | 5.30     | 2.87  | 0.10  |
| St. 3.2 | 5.86     | 3.04  | 0.11  |
| St. 3.3 | 6.14     | 1.98  | 0.10  |

Based on the research results, the concentrations of heavy metal cadmium in Ketingan Estuary in each station showed that cadmium content was above the threshold in the water. The threshold is set by the Decree of the Minister of the Environment No. 51 of 2004, regarding quality standard of seawater (seawater biota) which allows threshold for heavy metal cadmium (Cd) between 0.001 to 0.015 mg/L. From the three observation stations, we found that concentration of cadmium heavy metal in seawater was an average of 2.44 mg/L. The lowest content of heavy metal cadmium (Cd) was at the station near the water port 1.74 mg/l and the highest was at the river downstream station 3.04 mg/l. The results of cadmium heavy metal concentrations in sediments from the three observation stations was an average 6.49 mg/L. The heavy metal content of cadmium (Cd) in the lowest sediment was at the station near the
fisherman-boat port of 5.18 mg/kg and the highest was at the station near the local housing 8.15 mg/kg. The concentration of metal cadmium (Cd) exceeded the sediment quality threshold set by the USEPA National Sediment Quality Survey (0.65-2.49 ppm) and The Ontario Ministry of Environment (0.5-2.5) mg/Kg.

Heavy metals enter into an organism in various ways, namely through its respiratory tract (gills), digestive tract (intestine, liver, kidney), food chain, and skin penetration (Zainuri et al., 2011). The results of cadmium heavy metal concentrations in the liver of two-spot catfish (Mystus nigriceps) from the three observation stations was an average of cadmium (Cd) heavy metal concentration 0.124 mg/L. The heavy metal content of cadmium (Cd) in the lowest sediment was found at the station near the fisherman-boat port 0.16 mg/kg and the highest was at the station near the local housing 0.10 mg/kg. The heavy metal content of cadmium (Cd) in two-spot catfish (Mystus nigriceps) in Ketingan Estuary Sidoarjo was above the quality standard threshold according to SNI 7387 (2009) of 0.1 mg/Kg.

**Figure 2.** Histopathology of the liver of two-spot catfish (*Mystus nigriceps*).

- **(A)** Picture of Normal/Control Liver
- **(B)** Image of Liver Station 1 (1000x)
- **(C)** Image of Liver Station 2 (1000x)
- **(D)** Image of Liver Station 3 (1000x)

Description:  
C : Congestion  
P<sub>H</sub> : Hepatocyte swelling  
A : Picture of Normal/Control Liver  
B : Image of Liver Station 1 (1000x)  
C : Image of Liver Station 2 (1000x)  
D : Image of Liver Station 3 (1000x)

The observation on damage to two-spot catfish’s liver (*Mystus Nigriceps*) was hepatocyte congestion and swelling. According to Van Dyk et al. (2005) one of the causes of congestion is due to exposure to chemicals such as cadmium, mercury and zinc. This happens because most of the toxins or toxic substances enter the body absorbed by the cell will then be taken to the liver by the veins, so the liver may become damaged. This finding was also supported by the study of Pantung et al (2008) which stated that fish exposed to cadmium cause hepatocyte congestion and swelling. This is reinforced by Hinton
and Lauren (1990) who reported that the exposure of cadmium to hepatocyte cells will cause swelling of hepatocytes as a direct result of toxic substances that affect directly on the mechanism of ion transport.

Cell swelling begins with the ability of cells to regulate osmotic regulation of the external environment by utilizing metabolic energy to pump sodium ions out of the cell. Heavy metals that accumulate in the liver cells affect the metabolic process to be disrupted so that the cell cannot pump enough sodium ions. The concentration of sodium ions in the cell is higher and water can enter the cell. This excessive concentration of water results in cell swelling consequently the process of exchange of nutrients and other substances will be disrupted (Fujaya, 2008).

In the liver cells, congestion is characterized by blood blocking. This is in line with the view of Sudiono (2003) who stated that congestion is characterized by swelling of the liver cells in which the blood is blocked due to disorders in blood circulation in sinusoids. The entry of excess water into the cell causes the cell swelling, so the size of the cell increases and sinusoids eventually becomes constricted. Sinusoid is a cavity found in the liver tissue that allows for nutrient exchange. If the sinusoid narrows due to cell swelling, the blood will be blocked in the liver tissue so the process of exchanging nutrients between blood and hepatocytes will be disrupted. Congestion in the liver can also cause damage to the work system in the liver which affects the detoxification system and digestive system in fish (Takashima & Hibiya 1995). Symptoms on histopathology in congestion is the presence of blood spots in blood vessels caused by exposure of chemical agents (Juhryyah, 2008).

**Table 2. Parameter measurement of water quality.**

| Sample | Temperature (°C) | pH | DO (mg/l) | Clarity (cm) |
|--------|------------------|----|-----------|-------------|
| ST1.1  | 26               | 7  | 4         | 20          |
| ST1.2  | 26               | 7  | 4         | 20          |
| ST1.3  | 26               | 7  | 4         | 20          |
| ST2.1  | 26               | 7  | 4         | 20          |
| ST2.2  | 25               | 7  | 4         | 20          |
| ST2.3  | 27               | 7  | 4         | 20          |
| ST3.1  | 26               | 7  | 4         | 20          |
| ST3.2  | 25               | 7  | 4         | 20          |
| ST3.3  | 25               | 7  | 4         | 20          |

The temperature in the waters around the entry of Ketingan Estuary ranged from 25 to 27°C which indicates that the waters were good. According to Sumijo (2011) a good range of temperature for fish in the tropic is between 25°C and 32. DO values or oxygen solubility in the waters around the entry of Ketingan Estuary were 4 mg/L which indicates that these waters had low dissolved oxygen. The value of dissolved oxygen in the waters should be between 6 to 8 mg/L (Barus, 2004). According to Rinawati et al. (2008), the minimum oxygen solubility that can add to heavy metal toxicity to the life of an organism is around 4.0mg/L. The pH value in the waters around the entry of Ketingan Estuary is 7 which indicates that the water pH was good. The ideal pH value for aquatic organism life in general is from 7 to 8.5. The increase in pH above neutral value will increase the concentration of ammonia which is very toxic to the organism (Barus, 2004).

Based on the results of examination of sediment, water, and liver of two-spot catfish from around the entry of Ketingan Estuary, Sidoarjo, the heavy metal content of cadmium (Cd) in the lowest water was at the station near the water port at 1.74 mg/l and the highest was at the downstream river at 3.04 mg/l. The heavy metal content of cadmium (Cd) in the lowest sediment was at the station near the fisherman-boat port of 5.18 mg/kg and the highest was in the station near the local housing 8.15 mg/kg. The heavy metal content of cadmium (Cd) in the lowest sediment was at the station near the fishing boat port of 0.16 mg/kg and the highest was the station near the residential settlement 0.10 mg/kg. The content of cadmium (Cd) has exceeded the threshold set by the Government and the Indonesian National Standard.
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