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The Effects of Mercury Chloride (Hgcl₂) on the Changes in Hematology and Blood Sugar Level in Carps (*Cyprinus carpio*)

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The Effects of Mercury Chloride (HgCl₂) on the Changes in Hematology and Blood Sugar Level in Carps (*Cyprinus carpio*)

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Abstract. One of the increasingly heavy metals concentration is mercury (Hg). Exposure to mercury (Hg) can cause changes to blood hematology of fish caused by accumulation of mercury (Hg) that occurs in various types of organs, especially in the kidneys and liver which can suppress hematopoiesis tissue activity. The purpose of this study is to determine the hematological changes, including erythrocyte, leukocyte, Hb, Hematocrit and blood sugar levels in carps. The method used was an experimental method with Completely Randomized Design (RAL) with 4 treatments and 5 replications at the doses of 0 (control), 0.05, 0.10 and 0.15 ppm. The results show that there is a significant difference in each treatment and parameter. There is a decrease of erythrocytes by 1,678,000 cells/ mm³ in treatment D (0.015 ppm), leukocyte increase by 16,360 cells/mm³ in treatment D (0.015 ppm), Hemoglobin decrease by 6.7g/dl in treatment D (0.015 ppm), hematocrit decrease by 10.24% in treatment D (0.015 ppm) and an increase in blood sugar by 99 mg/dl in treatment D (0.015 ppm).

Keywords : Mercury chloride, Hematologic, Blood Sugar, *Cyprinus carpio*.

1. Introduction

1.1. Background of the Study

There are many industrial wastes dumped directly into rivers or surrounding waters today. The presence of heavy metals in water affects the life of aquatic biota, because of the ability of biota to accumulate heavy metals in water (Jakfar et al., 2014). One of the heavy metals that continues to increase in concentration is mercury (Hg) (Widyaningrum and Tutik, 2011). Mercury has been widely used in the fields of medicine, agriculture, industry and mining activities. The waste products can pollute the waters and can accumulate in sediments, in the body of fish and other aquatic biotas (Nirmala et al., 2012).

Mercury chloride (HgCl₂) includes inorganic Hg which is very toxic, caustic and is used as a disinfectant. This type of mercury can cause severe acute toxicity. Water contaminated with heavy metals for long periods of time can cause changes in the immune system, picture of blood and tissue structure/organs of fish (Yuniar, 2009). Exposure to mercury (Hg) can cause changes to the hematology of fish blood caused by accumulation of mercury (Hg) that occurs in various types of organs especially in the kidneys and liver which can suppress hematopoiesis tissue activity (Kondera et al., 2012).

Carp (*Cyprinus carpio*) is an important commodity fish, in 2013 its production reached 500,000 tons with an average increase of 57.21% in 2010-2013 (KKP, 2013). According to Cobalt et



al., (2013) carp is a water biota that has fulfilled the requirements for bio-indicators because carp are adaptable to polluted waters.

If the community continuously consume mercury contaminated fish, the mercury contained in these fish will significantly accumulate in their bodies (Ciptadi et al., 2015). In the case of Minamata disease which began to spread since 1958 in the city of Minamata, Japan is an example of the excess mercury content originating from the Chisso battery industry which caused poisoning and even caused hundreds of people to die and experience nerve paralysis (Yudhiarti et al., 2013) The dead fish around the Minamata bay have methyl mercury levels of 9 to 24 ppm (Putranto, 2011). Meanwhile according to the Food and Drug Administration (FDA) and Indonesian National Standard (SNI), the tolerance limit for consuming mercury in food is 0.3 mg/week or 0.042 mg/day (Ciptadi et al., 2015).

2. Research and methodology

2.1. Time and place

This research was conducted in the laboratory at Faculty of Fisheries and Marine, Universitas Airlangga in April 2017.

