Relationship Between the Concentration of Mercury (Hg) Along Kahayan Watershed Central Kalimantan with the Bioaccumulation, Morphological and Cytological Changes of Catfish (Pangasius pangasius)

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Abstract
Kahayan River is known to be polluted by mercury due to the gold mining activities which use the mercury in the amalgamation (gold purifying) process. The water and fish in this river are consumed by most of the community live in the river basin. The fish consumed by Central Kalimantan people generally come from Kahayan River. Catfish (Pangasius pangasius) is one of the fish which is often consumed by Central Borneo people. They like the fish because of its delicious taste and its fat flesh. Besides, the fish bones are quite big and do not reach its body flesh. Generally, the yellowtail catfish is also raised by the local citizen inside baskets called karamba along the Kahayan watershed. The purposes of this research are to know: 1) the connection between the concentration of mercury along the Kahayan watershed and the mercury bioaccumulation contained in the catfish’s gill, liver, kidney, and muscles, 2) the catfish’s morphological changes related to the mercury bioaccumulation, and 3) the catfish’s cytological changes related to the mercury bioaccumulation. The results of the research are 1) there is connection between the mercury concentration along the Kahayan watershed and the mercury bioaccumulation contained in the catfish’s gill, liver, and muscles or Ho acceptance, 2) the morphological changes in the fish’s organs are: (a) the gill are experiencing colour changes from red with no spot into pink with spots, (b) the catfish’s liver are experiencing colour changes from brownish into dark red, and shape changes from compact and not wrinkled into a little bit wrinkled, (c) the fish’s kidney are experiencing colour changes from red into dark brownish red, and shape changes from oval into wrinkled, (d) the fish’s muscles are not experiencing morphological changes either the colour or the shape, there are only some spots in the middle part of the muscle, 3) the cytological changes in the organs cells of the catfish are: (a) the changes in the gill’s cells are in the form of lamella edge swelling, lamella epithelium cell spreading, and nucleus disappearing; these indicate that there is necrosis in the gill’s cells; (b) the necrosis in the heart cells are marked with the cells colour absorption as the consequence of cells permeability declining and oedema (cell swelling); (c) the necrosis in the kidney cells are marked with the cells colour absorption as the consequence of cells permeability declining and oedema (cell swelling); (d) the cytological changes in the muscles cells of the catfish are not clearly seen, but the blood smear of the sample fish from the villages whose river water indicated to be polluted by mercury like Tumbang Anjir village is having necrosis, indicated by a strong colour absorption as the consequence of cells permeability declining. From the research results, it can be concluded that the mercury bioaccumulation in the Kahayan watershed related to the catfish’s morphological and cytological changes is as the result of the toxic nature of mercury and there is direct connection between the mercury concentration along the Kahayan watershed and the mercury bioaccumulation inside the catfish’s body.

Keywords: bioaccumulation, catfish, cytological change, mercury, morphological change

Introduction
Mercury (Hg) is one of the heavy metals B3 (Hazardous and Toxic) widely used in life for various purposes such as a mixture of cosmetics, contents of barometer-thermometer aerometer, electric industry, and refining of gold [1]. Mercury is a chemical substance with the characteristics of high toxicity level, volatile (easily to be evaporated), has a boiling point of 356.58 ° C and 38.87 ° C melting point. Mercury generally are: 1) a liquid, so it's easy to spread on the surface of the water and difficult to collect, 2) can be transformed by microorganisms in the water (sea, river or lake) into a component of methyl mercury (CH3Hg+) which is highly toxic, with the existence of food chain that allows it accumulate in the body of animals and humans, and 3) can undergo relocation (translocation in plants and animals) [2].
These characteristics, when methyl mercury is in the water, may cause disturbance toward aquatic life, whereas aquatic biota such as fish is the primary source for human consumption. Methyl mercury can be absorbed by fish through the gills or skin, the result will be accumulated in their body, and if the fish are eaten by humans, they can cause diseases such as Minamata case.

