Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan (2022) 11(2): 148-152

DEPIK

Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan

Journal homepage: www.jurnal.unsyiah.ac.id/depik

Metallothionein levels on the gill and liver of *Mystus nigriceps* (Valenciennes, 1840) in Welang River, Pasuruan City, East Java

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ARTICLE INFO

Keywords:
River
Metallothionein
Cadmium

DOI: 10.13170/depik.11.2.24748

ABSTRACT

This research was conducted in October 2021 using a survey method. Sampling was carried out three times with a span of every 2 weeks at 3 stations along the lower reaches of the Welang River Pasuruan Regency. Parameters observed included cadmium metal content in fish gills and liver, metallothionein levels in gills, and liver of *M. nigriceps*. Fish and the relationship between cadmium metal and metallothionein levels in the gills and liver of *M. nigriceps* in the Welang River. The average yield of cadmium metal content in the gills of *M. nigriceps* is 5.943-8.103 mg/l. Meanwhile, the average result of heavy metal content in the liver of *M. nigriceps* is 6.397-9.997 mg/l. Measurement of metallothionein levels showed that the average level of metallothionein in the gills of *M. nigriceps* was 5.860-8.688 ng/mL. Meanwhile, the average level of metallothionein in the liver of *M. nigriceps* is 7.110-8.334 ng/mL. And the results of the linear regression test showed that the average content of cadmium in the gills and liver with the average metallothionein levels in the gills and liver of catfish was significantly correlated.

Introduction

Rivers are open waters that flow from upstream to downstream and also the entry of waste originating from human activities in residential, agricultural, and industrial areas in the surrounding area. The entry of waste into river bodies can cause changes in physical, chemical, and biological factors in the waters (Sahabuddin *et al.,* 2014). The Welang River is the main river with a catchment area of 518 km^2, and one of the longest rivers, which is 36 km with a width of 35 m, and is located in the city of Pasuruan. This river empties into Pulokerto Village, Kraton District the Welang River is also a border river that borders Pasuruan City and Pasuruan Regency. Survey results during research, the Welang river is also a body of water that receives wastewater from various industries and domestic waste from the surrounding community into the river body, resulting in a decrease in river water quality and affecting the health of organisms in the river water.

The quality of the river would experience changes under environmental developments that are influenced by various human activities. River pollution is certainly caused by the life around it, both on the river itself and human activities as river users (Mardhia and Viktor, 2018). Results Activities originating from industrial, domestic, or around the Welang river can produce waste in the form of heavy metals that enter the aquatic ecosystem either directly or indirectly. The aquatic environment that is polluted by heavy metals would accumulate in the body of the fish (Tchounwou *et al.,* 2012). Heavy metals that already exist in these waters would be easily absorbed and buried in phytoplankton where this is the starting point of a food chain process, then it would continue to other organisms. Heavy metals that are contaminated with soil in the waters would reach the food chain and in the end can endanger the life of fish in these waters (Yusuf, 2011).

The increase in heavy metal levels in water is followed by an increase in heavy metals in the bodies

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p-ISSN 2089-7790; e-ISSN 2502-6194
Received 9 February 2022; Received in revised from 4 April 2022; Accepted 25 June 2022
Available online 19 July 2022

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 11, Number 2, Page 148-152

of fish and other biotas in these waters, so water pollution by heavy metals would result in polluted fish living in them. Heavy metal levels such as cadmium are often detected in the gills, kidneys, and liver of fish (Thophon *et al.,* 2003). Fish gills are the main organ that acts as a surface diffusion mechanism of respiratory gasses (oxygen and carbon dioxide) between blood and water, thus changes in the aquatic environment would directly affect the structure (Saputra *et al.,* 2013). The liver is the main organ in the fish body that has the function to maintain homeostasis through metabolism and nutrient storage and detoxification in fish. In addition, the liver is also a target organ for toxicants including heavy metals (Sari *et al.,* 2016).

Metallothionein is a universal biomarker that functions as an early warning against pollution by exposure to heavy metals (Cd, Hg, and Pb) at the subcellular level, the initial reaction before the response occurs at a higher biological organism (spectrum) level (Dewi *et al.,* 2014). In general, Metallothionein has a basic structure in the form of metal elements and amino acids as a constituent of the protein body. Metallothionein induction depends on the amount of oxidative heavy metals and glucocorticoids in the body. In general, high amounts of metallothionein are synthesized in the liver and kidneys as scavengers in cells due to the presence of cysteinyl thiolate groups (Nedecky *et al.,* 2013).

Exposure to metal cadmium, lead and mercury (Cd, Pb, and Hg) whose levels are still below the quality standard has been able to induce metallothionein synthesis in liver tissue so that metallothionein appears. Metallothionein formed would function as detoxification of heavy metals. In other words, if there is exposure to heavy metals that have a high affinity for thioenin, the metal has a high ability to induce metallothionein, thus forming metallothionein, and the metal would be detoxified immediately, resulting in no metal accumulation in the body that has the potential to exceed the threshold (Dewi *et al.,* 2014).