2.2. Tools and materials

The equipment used in the maintenance and treatment of carp (*Cyprinus carpio*) for 6 days were: an aquarium measuring 40 x 50 x 35 cm³ with a volume of 40 liters of water as many as 20 pieces each with aerators, hoses and large-sized fishing nets. The equipment used to measure water quality during maintenance and treatment were a thermometer, pH meter and DO-meter. The equipment used in making and observing hematology were 1 ml injection syringes, microtube, Sahli pipette, hemoglobin tube, haemometer sahli, microhematocrit tube with capillary system, hematocrit centrifuge, microhematocrit reader, hemocytometer, glass cover, leukocyte pipette, haemocytometer, glass object, easy touch glucose, Reagent strips and digital cameras.

The materials used in the study were water, mercury chloride, and carps. The ingredients for measuring hematological changes and blood sugar levels were liquid EDTA, blood of Carp (*Cyprinus carpio*), HCl 0.1 N, Hayems solution, Turk's solution, Giemsa 10% and aquadest.

2.3. Work procedures

2.3.1. Preparation of Containers and Test Fish

Research preparation was started by cleaning the equipment used. The aquarium used for maintenance was washed with soap, rinsed with clean water then disinfected in the form of chlorine for 24 hours and dried under the sun. The dried aquarium was each filled with 40 liters of water and aerated. Carps (*Cyprinus carpio*) were acclimatized in order to adapt to the new environment before treatment with exposure to mercury (Hg). Each aquarium was filled with 12 carps (*Cyprinus carpio*).

2.3.2. The Making of Test Media

Making test media was done by calculating each dose of mercury to be dissolved and used as a test medium for each treatment. The concentration of mercury chloride (HgCl₂) or mother liquor at 1000 ppm and the dose used was at 0 ppm (control), 0.05 ppm, 0.10 ppm and 0.15 ppm, each of which was dissolved in 40 liters of water. In making test media, the dilution formula was used (Ezraneti, 2011) :

$$V_1.N_1 = V_2.N_2 \quad (1)$$

Description:

N1: Mercury concentration in stock solution (mg/l)

V1: Volume of stock solution to be collected (ml)

N2: Desired concentration of mercury in water media (mg/l)

V2: The desired volume of research water media (ml)

2.3.3. *Exposure of Mercury to Carp*

The duration of mercury exposure to carp was based on Masud et al., (2009) which stated that a dose of 0.10 ppm mercury chloride can cause changes in body color and hematological response of carps (*Cyprinus carpio*). So, the doses used in this study were 0 ppm (control), 0.05 ppm, 0.10 ppm and 0.15 ppm. During exposure of mercury, the carps were given artificial feed in the form of pellets with the frequency of feeding as much as twice at 08.00 and 16.00 WIB and water quality was measured (pH, DO and Temperature) every day.

2.3.4. *Carp blood sampling*

Blood was taken using a syringe that has been given liquid EDTA as an anticoagulant and inserted into the vertebral bone where caudal vein exists. The blood was allowed to flow in a capillary manner and then sucked by slowly pulling it. The collected blood was inserted into the microtube to be immediately observed for its hematological response.

2.3.5. *Hemoglobin measurement*

Hemoglobin level measurement was done using Sahli method. The principle of this method is to convert hemoglobin in blood to hematinic acid by hydrochloric acid. The blood was sucked using a Sahli pipette to a scale of 20 mm³, the tip of the used pipette was cleaned with tissue paper. The blood was then transferred into the hemoglobin tube containing HCl 0.1 N to scale 2, then left for 3-5 minutes so that hemoglobin reacted with HCl to form hematinic acid. The blood was then stirred and added with distilled water until the color became the same as the standard color. Scale reading was done by looking at the surface height of the solution matched with a G% lane scale, which showed the amount of hemoglobin in grams per 100 ml of blood (Wedemeyer and Yasutake, 1977).

2.3.6. *Hematocrit Measurement*

The blood was sucked using a heparin-coated microhematocrit tube with a capillary system. The function of heparin is to prevent blood clots in the tube (Amlacher, 1970). After the blood reached $\frac{3}{4}$ part of the tube, then one end of the tube was blocked with cristoseal. The capillary tube which was filled with blood was then rotated using centrifuge at 6000 rpm for 5 minutes. Measurements were done by comparing blood body volume to the volume of all blood using a hematocrit scale (Anderson and Siwicki, 1993).

