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The Effect of Heavy Metals on Tissue Damage in Different Organs of Goldfish Cultivated in Floating Fish Net in Cirata Reservoir, Indonesia

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ABSTRACT

Cirata Reservoir receive the antrophogenic wastes that cause pollution. The amount of heavy metals contamination in the reservoir need to be considered. This research was aimed to see the environment quality, accumulation of heavy metals and the histopathology of organs of goldfish (Cyprinus carpio) which cultured in the floating net cage. This study was conducted to determine: the water quality, the heavy metals in the waters and in the organ of fish, and analyze the histopathological preparations of these organs. The result showed that, the water quality was still in a good condition, although the heavy metals concentrations of Pb, Cr, Cd, and Hg were higher than Indonesian Guidelines. The average of heavy metals concentrations in liver, kidney and spleen were higher than Netherland's Guidelines. Histopathological analysis of the liver, kidney, and spleen showed that these organs relatively had experienced the abnormalities caused by the heavy metals.

KEYWORDS Cirata Reservoir, heavy metals, goldfish, histopathology, gill, kidney, liver, spleen, abnormality.

Introduction

Cirata Reservoir is a multipurpose reservoir used for a various activities. One of the activities that is noticeable in this reservoir is fishing activity, in view of the fishing activity in the form of fish culture in the floating net cages in this reservoir is very dense, it reaches to 53.031 units (communication with the Incorporated Company of PJB UP Cirata and the Management Office of Cirata Reservoir, 2013), so that has exceeded the normal capacity of floating net cage in Cirata Reservoir which should be only 12.000 units.

The ecosystem of Cirata Reservoir utilized for the fish culture has been polluted by the hazardous and toxic subtances, particularly the heavy metals such as mercury (Hg), lead (Pb), and cadmium (Cd) are mainly derived from the liquid waste of the industrial activities, urban activities, and others that are present in the upstream and around reservoir (Riani, 2010 a dan b). The polluting of Cirata Reservoir by mercury (Hg), lead (Pb), and cadmium (Cd) is the problem that requires a serious attention. This is due to the heavy metals are toxic and can be accumulated in the body of organism as well as cause the health problems and the abnormality of a various organs (Mason 1981; Moore and Ramamoorthy 1984; Klaassen et al., 1986; Guven et al., 1999; Freedman 1989 in Ashraf et al., 2011; Riani, 2012).

However, the research on the organ abnormality in the fish body, especially the goldfish cultured in the floating net cage has never been done before. The research that had been done in Cirata Reservoir generally more to be the study of the water quality, sedimentation rate, management of farming in floating net cages, estimation of carrying capacity of environment, which showed that Cirata Reservoir has been contaminated specially by the organic waste. The researchs were conducted in Cirata Reservoir included the study of Kartamihardja et al. (1999) on the sedimentation rate, nutrients of sediment and nitrification capability test of Nitrosomonas bacteria for restoration of water quality in the farming of floating net cage. Krismono (1999) concerning the environmental management of fish culture in the floating net cage. Sukimin (2008) on the subject of the environmental carrying capacity estimation model based on the phosphorus waste. Insan (2009) about the trophic status and the carrying capacity of floating net cage in Cirata Reservoir. Saputra (2009) on the bioaccumulation of heavy metals in the catfish cultured in the Cirata Reservoir and in the Laboratory. Maulana (2010) regarding the carrying capacity of the floating net cage in Cirata Reservoir. The study of Riani (2010b) on the heavy metals contamination of fish cultured in the floating net cage in Cirata Reservoir. Ardi (2012) concerning on the dynamic control of the activity of phosphorus source (P) in the reservoir in an integrated and environmentally sound: study case of Cirata Reservoir, West Java. Rachmadianti (2013) on the topic of the analysis of the heavy metals concentrations of zinc (Zn) and copper (Cu) in the tilapia fish and Cirata Reservoir, Purwakarta, West Java. Nurfaradilla (2013) on the financial feasibility analysis of the business of floating net cage in Cirata Reservoir with flushing cost internalizing. Radityo (2013) on the economic impact of the water pollution on the fisheries of floating net cage system in Cirata Reservoir, West Bandung Regency. Aditriawan (2013) about the accumulation of copper (Cu) in the tilapia fish (Oreochromis niloticus) raised in the media containing sediment from Cirata Reservoir. Wiradisastra (2014) on the topic of the biodiversity of non-cultured fish in Cirata Reservoir, Cianjur, West Java. Listya (2014) on the release of phosphorus from the floating net cage of pomfret fish (Colossoma macropomum) in Cirata Reservoir. Anhar (2014) about the estimation of the economic losses value due to the pollution water in Cirata Reservoir, Cianjur Regency, West Java Province. Rahmaniah, (2014) estimated the value of willingness to pay and identified the economic behavior of floating net cage fish farmers in Cirata Reservoir. Riani et al. (2014) about the unviable larvae of the chironomidae in Saguling Reservoir that located just in downstream Cirata Reservoir. Hamzah et al. (2014) on the water quality of Jatiluhur Reservoir (downstream Cirata Reservoir), etc.

