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## Bioaccumulation and histopathological effect on the gills and liver of silver barb (*Barbonymus gonionotus*) exposed to the heavy metal nickel

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# Bioaccumulation and histopathological effect on the gills and liver of silver barb (*Barbonymus gonionotus*) exposed to the heavy metal nickel

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**Abstract.** The silver barb (*Barbonymus gonionotus*) is one of the freshwater fish which is often found in river, specifically the Java and Sumatra islands in Indonesia. A water environment that has been exposed into nickel will cause fish bioaccumulation and cause histopathological changes in the gills and liver of the fish, especially in the silver barb. This research aimed to observe the bioaccumulation and histopathological effect on the gills and liver of silver barb from nickel (Ni) under different concentration treatments. This research used the experimental method where the parameters observed were the nickel level of content in the gills and liver, as well as the histopathological changes after the nickel exposure. The bioaccumulation level was measured using the AAS (*Atomic Absorption Spectrophotometric*) method. The result showed that the nickel concentration given affected the bioaccumulation level and caused histopathological changes in the gills and liver of the silver barb. The highest bioaccumulation level was observed in the nickel exposure using a 1.8196 ppm dose resulting in a 1.85095 ppm accumulation in gills and 4.3982 ppm accumulation in liver. The changes that occurred in the gills were oedema, hyperplasia, and lamellae fusion, while the changes in the liver included vacuolization, congestion, and necrosis.

## 1. Introduction

The development of industry in Indonesia has had a lot of impact in the environment, primarily the water environment. Based on the data from the Ministry of Industries' performance report in 2015, there was an increased growth in the heavy metal industries in 2014 by 2.94% and this went up to 7.83% in 2015. These industries produce heavy metal waste, including nickel. The diverse use of nickel contains household furnishings and electronic components, as well as car, boat, and motor spare parts, and the basic material for coin money [1]. Aside from the heavy metal industries, nickel waste is also produced in the mining activity of nickel seeds. Any nickel (Ni) that goes into the water will accumulate in the sediments and organisms that exist there.

Nickel is one of the heavy metals that possess toxic properties. Nickel in a high concentration can cause problems in the blood, interfere with the respiratory system, damage the tissue and mucous membranes, and change the cells and chromosome system. Therefore, since 2006, the European Union had proposed WTO in order to establish nickel as a dangerous substance [2].



The bioaccumulation of nickel (Ni) in fish can be observed by the means of the measurement of the level of Ni in the fish organs. The organs that accumulate Ni incur histopathological changes. The analysis of the histopathological changes was used to learn of the fish's health description through the structural changes that occurred in the main pollutant target organ [3]. The fish accumulated heavy metals in the body through multiple ways, such as in the respiratory system and through digestion, or directly through the skin [4]. Silver barb fish (*B.gonionotus*) are known as being sensitive to environmental change, so they are excellent as a bioindicator of the changes that occur in the aquatic environment [5].

Bioaccumulation in the fish's body will cause histopathological changes of some of the organs, including the liver and gills. The gills are a respiration organ which has a wide surface area and is easily exposed in the environment, which means that this organ is often attacked by toxic material in the water [6]. The liver is a vital organ that serves as a detox mechanism that secretes some of the chemicals used in the digestion process. The liver plays an important role in the process of metabolism and contaminant transformation. Therefore, the liver often accumulates toxins, which are easily exposed to toxic effects [7]. Based on the above issues, further research was necessary to be conducted in order to observe the bioaccumulation level and the histopathological sample disruption in the liver and gills of silver barb fish. This is expected to be used as the early handling stage of the contaminant presence in the rearing media of fish farming in order to minimize mass mortality.