2.3.7. *Calculation of Red Blood Cell Count (Erythrocytes)*

Observation procedures and counting of red blood cell counts in this study were based on Blaxhall and Daisley (1973). The calculation was done by thinning the blood with Hayem's solution in a maximum scale mixing pipette 101. There is a red grain inside this pipette that functions as a stirrer.

The blood was extracted with a mixing pipette up to a scale of 0.5, then with the same pipette smoked Hayem solution to scale 101. The pipette was then shaken to form an eight for 3-5 minutes so that the blood is evenly mixed. The first drop was removed and the next drop was dropped into the hemocytometer and covered with a cover glass. Calculations were carried out in 5 small boxes, namely in the upper left corner, upper right corner, lower left corner, lower right corner and in the middle (Sahetapy, 2012). The calculated number of red blood cells was converted by the formula:

$$\text{Red blood cell count} = \sum \text{counted red blood cells} \times 10^4 \text{ cells/mm}^3 \quad (2)$$

2.3.8. *Calculation Method for Leukocyte Amount*

The method of calculating total leukocytes is explained by Blaxhall and Daisley (1973) that a blood sample is sucked with a pipette containing a white stirrer scale up to a scale of 0.5, then Turk's solution is added to a scale of 11. Stirring was done in a pipette by swinging a hand holding a pipette form an eight for 3-5 minutes until the blood is evenly mixed. The first drop of the blood solution in the pipette was removed, the blood sample was dropped on the haemocytometer, which was then closed with a lid. The total number of leukocytes was 5 boxes with the following formula:

$$\text{The number of calculated leukocyte cells} \times 50 \text{ cells/mm}^3 \quad (3)$$

2.3.9. Counts of blood sugar

Blood glucose measurement in carps was carried out using the Easy Touch Glucose tool. The collected fish blood samples were then dropped into the glucose test strip, then a test strip was inserted into the glucose meter so that the blood glucose results can be read (Samsisko, 2013).

2.4. Research Parameters

The main parameters in this study were the number of erythrocytes, the number of leukocytes, hemoglobin levels, hematocrit levels and blood sugar levels. Supporting parameters included temperature, DO and pH.

2.5. Data Analysis

This study used a method of analyzing data using Variant Analysis (ANOVA) with the study design of a Completely Randomized Design (CRD). If there is a difference, it will be further tested by using the Duncan Multiple Distance Test with a confidence level of 0.05 to determine the differences between all treatments.

3. Finding and discussion

3.1. Erythrocytes of Carps (*Cyprinus carpio*)

The ANOVA test results on Carp erythrocytes (*Cyprinus carpio*) for 6 days show that the increase in exposure to mercury chloride show significantly different results ($p > 0.05$) to changes in erythrocytes in Carps (*Cyprinus carpio*), so that it could be continued with multiple distance tests of Duncan (Duncan's Multiple Range Test). The average graph of erythrocyte values at the level of exposure to mercury chloride can be seen in Figure 5.1

Table 5.1. The average and standard deviation of erythrocyte values for 6 days.

Treatment	$(\bar{x} \pm SD)$
Control	1826000 ^a \pm 73006.85
Mercury Chloride 0.05ppm	1770000 ^{ab} \pm 60827.63
Mercury Chloride 0.10ppm	1768000 ^{ab} \pm 142548.2
Mercury Chloride 0.15ppm	1678000 ^b \pm 82280.01

The same superscript shows a real difference ($p > 0.05$)