This study aimed to analyze the levels of metal cadmium in the gills and liver of *M. nigriceps* levels of metallothionein in the gills and livers of fish, and the relationship between cadmium metal and metallothionein levels in the gills and liver of *M. nigriceps* in the Welang river, Pasuruan district.

Materials and Methods

Location and time of research

This research was conducted in October 2021, which is located on the Welang River, Pasuruan Regency (Table 1 and Figure 1). The method used in this research is a descriptive survey method. Determination of sampling stations based on purposive sampling method by taking into account certain characteristics and land use. The first station is located near PT Carma Wira, the second station is an agricultural waste disposal area and a community boat dock of Tambakrejo Village, and the last station is in the shrimp farming area.

Table 1. Positions of three stations on the Welang River.

Station Names	Coordinates
Station 1	7⁰37’42.4”S and 112⁰52’29.4”E
Station 2	7⁰37’00.0”S and 112⁰52’32.2”E
Station 3	7⁰35’48.7”S and 112⁰52’10.8”E

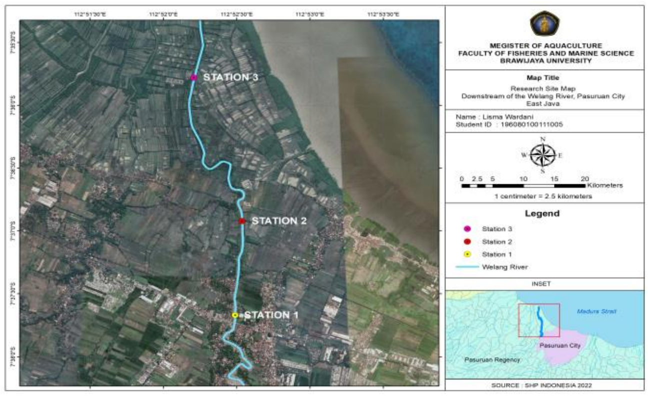

Figure 1. Image map of the research location downstream of the Welang River.

The method of observing cadmium in target organs using the AAS method and analysis of metallothionein levels in the gills and liver of *M. nigriceps* using the ELISA (enzyme-linked immunosorbent assay) method. ELISA is a plate-based assay technique designed for the detection and quantification of peptides, proteins, antibodies, and hormones. The procedure for measuring metallothionein levels is starting by taking samples of fish gills and livers, homogenization stage, extraction stage, purification stage, and metallothionein quantification. This procedure is following the ELISA method (Booster Biological Technology, 2020).

The data of cadmium metal levels and metallothionein levels in the gill and liver were analyzed using simple linear regression correlation using SPSS. This is to see the relationship between cadmium metal and metallothionein levels in those target organs.

Results

The heavy metal content of cadmium in the gills and liver of in *M. nigriceps* the Welang River can be seen in Figure 2. and the results of the average levels of metallothionein in the gills and liver can be seen in Figure 3.

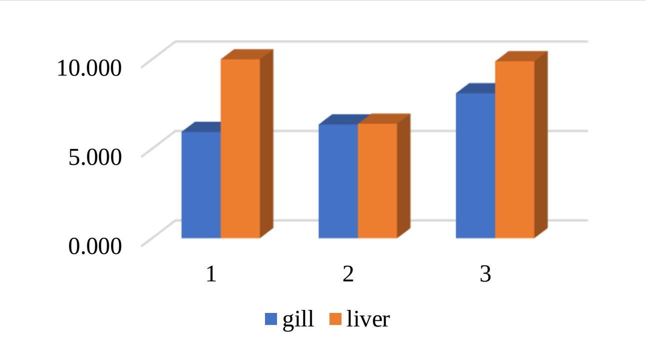

Figure 2. The content of heavy metal cadmium in the gills and liver of *M. nigriceps*.

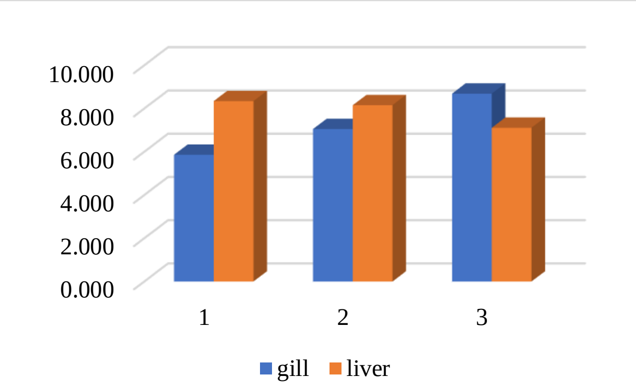

Figure 3. Average levels of metallothionein in the gills and liver of *M. nigriceps*.

Cadmium metal content in Figure 2 The results showed that the average metal content of cadmium in *M.nigriceps* fish was 5.943-8.103 mg/l. The highest cadmium metal content in fish gills was found at station 3 namely 8.103 mg/l, and the lowest cadmium metal content was found at station 1 which was 5.943 mg/l. While the average content of heavy metals in the liver of *M. nigriceps* is 6.397-9.997 mg/l. The highest cadmium metal content in the liver of *M. nigriceps* was found at station 1 which was 9.997 mg/l and the lowest cadmium metal content was found at station 2 which was 6.397 mg/l.