The data above shows that the research regarding the condition of the goldfish organs affected by the pollution of the hazardous and toxic substances (heavy metals), its data is relatively undiscovered. Therefore it needs some actions to obtain the information on the subject of the effect of the hazardous and toxic substances (heavy metals) on the damage of a various organs tissues of goldfish (Cyprinus carpio) cultured in the floating net cage in Cirata Reservoir. The aim of this study is to examine the condition of environment quality, the accumulation of heavy metals and the example of the abnormality on the tissues of the gill, spleen, kidney, and liver organs of goldfish cultured in the floating net cage caused by the contamination of the hazardous and toxic waste (heavy metals) through the histopathologic analysis.

Materials and Methods

This study was conducted in the waters of Cirata Reservoir, West Java. The animal testing in this study used the goldfish (Cyprinus carpio) cultured in the floating net cage in Cirata Reservoir. The chemicals used were the materials for the analysis of water quality and heavy metals. For the purpose of the heavy metals analysis, it was used the preservative solutions of H2SO4 and HNO3, while for the histopathological preparation making was the solutions of bouin, alcohol, xylol, paraffin, hematoxylin-eosin dye, object glass, cover glass, etc. As for the selection of floating net cage and the fish sampling in floating net cage were done in randomized way.

The tools used in this study were the Nansen bottle, Camerer bottle, polyethylene plastic bottle, freezer, cooler box, AAS (Atomic Absorption Spectroscopy). The others were the oven, microtome, glasses for the dehydration and rehydration processes, glasses for the dyeing process, water bath, microscope, thermometer, pH meter, DO meter, and spectrophotometer.

In this study, it was analyzed the heavy metals of Pb, Cr, Hg, and Cd presented in the water and fish organs (spleen, liver, and gills). In the analysis of heavy metals in the organs, the taking of spleen, liver, and gills were done first, 5 grams respectively. Subsequently, the dry ashing was done, so that the organic material was lost, and then its residue was dissolved in the dilute acid. The solution was set up in a flame in the AAS so the absorption or the emission of the metals can be analyzed and measured at the specific wavelength. Furthermore, it was conducted the determination of the metal concentration in the samples from the standard curved that was obtained:

If :

Weight of sample (g) = W

Volume of extract = V

Concentration of sample solution (μg/mL) = a

Concentration of blank solution (μg/mL) = b

$$= \frac{(a-b)\times V}{0\ W}$$

Content of metal (mg/100g)

The histological preparations of organs were made by the fixation of organs in the Bouin solution for 24 hours. Then, the dehydration process was done in a series of gradual alcohol concentrations. Subsequently, the preparation was cleared (clearing) with the solution of xylol, then the tissue was embedded into the paraffin (embedding) and the blocking process by planting the preparation into paraffin that had been melted with heat, and then frozen with regular cooling to form a solid block. A solid block that had been really dry was cut (sectioning) with the 5 μm thick microtome, then the preparation was deparaffinized, by way of gradual immersion in the solution of xylol, and continued with the rehydration process, in gradual alcohol concentration. The process followed by the dyeing (staining) using the hematoxylin dye (1 minute) and next using the eosin (3 minutes), then the dehydration process was done in a series of gradual alcohol; and the process continued with the clearing by soaking the preparation in the xylol (15 minutes) with three replications. The next step was mounting, after that the samples were left for at least 24 hours. The preparation that had been set then was analyzed under the microscope and compared with the Atlas of Histology as a normal control.