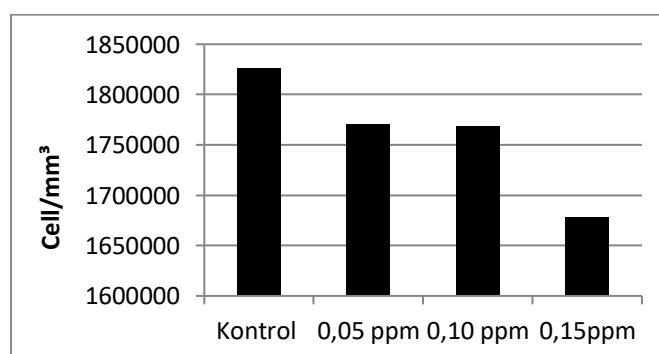


Figure 5.1. Graph of the Average Erythrocytes in Carp

Based on Figure 5.1, changes in erythrocytes in Carp (*Cyprinus carpio*) exposed to mercury chloride (HgCl_2) are decreased along with the increasing dosage. Reduction of erythrocytes occurs in each treatment. As for the control treatment, B (0.05 ppm), C (0.10 ppm) and D (0.15 ppm) show 1,826,000 cells/ mm^3 , 1,770,000 cells/ mm^3 , 1,768,000 cells/ mm^3 and 1,678,000 cells/ mm^3 respectively.

A decrease in the number of erythrocytes occurs because exposure to mercury chloride (HgCl_2) causes reduction in erythropoiesis and inhibits the formation of red blood cells. Metal accumulation of Hg occurs in various types of organs, especially in the kidneys and liver, which allows it to suppress haematopoietic tissue activity. Significant decreases in haematological parameters occur due to the increasing number of destroyed red blood cells (Kori-Siakpere et al., 2009) or haemodilution (Adeyemo, 2005) or damage to the haematopoietic system (Singh et al., 2008). The reduced number of erythrocytes causes the fish to become anemic. Al-Attar (2003) believed that anemia occurs because of the possibility of increased erythrocyte damage or reduced release of erythrocytes in the blood circulation.

3.2. Carp Leukocytes (*Cyprinus carpio*)

The ANOVA test results on Carp (*Cyprinus carpio*) leukocytes for 6 days show significantly different results ($p < 0.05$) on leukocyte changes in Carp. Based on the results of Duncan's multiple distance test, it can be seen that the lowest leukocyte value is seen in the control treatment with an average of 14,050 cells/ mm^3 . The highest leukocyte value is found in treatment D (Mercury Chloride 0.15 ppm) with an average of 16,360 cells/ mm^3 . The graph of the average leukocyte value at the level of exposure to mercury chloride can be seen in Figure 5.2.

Table 5.2. Average and standard deviation of leukocyte values for 6 days.

Treatment	$(\bar{x} \pm SD)$
Control	14050 ^a \pm 2969.26
Mercury Chloride 0.05ppm	15050 ^b \pm 1006.23
Mercury Chloride 0.10ppm	16160 ^c \pm 204.33
Mercury Chloride 0.15ppm	16360 ^c \pm 296.65

Different superscripts show significant differences ($p < 0.05$)

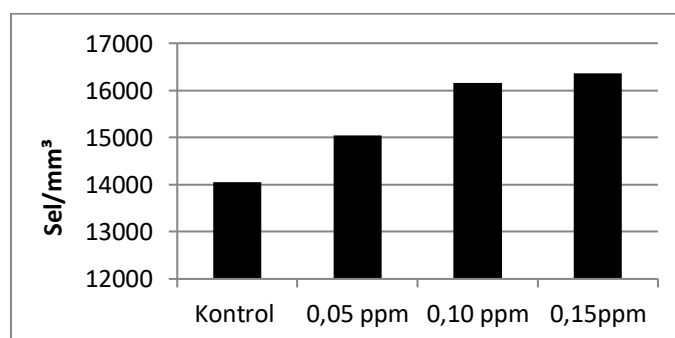


Figure 5.2. Graph of Average Leukocyte in Carp

Figure 5.2 shows that the increase in exposure to mercury chloride (HgCl_2) is directly proportional to the increase in leukocytes in Carp (*Cyprinus carpio*) which is indicated by the average value of the control treatment of 14,050 cells/ mm^3 increased to 15,050 cells/ mm^3 , 16,160 cells/ mm^3 and 16,360 cells/ mm^3 at each increase of 0.05 mercury chloride (HgCl_2).