Reports of some researches showed several regions in Indonesia have been polluted by mercury in the last 20 years. The rivers in Kalimantan are also reported to have suffered contamination by mercury particularly in Kapuas and Kahayan rivers. Mercury pollution in Kahayan has happened for decades, even in the year 1997-2002 it suffered of severe mercury pollution [3].

One of the most prominent activities which appears in the rivers of Central Kalimantan is a gold mining which is traditionally done by the community, especially for the upper and middle watershed area (DAS) of Kahayan river. Gold mining waste is usually disposed into Kahayan river directly. In fact, the quality and quantity of river water should be maintained to be eligible for long-term use of human survival surroundings.

The level of mercury pollution in the Kahayan watershed in May and June 2004 was estimated to remain five times above the threshold value (TV), which was 0.005 to 0.008 mg/l. Food and Drug Administration (FDA) has determined the TV standardization of mercury levels in water should not be more than 0.001 mg/l, sediment 0.005 m/l, and the fish of 0.5 mg/kg (WHO). If exceeding that value could lead to increased pollution of river water polluted by mercury compounds as well as a number of fish commonly consumed by the public [3].

Central Kalimantan people generally consume freshwater fish from Kahayan river. The fish are a lot more coming from cages (167tons) than from the pool (15ton) [4]. Nutrition surveys conducted in 1995 reported an average fish consumption of communities in Central Kalimantan is 99.5g/person/day. Whereas in 2002 reached 48.36kg per capita/year or well above the national average of 29kg per capita/year. This is the highest fish consumption in Indonesia compared to other provinces [5].

The process of entry of mercury into the body of the fish is due the nature of fish which swim actively and continuously the whole day and generate flow power to against the flow of water. Along with these activities, the fish will absorb as much oxygen through gills which simultaneously also absorbs heavy metal compounds dissolved in the water. Fish also has the ability to absorb the chemicals dissolved in the water through the process of bioconcentration. The entire aquatic biota that have special organs such as gills will be able to absorb oxygen by diffusion through the outer membrane, the surface is usually permeable to materials and chemical compounds [6].

Catfish (*Pangasius pangasius*) fish is one of the most widely consumed by the people of Central Kalimantan. This fish is preferred because it tastes good, its muscles are soft and slightly greasy. Many catfish live in the waters along the watersheds in Central Kalimantan, when it is contaminated and eaten by humans, the polluting material will come to human organs and attack their health.

Fish which are often contaminated by mercury compounds can result the accumulation of mercury in fish body comparable with its expression level. Organic mercury which poisoned fish body can cause pathological changes and disorders such as gill hyperplasia, where morphological changes and sitologis of the fish occur. Other pathologic changes of the fish can be liver dissosiasi, fatty infiltration, necrosis (cell death), and declining rates of glycogen [1]. Based on this case, the formulation of the problem is as follows.

1. Is there any connection between mercury concentrations along the Kahayan watershed with the bioaccumulation of mercury in gills, liver, kidney and muscle of catfish organs?
2. How catfish morphological changes associated with bioaccumulation of mercury?
3. How catfish cytological changes associated with bioaccumulation of mercury?

The hypothesis of this study is: there is a connection between mercury concentration along the watersheds of Kahayan river, Central Kalimantan with the bioaccumulation of mercury in gills, liver, kidney and muscle of catfish organs.
**Research Methodology**

This study was ex post facto, because it was aimed to see and reveal morphological and cytological changes in catfish due to exposure to mercury concentrations found along the Kahayan watershed. Catfish were taken from the cages along the Kahayan watershed in six districts, two villages were taken from each district as the sampling site. The population in this study were all catfish in the Kahayan watershed. Population was determined based on the characteristics and habits of catfish found in the waters along the Kahayan watershed whose numbers are not certainly known.

The sample in this study were catfish in cages at each sampling site. Samples were taken from the cages as many as 3 catfish with sampling criteria as follows: 1) weighted between 700-1000 grams, 2) age between 2-3 months, and 3) living in cages as long as 3-4 months. The number of samples taken from the cages along the Kahayan watershed was 36.