Results and Discussions

Cirata Reservoir was the waters colored green and had a low transparency level that was 63 – 178 cm. Its greeny water was caused by the high abundance of phytoplankton which resulted in low water transparency. The values of water quality parameters of Cirata Reservoir such the turbidity, temperature, pH, dissolved oxygen (DO) during the day, total of phosphorus, nitrite, nitrate, ammonia and H_2S were 135 – 270 NTU, 25 – 32^{o}C, 3,34-7,8; 6,55-7,88 mg/L, 0,17 – 0,38 mg/L, 0,01 – 0,07 mg/L, 0,17 – 0,34 mg/L, 0,04 – 0,24 mg/L and 0,02-0,16 mg/L, respectively. These data showed that the temperature, pH, and dissolved oxygen in the water were enough to support the lives in it, including the life of goldfish cultured in floating net cage. However, the contents of total phosphorus, nitrate, nitrite and ammonia in this waters had exceeded the maximum permissible limit settled. This high nutrient and toxic gases around the floating net cage showed that the surrounding waters of floating net cage had been polluted by the organic materials that suspected to originate from the rest of the fish feed and from the anthropogenic activities on the land.

The Cirata Reservoir waters was also contaminated by the heavy metals of Pb, Cr, Cd and Hg with the average of concentration can be seen in Table 1, while the concentration of heavy metals in a various organs of fish is presented in Table 2.

Table 1.

Table 2.

The concentration of Pb, Cd, and Hg contained in around the floating net cages were relatively quite high (Table 1), exceeded the concentration limits (guidelines levels) were established. The high content of Pb, Cd, and Hg caused by the high input of the liquid waste came from a various anthropogenic activities that were around the Cirata Reservoir, and also from the input of the upstream areas (Riani, 2010b). It was in accordance with the statement of Rosella et al. (2009) which said that the contamination of heavy metals in the waters sediment derives from the anthropogenic activities. The anthropogenic activities that contribut the heavy metals into the waters can be in form of the activities of industrial, transportation, agricultural, domestic household, etc (Shea, 2010). According to Chapman et al. (1998) the sources of metal contamination in the sediment vary widely, such as there are sourced from the nature, and also from the anthropogenic activities. However, the metals naturally present in the nature, but the amount of heavy metals are relatively low (Ogoyi et al. 2011).

The heavy metals concentrations in the fish body were much higher than in the waters (Table 1 dan 2). This was due to the variety of occurrences on fish body and other aquatic biota, including the regular diffusion, biomagnifications, and bioconcentration (Forstner dan Prosi, 1979; Klaassen et al., 1986; Boisson et al., 2003) in goldfish. According to Squadron (2013) those heavy metals entered the body through three ways, i.e. through the ingestion, during the transfer of metal ions through the lipophilic membrane and through the adsorption on the lipophilic membrane. On the other side, the heavy metals can be accumulated in the body of organism (Klaassen et al., 1986; Horng et al., 2009), including in the goldfish cultured in the floating net cage in Cirata Reservoir.

The histopathological analysis of the liver, gills, kidney, and spleen organs of goldfish culture in floating net cage shown in Figure 1, 2, 3, and 4. The analysis result showed that the gills of goldfish were generally in a normal condition (Figure 1). This proved that although the waters of Cirata Reservoir contaminated by the heavy metals of Pb, Cr, Cd, and Hg which are the hazardous and toxic substances, but these heavy metals relatively has not interfered with the gills organ. This was seen clearly in the result of histopathological analysis that did not show the significant abnormality on the gills of the goldfish (Figure 1).

The histopathological analysis of goldfish liver showed the deviation from a normal condition as can be seen in Figure 2. In Figure 2 it is seen that there are quite a lot of liver cells were degenerated, even from the liver preparations also appeared some parts of liver had already had necrosis (dying). The infiltration of limphocytes also occurred in the liver. The occurrence of this lymphocytes infiltration was an indication that in the liver organ had arised a liver inflammation or cirrhosis (Figure 2). This result showed that the liver organ of goldfish cultured in Cirata Reservoir had suffered more damage than other organs, so it will interfere with liver function and detoxification will not take place properly

Figure 1.
Figure 2.
Figure 3.
Figure 4.