Metallothionein levels are shown in Figure 3. The results of the study in the Welang River showed that the average level of metallothionein in the gills of *M. nigriceps* was 5.860-8.688 ng/mL. The highest average metallothionein level in the gills of *M. nigriceps* is found at station 3 which is 8.688 ng/mL and the lowest average metallothionein level is at station 1.

The average level of metallothionein in the liver of *M. nigriceps* is 7.110-8.334 ng/mL. The highest average metallothionein level was found at station 1 namely 8.344 ng/mL, and the lowest average metallothionein level in the liver was obtained at station 3.

Based on Table 2, the linear regression test shows that the variable X which has a significant value of <0.05 is cadmium metal gills and liver of catfish (*M. nigriceps*). This shows that cadmium metal in the gill and liver has a significant effect on the amount of metallothionein. Based on the value of the correlation coefficient (R) also shows that cadmium in the gills and liver has a strong correlation with metallothionein. The results of the regression equation on cadmium metal in the gills and liver have a directly proportional relationship, this can be seen from the magnitude of the effect of the average content of cadmium metal in the gills on the average metallothionein content in the gills is 1.363 ng/mL. Meanwhile, the average effect of cadmium metal content in the liver on metallothionein levels was on average 0.953 ng/mL. These results indicate that when cadmium in the gills and liver increases, the amount of metallothionein in the gills and liver will also increase.

Table 2. Linear regression test of cadmium metal in gills and liver with metallothionein levels in gills and liver of fish *M. nigriceps*

Parameter	Sig.	Correlation	regression equation
Gill	0.013	0.781	Y= -2.071+1.363x
Liver	0.040	0.690	Y= -0.477+0.953x

Discussion

Metallothionein protein which acts as a metal-binding protein can be used as an indicator of pollution because the presence of metallothionein in oysters functions as a binder of heavy metals that accumulate in the body (Rumahlatu *et al.,* 2012). Metallothionein is found in all body tissues of fish. The gills and liver are generally the most studied organs for metallothionein induction in fish because they have their respective roles in the absorption and detoxification of heavy metals in water (Costa *et al.,* 2008). This is in accordance with the results of research in the Welang River where the more cadmium metal content that accumulates in the gills and liver, the more metallothionein protein expression in the fish organs increases.

Metallothionein synthesis in fish is associated with organs involved in metal absorption, metabolism, and excretion such as gills, liver, kidneys, and intestines (Sevcikova *et al.,* 2011). The liver is the main organ in the fish body that has the function to maintain homeostasis through metabolism and nutrient storage and detoxification in fish. In addition, the liver is also a target organ for toxicants including heavy metals (Yona *et al.,* 2020). The results of this study showed that the highest metallothionein levels in the liver can be seen in Figure 2. Because the heavy metal content in the liver is higher than the cadmium metal content in the gills. These results are in accordance with the opinion of Siscar *et al.* (2014), that the liver functions as a detoxifier that can bind pollutants in the water which is characterized by high metallothionein.

The binding of heavy metals in metallothionein is believed to be a defense mechanism to protect important proteins in the metabolic processes of the fish body from the influence of heavy metals (Legras *et al.,* 2000). If the speed of heavy metals entering cells exceeds the rate of metallothionein synthesis, the liver's ability to detoxify will decrease, so that excessive heavy metals will be distributed throughout the body through the blood vessels (Suratno *et al.,* 2017). In this study, metallothionein levels were found in different fish organs where the highest metallothionein levels were found in fish livers compared to metallothionein levels in gills. However, at the last station, the metallothionein levels in the fish liver were lower than in the gills. These results are in accordance with the statement of Gonick (2011), that different levels of metallothionein in fish body tissues accumulate depending on the increase in the amount of metallothionein mRNA in tissues exposed to heavy metal cadmium in response to different flow rates of heavy metals in tissues. Different levels of metallothionein are also associated with other metal-binding proteins. metallothionein (MT) can decrease the elimination of cadmium through bile and is the main agent for tissue cadmium retention (Shamsi and Fatimah, 2014).

Conclusion

The content of cadmium in the Welang River has exceeded the quality standard. The relationship between cadmium metal with metallothionein levels in the gills and liver of *M. nigriceps* has a strong relationship, the higher the cadmium metal content that accumulates in the gills and liver, the metallothionein protein expression in fish organs increases. Therefore, this measurement of metallothionein proved to be an effective heavy metal biomarker.

Acknowledgments

Researchers would like to thank various parties, especially supervisors, comrades in arms, and the community around the Welang river, Pasuruan Regency, East Java, and local fishermen who have facilitated the place during the research so that it can be carried out properly.

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How to cite this paper:
Wardani1, L., E.Y. Herawati, A.M. Suryanto. 2022. Metallothionein levels on the gill and liver of *Mystus nigriceps* (Valenciennes, 1840) in Welang River, Pasuruan City, East Jawa. Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan, 11(2): 148-152.