Exposure to mercury chloride (HgCl_2) causes leukocyte count to increase with high concentration. The toxicity of mercury chloride (HgCl_2) causes stress so that a protective response occurs from the

fish's body which results in an increase in the number of white blood cells (Yuniar, 2009). Leukocytes are blood cells that play a role in the immune system (Mahasri et al., 2011). Improvement is caused because fish can defend themselves from bad conditions due to exposure to heavy metals mercury chloride (HgCl_2) so that the number of white blood cells continues to increase and the fish's body will form antibody. Increased white blood cells occurred through stimulation of the leucopoietic process (Nirmala et al., 2012).

3.3. Carp Hemoglobin (*Cyprinus carpio*)

The results of the ANOVA test on hemoglobin of Carp (*Cyprinus carpio*) for 6 days show significantly different results ($p < 0.05$). Based on the results of Duncan's multiple distance test, it can be seen that the lowest hemoglobin value is seen in treatment D (Mercury Chloride 0.15 ppm) with an average of 6.7 g/dl. The highest hemoglobin value is found in the control treatment with an average of 7.6 g/dl. The graph of the average value of hemoglobin at the level of distribution of mercury chloride (HgCl_2) as shown in Figure 5.3.

Table 5.3. Average and standard deviation of hemoglobin values for 6 days.

Treatment	$(\bar{x} \pm SD)$
Control	$7,6^a \pm 0.24$
Mercury Chloride 0.05ppm	$7,28^{ab} \pm 0.48$
Mercury Chloride 0.10ppm	$6,9^{ab} \pm 0.71$
Mercury Chloride 0.15ppm	$6,7^b \pm 0.22$

Different superscripts show significant differences ($p < 0.05$)

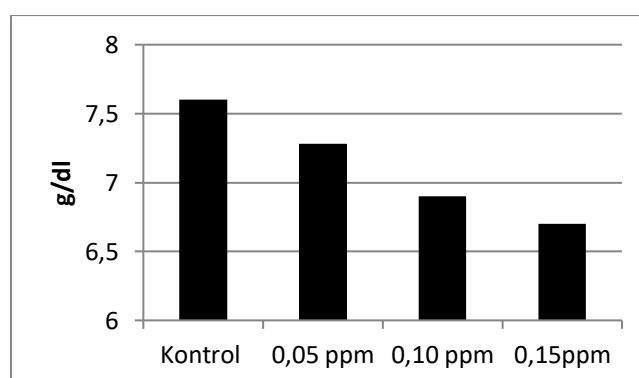


Figure 5.3. Graph of Average Hemoglobin in Carp

Figure 5.3 shows that the increase in exposure to mercury chloride (HgCl_2) is directly proportional to the decrease in hemoglobin in Carp (*Cyprinus carpio*) which is indicated by the average value of the control treatment of 7.6 g/dl decreasing to 7.28 g/dl, 6.9 g/dl and 6.7 g/dl at each increase of 0.05 mercury chloride (HgCl_2).

Results of the study shows that there was a decrease in hemoglobin levels along with the increasing concentration of mercury chloride (HgCl_2). According to Maheswaran et al., (2008), a decrease in hemoglobin levels in fish exposed to heavy metals can be caused by inhibition of the formation of red blood cells due to the direct influence of the metal on the formation of blood cells (kidney or lymph),

increased red blood cell damage due to changes in membrane permeability and increased pressure on the membrane and disruption of absorption of Fe. Decreasing hemoglobin levels indicates that the ability of fish to provide sufficient oxygen for body tissues is limited, resulting in a decrease in physical activity (Scott and Sloman, 2004).