The other organ that had been damaged was kidney. There were a degeneration of cells and the infiltration of lymphocytes in the kidney of goldfish. In the histopathologycal preparation of kidney was also found a set of erythrocytes (shown by the arrow in Figure 3A). In the higher animal (including fish) it has a closed circulatory system of blood, so that the erythrocytes (and blood in general) have never been out of the vessels, but in this preparation it was found a set of erythrocytes were between cells. The presence of these erythrocytes between cells was predicted due to the delicate blood vessels in the kidney, so that the blood vessels were easily torned, and resulted in release of erythrocytes from the blood vessels, into the insterstitial part (Figure 3). This condition showed that there was an indication (relatively) that the inflammation of kidney (nephritis) had occurred, so the kidney functioned as a filter (filtration) and disposed the substances that harm the body fish, will be interrupted.

In the spleen, it was found the fractions of haemoglobin. This indicated that the spleen had suffered the erythrocytes destruction (Figure 4). In the spleen also occurred the hyperemia, this is the tissue or part of tissue that reddened. This showed that the function of spleen of goldfish cultered will be interrupted.

The level of damage to the liver organ is greater than other organs, followed by the level of damage to kidney and spleen. The occurrence of damage in the organs was predicted due to the heavy metals (Pb, Cd, and Hg) that entered the body through the biomagnifications (eat-eaten process) (Klaassen et al., 1986). This strengthen by the data of concentrations of heavy metals in the waters that amount exceeded the maximum permissible limit had been established, so that one at a time there will be an accumulation in the organs as seen from the high concentration of those heavy metals in the body organs of goldfish (Forstner and Prosi, 1979; Klaassen et al., 1986; Ahalya et al., 2004 and Gawad et al., 2010) (Table 2).

Conclusions

1. The water quality (except the parameter of heavy metals) of Cirata Reservoir is still in a good condition.
2. The average of heavy metal concentration of Pb, Cd and Hg in the waters of Cirata Reservoir had exceeded the maximum permissible limit settled.
3. The average of heavy metal concentration of Pb, Cr, Cd and Hg in the kidney, liver, and spleen organs of goldfish cultured in the floating net cage in Cirata Reservoir had exceeded the maximum permissible limit settled.
4. Based on the histopathologycal analysis, there had been the damage in the liver, kidney and spleen organs of goldfish cultured in the floating net cage in Cirata Reservoir. The damage of these organs allegedly due to the accumulation of heavy metals in the organs that its concentrations had exceeded the maximum permissible limit settled.

Tables

Table 1. The average of heavy metal concentrations in Cirata Reservoir waters

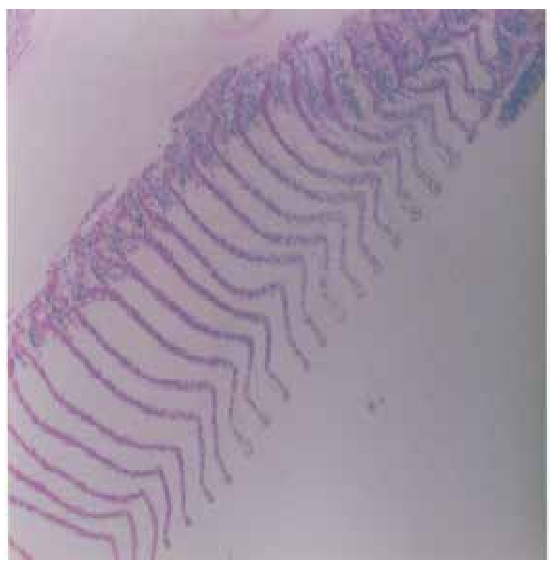
No	Sample	Heavy metal content (ppm)			
		Pb	Cr	Cd	Hg
1	Water	0,036	0,045	0,032	0,011
2	Indonesian Guidelines Levels The Government Regulations of No. 82 Year 2001 Class I	0,030	0,050	0,010	0,001