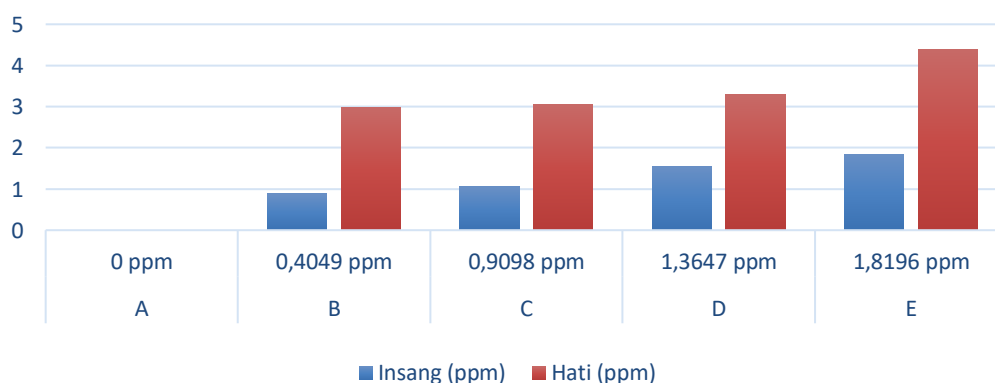
## 2. Materials and methods

This research used an experimental method conducted by using different concentrations of Ni on silver barbs; we then examined the Ni content, as well as observing the histopathological changes in the gills and liver of the fish. The research design used in this research was a complete randomized design (CRD). This research used five Ni exposure level treatments with one negative control for the comparison treatment. The replications given for each treatment were times four with random aquarium placement. Based on the range and acute toxicity test that was conducted, the treatments given for the sub-chronic effect test were A (0 ppm), B (2% of  $LC_{50}$  or 0.4549 ppm), C (4% of  $LC_{50}$  or 0.9098 ppm), D (6% of  $LC_{50}$  or 1.3647), and E (8% of  $LC_{50}$  or 1.8196 ppm).

The silver barb fish used were retrieved from fish farmers in Dukuksampeyan, Gresik, East Java, Indonesia. The rearing activity was conducted for 15 days. The fish gills and livers were taken and analyzed using Atomic Absorption Spectrophotometric (AAS) to observe the Ni concentration that had accumulated on the gills and we also prepared the histopathological sample observation using haematoxylin-eosine coloration.

## 3. Results and discussion

The examination results of the Ni bioaccumulation in the gills and liver of the silver barb exposed for 15 days has been explained in Figure 1.



**Figure 1.** Ni bioaccumulation in the gills and liver of silver barb

Figure 1 shows that all of the treatments showed there to be bioaccumulation in the gills and liver to different levels. The bioaccumulation of Ni on the gills and liver caused histopathological changes in both organs.

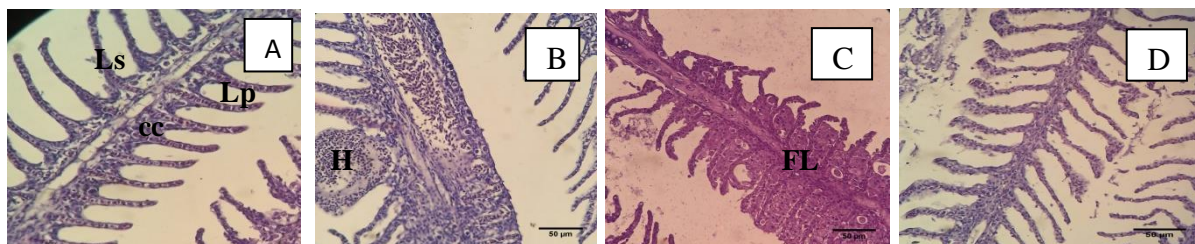
The observation of the gill and liver histopathological changes in the silver barb (*Barbonymus gonionotus*) showed that all of the treatments experienced changes or damage to the gill and liver tissue due to exposure to the Ni heavy metals. The observation of the gill tissue damage found there to be histopathological changes in the form of edema, hyperplasia and fusion lamella, while in the liver, the damage was in the form of vacuolization, congestion, and necrosis. The data on the levels of gill and liver tissue damage have been described in Table 1.

The histopathological changes observed in the gills and liver showed that all of the treatments resulted in tissue disruption caused by Ni exposure. The gills disruptions observed were oedema, hyperplasia and lamellae fusion, while the liver disruptions were vacuolization, congestion, and necrosis. The histopathological tissue changes in the gills and liver have been presented in Table 1.

**Table 1.** Histopathological disruption of the gills and liver in silver barbs

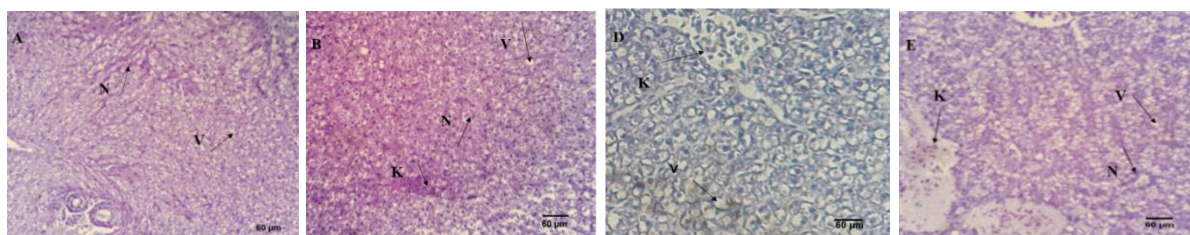
Treatment (ppm)	Disruption					
	Gills			Liver		
	Oedema	Hyperplasia	Lamellae fusion	Vacuolization	Congestion	Necrosis
0	++	+	+	+	+	+
0.4549	++	+	+	++	++	+
0.9098	++	+	+	++	++	++
1.3647	++	+	++	++	++	++
1.8196	++	++	++	+++	+++	+++

Scoring calculation of the histopathological change where 0 showed that no damage occurred, + showed 0-30%, ++ showed 30-70%, and +++ showed 70% damage in one observational field [8]. The histopathological figures of the gills exposed to Ni have been presented in Figure 2.



