

Bioaccumulation of Heavy Metals in Fish (*Barbodes sp.*) Tissues in the Brantas River, Indonesia

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Received: November 12, 2016

Accepted: January 28, 2017

ABSTRACT

This study aims to determine the content of heavy metals and their effects on fish (*Barbodes* sp.) tissues in the Brantas river. Sampling was performed three times, namely in March, June, and September 2016. The samples of fish in this research that the upstream (Reservoir Karangates) and downstream of the Brantas river (Kali Surabaya and Kali Jagir). Fish tissues (liver, gonads, and gills) were collected, analyzed the heavy metal and tissues histopathology, stained with Haematoxylin-Eosin. The results showed that all the tissues of fish that live in the upstream and downstream of the Brantas River contain high heavy metals (Pb and Cr). The highest levels of heavy metals found in succession in the tissues liver, gonads, and gills. High levels of heavy metals that damage the structure of cells and tissues of all tissues of the fish. The most damage found in tissues containing the highest levels of heavy metals. High of heavy metals in the cells and tissue caused damage and necrosis cells.

KEYWORDS: Brantas river, heavy metal, liver, gonads, gills.

1. INTRODUCTION

Brantas River is located in East Java, is the second longest river in Java after the Bengawan Solo. Brantas River is a very important densely populated area in East Java, Indonesia for agriculture [1]. The river plays a role in irrigation along the stream and is habitat for a survival of aquatic biota, including fish [2]. Determination of contamination level of heavy metals throughout the river is important and has a major role in controlling the ecological conditions of its environment [3,4]. Clean up of the contaminations caused by heavy metals based on the natural methods including use of plants such as beech leaves will result in a healthy surrounding life while leaving no negative effects [4]. Biota water that serves as a provider of nutrition for the community and as biological indicators of the health of the fish that ecosystem waters [5]. In the river contaminated by heavy metals may lead to biomagnification and bioaccumulation in the food chain of an water environment. Such contaminants transported from its sources through river system and deposited downstream. Since most of the pollutants could be mixed and became suspended in bottom sediment [6]. The presence of heavy metals in the river can lead to greater environmental problem when the contaminated sediments resuspended and such metals are uptaken by fish. Hence, consumption of such kind of fish may form a significant pathway to metals contamination in the human being and eventually poses greater health risk.

Brantas river has detected heavy metals (Pb, Cu, Cr, and Cd). Some heavy metals in waters that enter the body organism is able to bind to receptors on the cellular level. Heavy metals are able to bind to estrogen receptors (metalloestrogens) have to be estrogenic, so it will disrupt and inhibit the function of the endocrine system [7]. In the reproductive system, the presence of heavy metals that are estrogenic can lower the reproductive capacity of the organism, if persists may lead to the extinction of an organism [8, 9, 10].

The existence of this precipitation causes the concentration of heavy metals in sediment is higher than the water above it. Presence of heavy metals in the water when it enters the body of fish can accumulate to all the tissues and tissues of fish [11, 12]. Gills and digestive tract is the first organ that is directly related to the heavy metal waters [13]. Furthermore, the flow of blood into the cells and tissues of the body, include the reproductive tissues [12]. Heavy metals are toxic and estrogenic can reduce the ability and the survival of fish [12]. Fish populations decline as many fish that heavy metal poisoning, if this was left unchecked can lead to extinct aquatic organisms.

The problem in this study are different levels of heavy metals (Pb and Cr) on the tissues of fish in the upstream and downstream Brantas river, difference in levels of heavy metals can damage cells of fish in the upstream and

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downstream Brantasriver. This study mainly aimed to investigate the content of heavy metals and their effects on fish (Barbodes sp) tissues in the Brantasriver.

2. MATERIALS AND METHODS

The chemicals used include paraffin, alcohol, paraformaldehyde solution, xylol, hematoxylin-eosin, tools sed are microtome, microwave, light microscope and inverted microscope (Olympus), and Cold Vapour Atomic Absorption Spectrophotometer (CV-AAS). Sampling was performed three times, namely in March, June and September 2016. The samples of fish(*Barbodes sp.*)collected from the upstream (reservoir Karangates) and downstream of the Brantas river (Kali Surabaya and Kali Jagir).