3.4. Carp Hematocrit (*Cyprinus carpio*)

The results of ANOVA test on Carp hematocrit (*Cyprinus carpio*) for 6 days show significantly different results ($p < 0.05$) on hematocrit changes in carp so that Duncan's multiple range test could be continued. Based on the results of Duncan's multiple distance test, it can be seen that the lowest hematocrit value is seen in treatment D (Mercury Chloride 0.15 ppm) with an average of 10.24%. The highest hematocrit value was found in the control treatment with an average of 13.24%. The graph of the average hematocrit values at the levels of distribution of mercury chloride (HgCl_2) can be seen in Figure 5.4

Table 5.4. Average and standard deviation of hematocrit values for 6 days

Treatment	($\bar{x} \pm SD$)
Control	13.24 ^a \pm 0.34
Mercury Chloride 0.05ppm	12.48 ^b \pm 0.32
Mercury Chloride 0.10ppm	11.1 ^c \pm 0.14
Mercury Chloride 0.15ppm	10.24 ^d \pm 3.34

Different superscripts show significant differences ($p < 0.05$)

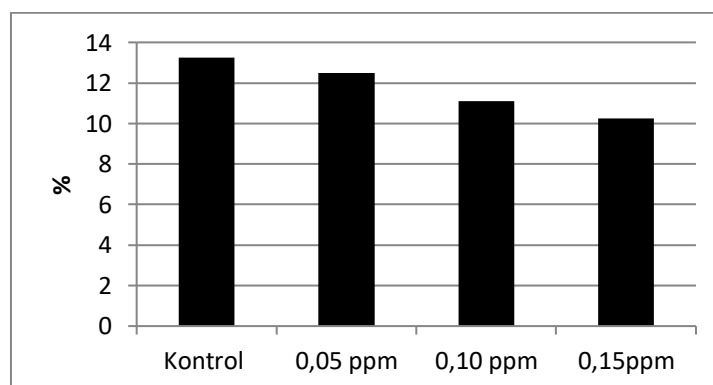


Figure 5.4. Graph of Average Hematocrit in Carp

Based on Figure 5.4 it is known that the increase in exposure to mercury chloride (HgCl_2) is directly proportional to the decrease in hemoglobin in Carp (*Cyprinus carpio*) which is indicated by the average value of the control treatment of 13.24% decreasing to 12.48%; 11.1% and 10.24% for each increase of 0.05 mercury chloride (HgCl_2).

The results show that the increase in exposure to mercury chloride (HgCl_2) is directly proportional to the decrease in hematocrit levels in Carp indicated by the average value of the control treatment of 13.24%, decreasing to 12.48%; 11.1% and 10.24% for each increase of 0.05 mercury chloride (HgCl_2). This decrease in hematocrit levels is due to exposure to heavy metals in fish as stressors have the possibility of causing osmotic imbalances and changing the ion exchange regulation system, which

ultimately reduces blood pH and erythrocyte volume and then decreases hematocrit (Singh et al., 2008).

Decreased hematocrit is directly proportional to the decrease in the number of erythrocytes. If erythrocytes in the blood decrease, the hematocrit levels in the blood will also decrease. Hematocrit values are influenced by several factors including erythrocytes (number, size, shape, comparison of anticoagulants with blood, storage area and homogeneity), environment, gender, species and age of fish at the time of blood sampling (Suhermanto et al., 2013).

3.5. Measurement of Blood Glucose Levels

The results of the ANOVA test on blood glucose in Carp (*Cyprinus carpio*) for 6 days show significantly different results ($p < 0.05$) on changes in blood sugar in Carp. Based on the results of Duncan's multiple distance test, it can be seen that the lowest glucose value is seen in the control treatment with an average of 50 mg/dl. The highest glucose value is found in treatment D (Mercury Chloride 0.15 ppm) with an average of 99 mg/dl. The average graph of glucose change values at the level of exposure to mercury chloride (HgCl_2) can be seen in Figure 5.5.

Table 5.5. Average and standard deviation of glucose values for 6 days.