**Figure 2.** Histopathological changes on the gills and liver of the silver barb. A. Normal gill, B. 1.81 ppm Ni exposure, C. 1.36 ppm Ni exposure, D. 0.909 ppm Ni exposure withn oedema occurred, Lp = primary lamellae, Ls = secondary lamellae, cc = chloride cell, E = oedema, FL = lamellae fusion, H = hyperplasia, HE coloration. 400x magnification. 50 µm bar.

Liver histopathological changes in the silver barb exposed to Ni as presented in Figure 3.



**Figure 3.** Liver histopathological changes in the silver barb. A. 0.45 ppm Ni exposure concentration, B. 0.40 ppm Ni exposure concentration, D. 1.36 ppm Ni exposure concentration, E. 1.81 ppm Ni exposure concentration, V=vacuolization, K=congestion, N=necrosis. HE coloration. 400x magnification. 60 µm bar.

Heavy metal bioaccumulation in organ tissue resulted in different levels for each part. The mechanism of Ni's entry into the silver barb fish was through the gills and skin, before entering the blood and being transported to the various tissues. Most of the toxicants that went into the body after epithelial cells absorption would be brought to the liver through the liver vein portal [9]. A high concentration of Ni in the gills and liver is due to the physiological function of both organs. The gills are the surface respiration organ that are exposed to direct contact with the water, and thus are potentially exposed to pollutants continuously. The liver is a vital organ that functions in the detoxification process [10].

The level of tissue damage on the gills and liver was different for each treatment. The Ni bioaccumulation was 0.83 ppm in the gills, which could disrupt the gill tissue, causing oedema, hyperplasia, and < 30% lamellae fusion. The 1.85 ppm accumulation caused the gills to form < 30% oedema, hyperplasia, and 30-70% lamellae fusion.

The oedema can cause tissue swelling as an inflammation effect due to fluid accumulation. Fluid accumulation caused the cell organelles to become distracted and decreased cell permeability, which slowly caused them to disappear and swell [11]. Excess oedema can cause the hyperplasia occurrence, which may lead to the interlamellae being clogged, causing the entire space to be filled with new cells, thickening the epithelium located at the base of lamellae [12]. Gill epithelium hyperplasia led to lamellae fusion. The epithelial cells which underwent hyperplasia and lamellae fusion can go on to cause a broad decreased gill surface for the respiration process, disrupting the blood flow in the gills, distracting the metabolism process and causing fish death [13].

The Ni bioaccumulation which occurred in the silver barb liver caused tissue damages, such as vacuolization, congestion, and necrosis. The liver tissue damage was increased along with the increased Ni bioaccumulation level. This was in accordance with [14], who stated that the metal-binding protein (metalothionine) presented in this occurrence showed at an increased level as the hepatocyte damage was exposed.

The Ni bioaccumulation at a 4.39 ppm concentration level in the liver caused tissue damage, i.e. vacuolization, congestion, and necrosis with a 2.15- 2.30 average scoring. Due to the exposure to Ni, this can affect the increased cell permeability, making the cells unable to maintain ion homeostasis, displacing the extracellular liquid into the cell. Homeostatic imbalances of ions and liquid cause swelling in the liver cells. The liver swelling is characterized by the presence of vacuoles or blank spaces, causing the cell cytoplasm to become murky with sinusoidal marrow. This causes the cell to swell, losing the membrane integrity. This removes the cell material, causing necrosis or cell death [15].

One factor influencing the heavy metal toxicity level was the exposure duration level, thus the higher and longer the exposure to a given level of heavy metal, the higher the heavy metal concentration in the organisms, causing a bigger toxicity effect. Variation in the heavy metal level was associated with the absorption process and sedimentation occurred in the dry season [16].

#### 4. Conclusion

The nickel (Ni) concentration affected the bioaccumulation level and caused the histopathological changes in the gills and liver of the silver barb fish. The highest bioaccumulation level was observed to be 8% of the Ni LC<sub>50</sub> exposure with 1.85095 ppm in the gills and 4.3982 ppm in the liver. The gill disruptions that occurred were oedema, hyperplasia and lamellae fusion, while the liver disruptions that occurred were vacuolization, congestion, and necrosis.

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