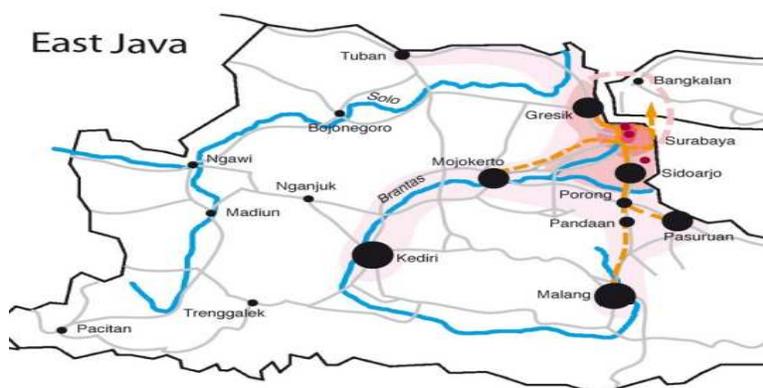


Figure 1. Sampling locations on the Brantasriver (◀)

Fish collection conducted by trawl nets. Specifications nets used to catch fish was as follows. Mesh size (mesh size) is divided into two distinct parts, namely the top and bottom. The upper part is made of yarn strings and has a mesh size of 2 cm, while the bottom nets made of nylon with mesh size 3 cm. Strings used thread size was 60. The difference in the size of the material and size of the net aims to simplify the process of catching fish. At the lower end of the net is equipped with chains bracelet made of iron and attached to each end of the net. The chain serves as ballast. Nets used has a radius of 6 m, with a net weight of approximately 20 Kg.

Fish tissues (liver, gonads, and gills) were collected, then some tissues analyzed lead(Pb) and chromium(Cr) content and others made preparations histopathology. Selected tissueswere dehydrated through graded series of ethanol, sectioned at 4-5 micrometer thickness and stained with Haematoxylin-Eosin. Measurement of levels of heavy metals in the tissues and tissues of the fish with CV-AAS method.

3. RESULTS

Concentrations of heavy metals (Pb and Cr) in the gonads, liver, and gills of fish from the upstream and downstream of the river Brantas in different species are presented in Table 1.

Table 1. Levels of heavy metals in the tissues (gonads, liver, and gills) fish in the upstream (Karang Kates reservoir) and downstream (Kali Surabaya and Kali Jagir) Brantas river

Stasion	Fish Organ	Pb (ppm)		Cr (ppm)	
		<i>Barbodesballeroides</i>	<i>Barbodesgonionotus</i>	<i>Barbodesballeroides</i>	<i>Barbodesgonionotus</i>
KarangKates Reservoir	Gonad	0.21 ± 0.2	0.20 ± 0.2	0.34 ± 0.5	0.28 ± 0.4
	Liver	0.16 ± 0.1	0.19 ± 0.2	0.14 ± 0.2	0.16 ± 0.1
	Gill	0.28 ± 0.3	0.41 ± 0.5	0.39 ± 0.5	0.40 ± 0.6
Kali Surabaya	Gonad	0.31 ± 0.2	0.31 ± 0.3	0.34 ± 0.4	0.36 ± 0.5
	Liver	0.26 ± 0.2	0.23 ± 0.2	0.27 ± 0.3	0.24 ± 0.3
	Gill	0.47 ± 0.5	0.41 ± 0.4	0.60 ± 0.7	0.41 ± 0.5
Kali Jagir	Gonad	0.30 ± 0.0	0.35 ± 0.2	0.25 ± 0.2	0.52 ± 0.7
	Liver	0.31 ± 0.1	0.30 ± 0.2	0.32 ± 0.4	0.26 ± 0.4
	Gill	0.50 ± 0.2	0.53 ± 0.3	0.46 ± 0.5	0.75 ± 1.1

The sensitivity of tissues of all fish species are *Barbodesballeroides* and *Barbodesgonionotus* to heavy metals (Pb and Cr) was almost the same. Accumulated Pb in the gonads of both species ranged from 0.21 to 0.35 ppm, the liver ranged from 0.16 to 0.31 ppm, and gill ranged from 0.28 to 0.53 ppm. While the highest Cr (0.75 ppm) was found on the gills *Barbodesgonionotus* that live in the waters of Kali Jagir, and the lowest (0.14 ppm) was found in the liver *Barbodesballeroides*.

The high levels of heavy metals (Pb and Cr) which accumulating in fish tissues can disrupt endocrine function of the hypothalamic-pituitary-gonadal. Steroid hormone synthesis and secretion decreased impact on the number of cells gametes. In the gonads of fish can change the structure of cells making up the gonads include spermatogenic cells. Spermatogenic cells exposed metal can inhibit gametogenesis process so that the production of spermatozoa decreased. Toxic nature caused the metal can damage the structure of the seminiferous tubule gonads (testes).