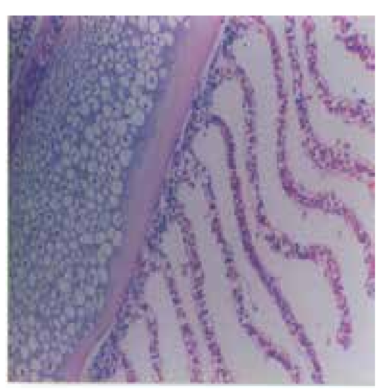
Table 2. The average of heavy metal concentrations in goldfish cultured in the folating net cage in Cirata Reservoir

No	Sample	Heavy metal content (ppm)			
		Pb	Cr	Cd	Hg
1	Goldfish : - Spleen - Liver - Gills - Flesh	 1,296 1,020 0,560 0,098	 1,398 1,098 0,654 0,060	 1,402 1,080 0,582 0,042	 1,084 0,984 0,540 0,046
2	Netherland's Guidelines Levels*	0,5	1,0	0,05	0,3

* The standard of heavy metal content allowed in the fish body and other fisheries products, especially for the fish consumed in Netherland

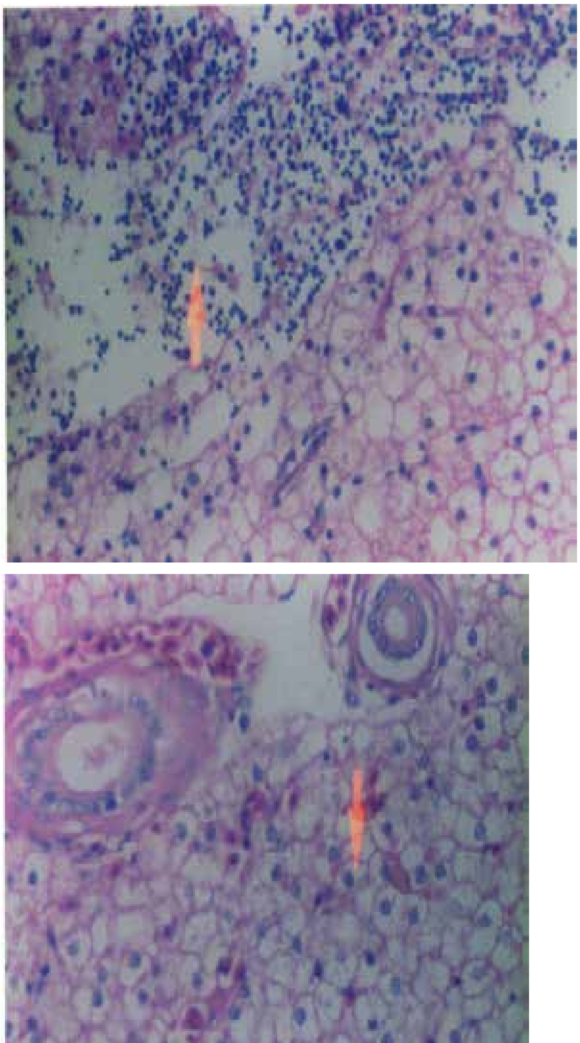
Figures and Figure legends





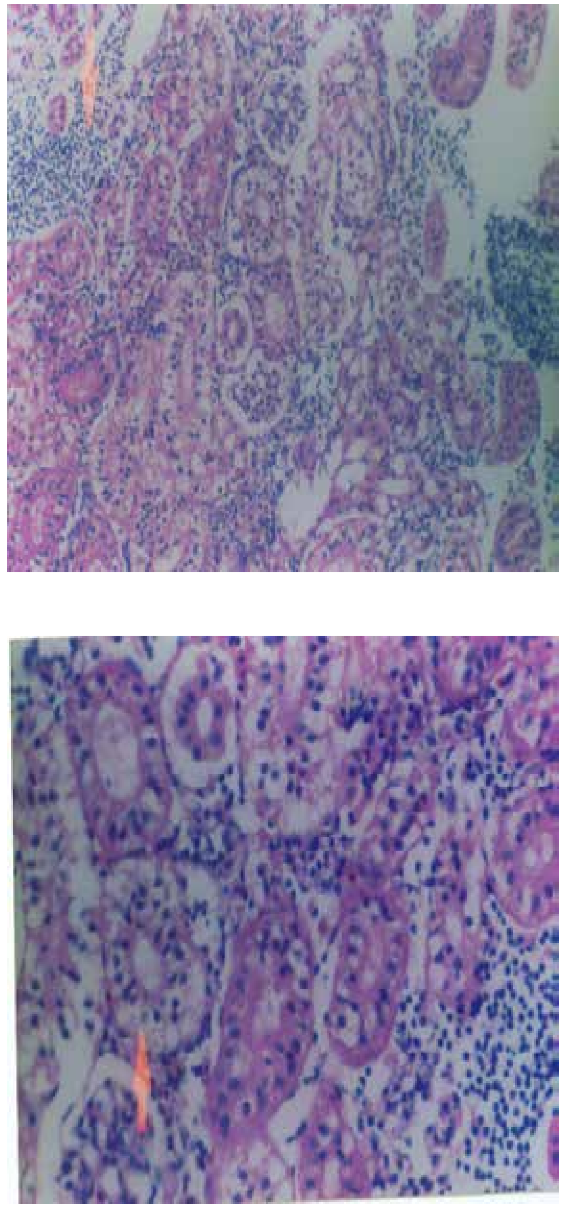
A. Magnification of 10 X 10 B. Magnification of 10 X 20

Figure 1. Gills microscopic structure of goldfish cultured in floating net cage in Cirata Reservoir (There is no abnormality found in A and B).



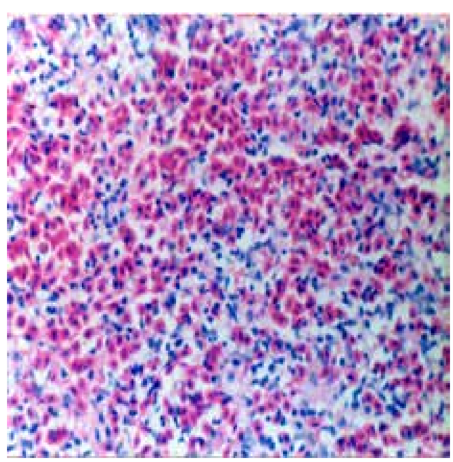