Treatment	$(\bar{x} \pm SD)$
Control	$50^a \pm 2.45$
Mercury Chloride 0.05ppm	$69.67^b \pm 3.65$
Mercury Chloride 0.10ppm	$78.33^c \pm 0.55$
Mercury Chloride 0.15ppm	$99^d \pm 9.80$

Different superscripts show significant differences ($p < 0.05$)

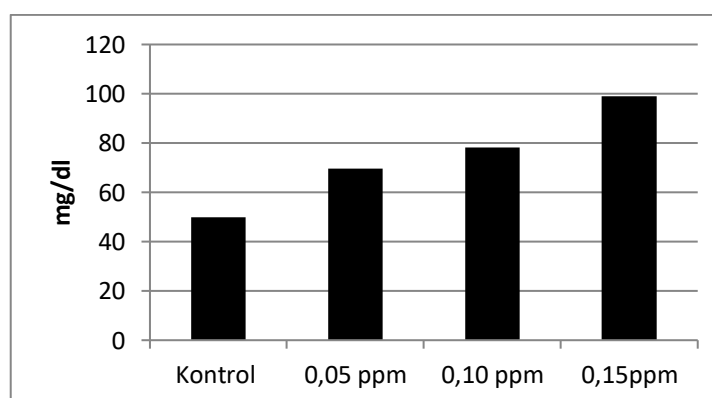


Figure 5.5. Graph of Average blood glucose level measurement

According to Figure 5.5, the increase in mercury chloride exposure is directly proportional to the increase in glucose in Carp (*Cyprinus carpio*) which is indicated by the average value of the control treatment of 50 increasing to 70.4; 78.4 and 99 at each increase of 0.05 mercury chloride (HgCl_2).

In observing blood glucose levels, it can be seen that an increase in blood glucose levels was directly proportional to the high concentration of mercury chloride (HgCl_2). Exposure to heavy metals causes fish to become stressed so that there is an increase in blood glucose levels in fish (Sahetapy,

2011). According to Barton (2002), when experiencing stress, fish will experience a primary and secondary response, an increase in blood glucose is a secondary response from fish experiencing stress, after the primary response is an increase in the number of stress hormones such as cortisol and catecholamines from internal cells. According to Rahmawati et al., (2010) in a stressful state there is an increase in glucocorticoids which results in an increase in blood glucose levels to cope with high energy needs when stressed.

3.6. Water Quality

Water Quality			
Day-	Temperature (°C)	pH	DO mg/L
1	28	7	3.8
2	28	7	3.8
3	28	7	3.8
4	28	8	3.7
5	29	8	3.5
6	29	8	3.5

Carp (*Cyprinus carpio*) can grow optimally at a temperature range of 23-30°C with a pH between 6.5-9.0. Carp can live in waters with low dissolved oxygen levels (0.3-0.5 mg. l⁻¹) (Flasjhans and Hulata, 2006). The data in the table shows that on exposure to mercury chloride (HgCl₂) for 6 days, the water quality, including temperature, pH and DO are in optimal conditions so that it is still able to support the survival of carp (*Cyprinus carpio*).

4. Conclusion

1. Exposure to mercury chloride (HgCl₂) at a dose of 0.05 ppm, 0.10 ppm and 0.15 ppm affects the hematological changes (erythrocytes, leukocytes, hemoglobin and hematocrit) and blood sugar levels in carp (*Cyprinus carpio*).
2. The toxicity of mercury chloride (HgCl₂) causes a decrease in the number of erythrocytes from 1,826,000 cells/mm³ to 1,678,000 cells/mm³ in treatment D (0.15 pmm), an increase in leukocytes from 14,050 cells/mm³ (control) to 16,360 cell/mm³ in treatment D (0.15 pmm), a decrease in hemoglobin level from 7.6 g/dl to up to 6.7 g/dl of treatment D (0.15 pmm) and a decrease in hematocrit from 13.24% to 10.24% D (0.15 pmm).
3. Exposure to mercury chloride (HgCl₂) causes an increase in blood glucose levels which is directly proportional to the increase in the dose of mercury chloride (HgCl₂) with an average of 50 mg/dl (control), 70.4 mg/dl (0.05 ppm), 78,4 mg/dl (0.10 ppm) and 99 mg/dl (0.15 ppm).

5. Suggestion

Based on the results of the study, it is recommended that further studies be conducted on the effect of exposure to mercury chloride (HgCl₂) at the same dose but by measuring hematological responses on a matter of hours to determine the minimum exposure time for mercury chloride (HgCl₂) that can affect hematological responses and blood sugar levels in carp (*Cyprinus carpio*).

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