The mechanisms by which heavy metals contaminants can affect fish reproduction are several. Some pollutants are known to influence directly the reproductive cycle of the adult fish. Pollutants accumulated in adult fish may be transported to the gonad during sexual maturation and result in a direct toxic effect on the developing gonad. High levels of heavy metals that damage fish testis (Figure 1).

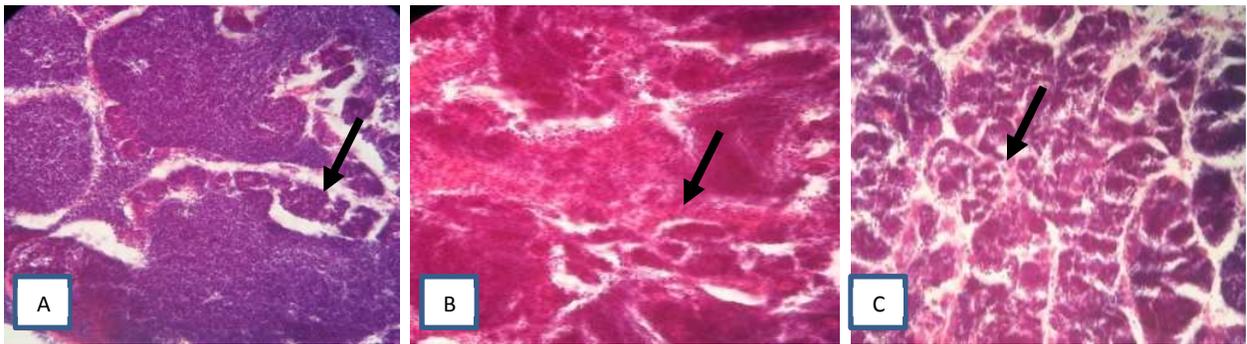


Figure 1. Structure of the gonads (testes) *Barbodes* sp. in the different retrieval station, A=Karangkates reservoir station, B=Kali Surabaya station, C=Kali Jagir station. Testicular tubular necrotic (↓)

Heavy metals in the form of ions into the cytoplasm of liver cells through the blood stream from the gills. Heavy metal ions in the cells of the liver can increase protein synthesis metalotionin (Mt). This protein will bind to the metal to reduce metal toxicity properties in the target cell. This is because the reactive metal ions can damage cells or necrosis. Hepatic function to neutralize or detoxify toxic materials toxic caused detectable levels of heavy metals lower gonads and gills of fish. However, when the metal content is very high (exceeding quality standards), cause structural changes in constituent cells of the liver (Figure 2).

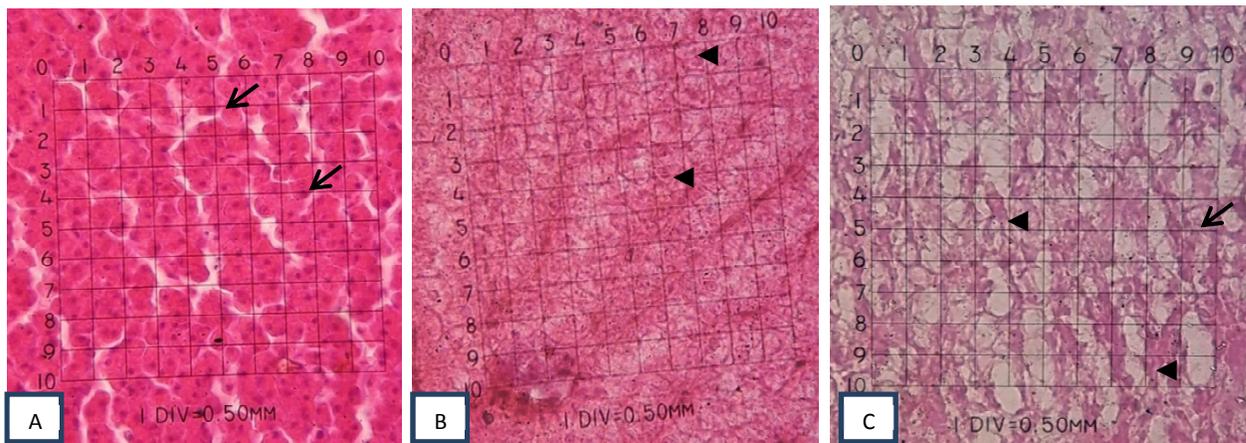


Figure 2. Structure of liver cells *Barbodes* sp. in the upstream and downstream of the river Brantas. A= fish in the upstream (Karangkates reservoir), B = downstream (Kali Surabaya), C= at river downstream (Kali Jagir). Cell necrosis (◄), cell swelling (↑)