Data retrieval is done by taking samples of surface water of river and catfish in 6 districts (two villages represented one district, where 3 catfish were taken from each village). Meanwhile the data collected after the measurement of the mercury concentration of the river water, measurement of the concentration of mercury in each organ (gills, liver, kidney, and muscle) of sampling catfish taken from the cages along the Kahayan watershed, as well as measurements of physical-chemical-biological data of the environment (as the supporting data) in 6 districts. Data retrieval is done using a sampling method based on demographic divisions. This is done because most of the gold mining and fish cages are not far from a village or settlement.

This study used two types of statistical analysis, descriptive statistics and nonparametric statistics. Descriptive statistical analysis was utilized to reveal the morphological and cytological change issues of catfish related to mercury bioaccumulation. Statistical analysis was used to examine the connection between mercury concentrations along the watersheds of Kahayan river, Central Kalimantan with the bioaccumulation of mercury in the organs (gill, liver, kidney, and muscle) of catfish. Pearson connection analysis was used to do this.

**Result of The Research**

Areas used as the main research site was watershed research only, that is Kahayan watershed. Kahayan watersheds ± 600km in length, ± 500m in width, and an average depth of ± 7m, the flow of the river are navigable in both wet and dry seasons along ± 500km. Measurements of temperature, pH, COD and BOD performed to determine the exact condition of the environment at the time of sampling, where water and catfish were collected, which was used as supporting data. Based on the measurements result that have been made, it can be presented in Figure 1 below.

![Bar Graph, Water Environmental Conditions Measurement Results at 12 Different Sampling Locations Along the watershed Kahayan Central Kalimantan](image)

**Details:**
Mercury concentration measurements was carried out to determine the concentration of mercury in Kahayan watershed during the research. There were 12 water sampling sites and samples of catfish along the Kahayan watershed, in which the concentration of mercury of the samples were then analyzed by using a spectrophotometer. Measurement or analysis of mercury concentrations in Kahayan watershed was conducted at Palangkaraya Health Laboratory. The measurement results can be presented in Figure 2 below.

![Bar Graph](image)

**Figure 2.** Bar Graph, Examination result of the Mercury concentration (mg/l) of the river water from 12 Different Sampling Locations Along the Kahayan watershed, Central Kalimantan. Details: 1a. Batu Nyiwuh village, 1b. Sarerangan village, 2a. Tumbang Anjir Village, 2b. Tewang Pajangan, 3a. Sepang Kota, 3b. Sepang Simin, 4a. Tangkahen Village, 4b. Bawan village, 5a. Bukit Rawi village, 5b. Tanjung Sangalang village, 6a. Pahandut Seberang, 6b. Tanjung Pinang.

Measurement of mercury level in catfish organs performed to determine the exact content of mercury in the fish sampled. Sample fish were taken at each sampling site consists of 3 fish. Organs measured for the mercury level were gill, liver, kidney, and muscle.

Graph of the results of the mercury levels found in each organ (gills, liver, kidney and muscle) of catfish at 12 sampling sites along the Kahayan watershed Central Kalimantan can be seen in Figure 3 below. Morphological condition of catfish catfish was observed through some organs; gill, liver, kidney, and muscle. The fish were taken from the cages at 12 different sampling sites along the watershed of Kahayan. The number of fish taken at each sampling site was 3.

Organs which did not undergo changes and those which underwent morphological changes after having the catfish organs observed where the samples were taken from the cages along the watershed of Kahayan at 12 different sampling locations are presented in Figure 4 - 7 below.
Figure 3. Line Graph of Examination Results of Mercury Level (mg/ kg weight) of catfish Organs from 12 Different Sampling Locations Along the watershed of Kahayan Central Kalimantan


Figure 4a. Gills are not experiencing Morphological Changes, and No Color Spot Gills Looks Fresh Red.