A. Magnification of 10 X 40 B. Magnification of 10 X 40

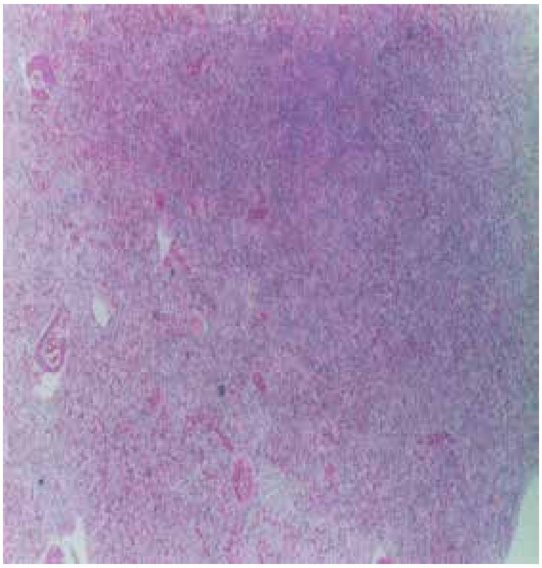
Figure 2. Liver microscopic structure of goldfish cultured in floating net cage in Cirata Reservoir (A. necrosis, degeneration, infiltration of lymphocytes, and B. degeneration, infiltration of lymphocytes).



A. Magnification of 10 X 20 B. Magnification of 10 X 40

Figure 3. Kidney microscopic structure of goldfish cultured in floating net cage in Cirata Reservoir (A. cells degeneration, lymphocyte cells infiltration, loss of erythrocytes from blood vessels, and B. cells degeneration.





A. Magnification of 10 X 40 B. Magnification of 10 X 4

Figure 4. Spleen microscopic structure of goldfish cultured in floating net cage in Cirata Reservoir (A. there are haemoglobin fractions and hyperemia, and B. hyperemia).

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