In the gills, the heavy metal entered through primary and secondary lamella simultaneously during respiration, then streamed through the bloodstream to the tissues of the fish. The gills are exposed to the metal can damage the gill lamellae. Its toxicity causes increased cell metabolism, so that the cells to swell. But when high metals can cause the fusion of secondary lamella. This situation occurs because of the swelling lamella lamella membrane and lysis of adhesions adjacent membrane, so that the lamella fusion into one. In high concentration of the metals could damage the membrane lamella, that was causing cell death or necrosis (Figure 3).



Figure 3. The secondary lamella structure of *Barbodes* sp. gills on different stations, A=atKarangkates reservoir, B=at Kali Surabaya, and C=at Kali Jagir. Proliferation of filamentous epithelium and edema (black arrow), fusion between two secondary lamellae (↓)

4. DISCUSSION

Heavy metal sources in the Brantasriver from various waste industry, the settlement and agriculture also contribute to increasing the levels of heavy metals in the Brantas river [1]. Karangkatesreservoir (upstream of Brantasriver) was a reservoir of water from several small streams. Lots of places suspected of being the source of the metal in this place, among which was a reservoir used as a cultivation of freshwater fish. Of fish feed was a source of organic material that is bonded to the metal, because the metal is in the form of compounds which bind water with organic material. Brantasriver downstream, the heavy metal content was higher than the upstream area. It is thought the number of waste disposed therein, so that the river Kali Jagir a final disposal site.

Accumulated heavy metals may lead to structure alterations in the tissues of fish [14]. In order to evaluate the adverse effects of the pollutants on aquatic organisms, there is trend to using biological parameters as biomarkers in aquatic pollution monitoring. As shown in Table 1, all fish organ in the Karangkates reservoir contained the lowest heavy metals concentration, while the highest concentration in Kali Jagir. The all fish contained the lowest concentrations of heavy metals in liver, while almost all fish species showed the highest concentrations in gills. In the organ all of the fish contained the Cr higher than the Pb. The accumulation of metals in *Barbodesballeroides* and *Barbodesgonionotus* showed different in all metals. However it can be noticed that, different tissues exhibited different patterns in heavy metals accumulation. In other words, no single type of fish showed the highest metals in all tissues. The result of this study showed the concentrations of heavy metals in the tissues both species was different.

The gonads, high levels of heavy metals can lower gonadal function in reproduction. Heavy metals exert multiple adverse effects through interfering with tissues functions, generating reactive oxygen species. The presence of ROS showed that heavy metals are toxic compounds that cause environmental pollution in the water. Heavy metals into the aquatic environment can accumulate in the body of aquatic organisms. Growth, osmoregulation and reproduction of these organisms will decrease [15]. In this study, heavy metal content in the liver is smaller than in other tissues (the gonads and gills). The process whereby heavy metals accumulate in the tissues and organs of living organisms dependent species and associated with detoxification mechanisms and metabolism [16]. Detoxification mechanisms that occur in liver cells, so that the heavy metal content in the liver to decrease.

The gill was the first organ that is in direct contact with the heavy metal in the waters. This interaction occurs when fish respiration, metals enter the body along with oxygen through their gills. The damaged gills showed an arrangement of filaments in double rows and the secondary lamellae arise from primer filaments. The gills of heavy metals exposed showed degenerative changes in epithelial cells of secondry filaments, necrotic changes and fusion in secondary lamellar epithelial cells. The gills of heavy metals exposed fish showed dilation

and edema in blood vessel of primary gill filament. Hyperplasia of epithelial cells between secondary lamellae led to fusion and separated from pillar system. Vacuolation and necrosis of gills lamellar epithelial cells in the damage gills. Edema and hyperplasia of lamellar epithelial cells was evident in gills of fish exposed to heavy metals. Some studies revealed that interstitial edema is one of the more frequent lesions observed in gill epithelium of fish exposed to heavy metals [14].

5. CONCLUSION

All the tissues of fish contained high heavy metals (Pb and Cr). The highest concentration of the heavy metal was in the gills, but the lowest concentration was in the liver. The heavy metals in the gonads and liver of fish caused damage and cell necrosis. But in the gills was caused fusion, proliferation of filamentous epithelium, and edema.

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