Figure 4b. Gills are experiencing Morphological Changes. Look at the spot and colour on gills

Details: Spot found on the gills

Figure 5. Catfish Liver

Details: a. Liver is not experiencing morphological changes
b. Liver is experiencing morphological changes
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Figure 6. Catfish kidney
Details: a. Kidney is not experiencing morphological changes
   b. Kidney is experiencing morphological changes

Figure 7a. Longitudinal pieces of catfish muscle morphology containing Mercury Levels in Muscle 0.001 mg/l
Figure 7b. Longitudinal pieces of catfish muscle morphology containing Mercury Levels in Muscle above 0.001 mg/l
Details: Spot found on catfish muscle

Catfish cytological observations undergone by preparat organ cells whose picture was taken with a light microscope brands Olympus DP 20 magnification 100x (to the gills) and 400x (to the gills, liver, kidney, and muscle). Photo organ cell preparat of gill, liver, kidney and muscle in 12 differentvillages (sampling locations) can be seen in Figure 8 - 12.

Figure 8a. Gill cells are not experiencing necrosis, observer under light microscope at 100x magnification
Figure 8b. Gill cells are experiencing necrosis, observer under light microscope at 100x magnification
Details: L = lamella
UL = Tip of lamella
UL hp = Tip of lamela experiencing hyperplasia (Look at figure 8b the tip of lamela gets swollen)
SE dl = Epithelial cell inside lamella (Look at figure 8b the epithelial cell inside lamella seems to spread and the cell nucleus seems to disappear)
SK = Capillary cells
Hypothesis testing of the research was done by using connection analysis to determine the connection between the concentration of mercury in watersheds along Kahayan, Central Kalimantan and bioaccumulation of mercury in the organs (gills, liver, kidney, and muscle) of catfish. Summary of connection analysis on each of organs where the samples taken from cages along the watershed of Kahayan, Central Kalimantan indicated in Table 2 s/d 5.

Based on the table above, it is known that there is a highly significant (1% level) connection between the concentration of mercury in watersheds along Kahayan watershed, Central Kalimantan with the bioaccumulation of mercury contained in each catfish organ. From these results it can be interpreted that the Hi was accepted, this means research hypothesis was accepted. This situation suggested there is a
connection between mercury concentrations in river water and the bioaccumulation of mercury in the organs of catfish along the watershed Kahayan.

Figure 12a. Muscle cells are experiencing the fewest necrosis, observer under light microscope at 400x magnification
Figure 12b. Muscle cells are experiencing the most necrosis, observer under light microscope at 400x magnification
Details: N = Necrosis (Observing necrosis on muscle cells is pretty complicated, ought to keep rotating themicro rounds on the microscope)

Table 2. Summary of Connection Analysis to Determine the Connection between Mercury Concentrations Along the watershed of Kahayan, Central Kalimantan and Mercury Bioaccumulation in Gills organ of catfish

<table>
<thead>
<tr>
<th>Mercury Concentration of Water River (mg/l)</th>
<th>Pearson Connection</th>
<th>Pearson Connection</th>
<th>Sig. (2-tailed)</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury level in Gills (mg/kg weight)</td>
<td>1</td>
<td>0.715**</td>
<td>0.000</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)

Table 3. Summary of Connection Analysis to Determine the Connection between Mercury Concentrations Along the watershed of Kahayan, Central Kalimantan and Mercury Bioaccumulation in livers organ of catfish

<table>
<thead>
<tr>
<th>Mercury Concentration of Water River (mg/l)</th>
<th>Pearson Connection</th>
<th>Pearson Connection</th>
<th>Sig. (2-tailed)</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury level in livers (mg/kg weight)</td>
<td>0.851**</td>
<td>0.851**</td>
<td>0.000</td>
<td>36</td>
<td>36</td>
<td>36</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)
Table 4. Summary of Connection Analysis to Determine the Connection between Mercury Concentrations Along the watershed of Kahayan, Central Kalimantan and Mercury Bioaccumulation in Kidneys organ of catfish

<table>
<thead>
<tr>
<th>Mercury Concentration of Water River (mg/l)</th>
<th>Mercury level in Kidneys (mg/kg weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury Concentration of Water River (mg/l)</td>
<td>Pearson Connection Sig. (2-tailed) N</td>
</tr>
<tr>
<td>Mercury level in Kidneys (mg/kg weight)</td>
<td>Pearson Connection Sig. (2-tailed) N</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)

Table 5. Summary of Connection Analysis to Determine the Connection between Mercury Concentrations Along the watershed of Kahayan, Central Kalimantan and Mercury Bioaccumulation in Muscle organ of catfish

<table>
<thead>
<tr>
<th>Mercury Concentration of Water River (mg/l)</th>
<th>Mercury level in Muscles (mg/kg weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury Concentration of Water River (mg/l)</td>
<td>Pearson Connection Sig. (2-tailed) N</td>
</tr>
<tr>
<td>Mercury level in Muscle (mg/kg weight)</td>
<td>Pearson Connection Sig. (2-tailed) N</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed)

Discussion

In general, the study site was 6 districts traversed by the flow of Kahajan from upstream to downstream, the Tewah District, Kuala Kurun, Sepang, Banana Tingang, Central Kahayan and Pahandut. Each district was presented by two to take the water sampling and catfish.

Based on the measurements that have been done, it is known that the average temperature of the water at the sampling site was 30.67 °C; mean of pH 6.31; COD average 15.58 mg/l, mean of BOD 7.71 mg/l (Figure 1). This shows the temperature of the water is warm enough as a place to live (habitat) for aquatic biota, the pH tends to be acidic, COD and BOD levels can be considered not to exceed the water quality standards that have been set.

The presence of mercury in watersheds throughout Kahayan was known by measuring the mercury concentration of river water samples taken from 12 villages different sampling locations (Figure 2). Measurement of concentrations of mercury river water showed Batu Nyiwuh Village, Sarerangan, Tewang Pajangan, Sepang Kota, Sepang Simin, Tangkahen, Bukit Rawi, Tanjung Sangalang, and Tanjung Pinang are not polluted by mercury or mercury concentrations in waters did not exceed the threshold value set, which is 0.001 ppm or 0.001 mg/l. While the other 3 villages, namely Village tumbles Anjir, Bawan, and Pahandut Seberang indicated as polluted, where they had mercury concentrations above the threshold value which has been determined. The difference is due to there are some villages that served as sampling locations such as Tumbang Anjir village, it was encountered many activities such us gold mining, while other villages do not have such activities.

Villages indicated as contaminated by such as Tumbang Anjir (0.011 mg/l) and Pahandut Seberang (0.007 mg/l), after examination of mercury levels in the catfish organs (gill, liver, kidney, and muscle), it showed catfish contained high levels of mercury accumulation (Figure 3). Mercury contained in the body of catfish caused by biota ability to absorb chemical compounds that are dissolved in the water through the process of bioconcentration. The entire aquatic biota having specialized organs such as gills, can absorb oxygen by diffusion through the outer membrane, this surface is usually impermeable to chemical compounds.[6]
According to Palar [7] Fish can absorb toxins from heavy metals such as mercury through the process of biomagnification in fish body. Biomagnification is the process by which pollutants (contaminants), through the food chain with trophic transfer satge at higher concentrations in the body of organisms of higher trophic levels.

Based on the connection analysis had been done, it appeared that the liver has a very significant correlation (Table 3) with mercury concentrations found along the watershed of Kahayan, Central Kalimantan. This is in accordance with the opinion of Darmono [1] that the highest metal accumulation usually found in detoxification organs (liver) and excretion (kidney). In both organs, usually metal also binds to various types of protein called metallothionein. Other than these two organs, the gill is an organ that is very sensitive to the influence of metal toxicity.

The presence of mercury in the gills is due to the role of gills as the first organ to take (uptake) metal compounds from the marine environment, so it can be said gill was the first organ contaminated. In the gill surface were found small epithelial cells, these cells will be in direct contact with a contaminated environment [7].

The presence of mercury content in the liver and kidneys because of the bond between the mercury with a protein called metallothionein. This process that helps the mercury detoxify mechanism in fish organs. This is in accordance with the opinion of Lasut [8] that the metallothionein is a binding protein a functioned in the binding process and the confinement of metal in the body tissues of every aquatic biota.

Changes that occur and were observed in catfish muscle just looks a little spot in the middle of the fish muscle (Fig. 7b). Spots are found in fish that have high levels of mercury bioaccumulation in their bodies such as catfish samples from Tumbang Anjir village. According to Avenant et al. in Salami et al. [9] Muscle fish usually will not show any significant change, although the concentration of heavy metals in waters is high and accumulation in the muscle organ is also high.

After the preparat observation had been done to the gill cells of catfish under a microscope, cytological changes were found in the cell. When compared to the gill organ cells which were not contaminated by mercury (Fig. 9a), the change in the forms of 1) The tip of lamella got swollen which was predicted to experience hyperplasia, 2) epithelial cells in the lamella spread, and 3) nucleus cells begin to disappear (Figure 9b).

Cytological changes in catfish liver cells could be observed by comparing the liver cells that were not contaminated by mercury (Fig. 10a) with liver cells that had been contaminated by mercury (Fig. 10b). In the picture shown necrosis characterized by strong color absorption in cells by decreasing the permeability of the cell so that the dye easily enters the cell and there is no nucleus in the cell.

Cytological changes that occur in the kidney of catfish can be seen from the comparison of kidney cells that are not contaminated by mercury (Fig.11a) with kidney cells contaminated by mercury (Fig.11b). From the observation that these shown presence of necrosis characterized by cells absorbing more of colors due to decreased cell permeability and edema cells.

Cytological changes in catfish muscle cells were not so obvious, but in some preparat which samples came from the village where the river was polluted by mercury such as Tumbang Anjir village (0.011 mg/l, Fig.12b) shown to experience necrosis in muscle cells, which was characterized by cells that absorb color more robust due to decreased cell permeability, compared to the muscle cells that had no cytological change.

According to Baraa [10] necrosis is due to external factors that can cause the membranes of cells undergo cell lyses, resulting in cell death. Cell death due to necrosis is characterized by the occurrence of edema (swelling = swelling) cells and changes in the mitochondria which was originally still reversible, and becomes irreversible and then breakdown the cell wall, so that the entire cell contents poured out of the cell.

Bowen and Lokckshin [11] found a variety of stress on cells caused by anything can cause cell death (necrosis). This situation can be attributed to what is happening on the organ of catfish undergo necrosis due to mercury contamination located along the watershed of Kahayan. In general, tissue and organs that were damaged will decrease physiological functions and metabolic processes such as respiration and excretion.
Conclusion
1. There is a connection between the concentration of mercury along Kahayan watershed with the bioaccumulation of mercury in organs; gills, liver, kidney and muscle of catfish,
2. Morphological changes that occur in organ of catfish are: (a) the gill are experiencing colour changes from red with no spot into pink with spots (b) the catfish’s liver are experiencing colour changes from brownish into dark red, and shape changes from compact and not wrinkled into a little bit wrinkled, (c) the fish’s kidney undergo colour changes from red into dark brownish red, and shape changes from oval into wrinkled, and (d) fish muscle do not undergo morphological changes in both color and shape.
3. The cytological changes in the organs cells of the catfish are: (a)the changes in the gill’s cells are in the form of lamella edge swelling, lamella epithelium cell spreading, and nucleus disappearing; these indicate that there is necrosis in the gill’s cells; (b) the necrosis in the heart cells are marked with the cells colour absorption as the consequence of cells permeability declining and oedema (cell swelling); (c) the necrosis in the kidney cells are marked with the cells colour absorption as the consequence of cells permeability declining and oedema (cell swelling); (d) the cytological changes in the muscles cells of the catfish are not clearly seen.

Suggestion
1. There needs to be an intensive outreach or counseling to the community, especially to those living closed to the Kahayan watershed from considering they are accustomed to catching fish, selling and distributing to public.
2. There needs to be an elucidation on the dangers of mercury to the environment and human health through leaflets.
3. There needs to be local government regulation concerning with environment disciplinary in order to be free of mercury pollution.

References